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BRUSSELS, BELGIUM • SEPTEMBER 16-18, 2021



ABSTRACT BOOK

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SYMPOSIUM LECTURE abstracts

THURSDAY, September 16, 2021

10:30 – 12:30 h

Symposium 1: ROOT CARIES AND NCCLS: CHALLENGES OF AN AGEING POPULATION (supported by *GC*)

Jo Frencken
Radboud University, Malden, Netherlands

The International Caries Consensus Collaboration (ICCC) has initiated the publication of a monograph about the 'Evolution of treating cavitated carious lesions' in 2018. The book presents modern cariological principles that have been derived from decades of research and is considered an essential part of the current philosophy of managing dental caries that is encompassed in Minimal Intervention Dentistry (MID). The aim of MID is 'Teeth for Life'. As the life expectancy of people is increasing over time, reaching 80-90 years, preservation of tooth tissue at a young age is essential in order for the elderly to have a sufficient number of functional teeth. The modern cariological principles have also implications for the treatment of carious lesions in elderly. The European Union (EU) has banned the use of amalgam for children under 15 years of age, breastfeeding and pregnant women. This ban poses the question: which restorative is the successor to amalgam? The question becomes even more important as many resin composites, one of the successors, contain monomers (BPA's) which are considered by the EU as an unwanted substance in the environment. This information makes glass-ionomer cement, another successor, a very interesting material to understand and discuss. The material has improved in mechanical and aesthetic characteristics over the last decades and is completely different from versions available in the 1980s and 1990s. The modern principles of cavity excavation and the use of modern glass-ionomers come together in the Atraumatic Restorative Treatment (ART). Not only is ART successfully used in children but it is also increasingly used in treating carious cavities in adults and elders. Because it uses hand instruments, ART is mobile and therefore, holds the opportunity to provide caries care for vulnerable elders at their homes, daycare centres and institutions. The survival of ART/HVGIC restorations meets those of resin composites.

Glass Hybrid as the Choice for Class v Restoration: Results of a Clinical Study

Falk Schwendicke
Chair and Head, Department of Oral Diagnostics, Digital Health and Health Services Research, Charite University, Berlin, Germany

For restoring cervical restorations, dentists have a range of options, differing in their application mode, physico-chemical properties, and costs. The presentation will first discuss the wider evidence base supporting different materials; second present the design and interim results of a randomized controlled trial comparing a glass hybrid and a composite material for cervical restorations in elderly individuals, and third evaluate the cost-effectiveness of different materials for this indication. The presentation targets researchers in gerodontology, restorative dentistry, and health services research as well as practitioners.

Optimizing Treatment Options for the Ageing Population – a Practical Approach

Gerald J. McKenna
Clinical Reader and Consultant in Restorative Dentistry, Centre for Public Health, Queens University Belfast, Belfast, United Kingdom

Objectives: To describe the changing oral epidemiology of older adults including increased natural tooth retention and increasing prevalence of chronic destructive dental diseases, particularly caries. This lecture will describe preventative and operative approaches for caries management in older adults.

Methods: Current research evidence will be summarised on population demographics; oral epidemiology and the aetiology of coronal and root caries. Particular attention will be drawn to the changing picture of oral health of dependent older adults within residential care and the challenges this poses for healthcare professionals. Practical solutions will be presented on preventative regimes and operative management of caries for older adults including the use of minimally invasive techniques such as Atraumatic Restorative Treatment.

Results: Research data will be presented from clinical trials on the effectiveness of Atraumatic Restorative Treatment for older adults in operative management of coronal and root caries. Data gathered from ongoing research will also be presented on development of preventative regimes for dependent older adults using a collaborative co-design approach with patients and carers.

Conclusions: As the oral health of the ageing population continues to evolve, dental professionals should be implementing effective preventative approaches alongside minimally invasive operative care, such as Atraumatic Restorative Treatment, for partially dentate older adults.

Effects of Calcium Silicates on Pulp Inflammation and Regeneration

Imad About
Aix-Marseille Université, Faculté d'Odontologie, Marseille, France

Pulp healing/regeneration following pathological conditions represents a real challenge in the provision of a suitable treatment that ideally leads to the induction of the dentin-pulp regeneration. However, the pulp response to tooth traumatic injury/carious lesions is highly dependent on the pulp inflammatory degree mainly due to its location within rigid dentinal walls. Thus, while the initial inflammation represents a pre-requisite for pulp healing, a rapid resolution of inflammation would favor the regenerative process which is key for a successful clinical outcome. Recent data have shown that injured pulp fibroblasts, which occurs under carious/traumatic injury, provide a series of biologically active molecules via Complement system activation. Among these active molecules, C5b and C5b-9 are involved in the local control of infection by killing cariogenic bacteria directly and inducing their elimination by phagoytosis. Other molecules such as C3a and C5a have shown a clear implication in pulp stem cell activation, proliferation and recruitment. These data clearly demonstrate that, upon injury, the pulp fibroblast exert a local control of the initial steps of inflammation and regeneration. In addition, the pulp cells control the inflammatory reaction by secreting multiple cytokines and growth factors that affect the balance between pulp inflammation and regeneration. Application of tricalcium silicate-based materials directly onto the pulp has been shown to modulate the inflammatory activity by decreasing pro-inflammatory factors and inflammatory cell recruitment. At the same time, the materials' hydration/by-products release and their interaction with the soft tissues induce stem cell proliferation and recruitment, thus, shifting the tissue response towards regeneration. This presentation will explain how a good knowledge of bioactive tricalcium silicates properties combined with the pulp anti-inflammatory/regeneration potentials can provide a rational in symptomatic carious exposure treatment as well as pulp exposure.

Calcium Silicates in Restorative Dentistry and Prosthodontics

Athina Bakopoulou
Department of Prosthodontics, School of Dentistry, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece

Preservation of pulp vitality in cases of deep carious lesions or pulp exposure after mechanical trauma is a critical factor determining the prognosis of teeth after restorative treatment. Calcium silicate-based bioactive cements have been proven successful when used for direct or indirect pulp capping (DPC/IPC) in Restorative Dentistry. In contrast, very limited clinical evidence exists on their efficacy in vital pulp therapy in Prosthodontics, either as cavity liners underneath indirect restorations or as core-build up materials of teeth serving as prosthetic abutments. This presentation will provide an overview of current clinical evidence on the outcomes of application of calcium silicates in vital pulp therapy (DPC or IPC) in restorative dentistry and prosthodontics. Besides, clinical cases demonstrating successful application of a calcium silicate-based cement (Biodentine™) in deep carious lesions of abutment teeth receiving indirect restorations or full-coverage crowns will be presented, and a step-by-step clinical protocol illustrated and discussed. Overall, factors in favor of application of calcium silicates in restorative dentistry and prosthodontics include their enhanced mechanical and handling properties, good sealing ability, and development of a firm bond with the underlying dentin substrate. Based on current evidence, calcium silicates are highly recommended for teeth of younger patients with mild symptoms of reversible pulpitis, following a strict IPC or DPC aseptic protocol.

Calcium Silicates in Regenerative Endodontics/Revitalization

Kerstin M. Galler
Department of Restorative Dentistry and Periodontology, University of Regensburg, Regensburg, Germany

To maintain or re-establish pulp vitality is an essential goal in conservative dentistry. Regenerative endodontics enable the replacement or healing of damaged structures of the dentin-pulp complex. After partial damage, vital pulp treatment is a means to restore the tissue's functionality. Advances in the fields of pulp biology and immunology, a deeper understanding of microbiological challenges and the availability of calcium silicates have improved the prognosis after vital pulp treatment considerably. Still, carious exposures are different from traumatic injuries, and different approaches (pulp capping, partial or full pulpotomy) have to be chosen depending on the clinical situation. After the complete loss of pulpal tissue, revitalization is a means to re-establish vital tissue in a formerly empty root canal. Revitalization is indicated in immature teeth with pulp necrosis and thus an alternative treatment option to the apical plug. Healing occurs after sufficient disinfection followed by provocation of bleeding into the root canal. New tissue formation occurs, which may involve a continuation of root formation. The use of calcium silicates is recommended for vital pulp treatment as well as for revitalization. This lecture will discuss biological and clinical aspects, cover procedural details and potential problems and highlight the influence of these materials on success and outcome.

13:30 – 15:30 h

Symposium 3: NEW ADVANCED GLASS CERAMIC TECHNOLOGY AND STATE-OF-THE-ART IN DIGITAL IMPRESSIONS (supported by *Dentsply Sirona*)

New Advanced Lithium Disilicate Ceramic – Clinical Experience

Sven Rinke
University of Göttingen, Göttingen, Germany

High-strength glass-ceramic materials offer the ideal combination of translucency and strength, especially for the fabrication of minimal-invasive restorations (veneers, partial crowns, table-tops). State-of-the-art materials such as Cerec Tessera and Celtra Press offer strength of up to 750 MPa (biaxial flexural strength). They are available for the production of chairside restorations (Cerec system) as well as for the conventional lab-supported fabrication process (labside). This allows for interesting ranges of application for minimal-invasive procedures such as extended ceramic veneers as an alternative to conventional crowns or adhesively luted partial crowns.

The improved mechanical properties of these materials provide for a minimal-invasive preparation for inlays and partial crowns, especially in the molar region, as the minimum material thickness can be reduced to 1 mm. Moreover, these materials offer improved optical properties and excellent polishing characteristics. They are, therefore, an excellent choice for monolithic restorations.

The all-ceramic treatment concept introduced in this webinar includes recommendations regarding indications and materials. Step-by-step instructions for every single clinical stage, from preparation to cementation, allow a direct implication of the content in daily routine. The concept covers both the fabrication of restoration in one appointment only (Cerec system) as well as the labside processing.

New Advanced Lithium Disilicate Ceramic – Development and Technical Insights

Erik Büchler
Development & Application Technology, Dentsply Sirona, Hanau, Germany

A new high strength glass ceramic with zirconia and virgilite crystals is presented. Discussion focuses on microstructure and the material's mechanical, chemical and fatigue test data. The resulting excellent machinability via CAD/CAM grinding and esthetics make the material perfect for high-end full-anatomical ceramic crowns. The high strength glass-ceramic is an integral part of a faster firing workflow when combined with a high-speed dental furnace. Fracture strength of the new high strength glass ceramics is above 700 MPa (biaxial). Wear of the material is in the range of human enamel. A CTE of 9,8 $x10^{-6}$ K^{-1} allows the material to be veneered as cut-back / correction with current veneering ceramics, if so desired. Two main types of crystal structures are embedded in an amorphous (glass) matrix: Lithium Disilicate crystals with a mean size of approx. 600nm and Virgilit with a mean size of approx. 200nm. Both crystal types are the source of an opalescence effect and together with oxides for pigmentation result in a natural tooth look. The material shows excellent marginal stability, allowing a reduction of wall thickness to min. 1,0 mm (adhesive) and min. 1,5 mm (glass ionomer) during CAD/CAM grinding. The minimum wall thickness for veneers is 0,4 - 0,6 mm. Dental restorations made from this new high strength glass ceramics require only a short glaze firing: When using a high-speed dental furnace the firing cycle time takes – even with stains – only 4,5 minutes. This new high-strength glass-ceramic is available under the tradename Cerec Tessera by Dentsply Sirona.

State-of-the-art in Digital Impressions

Andreas Ender
University of Zurich, Zurich, Switzerland

Digital impressions are constantly evolving and thriving more and more in the dental office. While the number of intraoral scanning devices is increasing and updates of current systems happen on a regular basis, the question is still how to use these, often expensive, devices to the best effort. Accuracy, ease of use, scanning technique, clinical indication are some of the keywords that always connect with digital impression systems. The aim of this lecture is to give an actual overview of digital intraoral impression systems. The level of accuracy and the comparison to well-established conventional impression techniques will be shown. The clinical indication based on accuracy and scan strategy can then serve as a guideline for the dentist, whether or not to use such systems in the dental office right now.

The Underlying Mechanisms of Pre- and Probiotics and Their Potential Role in Oral Health

Egija Zaura
Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, Netherlands

At health, microorganisms and their human host live in symbiosis: the microbes get the right nutrients and environment for their growth, in turn providing the host with the benefits for his or her health. This host-microbe relationship evolves in the first years of life, and, once established, remains stable. The specific properties of the host such as salivary pH and composition and the behavior of the host such as smoking, hygiene habits or intake of fermentable carbohydrates will shape the oral microbial community and its activity, resulting in a highly individual oral microbiome. In some cases, the interplay of these factors may lead to dysbiosis – a disturbed relationship between the host and its microbes - and even disease. If the host-microbe relationship is so stable, is it then possible to return to the balanced situation once it has entered the state of dysbiosis? In this presentation, two approaches gaining popularity in microbiome modulation – adding beneficial microbes (probiotics) to the oral ecosystem or promoting their growth with specific substrates (prebiotics) will be discussed. The current hypotheses on the potential underlying mechanisms of these two approaches will be critically assessed. Additionally, directions for further research will be highlighted.

Current Evidence of Using Pre- and Probiotics in the Prevention and Management of Caries and Oral Candidiasis

Svante Twetman
Dept of Odontology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen N, Denmark

Recent insights on the decisive role of the oral microbiome in health and disease have paved the way for pre- and probiotics in the prevention and management of oral diseases. Pre- and probiotics are however not separate entities as they always act in concert. For dental caries and oral candidiasis, local mechanisms of action in the biofilm seem predominant; growth inhibition, release of antibacterial substances and reduction of metabolic biofilm activity. Among the traditional prebiotics, arginine and xylitol supplements are effective for the ecological management of dental caries. Due to risk of bias in many of the clinical trials, the certainty of evidence is so far low. For probiotics, the first 1000 days of life provide a window of opportunity for modulating the microbiota through interventions with beneficial bacteria. Consequently, multiple studies have addressed early childhood caries prevention with milk containing probiotic bacteria served at daycare centers. A systematic review covering seven trials have indicated a prevented fraction of 43%. Studies are also available from caries-active schoolchildren and frail elderly with promising but yet not confirmed results. Probiotics exert excellent antifungal activities and may be suitable for ageing populations with mycosis due to chronic diseases and polypharmacy. Systematic reviews indicate that probiotics are superior to the placebo for the prevention and treatment of oral candidiasis in the elderly and denture wearers. Further clinical trials on the synergistic effects of pre- and probiotics are welcome to optimize the applications for oral health.

Current Evidence of Using Pre- and Probiotics in the Prevention and Management of Periodontal Diseases and Halitosis

Wim Teughels
KU Leuven, Leuven, Belgium

Periodontal diseases and halitosis are both associated with dysbiotic biofilms that are located on either the tongue or in the subgingival area. For both diseases, often antimicrobials are used as adjuncts in their treatment or prevention. However, current evidence shows that these antimicrobials are not without risk. Therefore, new treatments are more and more focusing on balancing the oral microbiome and bringing it into a state of homeostasis. To achieve this, probiotics and potentially prebiotics can be used. This presentation will focus on the current evidence on the use of probiotics for periodontal health and halitosis and will introduce the emerging knowledge on prebiotics for periodontal diseases.

The Properties of Zirconia from a Clinical Perspective.

Per Vult von Steyern
Odontology, University of Malmö, Malmö, Sweden

To gain aesthetic properties resembling the natural teeth, zirconia reconstructions were initially veneered with porcelain, with the consequence that chipping became a commonly seen drawback in the early adoption of the material. Interest in further developed zirconia materials, more translucent ones that could be used monolithically, without the fragile porcelain was therefore raised. Today, several types of translucent zirconia material can be used for different clinical purposes, but they all have that in common that increased translucency comes with the price of decreased strength and toughness. In the clinical situation, however, there are many options where 3Y-TZP, 4YSZ, 5YSZ etc. can be used depending on expectations on aesthetic- and mechanical properties in combination with design strategies aiming at the best clinical outcome. The objectives of the first part of the lecture will be to address reconstruction designs, material combinations, and material properties from the clinical perspective.

Zirconia: Fundamental Aspects and Practical Implications

Amund Ruud
NIOM Institute, Oslo, Norway

Zirconia is one of the most commonly used restorative dental materials. Through material development, improved strength and esthetics have resulted in increased adoption. The fundamental understanding of zirconia is a crucial part of this progress. The manipulation of the doping levels, typically in the range 3 – 6 mol%, of Y_2O_3 in ZrO_2 is the most important factor in this optimization, with increasing Y_2O_3 levels associated with improved esthetics and reduced strength. Its crystal structure is at the center when evaluating these factors for dental zirconia. Typically, one takes basis in the pure ZrO_2 system and its three known crystal structures: cubic (*c*), monoclinic (*m*), and tetragonal *(t*). This includes the well-known martensitic phase transformation, $t \rightarrow m$, toughening the material during mechanical stress. The introduction of Y_2O_3 increases the complexity of this system, however, with another tetragonal phase, *t'*, occurring. In order to understand the clinical effects such as low-temperature degradation, translucency, and strength, increasing the fundamental understanding is essential. The aspect of sintering conditions is crucial, with the manipulation of time, temperature, and atmosphere all being important factors when evaluating esthetic and mechanical properties.

What is New on the Transformation of Zirconia Ceramics for Dental Applications?

Fei Zhang[2, 1], Jérôme Chevalier[3], Maoyin Li[2, 1], Bart Van Meerbeek[1], Jef Vleugels[2]
[1]Department of Oral Health Sciences & Department of Materials Engineering, KU Leuven, Leuven, Belgium, [2]Department of Materials Engineering, Kasteelpark Arenberg, KU Leuven, Leuven, Belgium, [3]MATEIS, University of Lyon INSA Lyon, Villeurbanne, France

Zirconia having a unique toughening mechanism through stress-induced transformation has been known for a long time since it was first reported as "ceramic steel" about forty years ago. In dentistry, zirconia, especially when stabilized by yttria, has become one of the most important ceramics to fabricate fixed dental prostheses (FDPs) and implants. The transformation is hereby still the subject of extensive research, as it plays a key role in the strengthening, toughening, reliability and long-term stability (longevity) of zirconia prostheses. With regard to dental restorations, zirconia of higher translucency is in need in particular for monolithic restorations, but there is always a compromise between translucency, mechanical resistance (potential of transformation toughening) and aging stability (water-induced transformation). A range of zirconia ceramics with different composition and microstructure is thus proposed. Here, we will discuss different dental zirconia, focusing on the influence of increasing yttria content - the most robust way of enhancing zirconia's translucency - on their strength, crack resistance and reliability, as compared to lithium-disilicate (LS_2) glass ceramic. Importantly, due to the lack of transformability, zirconia stabilized with a higher yttria content showed not only reduced loading capability (using standardized bars and a four-unit FDP) but also lower reliability, while 5 mol% yttria-stabilized zirconia may approach the bottom limit of the window between conventional 3Y-TZP and LS_2 glass ceramic. On the other hand, the lower requirement on translucency for dental implants opens up the possibility of using alternative stabilizers, such as ceria-stabilized zirconia. The most intriguing properties obtained in our developed Ce-TZP-based ceramics are their exceptionally high reliability with limited strength scatter and damage tolerance. This is attributed to the benefits from transformation-induced plasticity and higher crack-initiation resistance. The feature of such materials will also be highlighted by its suitability for additive manufacturing.

16:00 – 18:00 h

YOUNG CED-IADR Symposium: NEW TRENDS IN TOOTH RESTORATION: ARE WE GETTING CLOSER TO CURING DENTAL DISEASE? (supported by *Colgate*)

Bacterial Interactions with Restorative Materials: how Close are we to Defeating the Invisible Enemy

Cher Farrugia

School of Clinical Dentistry, University of Sheffield, Sheffield, United Kingdom

Secondary caries is the main reason of failure of composite resin or glass-ionomer cement restorations and is most common at the interface between the restoration and the prepared cavity. The tooth structure is demineralized following invasion of acid producing bacteria, when fermentable carbohydrates are present. An effective antibacterial/bactericidal restorative material would therefore be in the ideal location to prevent secondary decay, especially since it has been shown that cariogenic bacteria, such as *Streptococcus mutans* can adhere to restorative materials. Although implementation of antimicrobial restorative materials is not a new concept, very few materials make it to market, partly due to the discrepancies between *in vitro* testing and the complex clinical scenario in which materials are used. Our research showed that processes employed in some antimicrobial tests such as sterilisation of the material prior to testing can alter material properties and therefore result in a material different to what is clinical used. In another series of studies we've shown that materials can behave differently after aging or after exposure to a clinically relevant environment (e.g. saliva, blood, dentine). Recent reviews also showed that most published research only challenges materials with *Streptococcus mutans* which isn't representative of the environment in the oral cavity and that most research doesn't assess other properties (physical, chemical or biological) that might be altered by the introduction of antimicrobial properties. This talk will highlight recent advances while discussing ways to potentially overcome current shortcomings in some of the experimental models used to test antimicrobial properties of restorative materials.

Regenerating the Dentine-Pulpcomplex Defect with Biomaterials: the Clinical Reality

Nastaran Meschi

KU Leuven, Leuven, Belgium

Post-natal wound healing is a combination of repair and regeneration. However, it has been described that, dependent on the tissue type, from a certain lesion size spontaneous wound healing is impeded. The latter is known as critical-sized defect and is the basis for tissue engineering and biomaterial development. Furthermore, it is crucial to know that the volume and quality of injury repair is dependent on the restorative material applied. The dental pulp is a small but complex entity. Even if in regenerative medicine many biomaterials and scaffolds have been developed and the adequate pool of mesenchymal stem cells and growth factors have been found to aid dental pulp regeneration, the critical-sized defect of the dentine-pulp complex is yet to be determined. Considerable efforts have been made to enhance the properties of hydraulic tricalcium silicates. Our research has shown that biomaterials aid bone regeneration in endodontic microsurgery, while repair is seen in revitalization procedures. Not only adequate disinfection remains an issue, but also a discrepancy in bioactivity potential exists when *in vitro* results are compared to *in vivo*. Natural scaffolds, such as blood platelet concentrates, are promising as they are autologous and hence inexpensive. Moreover, leukocyte and platelet rich fibrin (LPRF) is promoted as a bioactive scaffold. Firstly, due to its favorable centrifugation protocol, not needing any additives. Secondly, due to its concentrated matrix of leukocytes and platelets, releasing growth factors over a longer period of time. Despite all, our clinical trials in endodontic microsurgery and revitalization procedures cannot recommend LPRF.

Pulp Response to Potential Threats: Walking a Tightrope Between Inflammation and Regeneration

Matthias Widbiller

Department of Conservative Dentistry and Periodontology, University Hospital of Regensburg, Regensburg, Bavaria, Germany

The dental pulp is an immunologically active connective tissue that defends itself against external stimuli such as caries. The demineralization of dentine matrix by bacterial acids and penetration of cariogenic microorganisms trigger various defence mechanisms. In the course of the disease, the odontoblasts as the most peripheral cells are the first to encounter carious stimuli and produce reactionary dentine in order to reduce dentine permeability and prevent penetration of microorganisms into the pulp. At the same time, pulp cells respond by secretion of proinflammatory cytokines and antimicrobial peptides, which leads to the recruitment of immune cells that aim to destroy invading pathogens. From a clinical perspective, the defence mechanisms of the dental pulp are of great importance and can be exploited in various ways. In addition to biochemical cues from dentine matrix and bacteria, bioactive dental materials used in deep cavities to protect pulp tissue stimulate dentine formation. Besides the restoration, the control of the pulp inflammation is crucial for regeneration. Contrary to earlier beliefs, a pulpitis cannot only be distinguished as reversible or irreversible but represents a dynamic spectrum with many biological and spatio-temporal intermediates. Whereas removal of the pulp and root canal treatment has traditionally been the only treatment, various innovative approaches, e.g. partial amputations, are conceivable today. As demonstrated by our and other scientific work, modulation of the inflammation through biologically based concepts of caries excavation and the use of bioactive materials, as well as selective amputation make it possible to maintain the vitality of the pulp in whole or in part.

Prevalence and Multifactorial Aetiology of Periodontal Recessions

Sonja Sälzer
University of Kiel, Kiel, Germany

Many patients demonstrate gingival recessions. Although the prevalence is increasing by age pronounced recessions can already be present in younger patients in particular with good oral hygiene. For prevention of advanced recessions, it is of major importance, that the dental team is familiar with the diagnosis of beginning clinical signs. Furthermore, with the knowledge of possible risk factors patients should conclusively be carefully instructed. The etiology is multifactorial and always a combination of various anatomical factors and the influence of pathological and physiological causes. Besides risk factors such as plaque, gingivitis, smoking, gender and age tooth brushing is discussed. In particular, a correlation of recessions with tooth brushing frequency, tooth brushing duration and brushing technique as well as bristle hardness and the frequency of brush changes has been found, whereby horizontal scrubbing is most strongly associated with high recession. Whether power toothbrushes pose a clinically relevant risk compared to manual toothbrushes was investigated in long-term studies with an observation period of one to three years in patients with pre-existing recessions and thus in a group of patients with a higher risk of further progression of recessions published. These studies found for power toothbrushes (sonic, multi-directional or rotating oscillating) as well as for the control manual toothbrush a slight but statistical decrease in gingival recession with no difference between manual and power brushes. Conclusively, not the brush itself but the way it is used seem to determine whether trauma occurs.

Treatment Choices of Recessions in the Context of Patient Esthetics

Virginie Monnet-Corti
University of Marseille, Marseille, France

The visibility of pink (the gum) during the natural and forced smile, periodontal health, gingival contour, gingival aesthetic line, the presence of papillae will allow to establish a gingival aesthetic score, to determine the origins of the disharmony and the envisaged treatments. We will see through many clinical cases how, and at what point in the overall treatment plan, additive or subtractive periodontal plastic surgery can modify the gingival appearance and morphology in order to restore a harmonious smile. Based on the new classification of gingival recessions, we will propose a very precise way of analyzing the case and a decision tree to choose the best surgical technique for root coverage. Our pink aesthetic goal and the patient's expectations should be achieved with "tailor-made" treatment plans that may consist of a purely periodontal approach or combine other odontological disciplines such as aesthetic (cosmetic) dentistry and orthodontics.

Surgical Treatment of Recessions and Maintenance of Clinic

Giulio Rasperini
University of Milan, Milan, Italy

In the last years the aesthetic demand from the patients has become the biggest challenge in Periodontology. Besides functional results, it is now important to achieve aesthetic success, particularly in the anterior areas, where the expectations of the patients are even higher. A consequence of infection control may be shrinkage of the soft tissue with obvious aesthetic impairment. In the past, soft tissue contraction was generally considered necessary to achieve periodontal health. Nowadays gingival recession can be partially prevented, diagnosing the tissues characteristics, the phenotype and selecting the proper instruments for both, home oral hygiene and professional. For all these reasons root coverage procedure is a very popular procedure nowadays in periodontology. In a RCT study that involved 60 patients, Acunzo et al., 2016 evaluated the efficacy of the powered toothbrush oscillating rotating when compared with the manual toothbrush. This study concluded that the “Use of an oscillating-rotating powered toothbrush with a soft-bristle head resulted in higher GM stability after root coverage procedures compared with the use of a manual soft-bristled toothbrush” In 2018 Rasperini at al shows the 9 years outcome of patients treated in a RCT with coronally advanced flap alone or with connective tissue graft and maintained with Powered Toothbrush oscillating rotating with a soft-bristle head. In the conclusion of the study was reported: “The protocol adopted for oral hygiene maintenance (2–3 times/ years professional recall and use of oscillating-rotating toothbrush for daily dental care) is suitable for the long-term maintenance of results.” In this presentation rationale and evidence for maintenance after mucogingival surgery will be discussed.

From Lab to Clinic - a Systematic Approach to Test Zirconia Materials

Siegward Heintze
Research & Development, Ivoclar Vivadent AG, Schaan, Liechtenstein

Translucent multilayered zirconia materials offer the advantage of providing monolithic restorations whose aesthetic and translucent qualities closely resemble those of restorations made of conventional glass-ceramic materials. However, many manufacturers recommend the same or similar design parameters for crowns and bridges, irrespective of the type of zirconia material used and irrespective of whether the restoration is placed on natural teeth or on implants. Yet, the higher the yttria-content and the lower the alumina-content is, the lower the mechanical strength of the zirconia material will be. Furthermore, the masticatory forces exerted on implants are higher than those on natural teeth. Therefore, the design criteria should be adapted in line with the mechanical strength of the material and the area of application. Another parameter that should be considered is the position of the restoration on the milling disc, as the translucency and mechanical strength of these discs vary across the different layers. This presentation examines the effect of different design criteria (occlusal thickness, connector size, positioning within the disc, crown and connector transition) on the mechanical strength of the restoration by looking at the results obtained in long-term dynamic loading experiments carried out on different translucent multilayered zirconia materials. A systematic approach to establish suitable design criteria will be presented, including a validated test method, qualified test equipment, clinically validated acceptance criteria and computational simulation (Finite Element Analysis).

Zirconia in Clinical Service – No More Limits?

Peter Rammelsberg
Prothetik, Poliklinik für Zahnärztliche, Heidelberg, Germany

Introduction: Metal-ceramic single crowns (SCs) and fixed dental prostheses (FDPs) were long the gold standard for fixed restorations. Zirconia frameworks combined with complete ceramic veneers have however become established as a metal-free alternative. Increased technical complication rates have however compromised the long-term performance of all-ceramic restorations.

Objectives: The objective of a series of clinical studies on tooth-supported and implant-supported restorations was to investigate the survival and complication rates of zirconia-based SCs and FDPs - including monolithic and partially veneered restorations compared to metal-ceramic restorations.

Methods: From several prospectively documented clinical studies, 652 implant-supported SCs and 434 FDPs (with observation periods of up to 12.8 years) were included. 417 tooth-supported FDPs and 148 SCs were also investigated. More than 40% of the restorations were made with zirconia frameworks including a considerable proportion of restorations with monolithic design or partial veneers restricted to the buccal surface. Kaplan-Meier analyses were performed to compare survival and the incidence of complications for different framework materials and veneering techniques. Cox regression analyses were used to isolate risk factors.

Results: FDPs predominantly failed because of biologic complications (loss of abutment teeth or implants and secondary caries). Fractures of ceramic veneers were the most frequent technical complications, whereby zirconia frameworks demonstrated significantly higher chipping incidences compared with metal-ceramic FDPs. Long-span FDPs (>3 units) did not differ from 3-unit FDPs. Implant-supported and tooth-supported SCs showed high 5-year survival rates (>95%). Technical complications accounted for most failures with SCs in contrast to FDPs. Again, zirconia frameworks with complete veneers demonstrated significantly higher chipping rates. However, zirconia SCs with partial veneers or monolithic designs exhibited the lowest technical complication rates.

Conclusions: The success rate of tooth-supported and implant-supported SCs and FDPs can be significantly improved if complete veneers are avoided. The clinical performance of highly translucent zirconia with reduced flexural strength has still to be evaluated.

Bonding Zirconia - Concepts and Challenges

Mutlu Özcan
Center for Dental and Oral Medicine, Dental Materials Unit, University of Zurich, Zurich, Switzerland

Due to the introduction of new materials used in conjunction with CAD/CAM technologies, along with advances in adhesive technologies, conventional prosthetic dentistry has evolved into a less invasive discipline. In this regard, zirconia became a frequent material of choice for either tooth- or implant-borne reconstructions. Besides its definite mechanical advantages, some aspects of zirconia such as “adhesion” hold potential for further development. Over the last few decades, numerous research papers have been published on the topic of adhesion to zirconia and some guidelines could be established. Yet, with the introduction of translucent zirconia and its minimally invasive applications, bonding zirconia became a focus of research again. The ideal adhesion of resin cements to both dental tissues and zirconia requires the meticulous application of conditioning and cementation protocols. This lecture highlights the clinical and technical parameters during adhesive cementation/repair of zirconia and proposes guidelines in a step-by-step fashion.

Why do we Invent a new Universal Adhesive and a new Resin Cement – the Company Perspective

Christoph Thalacker
3M Oral Care, 3M Deutschland GmbH, Seefeld, Bavaria, Germany

Universal adhesives and simplified cementation systems have become increasingly popular. This presentation highlights some recent work in this area. A novel radiopaque resin has been developed which can increase the radiopacity of an adhesive to the level of dentin. This helps avoid questionable radiolucent areas on radiographs in the case of adhesive pooling. Spurred by the growing importance of minimally invasive dentistry, an adhesive formulation was developed that can bond and seal caries affected (CA) dentin. Shear bond strength to human sound and CA dentin was found to be statistically the same. Scanning Electron Microscopy of the bond – dentin interface revealed a continuous, well-defined hybrid layer on both sound and CA dentin. Further research activities included an improved silane mixture for bonding to glass ceramic, a formulation free of Bisphenol A derivatives, and integration of a dual cure accelerator. In the area of resin cements, efforts have been made to develop a universal cement that works both as a standalone, self-adhesive cement and as an adhesive cement when combined with a universal adhesive for further increased bond strength. A main focus was on a new amphiphilic redox initiator system which enables a very high degree of conversion especially at the challenging hydrophilic-hydrophobic dentin-cement interface. In turn, high bond strength and mechanical properties were obtained even without light cure. This enabled adaptation of the photoinitiators for an easier excess clean-up. Optimization of the esthetic properties resulted in a fluorescence close to human teeth and high discoloration stability. Finally, a new automix delivery system was developed for improved handling, better hygiene, and an 80% reduction in cement waste per application. This work resulted in 3M™ Scotchbond™ Universal Plus Adhesive and 3M™ RelyX™ Universal Resin Cement. In several in vivo evaluations by general practitioners, both received a satisfaction rate of over 90%.

Laboratory Performance of a New Universal Adhesive to Tooth Tissue and Glass Ceramics

Chenmin Yao[2], Mohammed H. Ahmed[3], Bart Van Meerbeek[1]
[1]CED-IADR, Leuven, Belgium, [2]KU Leuven (University of Leuven), Department of Oral Health Sciences, BIOMAT, Leuven, Belgium, [3]Faculty of Dentistry, Tanta University, Leuven, Flemish Barbant, Belgium

Universal adhesives (UAs) enable dentists to opt for an etch-and-rinse or self-etch mode to directly bond resin-based materials to enamel and dentin or to adhesively lute semi-direct and indirect ceramic or composite restorations. Many of todays UAs contain 10-MDP, not only considered the most efficient functional monomer to bond to tooth tissue, but also to bond to sandblasted zirconia ceramic. Some UAs contain a silane-coupling agent to simplify adhesive luting of glass-ceramic restorations. While a separate silane ceramic primer is claimed no longer needed, silane's instability in an acidic aqueous solution has been shown to seriously reduce UA's silanization efficiency. New combined silane technology, consisting of 3-(aminopropyl)triethoxysilane (APTES) and γ-methacryloxypropyltriethoxysilane (γMPTES), the latter providing polymerizable vinyl groups, was incorporated into an experimental UA formulation, being referred to as ADH-XTE and being the pre-cursor of the currently commercially available 3M™ Scotchbond™ Universal Plus Adhesive. Direct bonding of ADH-XTE to enamel and dentin appeared as effective as obtained by its predecessor 3M™ Scotchbond™ Universal Adhesive ('SBU'; 3M Oral Care), with a significantly lower bond strength recorded when applied in SE than E&R mode after 1-week and 50k thermocycles. Without separate prior silanization, ADH-XTE significantly outperformed the glass-ceramic (IPS e.max CAD, Ivoclar Vivadent) bonding efficiency of its γ-methacryloxypropyltrimethoxysilane (γMPTS)-containing SBU precursor, while it performed equally effective as SBU applied with prior silanization (3M™ RelyX™ Ceramic Primer). Hydrofluoric-acid etching still provided the best glass-ceramic bonding receptiveness, significantly better than tribochemical silica-coated glass-ceramic surfaces. Solely chemical bonding to as-milled and mirror-polished glass-ceramic surfaces without micromechanical interlocking remained insufficient to achieve durable bonding to glass ceramics, irrespective with/without prior silanization. In conclusion, the glass-ceramic bonding capacity of the new combined APTES/γMPTES silane-containing UA ADH-XTE surpassed that of its γMPTS-containing SBU precursor.

Longterm Laboratory Performance of a New Resin Cement Regarding Tooth Tissue and Fiber Post Cementation

Lorenzo Breschi
Department of Medical Sciences, University of Bologna, Trieste, Italy

Objectives: To evaluate push-out bond strength (PBS) and interfacial nanoleakage (IN) of a new self-adhesive resin cement compared to commercially available dual-cure materials for fiber post cementation with or without light curing.
Methods: Root canal treatment was performed on 100 extracted monoradicular human premolars. Standardized 8mm post space was prepared for luting fiber posts (RelyX™ FiberPost, 3M ESPE); following manufacturers' instructions, no fiber post surface pretreatment was performed only for RelyX™ Universal (RXU) groups. The teeth were randomly assigned to five groups (N=20) according to the adhesive treatment: RXU (3M ESPE); LUX-(LuxaCore Z Dual, DMG); MAX–(Maxcem Elite™ Chroma, Kerr™); CAL–(Calibra® Universal, Dentsply Sirona); MUL–(Multilink®Automix, Ivoclar Vivadent). Each group was further divided into two subgroups according to the polymerization protocol: light-curing for 60s or self-curing for 2h. Additional 6 teeth per group were used for IN analysis. PBS test and IN evaluation were performed at baseline (T_0) and 1-year (T_1) storage in artificial saliva. Results were statistically analyzed with three-way ANOVA and Chi-square test. Statistical significance was set at α=0.05.

Results: Statistical analysis revealed that variables “cement” and “aging” significantly influenced PBS ($p<0.05$), but not “polymerization” and “root region” ($p> 0.05$). Significantly lower PBS values ($p<0.05$) were detected in CAL groups compared to other cements, while RXU groups performed equally well ($p>0.05$) or better than other cements ($p<0.05$). At T_1, PBS values increased in the majority of groups, irrespective of root region ($p<0.05$). In general, the aging process produced an increase in marginal infiltration; the results were as follows: MAX=CAL>MUL=LUX>RXU ($p < 0.05$), irrespective of the polymerization condition and root region.

Handling Evaluation of a New Universal Adhesive and a New Resin Cement

FJ T. Burke

Dental School, University of Birmingham, Birmingham, United Kingdom

It has been considered that clinicians prefer to use an easy to handle material as they consider that this will encourage an optimal clinical outcome. A “handling” evaluation of a new material is therefore of relevance. This study by a UK-based practice-based research group, the PREP (Product Research and Evaluation by Practitioners) Panel therefore describes the handling of a novel universal resin luting cement and its associated newly introduced universal adhesive. In this regard, a questionnaire was designed to elicit information on the handling of the new materials. Eleven evaluators from the PREP Panel were sent explanatory letters, a pack of the materials under investigation and a request to use them, where indicated, for 10 weeks and then to complete the questionnaire. A total of 217 restorations were placed: the results from the questionnaire indicated that the novel resin luting cement scored 5 out of 5 on a VAS scale for ease of use (where 5 = maximum ease of use), compared with 4.1 out of 5 for the resin luting cement used prior to the study. For combined use with the newly introduced universal adhesive, the score was 4.9 out of a maximum of 5. Ease of clean up of the luting cement and design of the mixing tips also scored highly. Overall, the results indicated strong acceptance of the ease of use of the materials under evaluation. 100 % of evaluators stated they would recommend them to their colleagues.

16:00 – 17:00 h

Symposium 9: MODERN DIAGNOSTICS FACILITATE MODERN THERAPY: HOW TRANSILLUMINATION AND ARTIFICIAL INTELLIGENCE ALLOW EARLY MANAGEMENT OF CARIES (supported by *DMG*)

Using Artificial Intelligence to Foster Minimal Invasive Care Management.

Falk Schwendicke

Dept. for Operative Dentistry, Charite University, Berlin, Germany

At the dawn of the data era, medicine is becoming the subject to vested interests from tech companies and digital giants. Artificial intelligence (AI) is a buzzword – as is big data. The presentation will discuss what AI is, how it works and why it may revolutionize medicine. AI as a means to leverage medical data will facilitate a more personalized, preventive care and will pave the way towards minimal invasive management of diseases, oral ones like dental caries among them. We will present specific use cases, highlight where they would fit into clinical practice but also demonstrate the limitations in current AI applications in dentistry.

The Contribution of Transillumination for Accurate Early Detection and Diagnosis of Enamel Hypomineralization.

Omar Marouane

Faculty of Dental Medicine, Monastir, Tunisia

Enamel hypomineralization often presents challenging biological, mechanical, and aesthetic treatment scenarios. In regard to that, accurate detection is fundamental to improve treatment outcomes and minimize the likely incidence of recurrent failure. Conventionally, the detection of enamel hypomineralization is based on visual examination under reflected light conditions. However, the observation of enamel hypomineralization in this lighting condition poses some constraints, for it implies the perception of subjective signals such as color or translucency that may be influenced by external light conditions. As a result of these limitations, many enamel lesions may be overlooked or underestimated, which may lead to undertreatment or a potential nontreatment. Therefore, more sensitive instruments and methods have been developed to better detect enamel hypomineralization. Among these methods, recent research has shown that transillumination represents a promising imaging method for detecting enamel hypomineralization. Throughout this presentation, we will see what transillumination is, how it works and why it is important to introduce this diagnosis tool in our daily clinical practice. The advantages, limitations, and future development of transillumination will also be further discussed.

The Oral Microbiome - a Work in Progress.

Don Morrison
Department of Biology, University of Illinois at Chicago, Illinois, United States

The oral microbiome is a plastic assembly of numerous microbial species and subspecies. It evolves and adapts not only by changes in its species composition, but also by exchanges of genes between members of a single species, or, indeed, more rarely, by exchanges of genes from one species to another. One mechanism for such 8ransfers is shared by the entire genus Streptococcus: natural genetic transformation. Transformation in an occasional member of the human oral microbiome, Streptococcus pneumoniae (pneumococcus), provided the earliest evidence that genes consist of DNA. It has since become a powerful tool for direct manipulation of the pneumococcal genome, accelerating study of this widespread and devastating human pathogen. Recent advances in the study of natural genetic transformation in this species reveal that the uptake of naked DNA frequently studied in the laboratory represents but one step in a highly orchestrated mechanism by means of which one cell lyses another, obtaining access to DNA sequences of diverse ancestry. Operation of this mechanism during mixed infections by disparate pneumococcal strains in the natural human host, for example, gives each strain ready access to the species' large pan-genome. Broad conservation of the genetic transformation mechanism across more than five dozen species of streptococci suggests that genome-wide plasticity has been important for long-term survival in many different oral microbial niches.

Off-Target Impact of Antibiotics

Fernanda C. Petersen
Institute of Oral Biology, University of Oslo, Oslo, Norway

For almost every drug, there is a trade-off between benefits and risks. For antibiotics, prescription to humans has been largely based on a «better safe than sorry» or «just in case» approach, with little attention paid to risks. In the awake of the antibiotic crisis and the increased focus on a balanced microbiome for health, our attention is now turning to risks, including the unwanted collateral effects on the microbiome. Although antibiotics are used to target specific bacteria in the site of infection, off-target effects are virtually unavoidable. What happens when you take antibiotics is that your microbiome is hit by different antibiotic concentrations. Although these are usually lower than in the targeted infection sites, these concentrations can affect the microbiome, and select for and promote the development of antibiotic resistance. Recent studies driven by advancements in metagenomics are already revealing the impact of antibiotics in enriching for antibiotic resistance among commensals and major pathogens found at off-target sites. As the field moves forward, we expect novel approaches to reduce collateral effects and an increase in studies providing high-quality evidence on the off-target impact of antibiotics.

Impact of Risk Factor Control in the Treatment of Periodontitis

Eva Maria Muñoz Aguilera
Periodontology, UCL Eastman Dental Institute, London, United Kingdom

Stablished and putative risk factors for periodontitis have detrimental effects not only on disease presentation in terms of extent/severity and progression, but also on the outcomes achieved following periodontal therapy. These factors are often shared with other non-communicable diseases, with an impact on the aetiology and management of these conditions. Therefore, studies investigating risk factor control strategies and their influence on periodontal outcomes when applied prior to or alongside periodontal care are constantly emerging. Current evidence supports the implementation of programs aiming at smoking cessation, diabetes control, fostering physical activity, attaining a healthier diet, and weight loss as health approaches to improve and sustain periodontal outcomes and quality of life. Yet, stronger evidence is available for effective health behaviour change interventions on smoking cessation and dietary counseling. Compared with current smokers or oscilators, those individuals that quit smoking present improved periodontal parameters such as greater probing pocket depth reduction and less clinical attachment loss. Similarly, promoting dietary interventions in patients with periodontitis and either systemically healthy or with other comorbidities lead to significant improvements on bleeding upon probing and pocket depths reductions. Comprehensive interventions often target more than one factor, with an enhanced overall effect. Thus, in periodontal patients, in addition to supporting effective plaque control measures, identifying, and tackling risk factors should be incorporated in the early stages of treatment planning with a view to improving periodontal outcomes. Additionally, the effect of lifestyle interventions on systemic health outcomes should not be underestimated.

***Surgical Versus Non-Surgical Subgingival Instrumentation in the Treatment of Periodontitis*.**

Eduardo Montero Solis
Department of Clinical Specialties, University Complutense of Madrid, Madrid, Spain

Professional elimination of supra- and subgingival plaque and calculus are essential for the successful treatment of periodontitis. In this context, cause-related periodontal therapy aims to mechanically remove subgingival biofilm and to control inflammation, either with non-surgical or surgical approaches, in order to arrest further attachment loss by reducing probing depth (PD), as sites with PD ≤4 mm are associated with lower risk of disease progression and tooth loss. Non-surgical periodontal therapy consists on subgingival debridement. Traditionally, this approach has been done by means of subgingival scaling and root planing (SRP). A consistent amount of evidence has indicated that subgingival debridement is effective in reducing bleeding on probing (BOP) and PD, and gaining clinical attachment levels (CAL). However, it is technically demanding, and complete calculus removal is difficult to achieve. Access flaps were introduced to improve the efficiency of subgingival debridement by gaining direct access to the root surface, root concavities and furcations in sites with residual pockets irrespective of the pattern of bone resorption. Ideally, the least invasive treatment with a better biological cost-effectiveness ratio should be used to restore periodontal health. The effectiveness of the mode of therapy is going to be dependent on the initial PD. Surgery has shown benefits in terms of PD reduction when treating initially moderate and deep pockets. When combining the results for PD reduction and CAL gain, subgingival debridement seems to be the choice at moderately deep pockets and access surgery at initially deep pockets. Long-term results seem to be related with other factors different than the type of debridement provided (surgical or non-surgical), such as hygiene measures or maintenance protocols, as differences between therapies tend to disappear throughout time.

Impact of Adjunctive Antimicrobials in the Treatment of Periodontitis

Paula Matesanz
Complutense University of Madrid, Madrid, Spain

Background: Periodontitis is the most common chronic inflammatory non-communicable disease of humans. It is a major public health problem due to its high prevalence, and it may lead to tooth loss and disability, negatively affecting chewing function and aesthetics. The new classification of periodontitis, introduced in 2017, linked periodontitis diagnosis with approaches to prevention and treatment, relating the severity and extension of the disease with the degree of complexity and an individual's risk. This classification awoke the need for a clinical guideline, evidence-based, that provided recommendations to treat periodontitis. This Clinical Practice Guideline comprises of four different steps, that develops an incremental philosophy of treatment, in which adjuvants are analyzed as a part of the cause-related therapy. Adjunctive antimicrobials, whether local or systemic, have been studied by means of multiple systematic reviews, and different recommendations based on these data and other issues of analysis related to these adjuvants were included in the Guideline. Despite the significant and clinically relevant impact that local or systemic antimicrobials may have on treatment goals, other aspects must be taken into account when designing a Clinical Guideline: harm/benefit rate, economic impact of the therapeutic approach, preferences of the patient or applicability of the strategy, as examples, need to be analyzed before displaying a definite recommendation.
Objectives: The aim of this presentation is to shed some light on the use of this kind of products as adjuvants to mechanical periodontal treatment and help clinicians how to design an effective evidence-based treatment planning.
Conclusions: Adjunctive antimicrobials should not be routinely used in the treatment of Peridontitis Stages I-III, although some room for specific indications is explained in the The EFP S3 level clinical practice guideline.

KEYNOTE LECTURE abstract

Thursday, September 16, 2021

9:15 – 10:00 h

ORAL CANCER AND PRECANCER: THE RELEVANCE OF EARLY DIAGNOSIS (supported by *Vigilant Biosciences*)

Oral Cancer and Precancer: Relevance of Early Diagnosis

Silvio Abati

School of Medicine and School of Dentistry, Cancer Center, IRCCS San Raffaele Hospital, University Vita-Salute San Raffaele, Milano, Italy

Over 500,000 estimated new cases of oral cancer are diagnosed each year. The incidence of oral cancer increases with age, however cases in people younger than 40 years are increasing. It is a largely preventable cancer since most of the different risk factors identified. Tobacco use, alcohol consumption, and betel nut chewing are behaviors that increase the likelihood of the disease. Given its high mortality, early diagnosis is of utmost importance. Prevention and the anticipation of diagnosis begin with identification of potentially malignant lesions of the oral mucosa and with local conditions promoting chronic inflammation. Therefore, every lesion must be recognized promptly and treated adequately. The clinical recognition and evaluation of oral mucosal lesions can detect up to 99% of oral cancers/premalignancies. Surgical biopsy remains the gold standard for diagnosis of oral cancer. Early diagnosis of oral cancer is essential to save a patient's life, minimizing at the same time the negative impact on quality of life that would arise from invasive surgical intervention. Nowadays, there are several diagnostic tools for screening that improve the ability of the clinician to characterize any suspicious lesion, as well as the availability of new point-of-care testing using salivary biomarkers that recognize the risk of malignant transformation. In the last decade, research has identified biomarkers in biological fluids, i.e., saliva, that might have the potential of increasing early diagnosis and detect a premalignant and malignant lesion that is asymptomatic or unnoticeable. Saliva contains many organic and inorganic molecules, proteins, peptides, and electrolytes. Since these methods are non-invasive, inexpensive, and easy to perform as point-of-care testing technique, they can be well-accepted by patients. The scientific community is constantly updating preventive measures and screening methods to detect oral cancer at an early stage to reduce the diagnostic delay that could save the patient's life.

ORAL PRESENTATION abstracts

Final ID number 0001

Influence of Fabrication Method on the Color of Zirconia Crowns

Manuela Manziuc[1], Cristina Gasparik[1], Alexandru Burde[1], Javier Ruiz-López[2], Diana Dudea[1]
[1]Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania, [2]University of Granada, Granada, Spain

Objectives: To assess the influence of the fabrication method on the color of anterior zirconia crowns.
Methods: Forty-five anterior crowns were fabricated from three types of translucent zirconia (Cercon, Katana, Nacera – A1 shade), using different methods: monolithic crowns (n=5), monolithic crowns with a cut-back and enamel layering (single-layer) (n=5), and monolithic crowns with cut-back with dentine and enamel layering (bi-layer) (n=5). The crowns were seated using a transparent try-in paste on a replica of the master prepared tooth, made in composite resin (ND1) and CIELAB color parameters were recorded in three areas of the crowns (cervical, middle, incisal) using a dental spectrophotometer (Spectroshade Micro). Color differences (ΔE_{00}) between the types of crowns, tooth areas, and zirconia materials were calculated using CIEDE2000(1:1:1) and compared with the perceptibility (50:50%PT=0.8) and acceptability thresholds (50:50%AT=1.8). Data were analyzed statistically.
Results: CIE L*, a*, b*, C*, h°, values differed significantly between types of crowns, areas, and materials ($p<0.001$). Considering the fabrication method, the greatest color differences were between monolithic and single-layer crowns (moderately unacceptable mismatch with ΔE_{00} values between 1.8-2.7). The lowest color differences were found between monolithic and bi-layer crowns, for all the areas and the materials (ΔE_{00}<AT). Chroma had the greatest influence on the color difference between crowns made with different technologies. Color differences between cervical and incisal areas were above PT (for Cercon and Katana) and above AT (for Nacera), for all three types of technologies, with lightness having the greatest influence. Among materials, color differences calculated between Cercon and Katana were the lowest (ΔE_{00}<AT), with lightness and chroma having the greatest influence on the color difference.
Conclusions: Perceptible color differences between the three types of zirconia crowns were found. Monolithic and bi-layer crowns showed the closest color match. The color differences were higher in the incisal area and were material dependent.

0002

Pigment Characterization of Highly Translucent Multilayered Monolithic Zirconia Ceramics

Stevan Cokic[1], Maoyin Li[2], Shuigen Huang[2], Jef Vleugels[2], Bart Van Meerbeek[1], Fei Zhang[1, 2]
[1]Department of Oral Health Sciences, BIOMAT – Biomaterials Research group & UZ Leuven (University Hospitals Leuven), Dentistry, KU Leuven (University of Leuven), Leuven, Belgium, [2]Department of Materials Engineering (MTM), KU Leuven (University of Leuven), Leuven, Belgium

Objectives: To evaluate optical and mechanical properties, microstructure and aging resistance, and to characterize the influence of pigments added to specific highly translucent multilayer monolithic zirconia.
Methods: Three pre-sintered 5-mol% yttria partially stabilized zirconia (5Y-PSZ) disks (Lava Esthetic A2 and Bleach, 3M Oral Care; Katana STML A2, Kuraray Noritake) were cut in plates, sintered according to the manufacturer's instructions and mirror-polished. The influence of pigment addition on translucency parameter (TP), fracture toughness (n=10/group), Vickers hardness (n=10/group), biaxial strength (n=20/group) and hydrothermal aging stability (n=2/group) was correlated with microstructure and chemical and phase composition. The pigment composition and distribution were thoroughly evaluated by light and fluorescence microscopy, electron probe micro-analyzer (EPMA) with WDS and nanoSEM with energy-dispersive X-ray spectroscopy (EDX). Chemical and phase composition were assessed using X-ray fluorescence (XRF) and X-ray diffraction (XRD) together with electron backscatter diffraction (EBSD), respectively.
Results: In contrast to Katana STML, pigments in the Lava Esthetic zirconia appeared as large yellow and blue fluorescent agglomerates consisting of small grains (0.54 µm and 0.36 µm, respectively, versus 0.86 µm for the surrounding grains), lower Y_2O_3 content and presence of Er, Hf and Al. EBSD confirmed the predominant tetragonal composition of the agglomerates. Lava Esthetic disclosed the lowest aging resistance with transformation degradation occurring exclusively within the pigment agglomerates. XRD confirmed that all zirconia grades contained a high amount of Y_2O_3 in the tetragonal ZrO_2 phase (4.2-5.7 mol%), which resulted in a higher cubic phase (42-55wt%). Although no statistical differences were measured for Vickers hardness and toughness, Katana STML showed significantly higher TP for 1-mm and 1.5-mm specimen thicknesses, higher flexural strength and lower mechanical reliability compared to the Lava Esthetic materials.
Conclusions: Rare-earth oxide-containing zirconia agglomerates added as color pigments to the multilayered monolithic zirconia Lava Esthetic could be the cause for their lower optical and mechanical properties and reduced aging resistance.

0003

Comparison of Slurry-Based Additive Manufacturing Techniques for Zirconia Ceramics

Evita Willems[1, 2], Bernardo Camargo[1], Fei Zhang[1, 2], Bart Van Meerbeek[1], Jef Vleugels[2]
[1]Department of Oral Health Sciences, BIOMAT – Biomaterials Research group & UZ Leuven (University Hospitals Leuven), Dentistry, KU Leuven (University of Leuven), Leuven, Belgium, [2]Department of Materials Engineering, KU Leuven, Leuven, Belgium

Objectives: Additive manufacturing (AM) of ceramics is intensively researched because it allows creating highly dense complex shaped objects without tool wear and lower material loss than conventional subtractive manufacturing (SM). Indirect slurry-based AM techniques create a green ceramic body containing organic binder that needs further de-binding and sintering to obtain the

final component. This study focuses on the possibility to produce 3 mol% yttria-stabilized zirconia (3Y-TZP) dental materials using material jetting (MJ) and stereolithography (SLA).
Methods: 3Y-TZP discs (n=30), bending bars (n=30) and crowns were produced using MJ (Carmel 1400, XJET) with a layer thickness of 10.5 µm and SLA (Admaflex 130, Admatec) using a layer thickness of 10-30 µm. The correlation between mechanical properties and microstructure was investigated.
Results: Near fully dense materials were obtained for sintered MJ (6.03±0.01 g/cm^3) and SLA (6.03±0.01 g/cm^3). MJ exhibited the lowest and isotropic shrinkage (17.6%), while SLA showed an anisotropic shrinkage, with the highest shrinkage along the building direction (22.3%). First results indicate a bending strength of 733±152 MPa for MJ and of 656±131MPa for SLA printed ceramics using 25-µm layers. Decreasing the building layer thickness in SLA however increases strength. The layer-wise build-up was no longer visible in the bulk of the material after sintering for both techniques. Delamination cracks, agglomerates and spherical pores were observed in all sintered parts, independent of the AM technology used. The main strength defining defects were pores for SLA and delaminations for MJ, strongly influencing the strength in the building direction. SLA has a faster printing time, but a slower post-processing process with longer de-binding cycles. Surface roughness of MJ was higher due to supporting structures.
Conclusions: Both SLA and MJ allow to print highly accurate dental restorations taking into account the shrinkage factors, but the mechanical properties are still inadequate compared to SM materials.

0004

Fatigue and Aging of a Novel 3D-Printed Zirconia Implant

Fei Zhang[1, 2], Benedikt C. Spies[3], Evita Willems[1, 2], Christian Wesemann[3], Jef Vleugels[1], Bart Van Meerbeek[2]
[1]Department of Materials Engineering, KU Leuven, Heverlee, Belgium, [2]Department of Oral Health Sciences, BIOMAT – Biomaterials Research group & UZ Leuven (University Hospitals Leuven), Dentistry, KU Leuven (University of Leuven), Leuven, Belgium, [3]Department for Dentistry, Oral and Maxillofacial Medicine, University Hospital Freiburg, Freiburg, Germany

Objectives: Most of the currently available zirconia implants are manufactured in a subtractive manner, while significant efforts are needed to modify the zirconia surface to speed up the bone-to-implant integration. Additive manufacturing (AM) on the other hand allows direct shaping of the implant with different surface topographies without the need for roughening procedures, along with the possibility to customize implants to fit individual oral conditions and clinical situations.
Methods: In this study, 3Y-TZP zirconia implants were manufactured by nano-particle ink-jetting (XJET Carmel 1400, Xjet) in the 0° building direction with a layer thickness of 10.5 µm. The fracture load of the printed and sintered implants was tested according to ISO 14801 before and after combined fatigue and aging testing in a chewing simulator (5 kg load, 5 million loading cycles, 2 Hz, 85°C water for 1 month; n=8/group). The performance was thoroughly assessed by micro-CT, optical microscopy, SEM fractography, XRD phase transformation, micro-Raman stress assessment and FIB-SEM subsurface analysis.
Results: The fracture load of as-sintered implants was 516 ± 39 N, which increased to 1037 ± 319 N after fatigue and aging. The enhanced strength was attributed to a novel seamless integration of porous and non-porous volumes at the implant surface. The core of the printed implant was nearly fully dense, whereas a 10-30 µm thick, porous layer was formed at the surface with inter-columnar pores surrounding zirconia columns of ~10 µm that were oriented nearly perpendicular to the surface. After chewing fatigue and aging, the 1-2 µm top surface along the zirconia columns in the porous layer was transformed and compressive stresses were generated just below the porous layer where fracture of all implants was initiated.
Conclusions: 3D-printed ceramic implants showed high potential to combine innovative surface topography with sufficient mechanical strength.

0005

3D-Printing and Milling Accuracy Influence Full-Contour Zirconia Crown Adaptation

Bernardo Camargo[2], Evita Willems[1], Wout Jacobs[2], Marleen Peumans[2], Jef Vleugels[1], Bart Van Meerbeek[2]
[1]Department of Materials Engineering, Kasteelpark Arenberg 44, B-3001 Leuven, Belgium, KU Leuven, Leuven, Belgium, [2]Department of Oral Health Sciences, BIOMAT – Biomaterials Research group & UZ Leuven (University Hospitals Leuven), Dentistry, Leuven, Belgium, KU Leuven (University of Leuven), Leuven, Belgium

Objectives: To correlate trueness and cement space of crowns fabricated using a chairside and a laboratory milling unit with an inkjet 3D-printer to assess whether the 3D-printing accuracy is acceptable for clinical use.
Methods: Thirty crowns (inLab 4.0, Dentsply Sirona) were either milled using a Cerec MC XL (Dentsply Sirona) unit from Cerec Zirconia Mono L (Dentsply Sirona) or a LX-O 5-axis industrial machine (Matsuura Machinery) from Initial Zirconia HT (GC), or 3D-printed using an inkjet Carmel 1400C (Xjet) printer using a 3Y-TZP C800 (Xjet) zirconia-ceramic slurry (n=10). Upon scanning (high-resolution light Compact 5 (GOM) scanner), the crown trueness was determined by correlating the original CAD design with each digitized crown. Divided into marginal, axial, and occlusal thirds, data were correlated with the 3D cement-space characteristics provided by micro-CT (Phoenix NanoTom). Statistics involved Kruskal-Wallis test (α=.05) with Bonferroni correction for multiple tests. Correlation was evaluated using the Spearman-correlation test.
Results: Crown trueness positively correlated with thickness and volume of the marginal and axial thirds, and all thirds combined. The 3D-printing results are in-between those of the two milling systems, revealing undercut values not statistically different from those recorded for the Cerec crowns and a low overcut level that was statistically similar to that obtained by laboratory milling. Laboratory milling revealed a significantly better marginal accuracy with a consequently lower cement-space thickness. A higher overcut level was recorded for the Cerec-milled crowns in the marginal and occlusal thirds, resulting in the significantly highest occlusal cement-space thickness and cement-volume percentage exhibiting a cement thickness above 120 µm (limit considered as clinically acceptable). No statistical difference in trueness was found for the external crown dimensions.

Conclusions: The 3D-printed monolithic zirconia crowns provided sufficient manufacturing accuracy for clinical use. Accurate milling and printing of the dental crown margins is primordial.

0006

Fatigue Resistance of CAD-CAM Composites and Lithium Disilicate Glass-Ceramics

Maher Eldafrawy[1], Amélie Mainjot[1, 2], Michael Sadoun[3]

[1]Dental Biomaterials Research Unit (d-BRU), Institute of Dentistry, University of Liège (ULiège), Liège, Belgium, [2]Department of Fixed Prosthodontics, Institute of Dentistry, University of Liège Hospital (CHU), Liège, Belgium, [3]Majeb, sprl, Liège, Belgium

Objectives: Materials fatigue resistance studies are sparsely reported, probably because they are time-consuming. However, fatigue resistance constitutes an important property to predict prostheses' clinical performance. This work aims to compare the long-term flexural fatigue resistance of commercially available CAD-CAM composite materials, an experimental Polymer-Infiltrated Ceramic Network (PICN) and a lithium disilicate glass-ceramic under flexural cyclic loading.

Methods: Five commercial CAD-CAM composites (Cerasmart, GC (CER); Brilliant, Coltene (BRL); Tetric CAD, Ivoclar Vivadent (TET); Katana Avencia, Kuraray Noritake (KAT); Grandio, Voco (GRN)), an experimental PICN (Majeb (EXP)), and a lithium disilicate glass-ceramic (IPSe.maxCAD, Ivoclar Vivadent, (EMX)), were tested for flexural fatigue resistance. Blocks were cut to produce bars of dimensions (1.6±0.1)-mm x (4.0±0.1)-mm x (17.0±0.1)-mm, which were polished with a diamond pad (10-µm) under water irrigation (n=15). EMX was then fired according to the manufacturer's recommendations. Bars (n=5/group) were tested for fatigue in a water bath at 36°C in 3-point bending at 100-, 150- and 200-MPa for 1-million cycles (1Hz) or until fracture. For tests at 200-MPa, means ± standard deviations of flexural fatigue results were compared by ANOVA-1, followed by Tukey's test.

Results: At 100-MPa, TET, KAT, GRN and EXP did not fracture after 1-million cycles. At 150-MPa, only EXP did not fracture after 1-million cycles and showed higher fatigue resistance than the rest of the materials. It was followed by GRN (506,960±280,249), TET (353,126±433,511), KAT (315,108±206,414), BRL (977,84±183,588), EMX (2,649±4,845) and CER (215±229). At 200-MPa, EXP showed the highest fatigue resistance significantly ($p<0.001$) (323,210±460,52) cycles, followed by GRN (101,403±73,612), KAT (74,765±96,456), BRL (54,521±95,157), TET (26,503±15,842), EMX (2,893±4,569) and CER (1±1).

Conclusions: EXP showed the highest fatigue resistance significantly. Results highlight that the flexural strength of materials is not predictive of their fatigue behavior, which should be considered in clinical practice.

0007

Evaluation of Mandibular Condyle of Patients with Cleft Lip / Palate

Hazal Duyan, Burcu Evlice

Oral and Maxillofacial Radiology, Cukurova University Faculty of Dentistry, Adana, Saricam, Turkey

Objectives: In patients with unilateral cleft lip and palate (UCLP), asymmetry is present on the affected side due to the development deficiency of many structures. The aim of this study is to evaluate mandibular condyle morphology and structural features of patients with UCLP using cone beam computed tomography images.

Methods: The study sample consisted of 15 patients with UCLP (9 females and 6 males; mean age: 12.33 ± 2.024) and 15 age and sex matched control patients (9 females and 6 males; mean age: 12.33 ± 2.024). Mandibular condyle morphology was classified as convex, angled, straight, and round. Mandibular condyle structural features were classified as normal, flattening, erosion and osteophyte. The distribution of these categorical variables according to the cleft / non-cleft sides of the patients with UCLP and according to the UCLP (cleft side) / control groups were analyzed using chi-square and Fisher's exact test. The effect of cleft presence on these variables was evaluated using logistic regression analysis. IBM SPSS 25.0 software (IBM Corp., Armonk, NY) was used for statistical analysis.

Results: The distribution of the parameters examined was statistically similar between both the cleft / non-cleft sides of the patients with UCLP and between the UCLP (cleft side) / control groups ($p>0.05$). There was no statistically significant effect of the presence of cleft on the parameters examined ($p>0.05$).

Conclusions: The presence of a cleft was not a risk factor for abnormal mandibular condyle morphology and structural features. This study was designed as a pilot study. Future studies with larger sample sizes are required.

0008

Reliability of Orthopantomograms for Detecting Tooth Status in Adults

Lukas Naktinis[1], Paulius Raškevičius[1], Indre Stankeviciene[1], Alina Puriene[1], Lina Stangvaltaite-Mouhat[2, 3]

[1]Institute of Dentistry, Vilnius University, Vilnius, Lithuania, [2]Department of Clinical Dentistry, The Arctic University of Norway, Tromsø, Norway, [3]Oral Health Centre of Expertise in Eastern Norway, Oslo, Norway

Objectives: To assess the reliability of orthopantomograms for detecting healthy, decayed, filled, missing or unerupted teeth in adults.

Methods: Ten randomly selected depersonalized orthopantomograms were assessed twice within two weeks interval by two dental students. Each tooth status was examined and scored as follows: '0' – healthy (H), '1'- missing (M), '2' – decayed (D), '3' – filled (F), '4' – unerupted (U). Then, students independently assessed 170 orthopantomograms. Kappa statistics were used to assess the inter- and intra-observer reliability.

Results: In total, 320 teeth were assessed for intra-observer reliability, which resulted in kappa values of 0.972 and 0.944. Observer 1 showed disagreement in: 2 cases in D-H, 1 in H-D, 1 in F-D, and 1 in F- H groups. Observer 2 showed disagreement in: 3 cases in D-H and 7 cases in F-D groups. For inter-observer reliability, 5312 teeth were assessed. Kappa value was 0.894. Disagreement in

assessment between observers were as follows: 173 in F-H, 107 in F-D, 54 in D-H, 18 in M-F, 10 in M-H, 1 in U-D, 3 in F-U groups, 3 in U-H and, 1 in D-M.
Conclusions: Intra-observer and inter-observer reliability was assessed as almost perfect. There are indications that orthopantomograms may be reliable for detecting tooth status in adults. Studies with higher number and different background observers are needed to validate these results.

0009

Investigation of Transfer Learning Strategies for Dental Radiographic Imagery

Lisa Schneider[1], Joachim Krois[1, 2], Keno K. Bressem[3], Stefan M. Niehues[3], Falk Schwendicke[1, 2]
[1]Department of Oral Diagnostics, Digital Health and Health Services Research, Charité-Universitätsmedizin, 14197 Berlin, Germany, [2]Topic Group Dental Diagnostics and Digital Dentistry, ITU/WHO Focus Group on AI for Health, 1211 Geneva, Switzerland, [3]Klinik für Radiologie, Hindenburgdamm 30, Charité – Universitätsmedizin Berlin, 12203 Berlin, Germany

Objectives: Deep learning models are often initialized with weights of pre-trained ImageNet models, which usually boosts performance compared with random initialization. Notably, features learned on ImageNet may differ from features on radiographs, with initialization on medical images being potentially more suitable. We aimed to compare transfer learning on ImageNet versus CheXpert, an open repository for chest radiographs, versus random initialization for the segmentation of anatomic tooth structures (enamel, dentin, pulpal cavity) on bitewing radiographs.
Methods: We built 12 segmentation models by combining U-Net with different backbones (ResNet, VGG, DenseNet) and applied three initialization strategies (ImageNet, CheXpert, random). The resulting 36 models were trained up to 200 epochs with the Adam optimizer (lr=0.0001) and a batch size of 16. Our dataset consisted of 1721 bitewing human-annotated bitewings. We utilized a train/validation/test split of 80%/10%/10%. Model performances were primarily quantified by the Dice score.
Results: Random initialization led to a mean (SD) Dice score of 0.843 (0.007), while ImageNet and CheXpert reached 0.856 (0.014) and 0.856 (0.01), respectively. The lower performance of a random initialization was statistically significant compared to training based on ImageNet (p=0.013/t-test) or CheXpert (p=0.001). No significant difference was observed between ImageNet and CheXpert (p=0.944/t-test).
Conclusions: Transfer learning boosts model performances. The origin of transferred knowledge seems less relevant. Dental segmentation models benefitted similarly from pre-training on RGB photographs or monochromatic chest radiographs.

0010

Prediction of Caries Lesion Surfaces on Bitewings Using Deep Learning

Aleksander Krasowski[1], Lisa Schneider[1], Joachim Krois[1, 2], Ben Feldberg[1], Martha Duchrau[1], Jonas A. Rodrigues[1, 3], Falk Schwendicke[1, 2]
[1]Department of Oral Diagnostics, Digital Health and Health Services Research, Charité–Universitätsmedizin Berlin, Berlin, Germany, [2]Topic Group Dental Diagnostics and Digital Dentistry, ITU/WHO Focus Group on AI for Health, Geneva, Switzerland, [3]Department of Surgery and Orthopedics, UFRGS, School of Dentistry, Porto Alegre, Brazil

Objectives: Deep Learning using Convolutional Neural Networks (CNNs) has been used for caries detection on bitewing radiographs of permanent teeth. So far, CNNs do not provide information on the lesion surfaces (distal, mesial or occlusal), which would be beneficial for reporting of findings. We applied a CNN aimed to assess the discriminatory ability for caries lesion localization on bitewing radiographs of permanent teeth.
Methods: The dataset contained masks for each tooth, restorations and caries lesions generated in a previous study from 3775 bitewing radiographs of permanent teeth. The corresponding caries lesion surfaces were labeled by dental experts for each image. Mesial and distal labels were translated to left and right to abstract complexity. Data was split randomly into training (70%), validation (20%) and test (10%) sets, stratified by labels (distribution: left: 41.6%; right: 40.1%; occlusal 18.3%). A ResNet-34 classification model pre-trained on ImageNet with the AdamW optimizer (lr=0.0001) was trained up to 50 epochs with a batch size of 32. To prevent overfitting, dropout and data augmentation methods such as horizontal flipping and image rotation were applied.
Results: The model achieved a high accuracy (total: 97.9%; left: 98.7%; right: 99.3% occlusal: 92.8%) and F1-score (total: 0.98; left: 0.98; right: 0.99; occlusal: 0.94) on the test set.
Conclusions: A ResNet-34 classification model trained on a limited amount of data showed high discriminatory ability to classify caries lesion surface locations on bitewing radiographs of permanent teeth.

0011

Does 3D Imaging Enhance Diagnostic Performance in Complex Trauma Cases?

Gertrude V. Van Gorp[1], Dominique Declerck[1], Marjan Lambrechts[1], Reinhilde Jacobs[2]

[1]KU Leuven Department of Oral Health Sciences and Department of Dentistry, Unit of Paediatric Dentistry and Special Dental care, University Hospitals Leuven, Leuven, Belgium, Leuven, Belgium, [2]OMFS IMPATH Research Group, Department of Imaging and Pathology, Faculty of Medicine, University of Leuven and Oral & Maxillofacial Surgery, University Hospitals Leuven, Leuven, Belgium, Leuven, Belgium

Objectives: The aim of this study was to evaluate the impact of complexity of dental trauma cases on paediatric dentists' performance when diagnosing traumatic dental injuries (TDI), and whether three- dimensional CBCT imaging techniques present added value over conventional two-dimensional imaging in this situation.

Methods: A panel of nine paediatric dentists was invited to detect, interpret and diagnose radiographic findings in twenty dental trauma cases involving young permanent teeth. Peri-apical radiographs and CBCT images of each case were presented in random order. Findings were recorded using structured scoring sheets. Recordings of an experienced paediatric endodontologist and specialist in dento-maxillofacial radiology were used as benchmark. Results were analysed using a generalized linear mixed model.

Results: Overall the results for detection, interpretation and diagnosis of findings were poor, both using 2D and 3D, with a clear impact of case complexity ($P<0.001$). Using 3D resulted in a significant higher sensitivity for detection and interpretation of findings, both in easy and difficult cases ($P<0.001$). This was not the case for correctly diagnosing, with poorer performance when using 3D in complex cases ($P<0.001$).

Conclusions: This study provides evidence that case complexity influences diagnostic performance. The use of CBCT enhances detection and interpretation of findings but adversely affects diagnostic performance in complex cases. It is clear that imaging techniques using the third dimension cannot compensate for al low level of training and experience.

0011.1

Osteomeatal Complex Variations in Pediatric Patients Using CBCT

Elif Meltem Aslan Öztürk[1], Eda Didem Yalcin[2]

[1]Dentomaxillofacial Radiology, Gaziantep University Faculty of Dentistry, Gaziantep, Turkey, [2]Dentomaxillofacial Radiology, Dokuz Eylül University Faculty of Dentistry, Izmir, Turkey

Objectives: The aim of this study was to evaluate the relationship between osteomeatal complex variations **(**OMC) and maxillary sinus pathologies in pediatric patients using cone-beam computed tomography (CBCT).

Methods: CBCT images of 72 patients (44 males and 28 females) aged 7-18 years were evaluated retrospectively. The presence of nasal septal deviation (NSD), nasal septal pneumatization (NSP), concha bullosa (CB), accessory maxillary ostium (AMO), agger nasi cell (ANC), Haller cell (HC), Onodi cell (OC), ethmoid sinusitis and maxillary sinus pathologies were investigated. Maxillary sinus pathologies were classified. Correlations of OMC variations with each other, maxillary sinus pathologies and ethmoid sinusitis were investigated. The Chi-square test was used to analyze the relationships among variables and distribution of parameters.

Results: NSD was determined in 70.8%, NSP in 40.3%, ethmoid sinusitis in 75%, maxillary sinus pathology in 34.8% of the images. OMC variations rates were detected as CB 31.3%, AMO 16%, ANC 16%, HC 24.3% and OC 18.8%. The most common maxillary sinus pathology was localized mucosal thickening, with a rate of 15.3% on the right and 22.2% on the left. Statistically significant differences were determined between almost whole OMC variations with each other, and between the anatomical variations in OMC with maxillary sinus pathologies except for NSP and AMO ($p < 0.05$). The presence of maxillary sinus pathology and ethmoid sinusitis were more common in males ($p < 0.05$).

Conclusions: The anatomical variations in OMC had no significant effect on maxillary sinus pathology except for NSP and AMO. Besides, the results revealed that most of the anatomical variations in OMC were statistically significantly correlated with each other. The use of CBCT in pediatric patients is quite successful in examining OMC with its low radiation dose, low cost and high image quality compared to computed tomography.

0011.2

Viscosity of Saliva as an Obstacle for Salivary Diagnostics

László Márk M. Czumbel[1], Petra Szalkai[2], Ágnes Sárádi-Kesztyûs[2], Anna Földes[1], Attila Bérces[3], Barna Vásárhelyi[4], István Antal[2], Gabor Varga[1]

[1]Department of Oral Biology, Semmelweis University, Budapest, Hungary, [2]Department of Pharmaceutics, Semmelweis University, Budapest, Hungary, [3]Omixon Biocomputing Ltd, Budapest, Hungary, [4]Department of Laboratory Medicine, Semmelweis University, Budapest, Hungary

Objectives: Saliva is a promising tool to identify oral and systemic diseases using DNA and RNA based diagnostics. But the high viscosity of saliva prevents its wide usage in routine laboratories. We aimed to study the rheological properties of human saliva and tested various intervention methods to decrease its viscosity enabling automated pipettors and other diagnostic instruments to use this fluid.

Methods: We collected whole unstimulated saliva from 40 healthy individuals. Initially, we measured its viscosity (η) (unit: Pa×s) over a wide range of shear rate (unit: s^{-1}) at room temperature using a Kinexus Pro^{+} rheometer (Malvern Instruments Ltd, UK). Then, fixed share rate was applied to test the effect of freezing/thawing at -80°C, 30 sec vortexing, 15 min enzymatic digestion with ProteinaseK (1,1mg/ml) and adding 0.1 ml 1% acetic acid to 1 ml saliva on viscosity. Mean ± SEM were given, non-parametric paired Wilcoxon tests were applied.

Results: We observed an extremely large variability in viscosity between samples ranging 0.2847 Pa×s – 4.167 Pa×s at shear rate 0.1 s^{-1} (n=20, mean= 1.174 Pa×s, 95% CI: 0.6636 – 1.683). The viscosity of saliva strongly depended on the applied shear rate, indicating non-Newtonian fluid characteristics (n=20, at 0.1 s^{-1}, 1 s^{-1} and 10 s^{-1} viscosity was 1.35 Pa×s ±0.153, 0.13±0.022 Pa×s and 0.018±0.006 Pa×s, respectively). Freezing and thawing did not change sample viscosity at the same three shear rates (P=0.8695, P=0.4980, and P=0.6742, respectively). Vortexing did not change the viscosity either (P=0.8408). On the other hand, ProteinaseK exposure significantly decreased saliva viscosity (P=0.0002), similar to acidification of the samples by acetic acid (P=0.0121), used as a positive control.
Conclusions: Whole saliva has an extreme high variance in viscosity between subjects, an obstacle for using saliva in routine diagnostic procedures. But ProteinaseK application is a promising method to improve the rheological characteristics of saliva.

0012

Cells Isolated from the Dentin-Pulp Interface Express an Odontoblast Phenotype

Helmut Schweikl[1], Marialucia Gallorini[2], Matthias Widbiller[1], Wolfgang Buchalla[1]
[1]Department of Conservative Dentistry and Periodontology, University Hospital Regensburg, Regensburg, Germany, [2]Department of Pharmacy, University "G. d'Annunzio" Chieti-Pescara, Chieti, Italy

Objectives: The lack of odontoblasts culturable in vitro is a drawback for the investigation of the molecular cross-talk underlying dentin formation, as well as immune and sensory functions. Therefore, we isolated cells from the dentin-pulp interface of third molars from young patients and characterized their morphological and biochemical properties.
Methods: After tooth extraction, pulp tissues were removed from the crowns and the structure of the dentin-pulp interface was analyzed by scanning electron microscopy (SEM). Dentin-adherent tissue was scraped off the pulp chamber wall, and cells obtained from these explants and from pulp tissue were harvested after several weeks in culture. Properties of these primary cells were compared with those of periodontal ligament cells, osteoblasts, skin fibroblasts, epithelial cells or HeLa. Cell morphology was studied by light microscopy. The expression of odontoblast markers DSPP (dentin sialophosphoprotein) and DMP1 (dentin matrix protein) as well as TRPV4 (transient receptor potential cation channel) and S100A4 (S100 calcium binding protein) was analyzed by Western blotting. Gene expression of S100A4 was investigated by RT-qPCR.
Results: A monolayer of odontoblast cell bodies was tightly attached to the pulp chamber wall. Cell processes enter dentin tubules on the predentinal surface characterized by a network of collagen fibres preceding mineralized dentin. Microscopic observations of cell cultures indicated cell polarity and secretory activity in cells from the dentin-pulp interface. Protein expression of DSPP, DMP-1, and TRPV4 was detected in all cell types. High expression of S100A4 was observed at the protein and gene level in two of three fractions of cells isolated from the dentin-pulp interface, but was absent or weakly expressed in pulp cells.
Conclusions: We provide experimental evidence that cells isolated from the dentin-pulp interface express an odontoblast phenotype. The expression of S100A4 is a potential marker for differentiating between pulp cells and cells of the odontoblast lineage.

0013

Macrophage-Fibroblast Interactions Modulate Dental Pulp Regeneration

Charlotte Jeanneau[1], Chloé Le Fournis[1], Sandra Roumani[1], Aurélie Cotten[1], Thomas Giraud[1, 2], Imad About[1]
[1]Aix Marseille Université, Centre National de la Recherche Scientifique, Institut des Sciences du Mouvement, Marseille, France, [2]Assistance Publique – Hôpitaux de Marseille, Hôpital Timone, Service d'Odontologie, Marseille, France

Objectives: Pulp regeneration is a complex process which requires intercellular communications among pulp cells. Indeed, depending on their stimulation recent data have demonstrated that fibroblasts regulate macrophage M1/M2 phenotype differentiation. Here we aimed at investigating the effects of macrophage-pulp fibroblast interaction on pulp stem cell (DPSCs) proliferation and recruitment as well as on angiogenesis.
Methods: Human pulp cells were isolated from third molars. DPSCs were separated from fibroblasts using magnetic cell sorting with STRO-1. Two types of stimulation were performed to simulate pulp injury: 1) to mimic a carious lesion, fibroblasts were incubated with Lipoteichoic Acid (LTA) and physically injured; 2) to simulate an inflammatory reaction, fibroblasts were physically injured without LTA. Fibroblast supernatants were then incubated with undifferentiated macrophages (M0) to induce their differentiation into M1/M2. VEGF secretion by macrophages was quantified by ELISA. The macrophage supernatants' effects on endothelial cell (HUVEC) proliferation were investigated with the MTT assay while neo-angiogenesis was studied after endothelial cell culture on Matrigel. DPSCs proliferation and recruitment towards macrophage supernatants were investigated with MTT and Boyden chambers respectively.
Results: M2 and macrophages incubated with injured fibroblast supernatants induced a significant increase of VEGF secretion and neo-angiogenesis as well as DPSCs proliferation as compared to M0, M1 and those incubated with LTA. An important level of DPSCs recruitment was observed with M0, M1 and macrophages incubated with LTA-stimulated fibroblast supernatants. This was significantly higher than that observed with M2 and injured fibroblast supernatants.
Conclusions: Within the limits of this investigation *in vitro*, when macrophages are incubated with fibroblast supernatants, they differentiate into M1 or M2 depending on the fibroblast stimulation type and modulate neo-angiogenesis and DPSC recruitment. These are two major steps in the pulp regeneration process. This fine balance between M1 and M2 phenotypes represents a pre-requisite for a successful regeneration process.

0013.1

Effects of Pulp Capping Materials on Macrophage Differentiation and Activity

Jean-hugues Catherine[1, 2], Chloé Le Fournis[1], Thomas Giraud[1, 3], Aurélie Cotten[1], Charlotte Jeanneau[1], Imad About[1]

[1]Centre National de la Recherche Scientifique, Institut des Sciences du Mouvement, Aix Marseille Université,, Marseille, France, [2]Service de chirurgie orale, Assistance Publique – Hôpitaux de Marseille, Marseille, France, [3]Service d'odontologie, Assistance Publique – Hôpitaux de Marseille, Marseille, France

Objectives: Pulp capping materials have been shown to modulate pulp inflammation and regeneration. Depending on their stimulation, pulp fibroblasts have been shown to interact with macrophages modulating their differentiation into pro-inflammatory M1 or anti-inflammatory M2 phenotypes. Thus, upon carious lesion simulation, these fibroblasts have been shown to induce the M1 phenotype. Here, we studied the effect of subjecting pulp fibroblasts to tricalcium silicate-based materials on macrophage differentiation by investigating their cytokine secretion profile and phagocytic activity.

Methods: Samples of Biodentine™ (Tricalcium silicate) and TheraCal LC® (Tricalcium silicate with resin) were placed in MEM medium (0.05cm^2/mL) for 24h to obtain the materials' extracts. Human pulp fibroblasts, obtained by the explant outgrowth method, were physically injured and incubated with Lipoteichoic Acid (LTA) to simulate a carious lesion, and incubated with the materials' extracts to simulate direct pulp capping. After 24h, fibroblast supernatants were harvested and incubated with undifferentiated macrophages (M0) to investigate their differentiation into M1/M2 phenotypes. Secretion of pro-inflammatory TNF-α and anti-inflammatory Il-10 were analyzed by ELISA; phagocytic capacity of *S. Mutans* was assessed using Gentamycin protection assay. M0 macrophages chemically induced into M1 or M2 phenotypes were used as controls.

Results: Both materials' extracts significantly decreased macrophage pro-inflammatory TNF-α secretion as compared to injured/LTA-treated fibroblasts and M1 macrophages. Conversely, a significant increase in IL-10 secretion was observed with the materials' extracts as compared to injured/LTA-treated fibroblasts and M1 macrophages but was 25% less than M2 secretion level. Use of materials' extracts significantly decreased macrophage phagocytic activity as compared to injured/LTA-treated fibroblasts and M1 macrophages, but remained was comparable to that of M2.

Conclusions: The macrophage cytokine secretion profile and phagocytic capacity upon use of Biodentine™ and TheraCal LC® was comparable to anti-inflammatory M2 phenotype. These findings indicate that calcium silicate-based materials modulate macrophage differentiation and may be of interest in targeting pulp regeneration.

0014

Clinical, Radiological and Histological Healing Patterns After Tooth Autotransplantation

Mostafa EzEldeen[1], Mariano Simón Pedano De Piero[1], Jan Wyatt[3], Bart Van Meerbeek[1], Ivo Lambrichts[2], Reinhilde Jacobs[1]

[1]KU Leuven, Leuven, Belgium, [2]U Hasselt, Hasselt, Belgium, [3]UZ Leuven, Leuven, Belgium

Objectives: This study aimed to evaluate the outcome of the cone-beam computed tomographic (CBCT)-guided tooth autotransplantation (TAT) compared to the conventional TAT protocol, and to assess the 3D and histological healing patterns after CBCT-guided TAT.

Methods: This study included 100 autotransplanted teeth in 88 patients. Each experimental group consisted of 50 transplants in 44 patients (31 males and 19 females). The mean age at the time of surgery was 10.7±1.1 years-old for the CBCT-guided group. This was 10.6±1.3 years-old for the conventional-group. The mean follow-up period was 4.5±3.1 years (range1.1-10.4 years). Two transplants were extracted after 12 and 24months were imaged using nano-focus computed tomography and processed histologically.

Results: Overall survival rate for the CBCT-guided TAT was 92% and success rate was 86% compared to 84% survival rate and 78% success rate for the conventional-group *(P>0.005)*. The following measurements were extracted from the 3D analysis: root hard tissue volume (RV), root length (RL), apical foramen area (AFA), mean and maximum dentin wall thickness (Mean-DWT & Max-DWT). Overall, the mean percentage of tissue change was: RV gain by 65.8%±34.6, RL gain by 37.3%±31.5, AFA reduction by 91.1%±14.9, Mean-DWT increase by 107.9%±67.7, and Max-DWT increase by 26.5%±40.1. Principle component analysis (PCA) identified the Mean-DWT, RV and Max-DWT as the parameters best describing the tissue change after TAT. Cluster analysis applied to the variables chosen by the PCA classified the CBCT-group into 4 distinct clusters revealing different patterns of tissue healing after TAT. Histological analysis showed tubular reparative dentin formation and loss of normal pulp-dentin complex architecture.

Conclusions: The CBCT-guided approach increased the predictability of the treatment. The 3D and histological analysis provided insights into the patterns of healing.

0015

Bone Regeneration in Apical Periodontitis

Teodora Karteva[1], Vasil Popov[2], Tatyana Todorova[3], Maria Kazakova[3], Ekaterina G. Karteva[1], Neshka Manchorova[1], Victoria Sarafian[3]

[1]Operative Dentistry and Endodontics, Medical University - Plovdiv, Plovdiv, Bulgaria, [2]Control Systems Department, Technical University of Sofia, Branch Plovdiv, Plovdiv, Bulgaria, [3]Department of Medical Biology, Medical University – Plovdiv, Plovdiv, Bulgaria

Objectives: It has long been discussed that the healing rate of asymptomatic apical periodontitis (AAP) is contingent on the treatment protocol in terms of number of visits, preparation techniques and medicaments. However, bone healing is an intricate multifaceted process of cellular proliferation. The aim of this study was to examine a potential link between patients' mitogen-induced inflammatory mediator release and the rate of bone healing following non-surgical endodontic treatment of AAP.

Methods: Patients (n=20) diagnosed with AAP were enrolled in the study and underwent non-surgical endodontic treatment. Small field-of-view cone beam computed tomography was used to assess the lesion volume prior to and 10 months following treatment. Pre-treatment peripheral blood samples were collected from the patients and peripheral blood mononuclear cells were isolated and cultured in two different media: RPMI 1640 and RPMI 1640 + PHA. The supernatant was collected at the 24th and 48th hour and the levels of IL-1β and PGE2 were assessed by ELISA. Individual mitogen-induced reactivity (MIR) was calculated for each molecule. Logistic regression, extra tree classifier, ANOVA and heatmap methods were used to assess the association between the levels of MIR of each molecule and the rate of healing.
Results: The whole set of used algorithms outlined MIR PGE2/24h, MIR PGE2/48 and MIR IL-1β/48 as strong predictors of the rate of bone healing.
Conclusions: A link between the host's mitogen-induced reactivity and the rate of bone regeneration was observed. Further studies can shed light on the underlying pathways of the observed phenomenon and translate these finding into the clinical practice.

0016

Osteoconductivity of BSA Incorporated Amorphous and Crystalline Calcium Phosphate Coating

Menghong Li, Gang Wu, Yuelian M. Liu
Department of Prothetics and Oral Implantology, ACTA, Amsterdam, Netherlands

Objectives: An early osteointegration facilitates early loading of orthodontic mini-screws and reduces risk of failure. Biomimetic calcium phosphate (CaP) coating is a well-established surface-modification method of metallic implants to promote osteogenic activity and carry various bioactive agents for further functionalization. And it has been shown that bovine serum albumin (BSA) may promote mineral deposition because they contain structures, such as -OH, -NH, and -SH, with strong affinities for inorganic cations and anions. In this study, we hypothesized that biomimetic amorphous or crystalline CaP coating with BSA facilitated an earlier osteointegration of titanium mini-implants.
Methods: Biomimetic amorphous and crystalline coatings with or without BSA incorporation were prepared on the mini-pins, and both naked and with adsorbed BSA were used as control groups. Enzyme linked immunosorbent assay (ELISA) was utilized to identify the loading and release of BSA. The mini-pins were implanted in the rats tibial for in vivo evaluation of the course of osseointegration. 3 days, 1 week, 2 weeks and 4 weeks post-surgery, the samples were retrieved and subjected into histological and histomorphormetrical analysis to determine bone to implant contact (BIC).
Results: The rate of BSA release from amorphous and crystalline coatings in vitro during the initial 3 days was 81% and 23% respectively, and by the end of the monitoring period (day 35), 96% and 52% of the incorporated BSA had been released. A significant increase of BIC occurred on the 14th day for the mini-pins without any coating and those with amorphous coatings, while the BIC increase occurred already on the 7th day for the mini-pins with crystalline coatings compared to the BIC on the 3rd day. The incorporation of BSA postponed significantly the increase of BIC to a later time point of 35 days.
Conclusions: We confirmed that biomimetic crystalline coatings can lead a slow release of the protein and facilitate an earlier increase of bone to implants contact.

0017

Effects of L-PRF, a-PRF+ and i-PRF on Human Osteoblasts *in Vitro.*

Konstantinos Kosmidis, Karishma Ehsan, Luciano Pitzurra, Bruno G. Loos, Ineke D. Jansen
Periodontology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit, Amsterdam, Netherlands

Objectives: Different PRF protocols are used in clinical dentistry the last years. A positive effect of PRF in wound healing for soft tissues is known. However, hardly any literature comparing the new protocols for PRF (the A-PRF+ and i-PRF) with the original protocol of PRF (L-PRF), is present for osteoblasts and hard tissues. The aim of this study was to investigate the differences between Advanced Platelet Rich Fibrin (A-PRF+), Leukocyte Platelet Rich Fibrin (L-PRF) and injectable Platelet Rich Fibrin (i-PRF) in osteogenesis of a human osteoblast-like cell line *in vitro.*
Methods: A-PRF+, L-PRF and i-PRF was prepared from 6 male donors and cultured with 10 mL culture medium for 6 days. Osteoblasts from the osteoblast cell line (U2OS) were seeded in a density of 5 x 10^3 cells and cultured either with conditioned medium derived from the different PRF conditions or with fresh culture medium. At 5 different timepoints (0, 7, 14, 21, 28 days) the osteogenic capacity of the cells was assessed with an alizarin red staining to visualize mineralization. Next to that the calcium concentration, alkaline phosphatase activity was measured. With qPCR the expression of 6 osteogenic genes was measured (alkaline phosphatase, osteocalcin, osteonectin, ICAM-1, RUNX-2, Collagen 1a).
Results: A-PRF+ induced more mineralization and calcium after 28 days of culturing compared to the controls ($p<0.001$). i-PRF induced more calcium deposition compared to controls ($p<0.05$). No significant differences were found in cell proliferation and differentiation between the different conditions. However, there were significant differences between cell proliferation at different timepoints (0,7,14) in all conditions. Expression of RUNX-2 and osteonectin was lower in the PRF-cultures compared to control only at timepoint 7 and 14. Expression of osteocalcin was higher in i-PRF and L-PRF at 21 days compared to 7 ($p<0.05$) and 14 ($p<0.01$). ALP activity was significantly higher in the group cultured with the i-PRF medium ($p< 0.01$).
Conclusions: We found that only in the cultures with A-PRF+ more mineralization and calcium was present. This suggests that in the A-PRF+ condition the osteoblast-like cells are more differentiated to bone forming cells.

0018

Osteogenic Potential of Human Periosteal-Derived Cells for Bone Regeneration

Sandra Roumani, Charlotte Jeanneau, Martine Pithioux, Imad About

Sciences Movement Institute UMR 7287 CNRS, Aix Marseille University, Marseille, France

Objectives: Critical bone defect regeneration is a challenging issue as it requires a scaffold to provide mechanical resistance and Mesenchymal Stem Cell (MSCs) recruitment to regenerate new bone. Bone marrow is known as the major source of osteogenic stem cells. However, despite the scant data available, periosteum stem cells represent a real interest for bone regeneration. This study was designed to characterize Periosteum-Derived cells and their osteogenic potential by studying their osteogenic differentiation and matrix mineralization abilities.

Methods: human Periosteum-Derived Cells (hPDCs) were obtained by the outgrowth method and characterized by immunofluorescence with antibodies to CD9, CD34, CD45, CD90, CD105. Their proliferation was compared to pulp fibroblasts at day 3, 5 and 7 with an MTT assay. To investigate their differentiation toward the osteogenic lineage, cells were cultured in osteogenic induction medium for 1 month. Alkaline Phosphatase (ALP) activity was quantified every week. Similarly, Alizarine Red Staining (ARS) of calcium depositions was performed and quantified every week to determine matrix mineralization.

Results: hPDCs displayed a cuboidal shape, characteristic of periosteum cells. They expressed CD9, CD90, CD105 MSC-specific markers, the osteoprogenitor CD34 marker and displayed a significantly higher proliferation rate as compared to pulp fibroblasts. Use of osteogenic induction medium did not modify ALP activity but significantly increased the formation of the mineralized matrix as measured by Alizarin Red at the 4th week.

Conclusions: This work demonstrates that human Periosteum-Derived Cells display a MSC phenotype as demonstrated by a high proliferation rate, expression of stem cell markers and an osteogenic differentiation potential. They also have an osteoprogenitor cell profile as demonstrated by their osteogenic differentiation and mineralized matrix synthesis which highlights a bone regeneration capacity. In conclusion, hPDCs hold promise for bone regeneration and may be of interest in the management of critical bone defects.

0019

Are Chlorhexidine Mouthrinses all Acting the Same Towards Oral Biofilms?

Naiera Zayed[1], Nico Boon[2], Kristel Bernaerts[3], Wannes Van Holm[1], Tim Verspecht[1], Wim Teughels[1]

[1]Oral health sciences, KU Leuven, Leuven, Vlaams-Brabant, Belgium, [2]Center of Micobial Ecology and Technology, UGent, Gent, Belgium, [3]Department of Chemical Engineering, KU Leuven, Leuven, Belgium

Objectives: Chlorhexidine mouthrinses are marketed in different formulations. Therefore, this study aimed at evaluating the *in-vitro* effects of different chlorhexidine mouthrinses on bacterial load, ecology, and community structure of synthetic multispecies oral biofilms after single or multiple rinses.

Methods: Nine chlorhexidine mouthrinses were selected for this study. Two of the tested products are formulated with an anti-discoloration system, two products contain alcohol, and three products contain cetylpyridinium chloride (CPC) combined with the chlorhexidine. Tested products were grouped according to CHX concentration into 3 groups: low (L), medium (M), and high (H) concentration. Phosphate buffered saline (PBS) was used as a negative control rinsing solution. Multispecies oral biofilms were grown on the surface of hydroxyapatite discs for 48 h, then rinsed with the corresponding product. Biofilms were collected every 24 h for DNA extraction and vitality quantitative PCR (v-qPCR).

Results: The tested mouthrinses provoked different effects in terms of change in total viable bacterial load (VBL), ecology and community structure of the rinsed multispecies oral biofilms. There was no relation between chlorhexidine concentrations, presence, or absence of cetylpyridinium chloride and/or alcohol, and the observed effects. Some chlorhexidine mouthrinses that strongly lowered the total VBL, disrupted biofilm symbiosis. On the other hand, other chlorhexidine mouthrinses with limited impact on total VBL improved the biofilm ecology and community structure. These different effects could not be related to the presence of CPC, alcohol or the concentration of chlorhexidine.

Conclusions: Not all chlorhexidine mouthrinses have the same effect on oral biofilms. Therefore, a chlorhexidine product should not be selected randomly based on its composition. Product selection should depend on the indication: short-term treatment, long-term maintenance, or pre-/post- operative care. Clinical assessment seems important for patient-specific recommendations of a product.

0020

Salivary Calprotectin and Neutrophils in Oral and Inflammatory Bowel Disease

Mirjam Majster[1], Sven Almer[2, 3], Sebastian Malmqvist[1], Annsofi Johannsen[1], Ronaldo Lira Júnior[1], Elisabeth A. Boström[1, 4]

[1]Department of Dental Medicine, Karolinska Institutet, Stockholm, Sweden, [2]Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden, [3]Department of Gastroenterology, Dermatology and Rheumatology, Karolinska University Hospital, Stockholm, Sweden, [4]Department of Orofacial Medicine, Folktandvården Stockholms Län AB, Stockholm, Sweden

Objectives: Calprotectin, or S100A8/A9, is a neutrophil-derived acute-phase protein complex and fecal marker of inflammatory bowel disease (IBD). We have shown that calprotectin is elevated in saliva during IBD, but it is also known to be affected by oral disease. Here we assessed the salivary concentration of calprotectin in IBD patients and controls, in relation to intestinal and oral diseases. Furthermore, we investigated salivary neutrophils as a source of calprotectin and compared their ability to secrete calprotectin between IBD patients and controls.

Methods: Twenty-one IBD patients (14 Crohn's disease, 6 ulcerative colitis, 1 IBD-unclassified) and 20 controls were orally examined and sampled for stimulated saliva. Data regarding IBD extent, activity, and treatment was retrieved from all patients,

and 15 IBD patients provided fresh fecal samples for calprotectin determination. Calprotectin concentrations were determined by an enzyme-linked immunosorbent assay and adjusted to salivary flow. Expression and secretion of calprotectin was evaluated by immunofluorescent staining and inflammatory stimulation of cultured salivary neutrophils from IBD patients and matched controls. Calprotectin abundancies in relation to intestinal and oral diseases were compared by two-sided non-parametric tests with statistical significance set at $p \leq 0.05$.
Results: Calprotectin was significantly elevated in stimulated saliva of IBD patients compared to controls ($p<0.001$), particularly in Crohn's ($p=0.002$), and concentrations were unaffected by caries or periodontitis. Salivary calprotectin did not correlate to fecal calprotectin, nor was it affected by activity, treatment, or location of IBD. Salivary neutrophils expressed calprotectin, and patients and controls comparably secreted calprotectin in a time-dependent manner.
Conclusions: Salivary calprotectin is elevated in IBD regardless of oral disease, indicating that the effect of intestinal inflammation on salivary concentrations of calprotectin exceed that of periodontal inflammation. Furthermore, salivary neutrophils express and secrete calprotectin, suggesting that oral neutrophils may be affected by chronic intestinal inflammation.

0021

Clade G6 Saccharibacteria ("Ca. Nanogingivalaceae") are Highly Divergent from Clade G1

Jonathon L. Baker
Genomic Medicine, J. Craig Venter Institute, La Jolla, California, United States

Objectives: Saccharibacteria have reduced genomes, a small cell size, and appear have a parasitic lifestyle, dependent on host bacteria. At least 6 highly diverse clades of Saccharibacteria inhabit the human oral cavity. However, all oral Saccharibacteria with currently available complete genomes or cultured isolates belong to the clade, G1, leaving clades G2-G6 poorly understood.
Methods: Nanopore sequencing was used to deliver the first complete genome of an oral Saccharibacteria outside of clade G1, the G6 (proposed name, "*Ca.* Nanogingivalaceae") taxon, JB001.
Results: Phylogenomic and pangenomic analysis showed that G6 Saccharibateria are quite divergent from the more well-understood G1 taxa and encode a distinct functional and metabolic repertoire.
Conclusions: These Results: indicate that G6 Saccharibacteria may have unique lifestyles and depend on their hosts to fulfill rather different metabolic requirements. If true, this would likely have significant implications regarding the evolution, ecological role, and impact on human health of clade G6 Saccharibacteria.

0022

Immunological Response in the Dental Pulp After Caries Treatment

Ana Sotirovska Ivkovska[3], Efka Zabokova Bilbilova[1], Zlatko Georgiev[1], Natasha Stavreva[2]
[1]Pediatric Department, Faculty of Dentistry, Skopje, Macedonia (the former Yugoslav Republic of), [2]Department of Prosthodontics, Faculty of Dentistry, Skopje, Macedonia (the former Yugoslav Republic of), [3]Pediatric Department, Faculty of Dentistry, Skopje, Macedonia (the former Yugoslav Republic of)

Objectives: This study investigated the responses of the immune system to carious lesions and how operative procedures influence the distribution of the immunocompetent cells.
Methods: We have examined 30 human teeth from patients at the age of 9 to 14 years. This study investigated the responses of the immune system under 3 different clinical conditions: shallow and deep cavities and treated caries. Teeth were extracted from various therapeutic reasons (mostly from orthodontic reason), and immediately cut longitudinally; pulp tissue was extirpated and fixed in formalin for 24 hours at 4°C. The specimens were embedded in paraffin, according to standardized laboratory procedure. Sections were cut at 5 µm thickness and stained by the streptavidin-biotin complex immunoperoxidase method. Cells were identified immunohistochemically by using the following monoclonal antibodies: HLA-DR (for dendritic cells), CD45RO (for memory T lymphocytes), and CD20 (for B -lymphocytes).
Results: Initial pulpal response was characterized by a localized accumulation of HLA-DR antibody-positive cells in the pulp tissue beneath the caries lesion. In the pulp of progressed caries, a large number of HLA-DR-positive cells were observed with a marked increase of CD45 and CD20 positive cells. This might indicate the occurrence of antigen presentation locally in the pulp tissue which is very important for the immune response. However, six months after treatment, clusters consisting of HLA-DR-positive cells and CD45-positive T lymphocytes were recognized locally in the pulp tissue, regardless of cavity depth. CD20-positive B cells were seen only under the deeper cavities.
Conclusions: The results of this study demonstrated that dental pulps respond to cavity preparation and restoration, and that antigen presentation and cellular or humoral immune responses persist for many months, even after caries treatment.

0023

Effects of Benzalkonium Chloride Mouthrinse in SARS-CoV-2-Positive Patients: an RCT

Fabian Cieplik[1], Barbara Schmidt[1], Franz Audebert[2], Henriette Lang[2], Felix Buder[1], André Gessner[1], Toni Luise Meister[3], Eike Steinmann[3], Stephanie Pfaender[3], Veronika Vielsmeier[1], Josef-Maximilian Gottsauner[1]

[1]University Hospital Regensburg, Regensburg, Germany, [2]Praxiszentrum Alte Mälzerei, Regensburg, Germany, [3]Ruhr University Bochum, Bochum, Germany

Objectives: Preprocedural mouthrinses have been discussed as protective measure before conducting intraoral procedures in SARS-CoV-2 positive patients. The aim of this prospective randomized clinical trial was to investigate the effects of a 0.1% benzalkonium chloride (BAC) mouthrinse on reducing the intraoral SARS-CoV-2 load and infectivity.

Methods: Patients tested positive for SARS-CoV-2 within the last 72 h were included in this study. Oropharyngeal specimens were taken at baseline, whereupon patients had to gargle mouth and throat with 20 mL of 0.1% BAC or placebo (0.9% NaCl) for 30 seconds. After 15 and 30 minutes, further oropharyngeal specimens were collected. Viral load was analyzed from all specimens by RT-qPCR and antigen tests (Roche) were performed. Furthermore, infectivity of oropharyngeal specimens was analyzed via infection of Vero cells and quantified by limiting dilution assay. Data were analyzed non-parametrically (Friedman and Mann-Whitney tests; a=0.05).

Results: 24 SARS-CoV-2 positive patients were included in this study and randomized to the BAC (18) or the placebo group (6), respectively. Neither BAC nor placebo led to a significant reduction of intraoral viral load as determined by RT-qPCR. Four patients harbored antigen-positive samples. In three patients of the BAC group, the amount of viral antigen decreased, whereas it increased in one patient of the placebo group. Infectious virus could be cultured in 5 BAC-patients and one placebo-patient at baseline. There was a mild reduction in viral infectivity after BAC treatment which remained constant for up to 30 min. In contrast, in one placebo-patient with detectable virus, no reduction in viral infectivity could be observed.

Conclusions: These results indicate that a preprocedural mouthrinse with 0.1% BAC only mildly affects viral loads and infectivity in SARS-CoV-2 positive patients, indicating a minor role towards potential reduction of transmission rates. Therefore, strict infection prevention regimens remain of paramount importance in dental practice.

0024

Aerosol Spatter Reduction Efficiency of Different Dry-Field Isolation Techniques

Cem Peskersoy[1], Makbule H. Sahan[2]

[1]Restorative Dentistry, Ege University Faculty of Dentistry, Izmir, Turkey, [2]Prostodontics, Ege University Faculty of Dentistry, Izmir, Turkey

Objectives: The COVID-19 pandemic has led to a great concern in dentistry about the risk of aerosol spread which possibly includes coronavirus pathogens that can remain airborne over a prolonged period of time. The current study aims to investigate the aerosol spatter reduction efficiency of different dry field isolation techniques in a simulated setup that simulates different dental procedures.

Methods: Six dry-field isolation techniques were tested in a setup with different aerosol producing dental equipments. A crown and a class II cavity preparation on a manikin was performed on both anterior (no:11) and posterior (no:46) teeth with both high-speed handpiece and Er-Nd:YAG laser as well as a full-mouth debridement and air-polishing. These procedures repeated 3 times for each setup. In each setup; a dry-field isolation method is applied to reduce aerosol spatter, including rubber-dam, two intra-oral, two extra-oral aspiration systems and a high volume evacuator. Environmental and operator face shield splatter was determined using a Laser Particle Counter while suction efficiencies of the systems were measured by a surgical aspirator. A generalized linear mixed model was used for each scenario and post-hoc Tukey analysis and pairwise comparisons were performed to evaluate the differences (*p=0.05*).

Results: The most intensive aerosol producing dental equipments are air-polisher and high-speed handpiece (*p=0.089*). The experimental setups showed significant variability in the suction flow rate, but this was not correlated to the aerosol spatter patterns. Both intra-oral aspiration systems provided significantly better suction performance (*p=0.009*) while extra-oral aspiration systems failed to achieve sufficient aspiration and aerosol spatter reduction (*p=0.001*). Results indicate that spatter reduction was significantly better amongst the systems in which high-volume evacuator was used (*p=0.025*). Er-Nd:YAG laser significantly reduced the aerosols spatter compared with high-speed handpiece (*p=0.018*).

Conclusions: The use of dry-field isolation systems is necessary to minimize airborne contamination especially during the COVID-19 pandemic.

0025

Worn Dentition No Prep Treatment with PICN Restorations: 4-yr Results

Julie Oudkerk[1, 2], Maher Eldafrawy[2], Sandrine Bekaert[1, 2], Charlotte Grenade[1, 2], Alain Vanheusden[1, 2], Amélie Mainjot[1, 2]

[1]Department of Fixed Prosthodontics, Institute of Dentistry, University of Liège Hospital (CHU), Liège, Belgium, [2]Dental Biomaterials Research Unit (d-BRU), Institute of Dentistry, University of Liège, (ULiège), Liège, Belgium

Objectives: To prospectively evaluate the 4-yr Results of the One-step No-prep treatment of full mouth-worn dentition, a minimally invasive and multidisciplinary approach using PICN CAD-CAM composite restorations without provisional phase.

Methods: Seven patients (n=192 restorations) with severe tooth wear were included (BEWE score >13). Patient data were recorded, and an occlusal analysis and a tissue-guided wax-up were realized. After replacement of old fillings, no prep Vita Enamic restorations (posterior restorations and palatal veneers) were bonded within 24-h. Direct composites were performed to mask

the buccal joint on anterior teeth. Maxillo-facial physiotherapy was performed. Restorations were evaluated following FDI criteria. Treatment influence on Oral-Health-Impact-Profile-49 (OHIP-49) score was assessed.
Results: Tooth wear etiology was related to soft drinks consumption and bruxism. The mean VDO increase was 5.09±0.85mm [M1] on the incisal pin. The mean restoration thickness on molars was 0.55±0.21mm, and the lowest was 0.11mm. The 4-year survival rate of restorations was 99.5% and the success rate was 91.66%, with 14 minor chippings, one debonding and one restoration loss. A significant improvement of the global OHIP-49 score and a positive effect on pain (teeth, head, neck and back) were observed after treatment. The 4-year evaluation confirmed high satisfaction rates of esthetic and functional results.
Conclusions: In this clinical study on high-risk patients, PICN restorations, applied in a minimally invasive way, showed high survival and success rates after 4 years, while minor chipping of very thin occlusal borders constituted the most frequent complication. The use of PICNs allows the development of no-prep and simple treatment protocols of worn dentition. Those materials exhibit several advantages compared to ceramics for this indication, such as the ability to be milled to a very low thickness, the ease of in-mouth adjustments, and their damping behavior, which is particularly interesting in cases of bruxism.

0026

Three-Year Clinical Results of Second-Generation Zirconia Monolithic Posterior Restorations

Vinciane Koenig[1, 2], Sandrine Bekaert[1, 2], Nathalie Dupont[1, 2], Stéphane Le Goff[3], Alain Vanheusden[1, 2], Claudine Wulfman[3], Amélie Mainjot[1, 2]
[1]Dental Biomaterials Research Unit (d-BRU), University of Liège (ULiège), Liège, Belgium, [2]Department of Fixed Prosthodontics, Institute of Dentistry, University of Liège Hospital (CHU), Liège, Belgium, [3]Unité de Recherches en Biomatériaux Innovants et Interfaces (URB2i) – EA4462, Faculté de Chirurgie Dentaire, Université Paris Descartes, Paris, France

Objectives: To present the three-year clinical results of a prospective study about second-generation zirconia posterior restorations, which includes bruxers.
Methods: A total of 87 posterior monolithic zirconia tooth-elements (78 on implants and 9 on natural teeth, 18.5% of restorations being fixed partial dentures (FPDs)) in 43 patients were included in an original research protocol, which comprises *ex vivo* analyses. Occlusal contact point areas were determined, and half of those areas were left unglazed and just polished. Restorations were clinically evaluated following FDI criteria at each evaluation time (baseline, 6 months, 1 year, 2 years and 3 years, respectively) and antagonistic teeth were examined. Glaze wear *ex vivo* analyses using SEM were performed, temporarily removing the prostheses.
Results: At 3 years, the survival rate of restorations was 90.4% (100% for FPDs) and the success rate was 75.3%, with 6 abutment debondings, 3 implant losses, 2 unscrewings, 2 tooth-supported crown debondings (provisional cement use), 1 restoration fracture, 1 minor chipping, 1 core fracture, 1 root fracture and 1 periodontal disease. 85.7% of catastrophic failures occurred in patients with clinical signs of bruxism (61.7% of patients). Complications were also observed on antagonistic teeth (3 catastrophic failures). Clinical evaluation of the restorations showed good results from the aesthetic, functional, and biological perspectives. Glaze wear was observed on all occlusal contact areas after 1 year.
Conclusions: The treatment success rate was not as high as expected in this sample including patients with bruxism clinical signs. Monolithic zirconia FPDs showed excellent results. Yet, further research is needed for single-unit restorations, taking into consideration samples which do not exclude bruxers. The present results could highlight, from the authors' point of view, a weakness of full-zirconia restorations, which are strong but stiff and unable to absorb stresses, the weak link being the restoration support or the antagonist tooth.

0027

Fast Curing Bulk-Fill Resin Composites: a Systematic Review.

Elisabeth Dursun[1, 2], Philippe Francois[1, 4], Jean-Pierre Attal[1, 3]
[1]Innovative Dental Materials and Interfaces Research Unit, University of Paris, Paris, France, [2]Henri Mondor Hospital, Créteil, Djibouti, [3]Charles Foix Hospital, Ivry-sur-Seine, France, [4]Bretonneau Hospital, Paris, France

Objectives: Which practitioner has not wished to reduce photopolymerization time? The three second power cure system, consisting in an adhesive, a bulk-fill resin composite and a high-power curing light, is claimed to maintain the reliability of the cure process in only 3s. The aim of this study was, through a systematic review, to take stock of this system.
Methods: An electronic search on the PubMed database attempted to identify all relevant studies regarding fast curing resin composites. Papers published in English were identified after a review of their titles, abstracts and full text. The inclusion criteria were all well-conducted studies on this system, i.e. the Adhese Universal (*Ivoclar Vivadent*) adhesive, the Tetric PowerFill (*Ivoclar Vivadent*) sculptable bulk-fill resin composite or the Tetric PowerFlow (*Ivoclar Vivadent*) flowable bulk-fill resin composite, light-cured with the Bluephase PowerCure (*Ivoclar Vivadent*) high-power curing light.
Results: Short light exposure time with high radiant exitance seems to have no adverse (or almost) consequences on: degree of conversion, depth of cure, flexural modulus, Vickers microhardness, polymerization shrinkage and shrinkage stress. The bulk-fill resin composites also show less or no internal defects. However, the shrinkage force kinetics is affected and the clinical tolerance for 3s irradiance should be limited to an exposure distance of 5mm, by avoiding angulation.
Conclusions: Taking into account the latter precautions, fast-curing resin composites seem to be relevant and reliable for clinical use.

0028

Long-Term Investigation of Mechanical Properties of Bioactive Restorative Materials

Karlo Posavec, Matej Par, Visnja Negovetic Mandic, Vlatko Panduric, Zrinka Tarle, Danijela Marovic
Department of Endodontics and Restorative Dental Medicine, University of Zagreb, School of Dental Medicine, Zagreb, Croatia

Objectives: To evaluate mechanical properties of a new commercially available bioactive restorative material and compare it to established bioactive and inert materials after artificial aging for up to 3 months.

Methods: Four materials were tested: alkasite Cention (Ivoclar Vivadent) in self-cure (Cen-SC) or light-cure (Cen-LC) mode, giomer Beautifil II (Shofu; BII), conventional glass ionomer Fuji IX (GC; FIX), and resin composite Tetric EvoCeram (Ivoclar Vivadent; TEC) as a control. For flexural strength (FS) and flexural modulus (FM) testing according to ISO 4049, Cen-LC, BII, and TEC were polymerized with 950 mW/cm^2 for 20 s in 6 overlapping exposures on both sides, while Cen-SC and FIX were left to set for 15 min. The specimens (n=60/material) were dark stored in distilled water at 37°C, divided into three groups (n=20) and tested at 24h, 3 months, and 3 months+3 days in ethanol. FS and FM were measured using a customized universal testing machine at 1mm/min crosshead speed. The data were statistically analyzed using the Kruskal-Wallis test, and one-way ANOVA with Bonferroni post-hoc test, $p < 0.05$.

Results: BII (121.8-142.1 MPa) and TEC (107.8-132.2 MPa) had the highest FS at all time points of measurement, followed by Cen-LC (84.0-104.6 MPa) and Cen-SC (62.1-86.9 MPa). FIX had significantly lowest FS (7.9-12.0 MPa) and the lowest FM (0.5-2.7 GPa). FS and FM decreased at 3mo and after additional aging in ethanol for all materials except BII and Cen-SC. Contrary to other materials, Cen-SC had an increase in FS and FM at 3mo and 3mo+3 compared to 24h.

Conclusions: In this study, BII behaved similarly to a composite material and had the best resistance to aging. Alkasite Cention showed better mechanical properties than FIX. Cention should be used in the light-cure mode. This study was supported by Croatian Science Foundation, grant IP-2019-04-6183.

0029

Mechanical Properties of a Graphene-Based Composite for Dental Restorations

Virginia Biferi[1], Francesco de angelis[1], Mirco Vadini[1], Michele Di Cosola[1], Giovanna Iezzi[1], Adriano Piattelli[1, 2], Camillo D'Arcangelo[1]
[1]University of Chieti-Pescara, Chieti, Italy, [2]Unit of Dentistry, S.S. Annunziata Hospital, Chieti, Italy

Objectives: Recently, graphene has attracted both academic and industrial interest because it showed to enhance mechanical, physical and chemical properties of biomaterials.

This work aimed at comparing the mechanical properties of a graphene-based composite and a conventional and commercially available nanohybrid resin-based dental composite, used as control.

Methods: The composites were subjected to the following mechanical tests: 3-point flexural strength (FS) test, compressive strength (CS) test and Vickers hardness (VH) test. For each different mechanical test, ten samples of each composite were prepared (n=10), according to following specimen design: bar-shaped (2 x 2 x 25 mm) for FS, cylindrical (4 mm diameter, 8 mm height) for CS, disc-shaped (4 mm diameter, 2 mm height) for VH. All tests were performed using a universal testing machine (Lloyd Instruments- LR30KPlus). Means (and standard deviations) were calculated and compared using the Student-t tests ($P< 0.05$).

Results: In all mechanical tests performed, the ultimate strength/hardness observed for the graphene-based composite appeared comparable or slightly increased compared to the nanohybrid resin-based dental composite. Moreover, both the flexural modulus of elasticity (recorded on the FS test) and the compressive modulus of elasticity (recorded on the CS test) were significantly reduced for the graphene-based composite, compared to the control.

Conclusions: The experimental graphene-based composite tested in this study showed ultimate strength and hardness at least as good as those observed for conventional dental composites. At the same time, the significantly reduced flexural and compressive moduli describe a considerably more elastic material. Further studies are needed, to definitely understand the clinical meaning of the mechanical differences herein observed.

0030

Elution of Monomers from Dental Composites for Provisional Restorations

Marko Turkalj[1], Siemon De Nys[1], Matteo Creta[2], Lode Godderis[2, 3], Jeroen Vanoirbeek[2], Bart Van Meerbeek[1], Kirsten L. Van Landuyt[1]
[1]KU Leuven (University of Leuven), Department of Oral Health Sciences, BIOMAT – Biomaterials Research group & UZ Leuven (University Hospitals Leuven), Dentistry, Leuven, Belgium, [2]KU Leuven (University of Leuven), Environment and Health, Department of Public Health and Primary Care, Leuven, Belgium, [3]IDEWE, External service for prevention and protection at work, Heverlee, Belgium

Objectives: Elution of substances from dental composites occurs for prolonged periods of time after intraoral placement and can cause various adverse reactions. The aim of this study was to assess the elution of monomers from composite materials employed for provisional restorations.

Methods: Cylindrical specimens (n = 10) were prepared from two dual-cure materials (*Integrity Multi-Cure, Dentsply Sirona; Tempsmart DC, GC)* and two chemically curing materials *(Protemp 4, 3M; Structur 3, VOCO)* in custom-made Teflon molds (5.5 mm internal diameter, 2 mm thickness). A conventional restorative composite (*Essentia, GC*) was used as a reference. The composites were cured according to the manufacturers' instructions, and dual-cure materials were prepared in both self and light-curing modes, respectively. Next, the specimens were put in glass vials and 1 mL of LC-MS grade water was added. The samples were divided in two groups to be incubated at 37 °C either for 24 hours or 28 days with extraction solvent being refreshed on a

weekly basis. The amount of eluted monomers BisGMA, UDMA, TEGDMA and BisEMA (-3 / -6 / -10) was quantified by UPLC-MS/MS.
Results: Monomer elution was detected for all materials in both groups. The amount of eluted monomers from *Protemp 4* was lower than the lower limit of quantification in both groups. When prepared in self-curing mode, significantly more monomers eluted from *Integrity Multi-Cure* and *Tempsmart DC* in both 24 hours and 28 days groups when compared to light-cured samples (two-way ANOVA, $p < 0.0001$). Compared to *Essentia*, only *Tempsmart DC* eluted significantly more monomers when prepared in self-curing mode in both groups and in light-curing mode in 24 hours group ($p < 0.0001$).
Conclusions: Elution patterns were material dependent. Moreover, when dual-cure materials were prepared in light-curing mode, monomer elution was lower than when the same materials were prepared in self-curing mode. Light curing of dual-cure provisional composites might improve their biocompatibility.

0031

The Effect of Local and Systemic Antimicrobials to L-PRF

Manoetjer Siawasch[2], Catherine Andrade[1], Marc Quirynen[2], Wim Teughels[2], Andy Temmerman[2], Ana Belen Castro[2]
[1]University of the Andes, Santiago, Chile, [2]KU Leuven, Leuven, Belgium

Objectives: In recent years, use of leukocyte- and platelet-rich fibrin (L-PRF) has gained increasing awareness for his biological- and mechanical properties. This study was designed to evaluate the effect of local and systemic administration of antimicrobials to L-PRF.
Methods: For part A 16 tubes of blood were collected from 8 healthy subjects. Prior to centrifugation for preparation of L-PRF membranes 12 of the 16 tubes were injected with 0.125ml, 0.25ml or 0.50ml intravenous Flagyl. One set of membranes was used to assess the release of major growth factors at indicated time intervals. Additional Flagyl release over time by L-PRF membranes was evaluated. The remaining L-PRF membranes were placed on the surface of agar plates inoculated with periodontal pathogens to determine their antibacterial activity. For part B another 6 subjects were enrolled with 3 subjects taking 2g amoxicillin and 3 subjects 500mg metronidazole as prophylaxis prior to surgery. Before and 2 hours after consuming one of the prescribed antimicrobials, three tubes of blood were collected for preparing L-PRF membranes. All membranes were used to measure the antibacterial activity against periodontal pathogens.
Results: The release in growth factors by L-PRF membranes was not influenced by the addition of Flagyl. The release of Flagyl could be detected up to day 3, however with the highest concentration during the first 4 hours. The concentration Flagyl was dose dependent. The antibacterial capacity of L-PRF membranes increased significantly for both the systemic intake and after the addition to the blood before centrifugation.
Conclusions: The antibacterial capacity of L-PRF against periodontal pathogens can significantly be enhanced by addition of antimicrobials, without disadvantage for the release of growth factors.

0032

Long-Term Survival of Teeth with Class III Furcation Involvement

Peter Eickholz[1], Maren Runschke[1], Bettina Dannewitz[1], Katrin Nickles[1], Hari Petsos[1], Bernadette Pretzl[2]
[1]Periodontology, Johann Wolfgang Goethe-University, Frankfurt/Main, Germany, [2]Conservative Dentistry, Section of Periodontology, Ruprecht-Karls-University Heidelberg, Heidelberg, Germany

Objectives: Evaluation of survival of teeth with class III furcation involvement (FI) ≥5 years after periodontal treatment and identification of prognostic factors.
Methods: All charts of patients that had accomplished active periodontal treatment (APT: step 1 and 2, if required step 3 therapy) at the center for Dentistry and Oral Medicine (Carolinum) Frankfurt, Germany beginning 2004 were screened for patients exhibiting teeth with class III FI. Patient charts had to document ≥5 years after accomplishment of APT. Charts were analysed for data (probing pocket depth) of class III FI teeth at baseline, T1 (examination after accomplishment of active periodontal treatment) and T2 (last supportive periodontal care: SPC). Further, baseline radiographic bone loss and treatment was assessed (systemic antibiotics during step 2, exclusive subgingival instrumentation, access flap, tunnelling, resective furcation surgery).
Results: One-hundred-sixty patients (age: 54.4±9.8 years; 82 females; 39 active smokers; 9 diabetics, 85 stage III, 75 stage IV, 59 grade B, 101 grade C) contributed 265 teeth with class III furcation involvement (2 maxillary premolars, 153 1st, 106 2nd, 4 3rd, 175 maxillary molars). Ninety-eight teeth (37%) were lost over a mean observation period of 109.0±33.5 months. Significantly less tooth loss (26%) was observed after adjunctive systemic antibiotic treatment than without (42%; $p = 0.013$). Baseline radiographic bone loss (BL) and PPD at start of SPC were less severe in retained (BL: 47.9±13.4%; PPD: 5.0±1.6 mm) than lost teeth (BL: 54.8±14.9%; PPD: 6.4±2.1 mm; $p < 0.001$).
Conclusions: Subgingival instrumentation with adjunctive systemic antibiotics seems to favor retention of class III furcation involved teeth. Baseline radiographic bone loss and PPD at start of SPC seem to deteriorate long-term prognosis.

0033

Evaluation of the Thermodynamic Effects of Diode Lasers Used in the Treatment of Periimplantitis

Fatma Çelik, Hasan Gündoğar
Periodontology, Gaziantep University, Gaziantep, Turkey

Objectives: One of the most important reasons for dental implant failure is peri-implantitis. Different treatment modalities were explained for peri-implantitis; one of them is lasers. Laser systems were used to clean the implant surface and to improve the

wound healing peri-implant soft tissues. This study aimed to investigate temperature change for used diode laser of different wavelength and power during laser irradiation to the dental implant.
Methods: In the present study, a sheep lower jaw was used, and implant was placed in premolar area after extraction. The implant inserting process was repeated separately for each wavelength (λ: 445 (± 5) nm, 660 (± 5) nm, and 970 (-10/+15) nm). Laser application was performed by Diode Laser (SiroLaser Blue), also power was 0.5mW-1W-1.5W-2W-2.5W-3W for 970 and 445nm, 25mW-50mW-100mW for 660nm. The temperature change from 2 points (apical, coronal) of the implant was recorded every 10s with a thermocouple during 60s.
Results: The critical threshold (10 ° C) was exceeded by different laser parameters; it was determined to be 7th second for 445 nm wavelength and 2.5W power, 13th second for 970 nm wavelength and 2W power. The use of other power settings for the 445 nm and 970 nm wavelengths were found to be reliable for 60s. It was observed that the critical temperature threshold was not exceeded at the apical point of the implant for the power settings (25mW-50mW-100mW) used for the 660 nm wavelength and after the application of all parameters.
Conclusions: Within the limits of this study, diode laser in dental implants is safe when used for a limited time at different wavelengths and powers, and when these times are exceeded, it has been observed it causes changes on the implant surface. Thus, diode laser can be used for peri-implantitis treatment with limited risk of dangerous effects.

0034

Surface Decontamination for Surgical Treatment of Peri-Implantitis: a Systematic Review
Giacomo Baima[1], Filippo Citterio[1], Federica Romano[1], Nurcan Buduneli[2], Mario Aimetti[1]
[1]University of Turin, Turin, Italy, [2]Ege Üniversitesi, Izmir, Turkey
Objectives: To assess the impact of different decontamination protocols and peri-operative systemic antimicrobials on the outcomes of surgical treatment of peri-implantitis.
Methods: Randomized clinical trials (RCTs) on surgical treatment of peri-implantitis were selected through an electronic search on Medline, Embase, Scopus, and Central databases. Only studies directly comparing two or more anti-infective strategies were included. Following data extraction, two different sets of meta-analyses were performed. Firstly, overall impact of different implant surface decontamination methods was assessed by comparing baseline values with outcomes at 6-12 months. Secondly, pairwise comparisons evaluated the potential benefit of adjunctive systemic antimicrobials over placebo. Results were expressed as weighted mean effect (WME), weighed mean difference (WMD) or risk ratio (RR).
Results: Sixteen RCTs were included. No pairwise comparisons were available for different surface decontamination methods. Use of curettes resulted in improved probing depth (PD) (WME = 2.14 mm), but the Results in terms of marginal bone levels (MBL) and percentage of disease resolution were unsatisfactory. Moreover, the potential benefit of adjunctive systemic antimicrobials over placebo was evaluated in two studies, representing a total of 178 implants. The meta-analyses identified a significantly larger improvement in MBL (WMD = 1.17 mm) and disease resolution (RR = 1.48) for test procedures but found no differences for PD reduction. None of the included studies addressed patient-reported outcome measures.
Conclusions: Although protocol heterogeneity was high, a combination of mechanical and chemical implant surface decontamination is recommendable, with titanium brushes and local delivery of minocycline showing encouraging results. Furthermore, there is evidence to support adjunctive usage of systemic antimicrobials together with the surgical treatment of peri-implantitis.

0035

Recession Coverage with or Without EMD: 5-Year Follow-up Results of a RCT.
Ho-Yan Duong, Alexandra Stähli, Jean-Claude Imber, Christoph Ramseier, Anton Sculean
Periodontology, School of Dental Medicine, University of Bern, Bern, Switzerland
Objectives: To investigate the 5-year results after recession coverage using the modified coronally advanced tunnel (MCAT) and a subepithelial connective tissue graft (SCTG) with or without an enamel matrix protein derivative (EMD).
Methods: The original study cohort consisted of 40 systemically healthy patients with single or multiple Miller Class I and II gingival recessions that were randomly assigned to either MCAT + EMD + SCTG (test group) or MCAT + SCTG (control group). The surgical approach for MCAT was identical in both groups. The following parameters were assessed at baseline, at 6 months and 5 years: mean and complete root coverage (i.e. MRC and CRC), probing depth (PD), clinical attachment level (CAL) and gain of keratinized tissue (KT). Esthetic outcomes were evaluated using the root coverage esthetic score (RES).
Results: After 5 years, 24 patients (n= 16/ females and n= 8/ males) with a total of 43 recessions could be evaluated (i.e. 14 patients with 22 teeth in the test and 10 patients with 21 teeth in the control group, respectively). After 5 years, mean recession coverage (RC) showed no statistically significant differences (p=0.8) between the groups with 69.8 ± 27.2% for the test and 73.3 ± 19.6% for the control group, respectively. Overall, 18 teeth or on patient level 9 patients reached 100% root coverage. After 6 months, MRC measured 78.0 ± 26.0% in the test and 77 ± 18% in the control group, respectively. The width of KT increased from 1.4 ± 1.1 mm at baseline to 2.2 ± 1.2 mm at 6 months and to 3.0 ± 2.0 mm at 5-years follow-up. At 5 years, mean RES was 7.17 while in 18 out of 43 teeth a maximal RES score of 10 was obtained.
Conclusions: Within their limits the present results indicate that over a period of 5 years, the use of MCAT with or without the additional application of EMD yielded similar clinical improvements.

0036

Synbiotic Stimulation of *Limosilactobacillus Reuteri* Strengthens Probiotic Effects.

Wannes Van Holm[1, 2], Tim Verspecht[1, 2], Naiera Zayed[1, 2], Nico Boon[2], Kristel Bernaerts[1], Wim Teughels[1]
[1]KU Leuven, Leuven, Belgium, [2]UGent, Ghent, Belgium

Objectives: The use of probiotics to influence the composition and formation of biofilms in oral diseases allows for an alternative for antibiotic and antiseptic use, but their effectiveness can be limited. The aim of this study was to evaluate the effect the synbiotic use of a prebiotic nutritional stimulant on increasing the probiotic effects.
Methods: Stimulation of *Limosilactobacillus reuteri* (ATCC PTA 5289) *in vitro* with glycerol was evaluated on pathogen growth on agar, growth & composition of a multispecies oral biofilm model on hydroxyapatite discs. In parallel, the inflammatory response of human oral keratinocytes (HOK-18A) to the probiotic and the biofilms (treated with and without probiotic) were also evaluated.
Results: Inhibition of periodontal pathogens on agar and in biofilms was significantly improved when the probiotic is supplied with glycerol, indicating improved antimicrobial production purely from glycerol. *L. reuteri* at approximately equal concentrations of bacterial cells to keratinocytes does not elucidate an inflammatory response and even reduced inflammation of multispecies biofilms.
Conclusions: Probiotics combined with a specific prebiotic can drastically improve their effectiveness, providing a higher likelihood of efficient treatment of oral disease.

0037

Periodontal Therapy Impact on MiRNAs in Periodontitis and Cardiovascular Diseases.

Gaetano Isola[1], Simona Santonocito[1], Alessandro Polizzi[1], Giuseppe Palazzo[1], Rosalia Leonardi[1], Ray C. Williams[2]
[1]Department of General Surgery and Surgical-Medical Specialties, Unit of Oral Surgery and Periodontology, University of Catania, Catania, Italy, [2]Department of Periodontology, University of North Carolina at Chapel Hill, Chapell Hill, North Carolina, United States

Objectives: Systemic inflammation may impair vascular function, and epidemiologic data suggest a possible link between periodontitis (PT) and cardiovascular disease CVD. This study was aimed to assess the effects of periodontal treatment of microRNAs (miRNA) expression in gingival crevicular fluid (GCF) in patients with PT and CVD as a measure of CVD risk.
Methods: For this 6-months prospective clinical trial, it were recruited healthy subjects (n=13) and patients with PT (n=13), CVD (n=13) and PT+CVD (n=13). Patients were assigned to receive full-mouth Scaling and Root Planing (FM-SRP) protocols. Patients were regularly monitored for clinical and GCF miRNA-7a, -21, -100, -125b and -200 changes at baseline and 30, 60, and 180 days follow-up. The primary outcome was the evaluation of GCF miRNA changes that were evaluated by real-time PCR.
Results: At baseline, in comparison with healthy subjects, patients with CVD had significant higher miRNA 200 ($p<0.05$) expression. However, in comparison with CVD patients, patients with PT and PT+CVD presented higher miRNA-21, -100 and -200 ($p<0.05$) and lower miRNA-125 expression. FM-SRP determined a significant reduction in clinical parameters between baseline and day 180 in all groups ($p<0.001$). Moreover, FM-SRP determined, at 180 days after therapy, a significant reduction of the miRNA 200 ($p<0.05$) in patients with CVD, of miRNA-21, -100 ($p<0.001$) in patients with PT and of miRNA -100, -200 and the upregulation of the miRNA-125 in patients with PT+CVD.
Conclusions: At baseline, compared with healthy subjects, patients with PT, CVD and PT+CVD presented significant dysregulated miRNAs GCF expression. Periodontal treatment with FM-SRP determined a significant reduction of GCF miRNA-21, -100 and -200 and the upregulation of miRNA-200 expression in the enrolled patients at 6-months follow-up. These results suggest that periodontal treatment could be important for the effective reduction of some GCF miRNA expression associated with augmented CVD risk.

0038

Periodontal Treatment and Systemic Blood Biomarkers: Systematic Review and Meta-Analysis

Marina Peric, Urska Marhl, Marco Nisi, Davide Cerreto, Stefano Gennai, Filippo Graziani
Department of Surgery, Medical, Molecular, and Critical Area Pathology, University of Pisa, Pisa, Italy

Objectives: To assess the effects of non-surgical periodontal treatment (NSPT) on changes on a vast array of serum biomarkers.
Methods: Randomized clinical trials (RCT), evaluating systemic blood biomarkers before and after the NSPT were searched by electronic databases and relevant hand-searched journals. Findings were summarized in evidence tables following the PRISMA statement. Quality analysis of RCTs was performed according to the Cochrane Reviewers' Handbook. Meta-analysis was performed for continuous outcomes using both fixed and random models with the OpenMeta [Analyst] and summarized in forest-plots.
Results: A total of 700 relevant citations were identified and 68 included in the final full-text analysis. Selected studies had a minimum of two months follow-up with at least 10 subjects enrolled. A statistically significant overall reduction was observed for serum levels of several inflammatory biomarkers: CRP, TNF-α, fibrinogen, IL-6 (all $p<0.001$), and IL-1β ($p<0.01$). Furthermore, glucose-related biomarkers, HbA1c and fasting glucose, presented a notable overall reduction after the NSPT ($p<0.001$ and $p<0.01$, respectively), especially in subjects with diabetes type 2. Moreover, a significant reduction in triglycerides ($p<0.05$) and an increase in HDL ($p<0.001$) levels was observed. Periodontal treatment triggers a systemic inflammatory reaction in the immediate post-operative phase; a significant increase of serum CRP levels of 5.00 mg/L (95% CI, 7.89 to 2.11; $p<0.01$) at day one after periodontal therapy was noted.

Conclusions: Periodontal therapy reduces the blood level of systemic inflammatory markers, glucose-related biomarkers and lipids. Present findings highlight further the importance to promote periodontal care in the management of subjects with concomitant medical pathologies.

0039

The Microbiome of Endodontic Infections and its Association with Clinical Features

Athina C. Georgiou[1], Suzette V. van der Waal[1, 2], Wim Crielaard[1], Egija Zaura[1], Bernd W. Brandt[1]

[1]Preventive Dentistry, Academic Centre of Dentistry Amsterdam (ACTA), Amsterdam, Netherlands, [2]Endodontology, Academic Centre of Dentistry Amsterdam (ACTA), Amsterdam, Netherlands

Objectives: To describe the endodontic infection of teeth with primary and secondary apical periodontitis and explore differences related to symptomatology.

Methods: Twenty-nine teeth with primary or secondary apical periodontitis were extracted and cryo-pulverized. Amplicons based on the V4 hypervariable region of the 16S rRNA gene were sequenced using Illumina MiSeq. The data was processed in line with the UPARSE pipeline and subsampled at equal depth (6400 reads/sample). The microbiome profiles were ordinated using Principal Component Analysis (PCA) and tested for differences between groups with permutational multivariate analysis of variance (PERMANOVA) using the Bray-Curtis distance. If significantly different, the microbial profiles were further analyzed using the LDA effect size biomarker (LEfSe) discovery tool.

Results: The 334 thousand sequences that passed quality filtering were clustered into 276 Operational Taxonomic Units (OTUs) and classified into 126 genera or higher taxa. The predominant genus in the entire sample set was *Fusobacterium*. The microbiomes of the endodontic infections were significantly associated with endodontic status (primary/secondary infection) (F=2.2, P=0.015) as well as with the presence or absence of pain (F=2.3, P=0.013). There were no associations between gender and microbiome or gender and the presence or absence of pain. The relative abundance of several OTUs differed based on pain existence. For example, *Streptococcus*, *Prevotella 7* and *Bifidobacterium* were more abundant in asymptomatic, while *Fretibacterium*, and *Peptostreptococcus* in symptomatic apical periodontitis. The *Streptococcus* OTU was further identified as *S. mutans* and even was the second most abundant OTU in the asymptomatic group. Secondary apical periodontitis showed a higher relative abundance of e.g. a *Tannerella* and *Mogibacterium* OTU.

Conclusions: The microbial profile of primary endodontic infection differed from that of secondary. The presence or absence of pain in apical periodontitis was related to the microbial ecology of the root canal. These results should be taken into consideration in future treatment strategies.

0040

Accuracy of Virtually Guided Access Cavity Preparation Through Augmented Reality

Andres Torres[1, 2], Marco Farronato[3], Mariano Simón Pedano De Piero[2], Reinhilde Jacobs[1]

[1]OMFS-IMPATH Research Group, KU Leuven, Leuven, Belgium, [2]Department of Oral Health Sciences, KU Leuven, Leuven, Belgium, [3]Department of Medicine, Surgery, and Dentistry, Università Degli Studi di Milano, Milano, Italy

Objectives: The aim of this study is to evaluate the accuracy of augmented reality (AR) for guided access cavity preparation in 3D printed jaws.

Methods: Two operators with different levels of experience in endodontics performed pre-planned virtually guided access cavities through markerless AR in 3 sets (upper and lower) of 3D-printed jaw models. The models were mounted on a phantom to mimic a real clinical situation. After treatment, a post-operative high resolution CBCT scan (NewTom, Verona, Italy) was taken for each model and registered to the pre-operative model. All access cavities were then segmented using 3-Matic Medical software 15.0 (Materialise, Leuven, Belgium). For anterior teeth and premolars the distance deviation in mm at the coronal entry point, apical point, and angular deviation of the access cavity was compared to the virtual planning. For the molars the distance deviation in mm at the coronal entry point was compared. Additionally, the surface area of all access cavities at the entry point was measured and compared to the planning. Descriptive statistics for each parameter were performed. A 95% confidence interval was calculated for both operators and each parameter.

Results: A total of 84 teeth and 90 access cavities (45 per operator) were drilled up to 4 mm depth inside the tooth. The mean deviation for the front teeth and premolars at the entry point was 0.51 mm and 0.77 mm at the apical point, with a mean angular deviation of 8.48° and a mean surface overlap of 57%. The mean deviation for molars at the entry point was 0.63 mm, with a mean surface overlap of 82%.

Conclusions: The use of AR for the drilling of access cavities on teeth teeth showed promising results and might have potential for clinical use. However, further development and research is needed before using it in-vivo.

0041

Design of Experiment: a Digital Tool to Optimize Apical Surgery

Raphael Richert[1, 3], Jean-Christophe Maurin[1], Jean-Christophe Farges[1], Jérome Molimard[2], Philippe Boisse[3], Maxime Ducret[1]

[1]Lyon Dental Hospital, Lyon, France, [2]Ecole nationale supérieure des Mines de Saint Etienne, Saint-Etienne, France, [3]INSA, Lyon, France

Objectives: The success rate of apical surgery has largely been improved in the last decades. Often proposed as last option, this therapeutic choice is however frequently limited by the mechanical resistance of the tooth, especially in cases of premolars. Four mechanical factors: the root-end filling material, the apical preparation, the root resection length, and the bone level were reported to influence the outcome. The current study aims to order their relative importance using a design of experiment (DOE).

Methods: Based on a statistically defined subset of factor combinations, sixteen finite elements models were generated to calculate the Von Mises stress criterion. The robustness was then validated using nine supplementary models. All finite element models were created based on a previously scanned intact maxillary premolar using Cone Beam Computed Tomography (CBCT). The different anatomical structures were segmented and modified to simulate the apicoectomy. The four mechanical factors extreme levels were defined using the existing literature (Table 1).
Results: The current study confirmed that factors preparation and bone highly influence the biomechanics of the resected tooth. However, DOE also revealed that half of the mechanical impact on the tooth was missed without considering interactions between factors, particularly between resection and preparation.
Conclusions: In conclusion, clinician could not rely on a single factor but should consider numerous potential interactions. Digital tools such as DOE appear to be well adapted to evaluate the mechanical impact of endodontic procedures. A future step would be to add the herein defined DOE in a three-dimensional imaging software to support clinician in their apicoectomy procedure.

0042

Pulpotomy as Irreversible Pulpitis Permanent Treatment: Short- and Long-Term Perspective

Julien Beauquis[1, 2], Julian G. Leprince[2, 1]
[1]DRIM/ LDRI, UCLouvain, Bruxelles, Belgium, [2]Dental Médecine, Cliniques universitaires Saint-Luc, Bruxelles, Belgium

Objectives: Irreversible pulpitis is a condition that causes patient a great deal of discomfort. Conventional treatment involves the removal of the entire dental pulp (pulpectomy). More conservative treatment alternatives such as pulpotomy are now considered as permanent treatment. The aim of this study was to analyze the short-term pain evolution and long-term treatment success of pulpotomy as permanent treatment of irreversible pulpitis on molars.
Methods: Patients consulting for irreversible pulpitis on a molar were considered eligible. Two treatment procedures were compared, first a conventional endodontic treatment performed by experienced endodontists in private practice (control group, n=42), and second, a full pulpotomy, followed by capping by Biodentine at root canal entrance, performed by practitioners with <3years experience (test group, n=43). Short-term pain was measured at 3 times point (t0=pre-treatment, t=24h, t=7d) by use of Heft-Parker pain scale. The data were submitted to chi-square analysis according to timepoint and treatment group with a significance level of $p<0.05$. Long-term treatment success was considered as absence of signs and symptoms combined with absence of periapical radiolucency. As long-term data collection is still ongoing, only preliminary results will be presented here, without statistical analysis.
Results: Complete follow-up was obtained for short-term outcome, while long-term data collection is still ongoing, with currently 79% and 60% follow-up, respectively for test and control groups. A significant reduction of pain was observed for both groups between timepoints ($p<0.0001$), while no significant difference could be identified between both treatment groups at each timepoint ($p>0.05$). The long-term success was 70% (median=26months) and 88% (median=25months), respectively for test and control groups.
Conclusions: The results of this prospective non-randomized study show that pulpotomy is an acceptable and more conservative permanent treatment option for irreversible pulpitis in molars. However, this needs to be confirmed after completion of long-term data collection and statistical analysis.

0043

Cross-Center Validity and Generalizability in Predicting Tooth Loss in Periodontitis

Lubaina T. Arsiwala[1], Falk Schwendicke[1], Joachim Krois[1], Amelie Bäumer-König[2], Bernadette Pretzl[2], Peter Eickholz[3], Hari Petsos[3], Thomas Kocher[4], Birte Holtfreter[4], Christian Graetz[5]
[1]Department of Oral Diagnostics, Digital Health and Health Services Research, Charité – Universitätsmedizin Berlin, Berlin, Berlin, Germany, [2]Department of Conservative Dentistry, Clinic for Oral, Dental and Maxillofacial Diseases, University Hospital Heidelberg, Heidelberg, Germany, [3]Johann Wolfgang Goethe-University Frankfurt/Main, Frankfurt/Main, Germany, [4]Department of Restorative Dentistry, Periodontology, Endodontology, and Preventive and Pediatric Dentistry, University Medicine Greifswald, Greifswald, Germany, [5]Clinic of Conservative Dentistry and Periodontology, University of Kiel, Kiel, Germany

Objectives: An increasing number of teeth are being retained in a growing population of older adults, resulting in expected increases in number of periodontally-affected teeth. The knowledge of an individual's predicted probability of losing periodontally-affected teeth would be instrumental in formulating prophylactic treatment plans. Hence, we aimed to predict tooth loss during supportive periodontal therapy (SPT) across four German university centers.
Methods: In our entire cohort (n=897), mean (standard deviation) age was 45 (11) years, with 59% females. Tooth loss in four centers (Kiel(KI) n=391; Greifswald(GW) n=282; Heidelberg(HD) n=175; Frankfurt/Main(F) n=49) during SPT was assessed. Our outcome was annual tooth loss per patient. Multivariable linear regression models were trained on data from 75% patients from one center and used for predictions on the remaining 25% patients of this center and 100% patients from the remaining centers. This procedure was performed for each center. The models' prediction error was assessed via root-mean-squared-error (RMSE) i.e., deviation of predicted estimates from the observed data.
Results: Annual tooth loss per patient differed across centers (median=0.00 (interquartile interval: 0.00, 0.17) in GW and 0.09 (0.00, 0.19) in F, $p=0.001$) Age, smoking status, and number of teeth present before SPT were associated with tooth loss ($p<0.03$) (Table 1). Predictions within centers showed RMSE from 0.14 to 0.30, and cross-center RMSE ranged from 0.15 to 0.31, indicating low generalizability (Table 2). Predictions had higher accuracy in F and KI than in HD and GW, while the center on which the model was trained had a less consistent impact on prediction estimates. None of the models evaluated showed useful predictive value.
Conclusions: Associations should be distinguished from predictions. Despite significant associations of covariates with annual

tooth loss, a clinically useful prediction was not possible, highlighting the need for further research to identify predictors of periodontally-affected tooth loss in adults receiving periodontal treatment.

0044

Periodontal Diseases and Awareness of Oral and Dental Health in Patients with Bipolar Disorder

Aysegul Sari[1], Hanifi Kokacya[2]

[1]Periodontology, Hatay Mustafa Kemal Üniversity, Faculty of Dentistry, Hatay, Turkey, [2]Psychiatry, Hatay Mustafa Kemal University, Faculty of Medicine, Hatay, Turkey

Objectives: Bipolar disorder is a chronic mood disorder characterized by dysfunction of psychological traits like self-esteem, speaking, thoughts, extreme shifts in mood, and sleep problems. It affects 2–3% of worldwide population. The aim of this study is to evaluate the association between periodontal diseases and awareness of oral and dental health in patients with bipolar disorder.

Methods: The study population consisted of 130 individuals who were 65 bipolar disorder patients and 65 healthy controls. All individuals underwent periodontal examination, and plaque index (PI), gingival index (GI), percentage bleeding on probing (%BOP), probing pocket depth (PD), and clinical attachment level (CAL) were recorded. The complaints of the patients about oral and dental health were enrolled and categorized as gingival bleeding, tooth sensitivity, halitosis, and aesthetic problems such as gingival recession, enlargement, tooth mobility, and abscess. The severity of depressive and manic episodes was assessed with Hamilton Depression Scale and Young- Mania Rating Scale, respectively in the bipolar disorder patients. Also, the Clinical Global Impressions Scale (CGIs) was used for assessing global illness severity in the patients group.

Results: PI, GI, BOP (%), PD, CAL (P=0.001), and missing teeth number (P=0.008) were higher in the bipolar disorder patients than the healthy controls. Prevalence of periodontitis was higher and periodontal health was lower in the patients with bipolar disorder than the healthy controls (P=0.001). Gingival bleeding (P=0.014), tooth sensitivity (P=0.001), halitosis (P=0.004), aesthetic problem (P=0.004), and tooth mobility (P=0.007) complaints were higher in the patients with bipolar disorder than the healthy controls. Positive correlations were detected between GCIs and CAL (p=0.04) in the patients group. GI, BOP (%), and CAL were higher in the patients with bipolar disorder who had aesthetic problem complaints than those without this complaint.

Conclusions: Patients with bipolar disorder can have a higher risk for periodontal disease than healthy controls. Since the symptoms in the presence of periodontal disease can become noticeable when they cause aesthetic problems by patients with bipolar disorder, periodontal diseases follow-up should be done regularly in these patients.

0045

BACH1 Binding Links the Genetic Risk for Severe Periodontitis with *ST8SIA1*

Avneesh Chopra[1], Ricarda Mueller[1], January Weiner[2], Jennifer Rosowski[3], Henrik Dommisch[1], Elisabeth Grohmann[4], Arne S. Schäfer[1]

[1]Department of Periodontology, Oral Medicine and Oral Surgery, Charité – University Medicine Berlin, Berlin, Germany, [2]Core Unit Bioinformatics, Berlin Institute of Health, Berlin, Berlin, Germany, [3]Department of Medical Biotechnology, Institute of Biotechnology, Technische Universität Berlin, Berlin, Germany, [4]Department of Microbiology, Faculty of Life Sciences and Technology, Beuth Hochschule für Technik Berlin, Berlin, Germany

Objectives: Genome-wide association studies identified various loci associated with periodontal diseases, but assigning causal alleles remains difficult. Likewise, the generation of biological meaning underlying a statistical association has been challenging. Here, we characterized the genetic association at the gene *ST8SIA1* that increases the risk for severe periodontitis in smokers.

Methods: We used CRISPR/dCas9 activation and RNA-Sequencing to identify genetic interaction partners of *ST8SIA1* and to determine its function in the cell. We used reporter gene assays to identify regulatory elements at the associated SNPs and to determine effect directions and allele specific changes of enhancer activity. Antibody electrophoretic mobility shift assays proved allele specific transcription factor binding at the putative causal SNPs.

Results: We found the reported periodontitis risk gene *ABCA1* as top up-regulated gene following *ST8SIA1* activation. Gene set enrichment analysis showed highest effects on integrin cell surface interactions (AUC = 0.85 ($q = 4.9x10^{-6}$) and cell cycle regulation (AUC) = 0.89 ($q = 1.6x10^{-5}$). We identified two associated repressor elements in the introns of *ST8SIA1* that bind the transcriptional repressor BACH1. The putative causative variant rs2012722 decreased BACH1 binding by 40%. We also pinpointed *ST8SIA1* as the target gene of the association.

Conclusions: *ST8SIA1* inhibits cell adhesion with extracellular matrix proteins and integrins, cell cycle, and enhances apoptosis. Likewise, tobacco smoke reportedly results in inhibition of cell adhesion, decrease in integrin-positive cells and cell growth. We conclude that impaired *ST8SIA1* repression, independently caused by reduced BACH1 binding at the effect T-allele as well as by tobacco smoke, contribute to higher *ST8SIA1* levels and in smokers who carry the effect T-allele, both factors would be additive with damaging effects on the gingival barrier integrity. The activity of *ST8SIA1* is also linked with the periodontitis risk gene *ABCA1*.

0046

Exome-Sequencing of 5 Families with Severe Early-Onset Periodontitis

Gesa M. Richter[1], Gunnar Wagner[2], Katharina Reichenmiller[7], Ingmar Staufenbiel[3], Orlando Martins[5], Britt-Sabina Löscher[4], Manuel Holtgrewe[6], Soren Jepsen[2], Henrik Dommisch[1], Arne S. Schäfer[1]

[1]Periodontology, Oral Medicine and Oral Surgery, Charité - Universitätsmedizin Berlin, Berlin, Germany, [2]Department of Periodontology, Operative and Preventive Dentistry, University of Bonn, Bonn, Germany, [3]Department of Conservative Dentistry, Periodontology and Preventive Dentistry, Hannover Medical School, Hannover, Germany, [4]Institute of Clinical Molecular Biology, Christian-Albrechts-University Kiel, Kiel, Germany, [5]Institute of Periodontology, Dentistry Department, University of Coimbra, Coimbra, Portugal, [6]Core Unit Bioinformatics – CUBI, Berlin Institute of Health, Berlin, Germany, [7]Private practice, Tübingen, Tübingen, Germany

Objectives: Periodontitis (PD) is characterized by alveolar bone loss leading to tooth loss. A small proportion of cases develop severe PD at juvenile or adolescent age without exposure to the main disease risk factors. It is considered that these cases carry mutations with large causal effects but they are largely unknown. To identify such mutations, we performed whole exome sequencing (WES) and targeted resequencing with 5 families with children that developed stage IV grade C PD between 3 and 18 years.

Methods: 5 families were selected for WES with stage III or IV, grade C PD diagnosed in their underaged siblings. The gene Cathepsin C (*CTSC)* was sequenced in 24 additional cases with PD stage IV grade C at ≥ 3 teeth with ≤ 25 years of age. We filtered mutations with a frequency < 0.002 and a Combined Annotation Dependent Depletion score (CADD) ≥ 20 from the WES data.

Results: In one family, we found compound heterozygous variants in the gene *CTSC* (p.R272H, p.G139R), one of which was previously identified in a family with prepubertal PD. Resequencing of *CTSC* in 24 cases (stage IV grade C) under 25 years of age identified the known mutation p.I453V (odds ratio = 4.06 (95% confidence interval = 1.6-10.3); P = 0.001) previously found in adolescent PD patients. A sibling of another family carried a homozygous mutation in *TUT7* (p.R560Q, CADD > 30), which is implicated in regulation of *IL6* gene expression. Two other siblings shared heterozygous mutations in the interacting genes *PADI1* and *FLG* (CADD = 36), which contribute to barrier integrity. We also found deleterious mutations in the PD risk genes *ABCA1*, *GLT6D1*, and *SIGLEC5*.

Conclusions: We conclude that the *CTSC* variants p.R272H and p.I453V have different expressivity and are of diagnostic relevance for prepubertal and adolescent PD, respectively. Additional putative causal mutations for early-onset PD located within genes that carry known susceptibility variants for common forms. The genetic architecture of juvenile PD is complex and differs between the affected siblings of the sequenced families.

0047

Evaluation of Apoptosis and Oxidation Mechanisms in Advanced Periodontitis

Tulin Cakir[1, 3], Meral Yuksel[2], Ali Cekici[1]

[1]Dental Faculty, Istanbul University, Istanbul, Turkey, [2]Health Services Vocational School, Marmara University, Istanbul, Turkey, [3]Dental Faculty, Istanbul Kent University, Istanbul, Turkey

Objectives: The aim of this study is to evaluate the apoptosis and oxidation mechanisms in systemically healthy Stage III and Stage IV, Grade C periodontitis patients in relation to clinical status. Additionally, correlation between clinical status, oxidative stress and apoptosis markers is examined.

Methods: This study was conducted with the permission of Istanbul University Clinical Studies Local Ethics Committee. Study group consists of 30 systemically healthy individuals diagnosed with Stage III and Stage IV, Grade C periodontitis between ages 18 and 45. Control group consists of 29 systemically and periodontally healthy individuals between ages 18 and 45. Apoptosis and oxidation events were screened by evaluating reactive oxygen metabolites, IL-1β, TNFα, Caspase-3, Caspase-8, Caspase-9, HIF-1α, total antioxidant and total oxidant levels in saliva and gingival crevicular fluid (GCF) samples. Clinical screening was performed by recording Plaque Index (PI), Gingival Index (GI), Probing Pocket Depth (PPD), Clinical Attachment Level (CAL), Bleeding on Probing (BOP) and tooth mobility. Kolmogorov-Smirnov test was applied to examine the distribution of the data. Mann-Whitney U test was used to compare clinical parameters and biochemical findings between study and control groups. Pearson Correlation Analysis was performed to examine the correlation between clinical and biochemical variables. All statistical analyzes in the study were performed using SPSS, v.22.0 statistical software. P <0.05 was considered statistically significant.

Results: CAL, PI, GI, PPD, OSI, TNF-α, Luminol-mediated and Lucigenin-mediated Chemiluminescence findings and Caspase-3 level were found to be strongly correlated[u1]. Age, PI, GI, PPD, BOP, CAL, Mobility, TNF-α, Caspase-3, TAOC, TOC, OSI, Luminol-mediated and Lucigenin-mediated Chemiluminescence findings were found statistically significantly different in the periodontitis group compared to the control.

Conclusions: Our findings show that hypoxia is associated with the increase of reactive oxygen molecules, which in turn result in the increase of apoptosis markers. Periodontal status was significantly correlated with oxidation and apoptosis in stage III and IV grade C periodontitis patients.

0048

Gingival Crevicular Fluid MicroRNAs as Diagnostic Biomarkers for Periodontitis

Benita Buragaite-Staponkiene[1], Kristina Snipaitiene[1, 2], Adomas Rovas[3, 4], Alina Puriene[3, 4], Egle Punceviciene[5, 6], Irena Butrimiene[5, 6], Sonata Jarmalaite[1, 2]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania, [2]National Cancer Institute, Vilnius, Lithuania, [3]Institute of Odontology, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, [4]Vilnius University Hospital Zalgiris Clinic, Vilnius, Lithuania, [5]Clinic of Rheumatology, Orthopedics Traumatology and Reconstructive Surgery, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, [6]Centre of Rheumatology, Vilnius University Hospital Santaros Clinics, Vilnius, Lithuania

Objectives: Periodontitis (PD) is a highly prevalent chronic inflammatory disease affecting tooth-supporting tissues and having a negative impact on overall health. Multiple mechanisms of pathogenesis of PD are epigenetically regulated and microRNAs (miRNAs) are considered as one of the key modulators that influence periodontal homeostasis. This study aimed to reveal PD-associated miRNAs in gingival tissue and gingival crevicular fluid (GCF) for subsequent use in non-invasive diagnostics of PD.

Methods: A total of 16 gingival tissue samples were used for genome-wide miRNA expression profiling. Following a thorough validation in an extended cohort of gingival tissue samples (N=80), miR-140-3p, -145-5p, -146a-5p, and -195-5p were selected for further analysis in GCF samples (N=210) by means of quantitative reverse transcription PCR.

Results: Microarray analysis revealed 140 miRNAs that were significantly upregulated in inflamed gingival tissues as compared to healthy controls. The levels of GCF miR-146a-5p were significantly lower in PD-affected individuals as compared to periodontally healthy participants (P<0.001). Severe forms of PD were associated with increased levels of GCF miR-140-3p and -145-5p and decreased levels of GCF miR-146a-5p (P<0.050). Moreover, the correlation between decreased levels of GCF miR-146a-5p and periodontal outcome parameters, indicating the worse clinical status of PD, were observed (P<0.010). The best diagnostic performance was demonstrated by a combination of the aforementioned miRNAs for diagnostics of both severe PD (AUC=0.709, P<0.001) and mild to moderate PD (AUC=0.612, P=0.018).

Conclusions: The current study revealed that miR-140-3p, -145-5p and -146a-5p were associated with PD and may be used as potential biomarkers for non-invasive diagnostics of PD.

0049

Systemic Perturbations Following Non-Surgical Periodontal Treatment in Hypertensive Patients.

Stefano Gennai[1], Morena Petrini[2, 1], Urska Marhl[1], Marina Peric[1], Rossana Izzetti[1], Marco Nisi[1], Filippo Graziani[1]

[1]University of Pisa, Pisa, Italy, [2]University of Chieti, Chieti, Italy

Objectives: Both, periodontitis and hypertension are associated with systemic inflammation. The relationship between periodontal disease and increased blood pressure (BP) values is related to the production of systemic inflammation mediators. Therefore, the aim of this study was to evaluate the acute-phase response following the two different non-surgical periodontal treatment (NSPT) protocols in patients affected by hypertension and periodontitis, namely Full-Mouth Scaling and Root Planing (FM-SRP) and Quadrant Scaling and Root Planing (Q-SRP), respectively.

Methods: Forty consecutive patients affected by hypertension and periodontitis were enrolled in the study. The patients were randomly assigned to either FM-SRP or Q-SRP group. Periodontal parameters and BP values were measured at baseline, 1 and 90 days after the NSPT. Blood collection was performed at each timepoint in order to assess the level of high-sensitivity C-reactive protein (hs-CRP) levels.

Results: Periodontal treatment was successful in both treatment groups, exhibiting improvements in all periodontal parameters. Statistically significant difference between groups was found in hs-CRP levels increase 24-h after the treatment, with the FM-SRP group presenting remarkably higher values (p<0.05). Mean BP values showed a significant decrease 3-months after the treatment.

Conclusions: FM-SRP determines an acute systemic inflammation response in patients affected by hypertension and periodontitis, with a peak in hs-CRP levels 24-h after the NSPT. Periodontal treatment can produce a beneficial effect on blood pressure.

0049.1

Bone Loss Estimation in Periodontitis Using Machine Learning on Periapicals

Nektarios Tsoromokos[1], Sarah Parinussa[2], Frank Claessen[2], David Anssari[2], Bruno G. Loos[1]

[1]Periodontology, Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, Netherlands, [2]Promaton, Amsterdam, Netherlands

Objectives: To develop an automatic analysis of periapical radiographs from periodontitis patients and non-periodontitis subjects for the % alveolar bone loss (ABL) on the approximal surfaces of teeth using a machine learning technique based on artificial intelligence, i.e. convolutional neural networks (CNN).

Methods: A total of 1546 approximal sites from 54 subjects on mandibular periapical radiographs were manually annotated (MA) for a training set (n=1308), a validation set (n=98) and a test set (n=140). The training and validation sets were used for the development of a CNN algorithm. The algorithm recognized the cemento-enamel junction, the most apical extent of the alveolar crest, the apex and the surrounding alveolar bone.

Results: For 140 unseen images in the test set, the CNN scored a mean of 23.1 ± 11.8 %ABL, while the corresponding value for MA was 27.8 ± 13.8 %ABL. The intraclass correlation (ICC) was 0.0601 (p<0.001). Further sub-analyses for various tooth types and various bone loss patterns showed that ICC's remained significant although the algorithm performed better on incisors than molars.

Conclusions: A CNN trained algorithm on radiographic images, showed a diagnostic performance with moderate to good reliability to detect and quantify %ABL in unseen periapical radiographs.

0050

Dynamic Changes in Orthodontic Tooth Movement in Rats: 3D Analysis

Chen Zong[2], Jeroen Van Dessel[1], Greetje Vande Velde[2], Guy Willems[1], Maria Cadenas de Llano Pérula[1]
[1]UZ Leuven, Leuven, Belgium, [2]KU Leuven, Leuven, Belgium

Objectives: To evaluate the changing pattern of orthodontic tooth movement (OTM) throughout time with a constant orthodontic force (OF) by an improved 3D analysis in a rat model, overcoming the limitations of previous 2D analysis.

Methods: Six adult male Wistar rats were randomly assigned to two groups by implanting a mini-screw in one hemimaxilla (split-mouth design). In Group 1 (n=6) a constant OF (25 g) was applied between the upper first molar (M1) and the mini-screw. In Group 2 (n=6) no force was applied on the contralateral hemimaxilla (control). All rats were longitudinally followed up with in vivo micro-CT before OTM (baseline, T0), after 10 (T1), 17 (T2), 24 (T3) and 31 days (T4). The OTM and bone mineral density (BMD) were measured with in 3D by a rigid voxel-based superimposition method. Values were compared between different timepoints and experimental sides by repeated measures MANOVA.

Results: M1 displaced significantly more in Group 1, while the other teeth didn't. Increased tipping of M1 was observed at T0-T1 and T3-T4, while at T1-T3 bodily movement was predominant. OTM had a significant effect on BMD (P=0.03*) but time did not (P=0.10). BMD was significantly lower in Group 1 at all timepoints except for baseline. Interestingly, BMD in Group 1 decreased at T1 but increased gradually from T1 to T4, parallel to the changing pattern of M1 displacement.

Conclusions: The changing pattern of OTM is not constant throughout time. Initially, M1 displacement was mainly due to tipping, while after BMD decreasing at T1, bodily movement dominated. The later increase of BMD from T2 to T4 led to a mix of bodily movement and tipping in M1 displacement.

0051

Comparison of Gene Profiling of Individuals with Oligodontia and Normal Dentition

Elif Mutafcilar Velioglu[1], S.Buket Bozkurt[2, 4], Erdogan E. Hakki[1], Seyit Ali Kayis[3], Sema Hakki[1]
[1]Department of Periodontology, Selcuk University Faculty of Dentistry, Konya, Turkey, [2]Department of Periodontology, Hacettepe University Faculty of Dentistry, Ankara, Turkey, [3]Department of Biostatistics and Medical Bioinformatics, Abant Izzet Baysal University Faculty of Medicine, Bolu, Turkey, [4]Research Lab of Dental Faculty, Hacettepe University Faculty of Dentistry, Ankara, Turkey

Objectives: Dentition is a complex adaptive system that is regulated by genetic, epigenetic, and environmental factors. Teeth development is directed by interactions between the ectoderm and mesenchyme and it is under strict genetic control. Oligodontia that is described as the absence of more than six permanent teeth excluding third molars can only be observed as isolated dental anomalies without any syndrome. However, several studies were performed on mutational analysis of dental anomalies, there is no report for comprehensive transcriptomic analysis. Therefore, we focused to compare whole genome expression analysis to find target genes and pathways related to developmental tooth anomalies.

Methods: Gender and age matched five individuals with nonsyndromic oligodontia and 3 individuals with normal dentition participated to this study. Total RNA was isolated for whole genome gene expression analysis from the blood of the participants. cDNA was synthesized and HumanHT-12 v4 Expression Bead Chip Direct Hybridization Assay was used in Illumina iScan platform for over 47.000 probes. Data were subjected to quantile normalization before analysis.

Results: Findings demonstrated that whole genome expression analysis could discriminate participants who have nonsyndromic oligodontia and normal dentition. While 2956 probes were up-regulated (≥ 1.5 fold changes), 2894 probes were down-regulated in oligodontia cases. Hence, in addition to mutational analysis, RNA analysis of differentially expressed genes could provide further valuable information on the mechanisms of nonsyndromic oligodontia.

Conclusions: Further confirmation studies are required for genes including MSX1, AXIN2, EDARADD, PAX9, EDA, EDAR which were previously associated with non-syndromic tooth agenesis. Studies with higher number of participants might better clarify the role of specific genes directly related with oligodontia (Selcuk University BAP).

0052

Skeletal and Soft Tissue Changes in Normal Occlusion Throughout Lifetime

Amit A. Rajbhoj, Marie Stroo, Giacomo Begnoni, Guy Willems, Maria Cadenas de Llano Pérula
Oral Health Sciences, Orthodontics, KU Leuven, Leuven, Belgium

Objectives: Age-related skeletal and soft tissue changes in human normal occlusion are important for orthodontic treatment planning and retention, especially due to the increase of adult patients seeking orthodontic intervention. The objective of this systematic review was to assess the available evidence in the literature on age-related skeletal and soft tissue changes in untreated normal occlusion.

Methods: An electronic search was performed until August 2020 using the terms 'Maxilla', 'Mandible', 'Lips', 'Ageing', 'Age groups'. Articles studying skeletal and soft tissue changes in orthodontically untreated subjects with neutro occlusion and comparing them between various age groups were included. Studies focusing on a single age group or written in languages other than English were excluded. The risk of bias of the studies was assessed with both the MINORS and ROBINS-I tools.

Results: From the initial 5539 studies, 52 were included. A high methodological heterogeneity was found. Mandibular plane inclination reduces from 7 years until 20 years. Facial height increases both anteriorly and posteriorly until 28 years of age. Anterior

cranial base length increases until 20 years and follows a slow increase until 46 years. Nasal width increases from 10 to 14 years, Nasolabial angle shows reduction from 8.8 to 17.8 years. Upper lip and lower lip length increase from 6 to 18 years along with protrusion of upper lip from 8 to 12 years and retrusion of the lower lip from 5 to 45 years.
Conclusions: Greater emphasis should be placed on the age-related soft tissue, skeletal and dental changes during diagnosis and orthodontic treatment planning to ascertain the available limits soft tissue adaptation to balance the given skeletal and dental changes made by the orthodontist. Well-designed prospective cohort studies with all the three dimensions of skeletal and soft tissues are necessary for confirmatory evidence. (PROSPERO: CRD42020203206)

0053

Resin-Infiltration of White-Spot-Lesions a Challenge for Orthodontic Bracket Bonding?

Anca Mesaros, Cristina Gasparik, Diana Dudea
Prosthodontics and Dental Materials, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Cluj, Romania

Objectives: Smile aesthetics has become a great concern for patients. White-spot-lesions on children's permanent dentition are often an aesthetic and structural concern for parents. However, nowadays treating these lesions with minimally invasive dentistry procedures by resin-infiltration is possible and frequently done as soon as the affected tooth finishes their complete eruption. Fixed orthodontic appliances are usually applied for tooth alignment and malocclusion correction also at a young age, but usually around the 10-16 years of age, when most of the permanent teeth are already erupted. Because in orthodontics there is a fair percentage of bracket bonding failure we wanted to see if some of it can be correlated to prior resin-infiltration procedures done on the teeth.
Methods: We conducted an observational retrospective pilot study on 120 orthodontic patients from a private practice and we found 8 that presented white-spot-lesions in their history, presenting a total of 27 affected teeth. Charts of these patients were studied for dates of resin-infiltration procedures, brand of orthodontic adhesive used and situations of bracket bonding failures during fixed orthodontic treatment. 5 of the patients, with 17 affected teeth underwent resin-infiltration procedures prior to their orthodontic treatment and 3 of them (with 10 affected teeth) opted to have orthodontics first.
Results: Although our study included only 27 teeth it was observed bracket adhesion failures more often and repeatedly in patients whom had previously had resin-infiltration procedures, and also using one particular type of adhesive, while no statistical differences have been found between the other adhesive technique used and patients whom didn't undergo resin-infiltration procedures prior to orthodontics.
Conclusions: Our preliminary results indicate the need for further research in the field of bracket bonding failures due to resin-infiltrations.

0054

Bisphenol-a Disturbs Bio-Mineralization in Mussels: Potential Implications for Molar-Incisor Hypomineralization

Fangfang Liu[1, 2], Franz X. Reichl[1, 2], Stefan Milz[3], Uta C. Wölfle[1], Jan Kühnisch[1], Christoph Schmitz[3], Juergen Geist[4], Christof Högg[1,2], Katharina Sternecker[3]
[1]University Hospital, Ludwig-Maximilians-University of Munich, Munich, Germany, [2]Walther-Straub-Institute of Pharmacology and Toxicology, Ludwig-Maximilians-University of Munich, Munich, Germany, [3]Institute of Anatomy, Faculty of Medicine, Ludwig-Maximilians-University of Munich, Munich, Germany, [4]Aquatic Systems Biology Unit, Department of Life Science Systems, Technical University of Munich, Munich, Germany

Objectives: Molar-incisor hypomineralization (MIH) is a global health problem and the etiology is currently unclarified. Recently, mussels were used as target organisms because of their bio-mineralization of carbonate shells during growth and ethical concerns regarding the use of vertebrate animals. The study aims to investigate the feasibility of zebra mussel (*Dreissena polymorpha*) as a new model species to screen potential factors of MIH.
Methods: 252 zebra mussels were randomly divided into 36 groups (n=7) for three experiments (12 groups/experiment). Zebra mussels of six groups per experiment were incubated with 100mg/l calcein (mineralization marker) solution for 96h, another six groups with tap water only (negative controls). Then zebra mussels with and without calcein pre-incubation were exposed to cadmium sulfate hydrate ($3CdSO_4 \bullet 8H_2O$, as positive control; 0, 0.01, 0.1, 1, 10 and 100mg/l), possible etiological factors of MIHincluding bisphenol-A (BPA; 0, 0.02, 0.2, 2, 20 and 200mg/l) and erythromycin (0, 0.1, 1, 10, 100 and 1000mg/l) as mineralization "disruptors" for 96h, respectively. After two weeks, mussels were sacrificed, the shells were collected and embedded with methylmethacrylate.
Results: Mortality was 100% in 20 and 200mg/l BPA groups. No difference with or without calcein incubation was observed. The median lethal concentration (96h-LC50) of BPA was 6.3mg/l with a 1.3-34.4mg/l confidence interval (CI) in zebra mussels. Moreover, the fluorescence intensity of calcein decreased ($p<0.05$) in both 2 mg/l BPA groups and 1 mg/l $3CdSO_4 \bullet 8H_2O$, whereas no increased mortality and decreased fluorescence intensity were found after erythromycin exposures. Mortality was 100% in 10 and 100 mg/l $3CdSO_4 \bullet 8H_2O$ groups (positive control), thereby the 96h-LC50 of cadmium was 3.1mg/l (95% CI, 0.7-10.5mg/l).
Conclusions: These results suggest that BPA may act as a potential causing factor for disturbed bio-mineralization. The bio-mineralization in zebra mussels seems to be an effective model for investigating potential causative factors of MIH.

0055

Oral Health Status and Tongue Biofilm in Neurological Patients: a Case-Control Study.

Pamela Pignatelli[1, 2], Raffaella Franciotti[3], Claudia Carrarini[3], Federica Maria Romei[1], Marco Onofrj[3], Adriano Piattelli[1, 4], Maria Cristina Curia[1]

[1]Department of Medical, Oral and Biotechnological Sciences, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Chieti, Italy, [2]Department of Oral Sciences and Maxillofacial Surgery, Sapienza University of Rome, Roma, RM, Italy, [3]Department of Neuroscience, Imaging and Clinical Sciences, "G. d'Annunzio" University of Chieti-Pescara, Chieti, CH, Italy, [4]Fondazione "Villa Serena" per la Ricerca, Città Sant'Angelo, Italy

Objectives: A bidirectional relationship was identified between the state of chronic oral inflammation and dementia in Alzheimer's Disease patients. The present study aimed to identify an association between oral health and neurological diseases.

Methods: Oral health status was recorded in 53 hospitalized neurological patients (not only with dementia) and in 31 healthy controls, using Plaque Index (PI), Gingival Index (GI), number of teeth (nbT), Healthcare Oral Hygiene Index (H-OHI), presence of Oral Infection (pOI), Lingual Patina Index, presence of fissured tongue, fixed/removable prosthesis. The abundance of *Porphyromonas gingivalis* (*Pg*) was determined in tongue biofilm by q-PCR. A Multivariate ANOVA was carried out, setting PI, GI, and nbT as dependent variable, and group as factor. A Generalized model was carried out, setting group as the dependent variable, and oral indexes as factors; AIC, R2, and p values were taken into account to rank the predictive role of the categorical indexes in distinguishing patients from control.

Results: A partial correlation with Spearman method, correcting for age, was used to test the association of *Pg* and oral indexes with systemic inflammatory indexes. Fold Change analysis revealed the increase for *Pg* (1.58), for GI (1.11), for PI (0.83), and −0.58 for nbT in neurological patients. MANOVA reported a significant difference between the two groups ($p<.001$, $f2=0.27$, $1-\beta=0.86$); the dichotomous pOI (OR: 10.286, 95%CI: 2.294 - 73.501, higher in patients) and the multinomial H-OHI (higher in controls) were the best variables. *Pg* was positively correlated with C-Reactive Protein (rho=0.55, p=.129) of patients, although not significantly.

Conclusions: The prevalence of oral infection, gingival inflammation, poor oral hygiene, tooth loss, higher Pg exposure was related to neurological disease. Compromised oral health status could increase the amount of tongue *Pg*, promoting an increase in systemic inflammation. Therefore, hospitalized neurological patients need major oral health care.

0056

Periodontal Diseases Increase the Oral Chronic Graft-Versus-Host Disease Occurrence

Cécile Olejnik[1], Thomas Muny[1], Leonardo Magro[2], Valérie Coiteux[2], David Beauvais[2], Micha Srour[2], Ibrahim Yagoub-Agha[2], Xavier COUTEL[1], Laurent Nawrocki[1]

[1]Univ. Lille, CHU Lille, Service d'Odontologie, F-59000 Lille, France, [2]Univ. Lille, CHU Lille, Service des Maladies du Sang, F-59000 Lille, France, F-59000 Lille, France

Objectives: Chronic graft-versus-host disease (cGVHD) is a frequent complication of allogeneic hematopoietic stem cell transplant (HSCT). The cGVHD commonly affects the mouth that leads to an alteration of the patient's quality of life and a potential malnutrition. The aim of this study is to evaluate the impact of oral factors on the development of oral cGVHD in patients who are candidate for HSCT for hematological malignancies.

Methods: An observational, retrospective, monocentric study was carried out in the Odontology Department of the CHU of Lille from February 2018 to March 2020. Both general factors (age, histocompatibility, allograft origin, conditioning treatment, hemopathy, preventive treatment) and oral factors (xerostomia, salivary pH, dental metal or resin filling, decay, apical infectious focus, periodontal disease) have been evaluated in order to determine their influence on cGVHD occurring.

Results: On 109 patients who are candidates for HSCT, 27 were excluded (dead at 3 months after HSCT or cancelled HSCT) and 11 were lost during the dental follow-up. Of the 71 patients, 22 developed oral cGVHD. The study showed that periodontal disease significantly differs between the cGVHD group and the no cGVHD group (p=0.0145), even if the periodontal disease factor was adjusted with known general factors as age, histocompatibility and allograft origin (p=0.0389). The periodontal disease factor significantly increased 4.173 times the risk of oral cGVHD occurrence.

Conclusions: These results highlight the relationships between the state of the oral cavity and the susceptibility to develop oral cGVHD. Collaboration between the hematologist and the dental surgeon is necessary to ensure good oral hygiene and a reduction in the inflammatory state of the oral cavity, and thus prevent the development of oral cGVHD.

0057

Radiographic Predictors of MRONJ in Patients Undergoing Tooth Extraction

Catalina Moreno Rabie[1, 2], Laurence Lapauw[1, 2], Hugo Gaêta-Araujo[3], André Ferreira-Leite[4], Reinhilde Jacobs[1, 2]

[1]Department of Imaging and Pathology, KU Leuven, Leuven, Flemish Brabant, Belgium, [2]Department of Oral and Maxillofacial Surgery, University Hospital Leuven (UZ Leuven), Leuven, Flemish Brabant, Belgium, [3]School of dentistry, Federal University of Alfenas (UNIFAL-MG), Alfenas, Minas Gerais, Brazil, [4]Department of Dentistry, University of Brasília, Brasilia, Brasilia, Brazil

Objectives: To identify local radiographic predictors of Medication-Related Osteonecrosis of the Jaw (MRONJ) in panoramic images of patients undergoing tooth extraction.

Methods: Through a retrospective search, patients were included if they were actively or previously treated with antiresorptive medication (ARM), underwent one or more tooth extractions, and had a pre- and postoperative panoramic radiograph. After data collection, blinded and independent observations were performed. Eleven distinctive imaging-related parameters were assessed preoperatively and five postoperatively, at every extraction site (Figure1). In addition to a case-control analysis, a subgroup analysis by ARM indication was performed assessing MRONJ development. Significance level was set at 5%.

Results: Between January 1st, 2010 and December 30th, 2019, 210 patients underwent tooth extraction from which 86 had an underlying oncologic diagnosis, and 124 had osteoporosis. The mean age of the oncologic and osteoporotic group was 68±12 and 69±11 years, the mean duration of the ARM treatment was 21 and 67 months, the number of extracted teeth were 269 and 383, with 69 (26%) and 16 (4%) of the teeth developing MRONJ, respectively. Additionally, a control group (n=108) that was age- (67.8± 10.7 years), number-of-extractions- (n=331), and sextant-of-extraction matched was selected. Preliminary results show that furcation involvement, caries, and changes in the bone pattern like sclerosis and heterogenous patterns were significantly more present in the MRONJ+ than in the MRONJ– group ($p \leq 0.05$). In addition, changes in the bone pattern, sequestrum formation, and crater-like defect showed an association with MRONJ+ in the postoperative parameters ($p \leq 0.05$).
Conclusions: MRONJ may be anticipated when recognizing furcation involvement, presence of pulpal caries, partial endodontic filling, and a sclerotic bone pattern in the to-be-extracted tooth. An heterogenous pattern, persistent alveolar socket, sequestrum formation, and crater-like defect could be signs of MRONJ after tooth extraction. This early identification encourages a patient-oriented decision making to avoid the onset of MRONJ.

0057.1

An Interprofessional Geriatric Assessment Project for Dental Undergraduate Students

Marc L. Auerbacher[1], Michael Drey[4], Birgit Wershofen[2], Yvonne Pudritz[3], Monika Fintz[5], Lydia Gebetsberger[1], Reinhard Hickel[1], Dalia Kaisarly[1]

[1]Department of Conservative Dentistry and Periodontology, University Hospital, LMU Munich, Munich, Germany, [2]University Hospital, LMU Munich, Institute for Medical Education, Munich, Germany, Munich, Germany, [3]Department of Pharmacy, University Hospital, LMU Munich, Munich, Germany, Munich, Germany, [4]Department of Medicine IV, Ludwig-Maximilians-University Munich, University Hospital, LMU Munich, Munich, Germany, [5]School of Nursing at the LMU University Hospital Munich, Munich, Germany

Objectives: Demographic changes lead to an increasingly elderly population in Germany. Thus, the number of elderly in need of dental care is constantly rising. Care of older people with complex health needs often require knowledge from multiple disciplines. Theoretical and practical learning in geriatric dentistry at dental schools is very important for young dentists to meet the special needs of older adults. Only a few universities in Germany incorporate geriatric dentistry into their curricula.
Methods: An Interprofessional geriatric Assessment Project (IgAP) was developed in which students of dentistry, medicine, pharmacy and nursing participated. Each group consisted of four students with one from each profession. The students performed various subject-specific assessments on geriatric patients in retirement homes. Dental students evaluated the oral health status using the Oral Health Assessment Tool (OHAT). The assessment findings were presented in small groups and individual treatment recommendations for each patient were proposed. The outcome of each profession was discussed within the interprofessional group of all students and with medical professionals. A survey was conducted at the end.
Results: The results of the survey showed that most dental students felt more comfortable regarding the treatment of geriatric patients after participating in the project (94.7%), became more familiar with the challenges faced by dentists providing dental care to vulnerable patients and considered the interprofessional collaboration very helpful (89.5%). All students were interested in further projects related to geriatric dentistry.
Conclusions: This project allows dental students to learn with, from and about other health professionals in order to improve collaboration and the quality of care. The dental students feel encouraged and motivated to provide oral health care for elderly with special health care needs.

0058

Resin-Modified Calcium-Silicate Cement Exhibits Antimicrobial Capacity Against *S. Mutans.*

Mariano Simón Pedano De Piero[1], Wannes Van Holm[2], Xin Li[1], Ivana Nedeljkovic[3], Kirsten L. Van Landuyt[1], Wim Teughels[2], Bart Van Meerbeek[1]

[1]Department of Oral Health Sciences, BIOMAT, KU Leuven and University Hospitals (UZ Leuven), Leuven, Belgium, Leuven, Belgium, [2]Department of Oral Health Sciences, Oral Microbiology, KU Leuven and University Hospitals (UZ Leuven), Leuven, Belgium, Leuven, Belgium, [3]Dental Materials Science, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, Amsterdam, Netherlands., Amsterdam, Netherlands

Objectives: To evaluate the antimicrobial capacity of freshly mixed resin-modified ('BioCal'; Harvard) calcium-silicate cement (CSC) as compared to that of the commercial resin-modified CSC TheraCal LC ('TheraCal'; Bisco) and resin-free CSC Biodentine (Septodont), versus that of set cements, this by means of turbidity and colony-forming unit (CFU) assays (n=3 per experimental group/condition). Ca/Si release and pH were also measured.
Methods: 1.5-cm^3 hCSC disks, placed in 24-well plates under sterile conditions, were allowed to set at 37°C/5%CO_2 for 24h. In addition to set CSCs, freshly mixed CSCs were immediately exposed to 1.5-ml brain heart infusion (BHI) per sample. Eluates from both conditions were collected after 24h and inoculated with *S. mutans* in 96-well plates, achieving a final bacterial concentration of $2x10^7$/ml. At 3h, 6h, 9h and 24h of incubation (37°C/5%CO_2), turbidity of cultures was measured spectrophotometrically (Varioskan, Thermo-Fisher). Fresh BHI, glucose-supplemented BHI, antibiotics-supplemented BHI and sterile material eluates served as controls. Subsequently, blood-agar plates were inoculated with 50 µl dilutions of the 24h cultures; after 48h of incubation (37°C/5%CO_2), CFU count was determined. To measure Ca/Si release and pH, the same setting was used as for the antibacterial assay but using sterile-deionized water instead of BHI. Ca/Si release and pH were measured with ICP-OES (Varian 720-ES, Agilent) and a pH meter (3210 WTW, equipped with a Hamilton MiniTrode electrode) respectively, both at 1h, 24h, 72h, 1w, 2w and 4w.

Results: Resin-modified 'BioCal' exhibited antibacterial activity against *S. mutans* following both the turbidity and CFU assays, in this order: Biodentine>BioCal>TheraCal ($p<0.05$). Fresh cements were more antimicrobial than set CSCs ($p<0.05$), corresponding to a higher pH and higher Ca/Si release from the freshly mixed than set CSCs ($p<0.05$).
Conclusions: BioCal revealed antimicrobial activity against *S. mutans*, which was related to its Ca/Si release and alkaline pH.

0059

Interfacial Characteristics of BIOfactor MTA and Biodentine with Dentin

Mehmet A. Soylu[1], Emine Simsek[2], Seref Nur Mutlu[3], Makbule B. Akbulut[4]
[1]Student, Necmettin Erbakan University Faculty of Dentistry, Meram, Konya, Turkey, [2]Dentist, Private Dentova Aesthetic Oral and Dental Health Polyclinic, Meram, Konya, Turkey, [3]Department of Dental Services, Necmettin Erbakan University Vocational School of Higher Education, Meram, Konya, Turkey, [4]Assoc Prof, Department of Endodontics, Necmettin Erbakan University Faculty of Dentsitry, Meram, Konya, Turkey

Objectives: Physical and chemical reactions between dentin and calcium silicate-based materials are expected to occur when these materials are used in perforation repair process. The objective of this study was to characterize the interface between dentin and MTA-Angelus (Angelus, Londrina, Brasil), Biodentine (Septodont, France) and BIOfactor MTA (Imicryl, Konya, Turkey) using Scanning Electron Microscopy (SEM) and Energy Disperse X-Ray Spectroscopy (SEM-EDS).
Methods: A total of fifteen dentin segments of 1.5 mm thickness were obtained from previously extracted single-rooted human teeth. Canal lumens were instrumented with diamond burs, and the smear layer was removed. The specimens were then randomly filled with MTA-Angelus, Biodentine or BIOfactor MTA (n=5) and were placed in distilled water for 28-days at 37°C. The samples were processed for SEM and three images from each specimen were randomly selected and examined. The samples were further subjected to SEM-EDS analysis to determine principal elemental composition of the material, dentin, and interfacial area. Elemental mapping was performed with each element being marked in a different color.
Results: Interfacial layer was evident in approximately 70% of SEM images in both MTA-Angelus and BIOfactor MTA samples. In the Biodentine group, interfacial layer was observed on more than half of the SEM micrographs. Tungsten was present only in MTA-Angelus-dentin interface while ytterbium was detected on BIOfactor MTA- dentin interface. Calcium levels in the interfacial layer between BIOfactor MTA and dentin were higher than levels in both dentin and cement. Silicon was evident in all material-dentin interfaces.
Conclusions: A distinguishable interfacial layer was observed in most of the samples within the BIOfactor MTA, MTA-Angelus and Biodentine groups. The elemental constitution of the interfacial layer was different from that of the materials in all groups. BIOfactor MTA as a novel root repair material exhibited promising characteristics.

0060

Effect of Endodontic Sealers on Periodontal Regeneration and Angiogenesis

Thomas Giraud[2, 1], Charlotte Jeanneau[2], Sandra Roumani[2], Imad About[2]
[1]Hôpital Timone, Service d'Odontologie, Assistance Publique – Hôpitaux de Marseille, Marseille, France, [2]Institut des Sciences du Mouvement, Aix Marseille Université, Centre National de la Recherche Scientifique, Marseille, France

Objectives: The major aim of root canal therapy is to achieve periapical tissue healing. Root canal sealing implies an interaction of the sealer with the surrounding tissues. This work was designed to investigate the effect of endodontic sealers on human periodontal (hPDL) cells' angiogenic and regeneration potentials.
Methods: Root canal sealers: Pulp Canal Sealer (PCS, zinc oxide eugenol), AH Plus® (AH, epoxy resins) and BioRoot™ RCS (BR, calcium silicates) were prepared and incubated in MEM medium (0.2mg/mL). hPDL cells, obtained by the explant outgrowth method, were incubated with Lipopolysaccharides (LPS) and the sealers' extracts. hPDL cells stimulated with LPS alone were used as control. VEGF, FGF-2 and TGF-b1 growth factor secretion was quantified by ELISA. hPDL cell colonization was evaluated using the scratch assay. Human Umbilical Vein Endothelial Cells (HUVECs) were used to evaluate the sealers' effects on neo-angiogenesis. HUVECs were incubated with hPDL cells supernatants, their proliferation was evaluated by MTT test, and neo-angiogenesis was assessed on Matrigel.
Results: All sealers increased VEGF secretion, but this increase was lower with BR and AH. FGF2 secretion increased with BR and AH while TGF-b1 increased only with BR. hPDL cell colonization significantly increased with BR and AH but decreased with PCS. Endothelial cell proliferation increased with BR and AH but this proliferation decreased with PCS. Neo-angiogenesis on Matrigel showed that that both PCS and AH decreased the number of tubular-likes structures as compared to BR.
Conclusions: This work highlights that endodontic sealers modulate PDL regeneration and angiogenic potentials. While BR induced hPDL cell colonization and growth factor secretion, stimulated endothelial cell proliferation and allowed tubular-like structure organization, these events were hindered with PCS and to a lesser extent with AH. These data suggest that the sealer's interaction with the surrounding tissue is of prime importance for periapical healing.

0061

Bone-Forming Genes Expression of Osteoblasts Cultured on Polymeric Nanosctructured Membranes.

Manuel Toledano-Osorio[1], Francisco Javier Manzano-Moreno[2, 3], Manuel Toledano[1], Antonio-Luis Medina-Castillo[4], Victor Javier Costela-Ruiz[3, 5], Concepcion Ruiz[5, 3], Raquel Osorio[1]

[1]Faculty of Dentistry, University of Granada, Granada, Spain, [2]Biomedical Group (BIO277), Faculty of Dentistry, University of Granada, Granada, Spain, [3]Instituto Investigacion Biosanitaria, ibs, Granada, Spain, [4]NanoMyP, Nanomateriales y Polimeros S.L., Spin-Off company of the University of Granada, Granada, Spain, [5]Biomedical Group (BIO277), Department of Nursing, University of Granada, Granada, Spain

Objectives: To evaluate the effect of polymeric nanostructured membranes in osteoblasts differentiation.

Methods: Nanostructured membranes were produced by electrospinning and functionalized with SiO_2-NPs (NanoMyP). They were then doped with doxycycline by immersion in an aqueous doxycycline solution. The following groups were established: 1) Undoped membranes (HOOC-M), 2) SiO_2-NPs functionalized membranes (HOOC-Si-M), 3) SiO_2-NPs functionalized membranes and doped with doxycycline (Dox-HOOC-Si-M). Membranes were subjected to MG63 osteoblast-like cells culturing (ATCC, Manassas, VA, USA) during 48h. Differentiation was assessed by real-time quantitative polymerase chain reaction (RT-qPCR) and Field Emission Scanning Electron Microscopy. In the RT-qPCR; TGF-β1, Runx-2, ALP, OPG, RANKL and BMP-2 were studied. Three membranes of each group were subjected to each test and both tests were performed in triplicate. Mean comparisons were conducted by one-way ANOVA and Tukey tests ($p<0.05$).

Results: The RT-qPCR Results are in the Table -means (nG of mRNA per nG of House Keeper gene) and standard deviations-. Letters indicate differences between membranes. All the studied genes were overexpressed in the Dox-HOOC-Si-M group, except RANKL which was downregulated, when compared with HOOC-M. The OPG/RANKL ratio, which expresses the bone-building activity of osteoblasts, was up-regulated in 28-fold change by the Dox-HOOC-Si-M when compared with HOOC-M group. The osteoblasts cultured on the modified membranes, showed an elongated spindle-shaped morphology, which has been associated with a more differentiated state.

Conclusions: The functionalization of the polymeric membranes with SiO2-NPs and Dox produced an increase of osteogenic gene expression on cells. Supported by Ministry of Economy and Competitiveness and European Regional Development Fund [MAT2017-85999P MINECO/AEI/FEDER/UE].

0062

The Effect of Bioactive Coatings on Enamel Microhardness

Ivan Šalinović, Valentina Brzovic Rajic, Ana Ivanišević Malčić, Ivana Miletic

University of Zagreb, School of Dental Medicine, Zagreb, Croatia

Objectives: The aim of this study was to determine the effect of three different varnish materials, containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), nano-hydroxyapatite and fluoride respectively, on enamel microhardness.

Methods: A total of 30 extracted human third molars were obtained, in order to prepare enamel specimens. They were immersed into autoploymerising acrylic resin and polished, exposing an enamel surface. The specimens were demineralized by applying 37% phosphoric acid for three minutes. Three experimental groups (n=10) were treated with 3M™ Clinpro™ White Varnish, MI Varnish® and Megasonex® toothpaste respectively at every 24 hours for 14 days, using micro brush. Between the treatments the samples were kept in saline. The microhardness of the specimen surface was determined by the Vickers method in triplicate (HV0,1), in three stages; baseline value, after demineralization and after the period of remineralization. Data were subjected to Scheffe post-hoc test.

Results: The mean microhardness values before demineralization, after demineralization, and after remineralization for enamel were 366,0±18,93, 190,30 ± 27,10, 236,57 ± 19,42 for M™ Clinpro™ White Varnish; 343,52 ± 26,66, 192,73 ± 16,37, 286,65 ± 34,71 for MI Varnish® and 393,05 ± 16,14, 201,90 ± 15,30, 237,97 ± 32,52 for Megasonex®. Microhardness values obtained for samples treated with MI Varnish® were higher compared to the other two ($p = 0.001$ for both comparisons), while the first and third group did not differ significantly from each other ($p = 0.97$).

Conclusions: The usage of MI Varnish® leads to higher microhardness values of the enamel after demineralization has occurred, compared to 3M™ Clinpro™ White Varnish and Megasonex® toothpaste.

0063

>3Years Follow-up of a Variable-Thread Tapered Implant.

Alexander De Greef[2], Greet De Mars[2], Charlotte Favril[2], Katleen Vandamme[1], Wim Teughels[2], Marc Quirynen[2]

[1]Department of Oral Health Sciences, University Hospitals Leuven, Leuven, Belgium, [2]Department of Oral Health Sciences, KU Leuven & Dentistry (Periodontology), Leuven, Belgium

Objectives: The aim of this prospective, single-center study was to document, for the NobelActive implant, survival rate, success rate and potential predictive variables for early bone loss (e.g. smoking, periodontal bone loss according to age, quality and quantity of bone, crestal gingival thickness, guided bone regeneration (GBR) procedures, ISQ and torque values).

Methods: From March 2011 to September 2016, 286 NobelActive implants were installed in 157 subjects. Separate analyses were performed for implants placed via a 1- or 2-stage procedure, and relative to the timing of placement/loading. At implant placement, abutment connection, functional loading, at 6, 12, 24 and up to 58 months post-loading, clinical and radiographic data were recorded.

Results: The cumulative survival rate after 12 months and 58 months of loading was respectively 99.7% and 98.6%. The mean marginal bone loss (MBL) of 276 patients between loading and 12 months was 0.05 mm (±0.77). 58 months post-loading, a mean bone loss of 1.05 mm (±1.43) was observed in 26 patients. Periodontal bone loss over age, smoking, crestal gingival thickness, torque values, bone quality, bone quantity and GBR influenced significantly the bone loss over time.
Conclusions: The presented 58 months (4.9 years) follow-up of functional loading data shows a divergent bone level around the NobelActive implants.

0064

Biofunctionalization of Dental Abutment Materials Support Stable Adhesion of Gingival Cells

Alena Palkowitz[1], Taskin Tuna[2], Shaza Bishti[2], Frederik Böke[1], Nathalie Steinke[3], Gerhard Müller-Newen[4, 5], Stefan Wolfart[2], Horst Fischer[1]
[1]Department of Dental Materials and Biomaterials Research, RWTH Aachen University Hospital, Aachen, Germany, [2]Department of Prosthodontics and Biomaterials, RWTH Aachen University Hospital, Aachen, Germany, [3]Flow Cytometry Facility, RWTH Aachen University Hospital, Aachen, Germany, [4]Institute of Biochemistry and Molecular Biology, RWTH Aachen University Hospital, Aachen, Germany, [5]Confocal Microscopy Facility, RWTH Aachen University Hospital, Aachen, Germany

Objectives: An essential criterion for the success of an implant-supported restoration is the quality of the adhesion between the abutment and the attached gingival tissue. Due to the lack of the natural 'epithelial junction', bacteria could penetrate into the interface if the adhesion is insufficient. To overcome this limitation, here we propose a novel method to effectively bioactivate abutment materials surfaces through the covalent conjugation of ECM proteins in order to enable a stable soft tissue adhesion.
Methods: A silane monolayer was applied on relevant implant materials (Y-TZP/Ti6Al4V) with subsequent coupling of the two extracellular matrix (ECM) proteins fibronectin and laminin via a specific crosslinker. The successful application was verified via XPS, FTIR-ATR, AFM and immunostaining. Retained function of the ECM-proteins after surface coupling was shown in a centrifugation-assay. Influence on proliferation and cell adhesion with and without saliva of gingival cells on the ECM-modified and non-modified specimens was investigated additionally. Moreover, integrin expression of gingival cells on the individual modified surfaces was assessed via confocal microscopy and flow cytometry.
Results: The centrifugation-assay showed that human gingival fibroblasts (HGFs) seeded onto the ECM-protein-coupled surfaces exhibited significantly higher adhesion ($p < 0.001$***) in comparison to non-functionalized controls. In addition, a proliferation assay (CCK-8) showed that significantly more cells ($p < 0.05$*) were evident after 7 days on ECM-modified surfaces compared to non-functionalized surfaces. Confocal microscopy revealed a much higher cell area (up to threefold) and enhanced expression of pFAK-Y397 on ECM-protein-coated surfaces compared to those seeded on native Y-TZP and Ti6Al4V. Moreover, HGFs on ECM-protein coated surfaces exhibited an enhanced integrin expression.
Conclusions: We showed that the covalent conjugation of ECM proteins via crosslinking on the dental abutment materials Y-TZP and Ti6Al4V enables a strong adhesion of HGFs. Therefore, the novel approach could improve the 'sealing' against bacteria at this interface and thus holds promise to potentially reduce or prevent peri-implant diseases in clinical application.

0065

5-Aminolevulinic Acid and red LED for Decontamination of Titanium Surfaces

Morena Petrini[1], Silvia Di Lodovico[2], Luigina Cellini[2], Federico Vecchiet[3], Giovanna Iezzi[1], Adriano Piattelli[1, 3], Simonetta D'Ercole[1]
[1]Medical, Oral and Biotechnological Sciences, University of Chieti, Chieti, Chieti, Italy, [2]Pharmacy, University of Chieti, Chieti, Chieti, Italy, [3]Unit of Dentistry, S.S. Annunziata Hospital, Chieti, Chieti, Italy

Objectives: This work aimed to evaluate the effectiveness of a novel photodynamic therapy protocol (ALA) against bacterial biofilm growth on two different titanium surfaces.
Methods: *Streptococcus oralis* has been cultivated for 24h on machined and double acid-etched (DAE, Resista, Italy) titanium discs that were previously incubated with saliva to permit the acquired pellicle formation. Then, half of the samples were treated with the ALA protocol. ALA comprehends the incubation of the samples for 45 minutes with a novel gel containing 5-aminolevulinic acid (Aladent, Alphastrumenti, Italy), followed by 7 minutes of red led irradiation at 630 nm and at 380 mW/cm^2. 4 different groups were tested: unexposed samples (MACHINED and DAE) and exposed ones (MACHINED ala and DAE ala). The colony-forming units (CFUs), the biomass, and the SEM observations were measured after 48 h.
Results: The exposed samples showed a significant reduction of the CFUs and biomass on both surfaces, without any statistically significant differences between MACHINED ALA and DAE ALA. The CFUs were significantly higher on unexposed MACHINED. DAE, DAE ALA, and MACHINED ALA showed a 29%, 84%, and 89% of bacterial reduction with respect to the unexposed MACHINED. SEM images showed that ALA gel trapped dead bacteria on the titanium surfaces, as demonstrated by the red dead cells visible at the live dead staining.
Conclusions: The results of this preliminary study confirm the effective bacteria-killing and biofilm reduction of this photodynamic protocol on titanium surfaces, making it suitable for the treatment of peri-implantitis.

0066

Effect of Vitamin-D_3 in HPDL-MSCs is Modified by Osteogenic Medium

Oleh Andrukhov, Christian Behm, Alice Blufstein, Andreas Moritz, Xiaohui Rausch-Fan
University Clinic of Dentistry, Medical University of Vienna, Vienna, Austria

Objectives: Vitamin-D_3 ($VitD_3$) is involved in bone metabolism, mineral homeostasis and regulating the immune response. Recent studies showed that the biologically most active $VitD_3$ metabolite $1,25(OH)_2D_3$ enhances the osteogenic differentiation of human periodontal ligament-derived mesenchymal stromal cells (hPDL-MSCs) *in vitro*. However, such an effect was not observed for $25(OH)D_3$, the biological precursor of $1,25(OH)_2D_3$ and the most common circulating $VitD_3$ metabolite. To understand this discrepancy, we have investigated the effect of osteogenic differentiation media components on the cellular effects of $1,25(OH)_2D_3$ and $25(OH)D_3$ in hPDL-MSCs.

Methods: Primary hPDL-MSCs were isolated from six different healthy donors and stimulated with either $1,25(OH)_2D_3$ (10 nM) or $25(OH)D_3$ (100 nM) in the presence or in the absence of dexamethasone (10-100 nM), β-glycerophosphate (1-10 mM), fetal bovine serum (FBS, 1-10 %) or ascorbic acid (5-50 µM). $VitD_3$-induced cellular activity was assessed by measuring the gene expression of osteocalcin (OC), which is directly regulated by the $VitD_3$ receptor (VDR). Additionally, the gene expression levels of CYP27B1, CYP24A1 and VDR, which are involved in the local vitamin D_3 metabolism, were measured.

Results: The gene expression level of OC was significantly enhanced by both $VitD_3$ metabolites. Dexamethasone inhibited $VitD_3$-induced OC expression in a concentration-dependent manner. Additionally, dexamethasone significantly increased the expression of CYP24A1, which is responsible for $1,25(OH)_2D_3$ inactivation. β-glycerophosphate slightly inhibited $VitD_3$-induced OC expression, which was also accompanied by the inhibition of VDR expression. $VitD_3$-induced OC expression was significantly inhibited by FBS in a concentration-dependent manner. This effect was more pronounced for $25(OH)D_3$ than $1,25(OH)_2D_3$ and was accompanied by the decreased expression of CYP27B1, which is involved in the local conversion of $25(OH)D_3$ into $1,25(OH)_2D_3$. Ascorbic acid had no significant effect on the investigated parameters.

Conclusions: Our data show that osteogenic medium components modify the effects of both $VitD_3$ metabolites in hPDL-MSCs. Translation of these data into the clinical situation implies that environmental factors could modify $VitD_3$ activity in the periodontium.

0067

Role of NLRP3 Inflammasome in Periodontitis: Friend or Foe?

Banndith Cheat[1, 3], Sarah Didi Alaoui[1, 3], Cyrile Wouatong[1, 3], Asmaa Foda[1, 3], Coralie Torrens[1, 3], Brigitte Baroukh[1, 3], Jeremy Sadoine[1,3], Lotfi Slimani[1, 3], Jérôme Bouchet[1, 3], Marjolaine Gosset[1, 2]
[1]URP 2496 «Orofacial Pathologies, Imaging and Biotherapies », Université de Paris, Montrouge, France, [2]Hôpital Charles Foix, Vitry-sur-, France, [3]Laboratoire d'excellence Inflamex, Paris, France

Objectives: Periodontitis are highly prevalent chronic inflammatory diseases triggered by the dysbiosis of oral microbiota and characterized by gingival inflammation and the irreversible destruction of the periodontium (*i.e.* cementum, periodontal ligament and alveolar bone). Among periodontal microbiota, *Porphyromonas gingivalis* (*P.g.)* is a keystone pathogen responsible for oral dysbiosis and tissue homeostasis disruption leading to periodontal diseases. NLRP3 inflammasome, a major determinant of inflammation, is overexpressed in periodontitis. Recently, it has been suggested that NLRP3 inflammasome may actively participate in the pathogenesis of periodontitis. The objective of the present study is to characterize the role of NLRP3 inflammasome in periodontal destruction and alveolar bone resorption during *P.gingivalis*-induced periodontitis.

Methods: Periodontitis induction was performed on WT and NLRP3 KO mice by mean of ligature soaked with or without *P.gingivalis* and subgingivally placed into the palatal sulcus of the first upper right molar. The left molar remains intact and served as control. We replaced the ligature twice a week during four weeks. The alveolar bone resorption was monitored by micro-computed tomography (micro-CT) through out the experiment.
All mice were sacrified and processed for histological analysis.

Results: Micro-CT analysis revealed a protective role of NLRP3 against bone loss in periodontitis. This result correlates with the protection against periodontal soft tissue destruction. Moreover, we show that NLRP3 inflammasome is overexpressed in the connective tissue of WT mice after periodontitis induction. This upregulation increases strongly the local production of the pro-inflammatory cytokine such as IL-1beta in WT mice, as compared to NLRP3 KO mice. This expression actively participates in the recruitment of polymorphonuclear cells which subsequently form a PMNs-protective wall at the site of periodontitis induction.

Conclusions: During periodontitis, the upregulation of NLRP3 inflammasome has a protective effect against periodontitis through recruitment of neutrophils. However, this protective role is subverted by the presence of *P.gingivalis*.

0068

Systemic Blood Markers in Periodontitis Affected Patients: Systematic Review and Meta-Analysis

Urška Marhl[1, 2], Marina Peric[1, 2], Marco Nisi[1, 2], Davide Cerreto[1, 2], Stefano Gennai[1, 2], Filippo Graziani[1, 2]
[1]Department of Surgical, Medical and Molecular Pathology and Critical Care Medicine, University of Pisa, Pisa, Italy, [2]Sub-Unit of Periodontology, Halitosis and Periodontal Medicine, University Hospital of Pisa, Pisa, Italy

Objectives: To assess the effects of periodontitis (PD) on systemic biomarkers blood levels in patients with or without comorbidities.

Methods: Observational studies, evaluating systemic blood biomarkers and periodontitis were searched by electronic databases and relevant hand-searched journals to answer the following PICO question: *Do subjects with periodontitis have elevated blood values of systemic inflammatory biomarkers when compared to controls without periodontitis?* The protocol was registered at

PROSPERO (CRD42019118831). Findings were summarized in evidence tables according to the PRISMA statement. The quality assessment of included studies was assessed using The Newcastle-Ottawa Scale (NOS) for case-control and cohort studies, and modified NOS for cross-sectional studies.
Results: A total of 712 Results was identified. After screening of titles and abstracts and further full-text analysis, 629 citations were excluded. The final 83 articles were analysed for methodological quality and availability of data for meta-analysis. The following biomarkers could be observed: inflammatory (C-reactive protein, Fibrinogen, Tumor necrosis factor-α, Interleukins IL-1β and IL-6), Glucose-related (HbA1c, Glucose) and Oxidative stress-related biomarkers (Ox-LDL, Total oxidant status), Lipids (Total cholesterol, HDL, LDL, VLDL, Triglycerides, Leptin), and leucocytes (lymphocytes, neutrophils). For all these biomarkers, subgroups between systemically healthy participants and those without any systemic comorbidities were observed. Higher blood levels in all biomarkers were observed in systemically compromised patients in comparison to systemically healthy ones.
Conclusions: Patients with PD show elevated blood levels of systemic inflammatory biomarkers. Therefore, the importance of periodontal care should be further promoted, ecpecially in patients with comorbidities.

0069

Fourteen-year Clinical Performance of a HEMA-Free One-step Self-Etch Adhesive

Marleen Peumans, Ellen Van de Maele, Kirsten L. Van Landuyt, Bart Van Meerbeek
KU Leuven (University of Leuven), Department of Oral Health Sciences, BIOMAT – Biomaterials Research group & UZ Leuven (University Hospitals Leuven), Leuven, Belgium

Objectives: The aim of this randomized controlled trial was to evaluate the 14-year clinical performance of a HEMA-free 1-step self-etch adhesive (1SEa) as compared with that of a 3-step etch-and-rinse adhesive (3E&Ra).
Methods: 267 non-carious cervical lesions in 52 patients were restored with the micro-hybrid composite Gradia Direct (GC), bonded in random order either with the HEMA-free 1SEa G-Bond ('GB'; GC) or the 3E&Ra Optibond FL ('OFL'; Kerr), the latter considered as gold-standard E&Ra (control). The restorations were followed up over 14 years for retention, marginal adaptation and discoloration, caries occurrence and tooth sensitivity. Statistical analysis involved a logistic regression model with generalized estimating equations (2-way GEE model).
Results: The patient recall rate at 14 years was 63.5%. In total, 82 restorations (41 GB, 41 OFL) failed because of retention loss (GB: 18.9%, OFL: 18.4%), severe marginal defects, discoloration and/or caries (GB: 24.3%; OFL: 23.9%). The overall clinical success rate was 56.8% and 57.7% for GB and OFL, respectively. The number of restorations with an unacceptable marginal defect increased during the last 5 years (GB: 18.2%; OFL: 20.5%). Twelve restorations (6 GB, 6 OFL) showed an unacceptable marginal defect due to recurrence of severe abrasion/erosion/abfraction. No significant difference in overall clinical performance was recorded between both adhesives, except for marginal discoloration at the incisal enamel side, which was observed significantly more for the 1SEa GB (72.8 %) than for the 3E&Ra OFL (44.9%) ($p<0.05$).
Conclusions: After 14 years, restorations bonded with the HEMA-free 1SEa performed clinically equal as those bonded with the 3E&Ra gold standard. Unacceptable marginal deterioration was the main reason for failure, followed by loss of retention. Although recurrence of severe abrasion/erosion/abfraction did not influence the bonding performance of the adhesive, it resulted in clinically unacceptable restorations.

0070

Enamel Shear Strengths and Failure Modes of Universal Bondings

Andreas Schedle, Shear Bond Group, Alexander Franz
University Clinic of Dentistry, Medical University of Vienna, Vienna, Austria

Objectives: The aim of the present study was to invesitgate the enamel bond strength and the failure modes of three self-etching universal bonding systems applied by inexperienced experimenters.
Methods: 288 bovine teeth were ground to expose an adequate enamel surface by 16 dental students in their regular courses. Composite cylinders (ø 4mm, Ceram.x Spectra ST, Dentsply-Sirona) were fixed onto the bovine teeth perpendicular to the exposed enamel surfaces using three universal bonding systems [Optibond Universal (Kerr), Scotchbond Universal (3M Espe), Prime & Bond active (Dentsply Sirona)]. Light curing was performed using LED Curing-Light (Premium Plus Int. Ltd.) in high mode. Shear bond strength was measured with a universal testing machine (Zwick/Roell). Data were analyzed by mixed-model ANOVA.
Results: The bond strengths were significantly different [$p<0.01$; Optibond Universal (18.9 MPa ± 4.4) > Scotchbond Universal (15.2 MPa ± 4.6) > Prime & Bond active (9.1 MPa ± 3.4)].
At the composite/enamel interface, 2 different types of fracture were recorded: cohesive (with enamel) and adhesive (between adhesive layer and enamel). Optibond Universal showed the highest number of cohesive fractures (12.5%), followed by Scotchbond Universal (2.1%). With Prime & Bond active, only adhesive fractures were observed.
Conclusions: The bond strength values of Optibond Universal were only slightly lower than those of Optibond XTR (20.1 ± 3.9), an established self-etching two-bottle bonding system tested in 2017 with the same experimental setup. In 2019, the dentin bond strength of the same universal bonding systems was determined. Dentin and enamel bond strengths were comparable for Optibond universal and Prime & Bond active, while slightly lower values were measured on enamel for Scotchbond universal.

0071

Acrylamide Monomers in Universal Adhesives

Mohammed H. Ahmed[1], Kumiko Yoshihara[2], Noriyuki Nagaoka[3], Chenmin Yao[1], Yasuhiro Yoshida[4], Bart Van Meerbeek[1]

[1]KU Leuven (University of Leuven), Department of Oral Health Sciences, BIOMAT, Leuven, Belgium, [2]Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Department of Pathology & Experimental Medicine, Okayama, Japan, [3]Okayama University Dental School, Advanced Research of Center for Oral and Craniofacial Science, Okayama, Japan, [4]Hokkaido University, Faculty of Dental Medicine, Department of Biomaterials and Bioengineering, Sapporo, Hokkaido, Japan

Objectives: The mono-functional monomer 2-hydroxyethyl methacrylate (HEMA) is often added to universal adhesives (UAs) to improve surface wetting and prevent phase separation. Nevertheless, HEMA promotes watersorption and hydrolysis at adhesive interfaces, hereby affecting long-term bonding to dentin. This study investigated if two acrylamide monomers could replace HEMA in an UA formulation applied in etch-and-rinse (2E&R) and self-etch (1SE) bonding mode.

Methods: Four experimental UAs were bonded to bur-cut dentin. In addition to 12wt% 10-MDP, 25wt% Bis-GMA and 10wt% TEGDMA as common monomer composition, the four formulations solely differed for either the acrylamide cross-linker monomer 'FAM-201' as TEGDMA alternative and HEMA replacement, the hydroxyethyl acrylamide monomer 'HEAA' as HEMA alternative, HEMA ('HEMA+'), or extra TEGDMA in a HEMA-free control ('HEMA-'), all added in a 15wt% concentration. The split-tooth study design involved application in 2E&R mode on one tooth half versus 1SE mode on the corresponding half. Micro-tensile bond strength of half of the micro-specimens was measured upon 1-week distilled-water storage ('immediate-1w μTBS'), with the other half measured after additional 6-month storage ('aged-6m μTBS'). Statistics involved linear mixed-effects (LME) modelling ($p<.05$). Mechanistic research involved thin-film (TF) XRD surface analysis, interfacial TEM characterization, cytotoxicity assay and LogP determination.

Results: FAM-201 revealed significantly higher μTBS than HEMA+ at 1w and 6m when applied both in 2E&R and 1SE bonding modes. HEAA's μTBS was significantly lower than that of HEMA+ at 1w when applied in SE mode. TF-XRD and TEM revealed similar chemical and ultrastructural interfacial characterization, including stable 10-MDP_Ca salt nano-layering, while interfacial interaction was not affected by the different monomer formulations. FAM-201 was least cytotoxic and presented with an intermediary LogP, while HEAA presented with the highest LogP, indicating high hydrophilicity and water-sorption sensitivity.

Conclusions: The acrylamide co-monomer FAM-201 could replace HEMA in an UA formulation, while HEAA not.

0072

Bonding of an Experimental Bioglass-Based 2-Step Universal Adhesive to Dentin

Caroline Mocquot[1, 2], Benjamin Mercelis[1], Mohammed H. Ahmed[1], Chuliang Tang[1], Brigitte Grosgogeat[3, 4], Pierre Colon[2], Bart Van Meerbeek[1]

[1]Department of Oral Health Sciences, BIOMAT – Biomaterials Research group & UZ Leuven (University Hospitals Leuven), Dentistry, KU Leuven (University of Leuven), Leuven, Belgium, [2]Department of Conservative Dentistry – Endodontics, Rothschild Hospital, Assistance Publique - Hopitaux de Paris / Multimaterials and Interfaces Laboratory (UMR 5615), BIOmaterials team, University of Lyon 1, Lyon, France, [3]Multimaterials and Interfaces Laboratory (UMR 5615), BIOmaterials Team, University of Lyon 1, Lyon, France, [4]Dental faculty - Hospices civils de Lyon, Dentistry, University of Lyon 1 (Claude Bernard), Lyon, France

Objectives: To determine the immediate dentin-bonding effectiveness of an experimental 2-step universal adhesive formulation to which experimental bioglass filler, being referred to as '0NaMBG', was incorporated or this bioglass filler was beforehand applied prior to adhesive treatment. The microstructure of the dentin surface, having received 0NaMBG, and of 0NaMBG itself was additionally characterized.

Methods: The immediate (1-week) micro-tensile bond strength (μTBS; n=10 teeth; two-way ANOVA: $p<0.05$) of an experimental BZF-29 (GC) adhesive, consisting of a 10-MDP primer and a hydrophobic adhesive resin, to which 5% 0NaMBG was self-added ('BZF-29_{0NaMBG}') and which was bonded in a 3-step etch&rinse (E&R) and 2-step self-etch (SE) mode, was measured to bur-cut dentin. The alternative bonding protocol involved the application of an aqueous 0NaMBG solution (0.015g/1.35mL distilled water) prior to BZF-29 bonding ('0NaMBG+BZF-29'). G2-Bond Universal ('$G2B_{uni}$', GC) and BZF-29 without filler ('BZF-29') served as controls. Additionally, 0NaMBG application on dentin was morphologically imaged by SEM and 0NaMBG's ultrastructure morphologically/chemically characterized using S(TEM)/EDXS.

Results: No significant difference in μTBS was recorded between BZF-29_{0NaMBG} and 0NaMBG+BZF-29, with E&R bonding significantly outperforming SE bonding (graph). When applied in E&R mode, BZF-29_{0NaMBG}'s μTBS was significantly higher than $G2B_{uni}$'s and BZF-29's μTBS. When applied in SE mode, no significant differences in μTBS were recorded. SEM revealed dentinal tubule obstruction by 0NaMBG filler. S(TEM)/EDXS revealed the presence of 50 to 100-nm beam-sensitive glass particles consisting of an ordered atomic network of several elements (Si, O, Ca and P).

Conclusions: The 0NaMBG-containing 2-step UA bonded equally well to dentin as when the bioglass filler was beforehand applied, hereby representing two options for a potential bioactive co-functionality. Research is ongoing to determine the aged μTBS and to characterize interfacial interaction by TEM.

0073

Improving the Wettability of Demineralized Dentin with Dimethyl Sulfoxide Pretreatments

Merve Uctasli[1, 2], Thiago H. Stape[1, 2], Arzu Tezvergil-Mutluay[1, 2]

[1]Department of Restorative Dentistry and Cariology, Adhesive Dentistry Research Group, University of Turku, Turku, Finland, [2]Turku University Hospital, TYKS, University of Turku, Turku, Finland

Objectives: This study assessed the effect of aqueous or ethanolic dimethyl sulfoxide (DMSO) dentin pretreatments and different drying methods on dentin wettability following the application of a hydrophilic or hydrophobic resin.

Methods: Standard smear-covered H_3PO_4-etched (32% wt, for 15s) mid-coronal dentin discs (2 mm thickness) were randomly divided into wet- or dry- groups depending on whether dentin surface were conventionally blot dried or air-dried for 30 s. Dentin discs were further assigned to subgroups pretreated with 50% solutions of DMSO/ethanol, DMSO/H_2O or no pre-treatment (control) followed by blot drying or air-drying. Dentin wettability was measured with contact angle measurements (Biolin Scientific, Espoo, Finland) using sessile drop method after dispersing a 3 µL drop of hydrophilic (Primer, Scotchbond Multi-Purpose, 3M-ESPE) or hydrophobic (Bond, Scotchbond Multi-Purpose, 3M-ESPE) resin mixture on dentin surface. The change in contact angle over 240 s time was evaluated using tangent drop shape analysis. Data (n = 8/group) were analyzed by factorial ANOVA followed by the Tukey test (α=0.05).

Results: Initial dentin hydration, pretreatment and moisture control after treatment significantly affected contact angles ($p < 0.05$). While DMSO pretreatments increased the wettability of the hydrophilic resin mixture to levels similar to wet dentin, contact angles of the hydrophobic resin were significantly lower than the no treatment control, regardless of initial dentin hydration or moisture control ($p < 0.05$).

Conclusions: DMSO pretreatments, especially DMSO/Ethanol, were not only able to reverse the wettability issues of air-dried dentin, but improved the overall wettability of the hydrophobic resin.

0074

Visualization and Biophysical Characterization of Dentin-adhesive Material Interfaces

Nadezda O. Bessudnova, Sergey Venig, Vladimir Senkin

Saratov National Research State University, Saratov, Russian Federation

Objectives: The aim of the research project is to develop methodology for in vivo visualization of dentin-adhesive material interfaces (AIs) in restorative dentistry.

Methods: 1. X-ray contrastive nano-particulate markers of Ag and Au were synthesized and embedded into adhesive material at the concentration levels of 0.5-30% by weight. Special methodology was developed to escape particle agglomeration in colloidal adhesive systems leading to their functional destabilization. 2. X-ray opacity of the samples of human dentin and adhesive system with different percentages of embedded nano-sized markers was evaluated using both X-ray and high-resolution CT imaging as well as a program calculating pixel intensity values that range from 0(black) to 255(white). 3. Thermocycling and cyclic mechanical loading machines were used to visualize artificial aging of AI. 4. Finite element analysis was applied to simulate and visualize different types of AI in restored teeth.

Results: 1. In the course of laboratory experiments it has been revealed that silver/gold nanoparticle concentration of 3-5% by weight is sufficient to make adhesive material X-ray contrastive without significant changes in its physical and chemical properties. 2. The developed colloidal system with embedded nanosized markers was applied to visualize and assess the penetration of adhesive systems of different generations in dentin. Adhesive systems of generation IV-VII were tested in the study. It has been evaluated the depth of adhesive penetration, the geometry and distribution density of adhesive tags, and, as a consequence, the quality of the formed AIs. 3.Tracing of nanoparticle migration in the process of AI aging made it possible to visualize adhesive material redistribution and reveal AI degradation in time. 4. Numerical simulation of chewing loadings on 3D models of restored teeth allowed to visualize and assess strain-stress responses in AIs to prognosticate possible cracks.

Conclusions: VIsualization in adhesive dentistry is a relatively new branch of scientific researches. The joint application of nanosized markers and modern methods of high-resolution CT imaging permits an objective assessment of AIs, whereas the use of numerical simulation at the preclinical stage provides an opportunity to virtual visualization of AIs to predict treatment outcomes.

0075

Shear Bond Strengths of Two Newly Marketed Self-Adhesive Resin Cements

Cansu Atalay[1], Uzay Koç Vural[1], Ivana Miletic[2], Sevil Gurgan[1]

[1]Department of Restorative Dentistry, Hacettepe University, Ankara, Turkey, [2]Department of Endodontics and Restorative Dentistry, University of Zagreb, Zagreb, Croatia

Objectives: The purpose of this in-vitro study was to compare the shear bond strength (SBS)s of two newly marketed self-adhesive resin cements to enamel, dentin and lithium disilicate (Lisi) glass ceramic block.

Methods: Forty-eight sound human molars were sectioned mesiodistally and 48 enamel and 48 dentin substrates were obtained. Additionally, 6x7x5mm -sized 24 specimens were produced from initial LiSi blocks. The prepared tooth specimens were randomly assigned into four groups(n=12) according to the surface treatments: (1) G-CEM ONE [GCO], (2)G-CEM ONE Adhesive Enhancing Primer [GCO-AEP]+GCO, (3)RelyX Universal [RXU], (4)Scotchbond Universal Plus [SUP]+RXU. LiSi specimens were randomly divided into two groups (n=12), as follows: (1)G-Multi Primer [GMP]+GCO, (2) Scotchbond Universal Plus+RXU. The resin cements were applied by a bonding jig with a cylindrical mold (Φ=2.38 mm). All specimens were kept in 100% humidity at 37°C for 24 h and then submitted for SBS testing in a universal testing machine(1mm/min). Data were analyzed by Welch's, one-way ANOVA and two

independent samples t-tests. The nature of failures was examined under a light microscope. One representative specimen from each group was evaluated for the bonding interface and failure mode using Scanning Electron Microscope.
Results: GCO and RXU showed similar SBS to enamel ($p>0.05$) and the use of GCO-AEP and SUP resulted in improved SBS($p<0.05$). No difference was detected between GCO-AEP+GCO and SUP+RXU. The GCO-AEP+GCO exhibited the highest SBS to dentin($p<0.05$), followed by GCOSUP+RXU>RXU($p<0.05$). There was no significant difference between SBSs of two resin cements to LiSi blocks($p>0.05$). No cohesive failure was observed for both groups.
Conclusions: The use of adhesives prior to the application of self-adhesive resin cements improved their bonding to tooth tissues. GCO demonstrated superior SBS to dentin, whereas both self-adhesive resin cements generated similar SBS to enamel and LiSi glass ceramic surfaces.

0076

Bond Strength of Temporary Luting Materials with Implant-Supported Provisional Crowns

Sarah M. Blender[1], Christina Maubach[1], Jochen Mellinghoff[2], Katharina Kuhn[1], Ralph G. Luthardt[1], Heike Rudolph[1]
[1]Department of Prosthetic Dentistry, Center of Dentistry, Ulm University, Ulm, Germany, [2]Private Practice, Ulm, Germany

Objectives: The cementation of dental restorations on implants can be performed by using definitive or temporary materials. Temporary cementation allows an easy removal of the restoration without damage and is available for future re-cementation. For this purpose, in addition to the established zinc oxide-based materials with and without eugenol, composite-based materials have recently become available. The aim of this study was to investigate the bond strength of these materials when provisional single-tooth crowns are bonded to one-piece zirconia implants.
Methods: Twenty provisional single crowns (3M™ Protemp™ 4, ESPE) were cemented onto one-piece zirconia implants (Z-Look3 Evo SLM, Z-Systems) using nine different temporary luting materials (see table below). After one day and seven days of water storage (37°C), the crowns were removed from the implants using a universal testing machine (Z010, Zwick/Roell). The maximum force was recorded (N), and the force per area was calculated (MPa). Statistical analysis was performed using univariate analysis of variance (SPSS version 27.0, IBM).
Results: The highest values were achieved for a composite-based luting material (Bifix Temp, VOCO GmbH) with 1,83 MPa (1d) and 1,61 MPa (7d). The lowest values were shown for a zink oxide-eugenol cement (TempoCem, DMG) with 0,69 MPa (1d) and a composite-based material (TempBond Clear,Kerr) with 0,57 MPa (7d). Whereas six out of nine materials showed no significant change in bond strength after seven days, a zinc oxide-eugenol-based cement (TempoCem, DMG) actually showed a highly significant ($p<0.001$) improvement in bond strength.
Conclusions: For temporary cementation of provisional crowns on one-piece zirconia implants, composite-based materials show the highest bond strength, followed by eugenol-free and eugenol-containing materials.

0077

Effects of Hypergravity on Proliferation, Differentiation and Circadian Rhythm of SCAP

Melania Toska, Athina Bakopoulou, Petros Koidis
Department of Prosthodontics, School of Dentistry, Faculty of Health Sciences, Aristotle University of Thessaloniki (A.U.TH.), Thessaloniki, Greece

Objectives: Aim of this study was to investigate the effect of hypergravity, applied for bone mass recovery after space flights or for treatment of chronic musculoskeletal diseases (e.g. osteoporosis), on viability/proliferation, osteo/odontogenic differentiation potential and circadian rhythm of oral stem cells.
Methods: Stem cells from apical papilla (SCAP) cultures were established from third molars of young healthy donors and characterized for stem cell-SC markers with flow cytometry. SCAP were exposed to hypergravity of 5g, 10g and 20g by utilizing centrifugation (Centrifuge 5804 R, Eppendorf) for 30 and 45 minutes. Viability/proliferation was evaluated by MTT assay compared with the control (1g). Real-time PCR was used to evaluate osteo/odontogenic markers' expression (DSPP, RUNX2, BMP-2, ALP), as well as circadian rhythm-related genes (CLOCK, BMAL1, CRY1, PER1, PER2).
Results: Results showed that application of 5g for 30 min had a significantly higher impact on increasing cell viability/proliferation compared with 1g. Application of 5g and 10g for 45 min also exhibited a significant increase in cell viability/proliferation at 72h. Gene expression indicated pronounced osteo/odontogenic shift of SCAP, evidenced by upregulation of DSPP, BMP-2, ALP, and RUNX2 particularly at 5g conditions. The expression of circadian-related genes showed differential, gravity-related alterations during differentiation, while the expression of CLOCK, BMAL1 and PER2 was significantly changed compared to 1g. Specifically, 5g and 20g induced downregulation of CLOCK and upregulation of PER2 compared with 1g, while BMAL1 increased significant at 5g. The expression of PER1 and CRY1 significantly decreased in all groups compared with baseline expression.
Conclusions: This is the first study validating the potential of hypergravity to influence proliferation, osteo/odontogenic differentiation, and circadian rhythmicity of SCAP. These results reveal that hypergravity engaging oral stem cells can be considered a promising new strategy towards dynamic tissue engineering of mineralized tissues, in addition to therapeutic application in management of bone-related diseases.

0078

Dental Organoids – a New Approach Towards Human Tooth Regeneration?

Lara Hemeryck[1], Florian Hermans[2, 1], Ivo Lambrichts[2], Annelies Bronckaers[2], Hugo Vankelecom[1]

[1]Department of Development and Regeneration, KULeuven, Leuven, Vlaams Brabant, Belgium, [2]Department of Morphology; Biomedical Research Institute, UHasselt, Diepenbeek, Belgium

Objectives: Tooth loss, mostly as a consequence of oral disease, is a major health problem worldwide. Replacing missing teeth with a biological tooth would be an interesting alternative to the current standard implantation of synthetic materials. Organoids, defined as self-forming 3D *in vitro* reconstructions of a tissue, provide a powerful means to pursue this goal.

Methods: In our lab, we succeeded in developing an organoid model derived from adult human dental tissue acquired following tooth extraction.

Results: These dental organoids express stem cell (e.g. SOX2) as well as tooth-related markers (e.g. amelogenin) and are long-term expandable. Interestingly, they show differentiation potential towards ameloblast-like cells when cultured in specified medium. Concordantly, single-cell RNA-sequencing reveals key features of amelogenesis and convergences with recently identified markers of mouse incisor mature ameloblasts. Finally, the organoids are able to deposit mineralized tissue when transplanted *in vivo*.

Conclusions: Taken together, we developed a new, unique organoid model which will provide a powerful tool to study human tooth development, pathology and repair, and may pave the way towards tooth regenerative replacement therapy.

0079

Mouse Molar and Incisor Organoids: Innovative Tools for Regenerative Medicine

Florian Hermans[1, 2], Lara Hemeryck[2], Ivo Lambrichts[1], Hugo Vankelecom[2], Annelies Bronckaers[1]

[1]Department of Morphology, Hasselt University, Hasselt, Belgium, [2]Department of Development and Regeneration, KU Leuven, Leuven, Belgium

Objectives: Worldwide, tooth loss, typically a consequence of oral or congenital disease or traumatic injury, is an important health problem, commonly treated by implantation of synthetic materials. Despite recent research efforts using different stem cells and biomaterials, complete regeneration of dental tissues is not yet possible. Being able to induce tooth regeneration with biological material, preferentially of biological origin, would resolve most of the limitations of current treatments based on synthetic components. Organoids, self-organizing stem cell-derived three-dimensional *in vitro*reconstructions of an organ, provide a powerful strategy to meet this objective.

Methods: Here, we embarked on the establishment of mouse tooth organoids as a tool to explore tooth biology and regeneration.

Results: We were able to establish organoids from early-postnatal mouse molars and incisors. The organoids were found long-term expandable, at present for more than 10 passages. Gene expression and immunostaining analyses revealed the presence of multiple dental markers, including amelogenin (AMELX). In addition, known dental epithelial stem cell markers (such as SOX2) were detected in the tooth organoids suggesting a prominent stemness character. Currently, we are testing the differentiation capacity of the molar- and incisor-derived organoids using *in vitro* and *in vivo* approaches. In fact, we have already obtained an improved *in vitro* protocol resulting in drastic increases in AMELX and ODAM, both at the gene and protein level.

Conclusions: Eventually, our study will help to unravel molecular and cellular aspects of tooth development, as well as of dental stem cell biology, in the end instrumental for pursuing tooth regenerative replacement therapy.

0080

Extracellular Matrix Remodeling in the Apical Papilla

Ronald B. Driesen, Tim Vangansewinkel, Ivo Lambrichts

Biomedical Research Institute, Cardio and Organ Systems, Hasselt University, Hasselt, Limburg, Belgium

Objectives: The apical papilla is a collagen and stem cell rich tissue which is involved in dental root maturation. Here, we investigate cellular heterogeneity in the medulla of the apical papilla and determine their role in extracellular matrix (ECM) remodeling.

Methods: Apical papillae were dissected from 13 human third molars (13-26 years), embedded in paraffin and stained for haematoxylin-eosin to investigate cellularity. Immunocytochemistry was performed to identify cellular phenotypes (vimentin, CD44), apoptosis (cleaved caspase-3), calcium and lipid binding (Annexin V), enzymes involved in ECM degradation (MMP-1, FAP-α) and collagen crosslinking (lysyl oxidase, osteopontin). Stem cells of the apical papilla (SCAPs) were isolated using the tissue explant method. Functional capacity of SCAPs to contract collagen was determined in 3-D floating collagen gels and second harmonic generation microscopy (SHG) was implemented to evaluate collagen type I organization.

Results: Macroscopical analysis revealed a reduction in apical papilla size during root elongation. Cellularity remained stable but decreased significantly when reaching full elongation. Regional phenotypical analysis identified vimentin+/CD44+ SCAPs in the outer medulla and vimentin-/CD44- as largest population in the inner medulla. High apoptotic activity as indicated by cleaved caspase-3 was shown in SCAPs and coincided with nuclear translocation of Annexin V. Cytosolic Annexin V expression was noted in the vimentin-/CD44- cell population suggesting differential calcium influx. SCAPs showed strong expression of lysyl oxidase and osteopontin *in situ* and *in vitro* but was absent in cells of the inner medulla. Moreover, SCAPs demonstrated a high contraction and crosslinking capacity after re-introduction in floating collagen gels. SHG imaging confirmed collagen crosslinking in the vicinity of SCAPs. Alternatively, SCAPs degraded ECM as indicated by the marked FAP-α and MMP-1 staining.

Conclusions: Our results revealed that SCAPs have the capacity to remodel the ECM during root elongation suggesting a novel role in dental root maturation.

0081

Potential Use of CLP Patient-Derived Fibroblasts for Alveolar Bone Reconstruction

Ludovica Parisi[1], Silvia Rihs[1], Giorgio C. La Scala[2], Isabelle Schnyder[3], Christos Katsaros[1], Martin Degen[1]

[1]Laboratory for Oral Molecular Biology, Dep. Orthodontics and Dentofacial Orthopedics, University of Bern, Bern, Switzerland, Switzerland, [2]Dep. of Pediatrics, Geneva University Hospitals, Geneva, Switzerland, [3]University Clinic for Pediatric Surgery, Bern University Hospital, Bern, Switzerland

Objectives: Autogenous bone graft is the gold standard for alveolar defects reconstruction in cleft lip and palate (CLP) affected patients. To relieve CLP individuals of the burden of another invasive surgery for harvesting bone, alternative strategies would be beneficial. Cleft lips are surgically corrected at the age of 3-5 months, which requires the excision of superfluous lip tissue reaching into the cleft. This tissue remnant represents an optimal source for the isolation of primary cells, including fibroblasts (Fb), which display compelling similarities to mesenchymal stem cells (MSCs) known to have bone regenerative potential. These observations prompted us to investigate the *in vitro* characteristics of CLP-Fb in regard to their potential future clinical use for regenerative purposes.

Methods: We employed an explant culture approach to isolate CLP-Fb. Therefore, we thoroughly assess similarities of CLP-Fb with bone marrow-derived MSCs, including the expression of MSCs markers (CD73, CD90, CD105, CD106 and CD146) and the capacity to differentiate into fat- and bone-forming cells.

Results: Both CLP-Fb and MSCs were plastic adherent and showed a spindle-like morphology, with comparable levels of CD73, CD90 and CD105. However, CLP-Fb showed considerably lower CD106 and CD146 levels than MSCs. When induced to differentiate into fat- and bone-forming cells, both CLP-Fb and MSCs expressed lineage-specific transcription factors as well as distinct tissue markers. CLP-Fb also possess additional MSCs characteristics, such as the ability to form colonies from single cells and the capacity to modulate the immune response. Further analysis revealed the presence of specific CD106-positive as well as CD146-positive subpopulations within our CLP-Fb cultures. While the CD106- positive population could be enriched by serum starvation, the CD146-positive was reduced by passaging, resulting in a reduced bone-forming capacity.

Conclusions: Our data show that CLP-Fb significantly share MSC-like characteristics and their use might represent a promising alternative approach for the repair of bone defects.

0082

4D Monitoring of Dentine Dissolution Using *in-Situ* Synchrotron X-ray Tomography

Nathanael Leung[1], Robert A. Harper[2], Bin Zhu[1], Richard M. Shelton[2], Gabriel Landini[2], Tan Sui[1]

[1]Department of Mechanical Engineering Sciences, University of Surrey, Guildford, Surrey, United Kingdom, [2]School of Dentistry, University of Birmingham, Birmingham, Birmingham, United Kingdom

Objectives: Dental erosion is one of the most common oral conditions, damaging the mechanical function and aesthetics of teeth by causing destruction of local dental hard tissues. Dentine can become exposed to acid if the erosion of enamel is severe or with gingival recession. Development of a treatment that can restore the structural integrity is therefore highly desirable. Such developments require a good understanding of the demineralisation process in dentine of which little is known regarding the progressive changes of the intertubular dentine (ITD) and peritubular dentine (PTD) microstructures. The aim of this study was to use high-speed synchrotron X-ray microtomography (SXM) to conduct the first *in-situ* investigation of the acid dissolution of human dentine to observe the time-resolved 3D (4D) microstructural changes of the ITD and PTD during simulated dental erosion.

Methods: SXM was performed on the I13-2 beamline at Diamond Light Source. During the experiment, dentine samples were exposed to 10 v/v% lactic acid (pH 2, 100 µL), whilst being continuously scanned, with a spatial resolution of ~0.325 µm and a temporal resolution of ~15 min.

Results: From the SXM data, different dissolution rates of ITD (1.79 µm/min) and PTD (1.94 µm/min) and their dimension profiles, measured to a depth of 325 µm below the original surface, were quantified.

Conclusions: The results provide some insight into the mechanisms of human dentine dissolution. This work also forms the basis for future time resolved SXM studies, using different acids, pH, and titration, that will provide evolving microstructural information that can aid the establishment of high-fidelity numerical models of the dentine dissolution process and its effects on the mechanical properties of dentine.

0083

The Effects of pH on Second Messengers in *S. Mutans*

Kasidid Ruksakiet[1, 2], Balázs Stercz[3], Pongsiri Jaikumpun[1], Orsolya Dobay[3], Ákos Zsembery[1], Gergo Tóth[4], Péter Horváth[4], Zsolt Lohinai[2]

[1]Department of Oral Biology, Semmelweis University, Budapest, Hungary, [2]Department of Conservative Dentistry, Semmelweis University, Budapest, Hungary, [3]Institute of Medical Microbiology, Budapest, Hungary, [4]Department of Pharmaceutical Chemistry, Budapest, Hungary

Objectives: Nucleotide-based second messenger (SC) signaling regulates biofilm formation and virulence of bacteria. Environmental factors including pH may affect SCs resulting in lifestyle transition of bacteria. We aimed to study the effects of different pH values on intracellular c-di-GMP, cAMP, and c-di-AMP levels related to biofilm formation in *Streptococcus mutans.*

Methods: *S. mutans* ATCC 35668 was grown in Brain Heart Infusion (BHI) broth pH 7.5 for 16 hours, then transferred to new BHI media with pH 4.5, 5.5, 7.5, or 8.0 and incubated for 3 h. The same amount of bacteria was obtained based on optical density (OD) for extraction of SCs. The extraction was performed by heat and ethanol while cell pellets were kept for total protein quantification by Quant-iT™ protein assay. HPLC-MS/MS was applied for SC determination. Biofilm formation was determined by crystal violet assay at 48 h.

Results: Normalized c-di-AMP level was 112.8±23.2 pmol/mg protein at pH 7.5, similar amount was observed at pH 5.5, but it decreased at pH 4.5 by 38.4% ($p<0.001$), or at pH 8.0 by 41.5% ($p<0.001$). The same tendencies were found in c-di-GMP levels (353.3±38.6 pmol/mg protein at pH 7.5). However, cAMP levels increased in stepwise manner toward lower pH, from pH 8.0 to pH 7.5 by 22.6% ($p<0.05$), to pH 5.5 by 44.8% ($p<0.001$) and to pH 4.5 by 36.9% ($p<0.001$). In contrast, biofilm formation decreased in stepwise manner from pH 8.0 toward acidic pH values.

Conclusions: In conclusion, pH 7.5 provides optimal condition for *S. mutans* SC homeostasis and biofilm formation. We speculate that *S. mutans* lacks acid adaptability after a single exposure to acidic shock resulting in low biofilm formation. Our results suggest that c-di-AMP and c-di-GMP are related to biofilm formation and cAMP to planktonic lifestyle in *S. mutans*.

0084

Masking Efficacy of Infiltration of (non-)Post-Orthodontic WSL or Fluorosis – Meta-Analysis

Richard Wierichs[1], Konstantinos Dritsas[2], Dimitrios Kloukos[2], Sotiria Bourouni[1]

[1]Department of Restorative, Preventive and Pediatric Dentistry, zmk bern, University of Bern, Switzerland, Bern, Switzerland, [2]Department of Orthodontics and Dentofacial Orthopedics, zmk bern, University of Bern, Switzerland, Bern, Switzerland

Objectives: The present review systematically analyzed clinical studies investigating the efficacy of resin infiltration on post-orthodontic or non-post orthodontic, white spot lesions (WSL) or fluorosis.

Methods: Five electronic databases (Central, PubMed, Ovid MEDLINE, Ovid EMBASE, LILACS) were screened. Article selection and data abstraction were done in duplicate. No language or time restrictions were applied. Outcomes were visual-tactile or DIAGNOdent measurements.

Results: Eleven studies with 1834 teeth being affected in 413 patients were included. Nine studies were randomized control trials, one a prospective cohort study and one had an unclear study design. Meta-analysis could be performed for 'resin infiltration vs.

untreated control', 'resin infiltration vs. fluoride varnish' and 'resin infiltration without bleaching vs. resin infiltration with bleaching'. WSL being treated with resin infiltration showed a significantly higher optical improvement than WSL without any treatment (standard mean difference (SMD) [95%CI] = 1.24 [0.59,1.88], moderate level of evidence [visual tactile assessment]) and with fluoride varnish application (Mean difference (MD) [95%CI] = 4.76 [0.74, 8.78], moderate level of evidence [DIAGNOdent reading]). In patients with fluorosis, bleaching prior to resin infiltration, showed no difference in the masking effect compared to infiltration alone (MD [95%CI] =-0.30 [-0.98, 0.39], moderate level of evidence).
Conclusions: Resin infiltration seems to be a viable option to aesthetically mask enamel white spot lesions and fluorosis. However, although the evidence was graded as moderate this conclusion is based on only very few well-conducted RCTs.

0085

Attitudes of Dutch Dentists Regarding the Treatment of Defective Restorations

Maxim Lagerweij[1], J.J. de Soet[2], Sophie Doméjean[3], Catherine Volgenant[1, 2]
[1]Department of Cariology, ACTA, Amsterdam, Amsterdam, Netherlands, [2]Department of Preventive Dentistry, ACTA, Amsterdam, Amsterdam, Netherlands, [3]Department Odontologie Conservatrice, University of Clermont Auvergne, Clermont-Ferrand, France
Objectives: The aim of this study is to assess the attitudes of Dutch dentists towards the treatment options of defective restorations, specific the replacement and repair.
Methods: A survey was sent to 700 Dutch dentists. Descriptive and chi-square tests were used to determine frequencies and differences in distribution. The study protocol was approved by the ethical committee of ACTA (reference 2018010).
Results: The response rate was 17% (120 dentists). When the dentists had to decide between repairing or restoring a restoration they choose mainly based upon the size of the defect, the size and material of the existing restoration and the accessibility of the lesion. When repairing a defective restoration, the type of material was less likely to affect the treatment choice of dentists with more experience as compared to dentists with less experience (graduated after the year 2000, $p = 0.014$). The estimated caries risk of the patient was less likely to affect the choice of dentists graduated in 2000 and after relative to dentists graduated before 2000 ($p = 0.048$). Most of the dentists were more inclined to replace small restorations (one or two surfaces, 58.3%), restorations made of amalgam (69.2%) and those of patients with a high caries risk (54.2%). Most dentists, however, were more inclined to repair large restorations (three or more surfaces, 58.3%), restorations made of composite (60.0%) and those of patients with a low caries risk (50.8%).
Conclusions: In this study it was found that the decision to repair or restore a restoration depended on the experience of the dentist, the material type of the restoration and the caries risk of the patient.

0086

The Influence of MI Prophy Paste on the Tooth Surfaces

Kyohei Toyonaga, Yusuke Sakaguchi, KATSUYUKI YAMANAKA, Futoshi Fusejima
R&D Department, GC Corporation, Tokyo, Japan
Objectives: Professional tooth cleaning (PTC) is an important treatment for preventing caries and periodontal diseases. We have developed a novel PTC paste (MI Prophy Paste) containing Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP, Recaldent™). The aim of this study is to evaluate the characteristics of MI Prophy Paste.
Methods: MI Prophy Paste (GC, MI), Nupro Prophylaxis Paste (Dentsply Sirona, NU), Proxyt (Ivoclar Vivadent, PR), Cleanjoy (Voco, CJ) Cleanic (Kerr, CL) were evaluated.
Surface roughness: Bovine teeth were embedded in resin and polished with silicon #4000-grit carbide paper. Each specimen was treated with the fine or coarse paste using a prophy cup (Nupro Revolv Soft; Dentsply Sirona) at 2,000rpm for 30seconds. Then, the surface roughness of each specimen was observed using a laser microscope (VR-X200; KEYENCE) (n=3). Stain removal: Bovine teeth were embedded in resin and polished with #4000-grit silicon carbide paper. Bovine teeth were immersed in staining liquid for 10minutes. Each specimen was polished using coarse pastes and a prophy cup (Nupro Revolv Soft) at 1,000rpm for 30seconds. The L*, a*, and b* values were measured by spectrophotometer (CMS-35FS; Murakami Color Research Laboratory) and ΔE was calculated (n=4). Gloss: Mirror polished bovine teeth were treated with fine pastes using a prophy cup (2,000rpm for 30seconds). The change of gloss value at measurement angle of 20°was measured by gloss meter (VG7000, Nippon Denshoku) (n=3).
Results: MI Coarse removed stain as well as other products. MI Coarse also showed least damage to tooth surface. MI Fine showed same or less damage to tooth surface than other products and showed highest gloss rate of the pastes.
Conclusions: MI Coarse polishes tooth surfaces gently while maintaining stain removal ability. MI Fine maintained gloss of tooth surfaces without damaging to teeth. These results indicate that MI Prophy Paste can be a clinically useful paste for professional tooth cleaning.

0087

Evaluation of Different Universal Bondings with Grape-Seed Extract Added

Ali Riza Cetin, Elif Can Simsek Balaban, Nurhan Ozoglu
Restorative dentistry, Selcuk University Faculty of Dentistry, Konya, Turkey

Objectives: Activation of matrix metallo proteinase (MMP) enzymes at the resin-dentin interface causes a decrease in dentin bond strength. Using MMP enzyme inhibitors can create a more stable linkage. In this study, the effect of grape seed extract (GSE) added into different universal adhesives on shear bond strength was evaluated.

Methods: Experimental bonding agents were prepared by adding GSE (0.5%) into different universal adhesives (Single Bond Universal,3M ESPE, USA;Nova Compo-B PLUS,IMICRYL,TRY;All Bond Universal,BISCO,USA). The occlusal enamel surfaces of the extracted human molar teeth were removed with diamond separe and smooth dentin surfaces were obtained. Samples were divided into 6 groups to about apply control or experimental adhesives. Test samples were restored with its own brand's composite (Filtek™ Ultimate,3M ESPE, USA; Nova Compo-C, IMICRLY, TRY; AELITE™ All-Purpose Body, BISCO, USA) to form 3 mm diameter cylindrical composite rods on dentin surface. After the samples were stored in water for 1 day, the shear bond strength test was applied at a rate of 1 mm/min. in universal test device. The obtained binding data were compared with One Way Anova test. (P=0.05)

Results: Among the control groups of the adhesives used in the study, the group with the highest shear bond strength data is Single Bond Universal (SBU) and the lowest is All-Bond Universal (ABU). When the control groups were compared with their experimental groups, no statistical difference was observed. In the SBU experimental group, compared to the control group, there was a decrease in the shear bond strength data, and an increase in the other two groups. Among the experimental groups, the group with the highest shear bond strength data is SBU and the lowest is ABU.

Conclusions: The addition of 0.5% GSE as MMP inhibitor into universal adhesives did not decrease the shear bond strength statistically.

0088

Does Humidity Influence Dentin Bond Strength of a Universal Adhesive?

Malte F. Peters, Silke Jacker-Guhr, Anne-Katrin Lührs
Hannover Medical School, Hannover, Germany

Objectives: The aim of this in vitro study was to determine the influence of dentin humidity on the microtensile bond strength (mTBS) of a universal adhesive applied either in self-etch (SE) or etch&rinse (ER) mode.

Methods: 60 caries- and restoration-free human molars were randomly distributed to 2 control and 10 experimental groups. After preparation of a flat dentin surface, a 5x5mm area was isolated by a perforated adhesive tape. For the control groups, a standardized volume of adhesive (15ml) was applied either immediately (SE-mode) or after etching with phosphoric acid (ER-groups). For the experimental groups, the surfaces were either wetted with 5µl of distilled water simultaneous to adhesive application ("over-wetting") or overdried for 10 seconds prior to adhesive application ("over-drying"). After placing a composite buildup, the teeth were sectioned for mTBS-testing. Half of the sticks (n=45) were tested after 24h, the other half (n=45) after thermocycling (TC, 15.000 cycles, 5/55°C). Data was statistically analyzed via ANOVA and Tukey HSD test (a=0.05).

Results: The one-way ANOVA showed significant differences between the groups ($p<0.001$). In SE mode, no significant differences were detected between the groups. In ER mode, over-wetting significantly reduced the initial mTBS compared to the control group ($p=0.038$). TC significantly affected the mTBS of the control group ($p=0.001$) and the over-dried group ($p=0.022$). After TC, no significant differences between the ER-groups were present. When comparing SE and ER mode, ER control and ER over-dried initially showed a significantly higher mTBS compared to the corresponding SE groups. After TC, the mTBS of ER over-wetted was significantly higher compared to all aged SE-groups.

Conclusions: The mTBS of a universal adhesive depends more on the application mode (SE vs. ER) and aging than on dentin humidity.

0089

Improvement of Bonding Effectiveness Using a new Hydrophobic Bonding Material

Azusa Yamanaka[1], Atsushi Mine[1], Ryosuke Hagino[1], Shintaro Ban[1], Masaya Ishida[1], Mariko Matsumoto[2], Bart Van Meerbeek[3], Hirofumi Yatani[1], Shoichi Ishigaki[1]
[1]Osaka university, Osaka, Japan, [2]Hokkaido univercity, Hokkaido, Japan, [3]University of Leuven, Leuven, Belgium

Objectives: This study evaluated the long-term bonding durability of a newly developed, two-step, hydrophobic bonding material.

Methods: Fifteen human molars were cut at the height of the contour and exposed dentin and then polished with #600 silicon carbide paper. Samples were randomly divided into three groups according to the bonding material used. A new two-step bonding system (BZF-29, GC; BZF group), a one-step bonding system (G-Premio Bond, GC; GBP group), and a two-step bonding system (CLEARFIL SE Bond 2, Kuraray Noritake Dental; SE2 group) were used following the manufacturers' instructions. A resin composite (CLEARFIL AP-X, Kuraray Noritake Dental) was built up by 2-mm-thick layers at a time and then light-cured. The specimens were stored in water at 37 °C for 24 hours and then sectioned into beams with a cross-sectional area of 1 mm^2. Micro-tensile bond strength (µTBS) was measured after storage in water for one year (n = 25). The fracture surfaces were then observed by scanning electron microscopy. Moreover, samples after 24 hours and one year of water immersion were observed by transmission electron microscopy.

Results: The µTBS values were 55.0 MPa, 13.8 MPa, and 41.3 MPa for the BZF, GPB, and SE2 groups, respectively. The µTBS value was significantly higher for the BZF group than for the GBP group ($p < 0.001$), whereas there was no significant difference between

the BZF and SE2 groups (p = 0.1). Interface failure was observed between the adhesive and resin composite in 52% of the samples in the GPB group (BZF: 0%, SE2: 8%), whereas cohesive failure in the resin composite or dentin was observed in 56% and 36% of the samples in the BZF and SE2 groups, respectively.
Conclusions: These results showed that the novel two-step system improves bonding between the adhesive and resin composite and has bond strength comparable to an existing two-step system.

0090

Shear Bond Strength of Different Composite Resin Materials to Biodentine

Miraç doğan, Zümrüt C. Özduman
Restorative Dentistry, Bezmialem Vakif University, Faculty of Dentistry, Istanbul, Turkey

Objectives: The aim of this study was to evaluate the shear bond strength (SBS) of different composite resins to Biodentine.
Methods: Sixty acrylic blocks with a central hole (2 mm depthx5mm diameter) were prepared (n=60). The holes were fully filled with Biodentine (Septodont, France) and the specimens were randomly allocated into 3 groups according restorative material [1-Conventional posterior composite, Estelite Posterior (EP)(Tokuyama, Japan) 2-Bulk fill composite, Filtek Bulkfill (FB) (3M ESPE, USA) 3-Short fiber reinforced composite, EverX Posterior (EX) (GC, Japan)] and 2 subgroups according to adhesive mode of application [self etch (SE) and total etch (TE)]. A universal adhesive (Single Bond Universal, 3M ESPE, USA) was applied on biodentine specimens. Then composite resins were applied on bonded area and light cured for 20 s (Valo, Ultradent, South Jordan, USA). SBS was evaluated using a universal testing machine (AGS-1000D, Shimadzu, Japan) with a crosshead speed of 1 mm/min, and the failure mode for each sample was recorded. Shear bond strength (MPa) data were evaluated with two-way ANOVA and Bonferroni Test (p<0,05).
Results: SBS of composite resins were higher when the adhesive was applied in TE mode. However, the difference between etching modes were not statistically significant (p>0.05). EP group yielded significant difference with FB group in SE mode (p<0.05). Predominant mode of failure was cohesive.
Conclusions: Type of composite resins might influence the SBS to Biodentine.

0092

Bond Strength of Veneer to Femtosecond Laser-Treated Monolithic Zirconia

Emmanouil-George Tzanakakis[1], Anastasia Beketova[2], Athanassios Rigos[2], Eleana Kontonasaki[2], Ioannis Tzoutzas[1]
[1]Operative Dentistry, National and Kapodistrian University of Athens, Athens, Greece, [2]Prosthodontics, Aristotle University of Athens, Thessaloniki, Greece

Objectives: Ceramic restorations with the new generation of high translucent zirconia (HTZ) have become an alternative to metal-ceramic due to their excellent aesthetic and mechanical properties. Despite their high translucency, they are often veneered with feldspathic glass-ceramics to enhance the aesthetic outcome, especially in anterior regions. However, limited scientific data exist about their bonding strength to veneer. The aim of this study was to evaluate the bond strength of HTZ to veneer after being subjected to novel femtosecond laser, compared with commonly applied surface treatments.
Methods: Fifty disk-shaped specimens (10mm X 5mm) were fabricated from a commercial 5 mole% yttria-stabilized zirconia (5Y-TZP). All specimens were polished and underwent different surface treatments: no treatment control (POL), air-abrasion with alumina particles 50um (AL50) and 90um (AL90), glass beads 10-60um (GL), and ablation with femtosecond laser (FEMTO). The specimens were veneered with a leucite reinforced dental ceramic (Creation ZI), mounted on a universal testing machine and subjected to shear force at a crosshead speed of 0.5mm/min until failure. One-way ANOVA was used to analyze the mean shear bond strengths values (SBS) with significance level set at p<0.05. A stereomicroscope was used at 20-60X magnitude to classify the mode of failure. The adhesive failure mode covering area (ADFM) was also analyzed with one way ANOVA (p<0.05) to reveal statistically significant differences among surface treatments.
Results: The highest SBS values were observed in Group FEMTO (33.21±9.99 MPa), however there were no significant differences compared to control and the other groups (p=0.051). The strength presented descending values from FEMTO to POL (FEMTO>AL90>AL50>GL>POL). The lowest percentage of adhesive failure was recorded in Group FEMTO (48±9,77%), which was statistically significantly different from all other groups.
Conclusions: Under the limitations of this in vitro study, femtosecond laser treatment of HTZ ceramics is a promising alternative method of surface processing before the application of the veneering ceramic.

0093

Cytotoxicity of Flowable Hydraulic Calcium Silicate-Based Cement Leachates

Paulius Tušas[1], Milda Alksne[2], Josette Camilleri[3], Saulius Drukteinis[1], Egle M. Jonaityte[1], Virginija Bukelskiene[2], Vygandas Rutkunas[1], Vytaute Peciuliene[1]
[1]Institute of Odontology, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, [2]Institute of Biochemistry, Life Sciences Center, Vilnius University, Vilnius, Lithuania, [3]School of Dentistry, University of Birmingham, Birmingham, United Kingdom

Objectives: Biocompatibility is an essential property for materials used in vital pulp therapy. The aim of this research was to evaluate cytotoxicity of flowable hydraulic tricalcium-silicate based cements (fHCSC) *in vitro*.
Methods: Tested groups/Materials: *MTA Flow Grey* and *White*: "Thick" consistency – fHCSCs; *ProRoot MTA White* – control; positive and negative control; Growth-medium (GM): αMEM supplemented with 10% FBS and 1% streptomycin/penicillin. The GM was left in contact with freshly mixed disks for 48h. Leachates were collected, pH measured, filtered through sterile 0.22µm pore filter and the 100, 50, 25 and 12.5% dilutions were made with GM. Human Dental Pulp Stem Cells (hDPSC; $1x10^4$ cells/cm^2;

3rd passage) were seeded in 96-well-plate and incubated for 24h in GM. Leachates transferred onto hDPSC and incubated for 24h. 0.2mg/ml MTT solution was added and the specimens incubated for 3h. Formazan crystals dissolved with DMSO. Absorbance measured spectrophotometrically at 570 nm. All measurements were determined in triplicates. Cell morphology was assessed by confocal microscopy. Data were evaluated using Shapiro-Francia test and ANOVA (R studio).
Results: Measured pH values: *GM* - 8.5, *IRM* – 5, *ProRoot* – 12.5, *MTA Flow Grey* - 9.5, *MTA Flow White* – 10. Cell viability and morphology were significantly affected by the presence of freshly mixed cement leachates and to different degrees depending on cement-leachate dilution ($p<0.05$). At 12.5, 25 and 50% concentrations, both fHCSCs and *ProRoot* determined cell viability above 80%. At 100%, all HCSCs revealed a lower cell viability than negative control ($p<0.05$). After 24h exposure, at 100% HCSCs leachates hDPCs showed drastic size reduction, rounded shape, which can be associated with cell death process. In other dilutions, hDPSCs had extended morphology, were less bipolar/multipolar, compared to negative control.
Conclusions: Freshly mixed *MTA Flow Grey* and *White* 100% leachates exhibited lower cytotoxicity than *ProRoot MTA White*, although morphologic changes of hDPSCs were seen in all groups.

0094

Diffusion of Calcium Hydroxide Mixtures on Dentine - a Preliminary Study

Beliz Ozel[2], Paul Anderson[1], Figen Kaptan[2]
[1]Dental Physical Sciences, Queen Mary University of London, London, United Kingdom, [2]Endodontics, Yeditepe University, Istanbul, Turkey

Objectives: Calcium hydroxide (CH) is an antimicrobial agent recommended for different clinical situations such as root canal infections, apexification and root resorption. The benefits of CH include antibacterial activities and tissue dissolving capacity. The extent of disassociation of CH into hydroxyl and calcium ions (Ca^{2+}) depends on the vehicle used in the mixture. The aim of this study was to use an ion-selective-electrode (ISE) method to measure the rate of diffusive transport of 'free' Ca^{2+} through radicular dentin exposed to three different CH mixtures.
Methods: Three extracted human lower premolar teeth with single-canal roots were included. The working length was determined with a #10 K-file and preparation was done using ProTaper Next (Dentsply, USA) files. Irrigation was performed with 5,25% NaOCl and for final irrigation, 17 EDTA% was used for 3 minutes. A cavity with a depth of 1 mm was created in the middle third of the mesial surface of all roots. The test CH mixtures were Multi-Cal (Pulpdent, USA), CH+distilled water, and CH + glycol. The test CH mixture of 0.5mL was inserted into one of the prepared root canals using a #25 Pastinject (MicroMega, France). The outer surface of each tooth (excluding the cavity) was coated with nail varnish, and each immersed in 10mL of distilled water. Ca^{2+}-ISE's (Nico2000, UK) was used to continually measure the 'free' Ca^{2+} concentration released into the solution from each sample for every 60s.
Results: Real-time ISE measurements were conducted over 6 days (140 hrs). The average rate of calcium ion release over 140h was 6.07×10^{-5} mmol/L/h for Multi-Cal, 5.02×10^{-5} mmol/L/h for the CH+glycol group, and 1.07×10^{-5} mmol/L/h for CH+distilled water. The total calcium ion release after 140h was 0.009 mmol/L for Multi-Cal, 0.01 mmol/L for the CH+glycol group, and 0.003mmol/L for CH+distilled water. The rate of release of calcium ion release was most stable from the Multi-Cal.
Conclusions: The Multi-Cal, a prepared composition of CH, has shown a steadier release of ions compared to others showing an irregular pattern, which may be related to the hand-mixing of the latter. Within the limitations of the study, a prepared composition of CH, with its steadier ion release, is a predictable and effective option in clinical use.

0095

Impact of S-PRG Elutes on Lipase Gene Expression in *Candida Albicans*

Watcharapong Tonprasong[1, 2], Masanao Inokoshi[1], Keita Hatano[1], Muneaki Tamura[3], Makoto Shimizubata[1], Shunsuke Minakuchi[1]
[1]Department of Gerodontology and Oral Rehabilitation, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan, [2]Restorative department, Naresuan University, Pitsanulok, Thailand, [3]Department of Microbiology, Nihon University School of Dentistry, Tokyo, Japan

Objectives: This study aimed to evaluate the effect of S-PRG elutes on the lipase gene expression in *Candida albicans.*
Methods: The S-PRG filler (Shofu) was immersed in distilled water, and the supernatant that was stirred for 24 hours was filtered and used as a sample. The media concluding sample at a final ratio of 1:16 and 1:32 media were prepared using a 2-fold concentration of YPG broth, inoculated with *C. albicans*, then, cultured at 37 ° C for 24 hours. After adjusting the fungal cell number, MagExtrator-RNA-kit (Toyobo Co., Ltd.) was used for RNA purification. Briefly, cultured pellet cells were treated with zymoliase, 700 µL of Lysis & Bonding solution containing 2-mercaptoethanol was added, and RNA was purified using wash solution I & II as instructed. The purified RNA was immediately converted to cDNA using the ReverTra AceTM qPCR RT Kit (Toyobo Co., Ltd.). Lipase gene family (*LIP*1–10) expression was confirmed with specific PCR primers. An untreated *C. albicans* was set as a control (n = 6/group). The data were statistically compared using the Shapiro-Wilk test followed by either one-way ANOVA with Tukey or Kruskal-Wallis test with Dunn's test ($\alpha = 0.05$) using the R software package.
Results: Kruskal-Wallis test followed by Dunn's test ($\alpha = 0.05$) demonstrated the S-PRG elutes at a concentration of 1:16 dilution suppressed the activity of *LIP*5 (0.54±0.25 U/L) and *LIP*8 (0.35±0.074 U/L), whereas we did not find any suppressed activity in the other genes. At a concentration of 1:32 of S-PRG elutes, there was no significant difference in the lipase gene expression when compared to the control.
Conclusions: Diluted S-PRG filler elutes (1:16) show a beneficial tendency to suppress the lipase activity of *C. albicans* via inhibiting the *LIP*5 and *LIP*8 genes which would relate to reducing the pathogenicity of *C. albicans*.

0096

Electrospun Polymeric Scaffolds as Carriers of Anti-Microbial and Anti-Inflammatory Agents

Konstantina Chachlioutaki[1], Elisabeth Adamoudi[2], Christina Karavassili[1], Athina Bakopoulou[2], Dimitrios Fatouros[1]

[1]Pharmaceutical Technology, School of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki, Greece, [2]Prosthodontics, School of Dentistry, Aristotle University of Thessaloniki, Thessaloniki, Greece

Objectives: Aim of the study was to evaluate the cell viability/proliferation/morphology and anti-inflammatory properties of engineered electrospun polymeric films [poly(lactic-co-glycolic) acid-PLGA and poly-vinyl alcohol-PVA] loaded with antimicrobial (ZnO nanoparticles-NPs and sericin) and anti-inflammatory agents (ketoprofen-KET).

Methods: Human Gingival Fibroblast-HGF cultures were established from gingiva biopsies of young healthy donors. Scaffolds were constructed through electrospinning in two formulations, one containing PLGA with 0.5%wt sericin and KET (0.05-0.5mg/ml), the other containing PVA combined with 1% or 3% Carboxymethyl-Cellulose-CMC, or 1% or 3% Hydroxypropyl-Methylcellulose-HPMC, and loaded with 0.5%wt ZnO NPs and KET(0.01-1mg/ml). PLGA scaffolds were seeded with HGFs and cell viability was assessed by MTT assay and compared to cells seeded on tissue-culture polystyrene (TCP-control). Live/dead staining evaluated cell viability and Scanning Electron Microscopy-SEM cell adherence/morphology. Anti-inflammatory properties were tested on Lipopolysaccharide-induced 264.7-RAW macrophages by analyzing the expression of pro-inflammatory markers by RT-PCR.

Results: Live/dead staining confirmed cytocompatibility, while SEM showed cell adherence and desirable morphology of HGFs on all PLGA compositions. PLGA combined with 0.5%wt sericin stimulated HGF proliferation/viability by 89% at 24h compared to TCP-control. Hence, this formulation was loaded with different concentrations of KET. MTT assay showed that KET does not profoundly influence HGF viability. Regarding PVA-based formulations, loaded with 0.1mg/ml KET produced the highest values of cell viability, reaching 146-172% at 24h compared to TCP-control, while higher KET concentrations decreased viability in a time- and concentration-dependent manner. RT-PCR showed that PVA-based scaffolds, loaded with 0.1mg/ml KET differentially decreased the expression of pro-inflammatory cytokines (IL-6/MMP-9/MMP-13/MCP-1) by macrophages, being more effective in the 3%HPMC-copolymer group.

Conclusions: Electrospun polymeric scaffolds support cell attachment and viability of HGFs, showing anti-inflammatory properties and can be further examined on their antimicrobial properties to be considered as tissue engineered-drug carriers in treating inflammatory oral diseases.

0097

In Vitro Study on Metal Ions Release from Orthodontic Archwires

Christos Gkanidis, Alexandros K. Nikolaidis, Christos Gogos, Elisabeth Koulaouzidou

Aristotle University of Thessaloniki, Thessaloniki, Thessaloniki, Greece

Objectives: The objective of this study was the detection and quantification of ion species that may be leached from different types of orthodontic archwires after short- and long-term storage in different environment acidity.

Methods: Dental archwires consisted of three different alloys (stainless steel, NiTi and CuNiTi) were purchased from Ormco and American Orthodontics. The samples were immersed in artificial saliva of varied pH values (3.5, 5 and 7) for either 7 days or 1 month at 37±1°C. The liquid extracts were analyzed by means of an axial viewing inductively coupled plasma-optical emission spectrometer (ICP-OES) system (Perkin Elmer, Optima 2100 DV). The optimum instrumental conditions were set as: 0.8 L min^{-1} nebulizer argon flow rate, 1300 W incident power and 1.5 mL min^{-1} sample flow rate. The ions detected and quantified were: Ni, Mn, Cr, Mo and Ti. Artificial saliva was measured as blank for baseline correction. Statistical analysis was applied to determine significant differences at level of significance set to 0.01, taking into account the parameters: pH and aging time. The values of the quantified concentrations represented as mean values ± standard deviation of three replicates.

Results: The results showed that the released ion species and the measured concentrations were not in accordance with manufacturers' data. The leachates were mainly enriched with Cr and Ni ions by decreasing the saliva pH, while most of the archwires released the highest amounts of Ni, Mn and Cr ions after 30 days aging at pH=3.5.

Conclusions: Overall, the total release of Ni and Cr ions was within the considered average dietary intake levels.

0098

Translucency, Crystallography and Flexural Strength of Multi-Layered Highly Translucent Zirconia

Masanao Inokoshi[1], Hengyi Liu[1], Makoto Shimizubata[1], Kosuke Nozaki[2], Shunsuke Minakuchi[1]

[1]Department of Gerodontology and Oral Rehabilitation, Tokyo Medical and Dental University, Bunkyo, Tokyo, Japan, [2]Department of Fixed Prosthodontics, Tokyo Medical and Dental University, Bunkyo, Tokyo, Japan

Objectives: To assess translucency, crystallography and flexural strength of multi-layered dental zirconia grades.

Methods: We investigated the following two multi-layered dental zirconia grades (KATANA YML, Kuraray Noritake; IPS ZirCAD Prime, Ivoclar Vivadent). Four layers (enamel, body1, body2 and body3) from KATANA YML and three layers (enamel, transition and body) from IPS ZirCAD Prime were used. Fully sintered zirconia square-shaped specimens (12x12x1.2-mm) from each layer were prepared. For each layer of the zirconia grades, the translucency parameter (TP) was measured using a colorimeter (n=5/group) and the zirconia grades were statistically compared using a one-way ANOVA followed by Tukey post hoc test (α=0.05). X-ray diffraction (XRD) with Rietveld analysis was used to assess the zirconia-phase composition (n=3/group). Fully sintered zirconia bar-shaped specimens (3.0x4.0x40-mm) from each layer were prepared. The 4-point flexural strength was measured using a universal material testing machine, followed by Weibull analysis (n=20/group).

Results: IPS ZirCAD Prime enamel had the significantly highest TP (27.8) followed by KATANA YML enamel (25.7) and body1 (25.2), whereas that of IPS ZirCAD Prime body was the lowest (22.4). XRD with Rietveld analysis revealed that KATANA YML enamel,

body1 and IPS ZirCAD Prime enamel had comparable *c*-ZrO_2 phase composition (45-50 wt%), followed by KATANA YML Prime body2 (32 wt%), body3 (32 wt%) and IPS ZirCAD Prime transition (33 wt%). Weibull analysis revealed that the Weibull characteristic strength of KATANA YML body1 (894.7 MPa), body 2 (908.7 MPa), body3 (907.1 MPa) and IPS ZirCAD Prime body (995.1 MPa) were comparable and higher than those of KATANA YML enamel (647.4 MPa), IPS ZirCAD Prime transition (586.5 MPa) and enamel (518.9 MPa).
Conclusions: For both zirconia grades, enamel layers contain higher amount of *c*-ZrO_2 phase, which resulted in significantly higher translucency than body layers. Moreover, the enamel layer had significantly lower flexural strength than the body layer.

0099

Long-Term low-Temperature Degradation Behavior of High-Speed Sintered Zirconia

Hengyi Liu[1], Masanao Inokoshi[1], Kosuke Nozaki[2], Makoto Shimizubata[1], Hiroto Nakai[1], Thaw Di Cho Too[1], Shunsuke Minakuchi[1]
[1]Department of Gerodontology and Oral Rehabilitation, Tokyo Medical and Dental University, Tokyo, Japan, [2]Department of Fixed Prosthodontics, Tokyo Medical and Dental University, Tokyo, Tokyo, Japan

Objectives: This study aimed to investigate the low-temperature degradation (LTD) of speed-sintered zirconia.
Methods: Five different yttria partially stabilized zirconia (KATANA HT: 4Y-PSZ, KATANA STML: 5Y-PSZ, KATANA UTML: 6Y-PSZ, all from Kuraray Noritake; Zpex 4: 4Y-PSZ, Zpex Smile: 5Y-PSZ, all from Tosoh) were investigated. Disc shape specimens (14.5 mm-diameter, 1.2 mm-thickness; n=15/group) were sintered either with conventional sintering protocols (approximately 7h) or speed sintering protocols (approximately 90min). Additionally, KATANA STML was sintered with 18 min sintering protocol. For the LTD test, specimens were hydrothermally treated with an autoclave at 134 °C under 2-3 bar in water vapor, and last for 2h, 5h, 10h, 15h, 20h, 30h, 40h. Before and after the LTD test, the crystalline structures of the specimens were evaluated using X-ray diffraction (XRD, D8 Advance, Bruker). The volume fraction of monoclinic zirconia (*m*-ZrO_2) content was statistically compared using either one-way ANOVA with Tukey post-hoc test or Kruskal-Wallis test with Dunn test.
Results: For KATANA HT, KATANA STML and Zpex 4, the higher monoclinic volume fraction was observed for the conventional sintering conditions than speed-sintered conditions. On the other hand, KATANA STML sintered for 18min and speed sintered Zpex 4 did not transform to monoclinic phase even after 40h of LTD. Speed-sintered (90 min) KATANA STML slowly degraded from 1.56±0.33 vol% of *m*-ZrO_2 at 0h to 4.52±0.92 vol% of *m*-ZrO_2 at 40h, and its transformation rate was just between conventional-sintering (10.10±0.58 vol%) and 18 min sintering (2.07±0.24 vol%). KATANA UTML and Zpex Smile did not show significant degradation up to 40h of the LTD test.
Conclusions: Significant degradation was observed in Y-PSZ with lower yttria content. No significant difference was found between conventionally sintered and speed sintered zirconia, except for KATANA HT, KATANA STML and Zpex 4, in which conventionally sintered zirconia had higher monoclinic volume fraction than speed-sintered zirconia.

0100

In-Vitro Performance of Wide-Span Multilayer, 4Y-TZP and 5Y-TZP Zirconia FPDs

Thomas Strasser, Alois Schmid, Martin Rosentritt
Department of Prosthetic Dentistry, UKR University Hospital Regensburg, Regensburg, Bayern, Germany

Objectives: Restorations milled from different positions within multilayer-blanks may vary in mechanical stability. This study investigated if high-translucent zirconia materials are applicable at four-unit molar fixed partial dentures (FPDs) and if positioning within multilayer blanks affects in-vitro performance.
Methods: Artificial molar teeth were milled (CORiTEC Disc PMMA, Imes Icore). Roots were covered in polyether impression material (Impregum, 3M Espe AG, D) and embedded in resin bases. Identical four-unit FPDs (n=8 per group) were fabricated from monolayer blanks and two different positions within a zirconia multilayer blank (Table). FPDs were adhesively luted (Panavia F2.0 TC, Kuraray Noritake, JP). Specimens were divided in control group (24h water storage at 37 C°) and TCML group (4x3000x5°C/55°C, 2 min, H_20 dist., $1.2x10^6$ á 50N). Fracture force after TCML was determined by central loading to failure at the pontic (v=1mm/min).
Results: Two 5Y-TZP FPDs failed during TCML. Mean fracture force values after 24h water storage varied between 804±172N (5Y-TZP) and 1237±408N ("above" position, priti multidisc ZrO_2). Mean fracture force after TCML varied between 714±191N (5Y-TZP) and 1154±233N ("bottom" position, priti multidisc ZrO_2). One-way ANOVA revealed significant differences ($p \leq 0.017$). Bonferroni comparison of fracture force showed significant individual differences ($p \leq 0.044$) between groups.
Conclusions: Milling from different positions within multilayer blanks led to minor differences in fracture force of four-unit FPDs. Multilayer and 4Y-TZP materials seem appropriate for clinical application at 4-unit molar FPDs.

0101

Practice-Based Clinical Evaluation of Chairside-Fabricated Partial Crowns - 5-Year Results

Sven Rinke[2], Emanuel Schmidt[2], Andreas Leha[3], Matthias Roediger[2], Dirk Ziebolz[1]

[1]Department of Cariology, Endodontology and Periodontology, University Medical Center Leipzig, Leipzig, Germany, [2]Department of Prosthodontics, University Medical Center Goettingen, Goettingen, Germany, [3]Department of Medical Statistics, University Medical Center Goettingen, Goettingen, Germany

Objectives: Prospective practice-based clinical evaluation of chairside-fabricated monolithic partial crowns made of zirconia-containing lithium silicate (ZLS) ceramics.

Methods: Between October 2013 and September 2014, 71 patients were restored with 92 partial crowns on vital or sufficiently endodontically treated teeth in three different private dental clinics (C1-C3). The monolithic restorations were fabricated chair-side (Cerec SW 4.2/Cerec MC XL) from a glaze-fired ZLS ceramic material (Celtra Duo, Dentsply Sirona). Adhesive cementation was performed in the total-etch technique with one of two dual-curing composite materials (Variolink (VL), Ivoclar Vivadent vs. Calibra (CAL), Dentsply Sirona). Modified USPHS parameters were evaluated annually. Moreover, the parameters "time-dependent survival" (in situ criterion), and "success rates" (event-free restorations) were evaluated according to Kaplan-Meier. Possible covariates of the survival (SVR) and success rates (SCR), evaluated in a Cox regression model, were restoration position (premolar/molar), cementation technique (VL/CAL), center (C1-C3).

Results: 57 patients with 71 crowns attended annual follow-up examinations (observational period: 58.0±15 months). Five complete failures (2 tooth-fractures, 2 restoration fractures,1 endodontic failure) were recorded. Additionally, four clinical interventions (2 endodontic treatments, 2 minor ceramic fractures requiring polishing) were necessary to maintain function. All failures and interventions during the 5-year follow-up period occurred in one of the three centers, leading to a significant difference (Log-Rank-Test: p=0.00067) in the center-specific 5-year success rate (C1 +C2: 100%; C3:71% / (CI95%): [0.55;0.87]). Restorations placed in C1 and C2 showed a significantly reduced risk for a failure or a clinical intervention (hazard ratio (HR)=0.103, p=0.026) compared with the restorations placed in C3.

Conclusions: Chairside-fabricated ZLS partial crowns show a good mid-term clinical performance. However, the clinical success rate is significantly influenced by the clinical settings and the operator (center effect). Additional clinical data from practice-based multi-center studies with larger study populations and longer observational periods are required for a more detailed investigation of this effect.

0102

Two-Year Clinical Performance of Novel Self-Adhesive-Composite Equivalent to Conventional Bulk-Fill-Composite

Konstantin J. Scholz[1], Karl-Anton Hiller[1], Julian C. Anthony[1, 2], Isabelle M. Tabenski[1, 3], Sarah Ettenberger[1], Fabian Cieplik[1], Marianne Federlin[1], Wolfgang Buchalla[1]

[1]Department of Conservative Dentistry and Periodontology, University Medical Center Regensburg, Regensburg, Bavaria, Germany, [2]Private Dental Practice, Potsdam, Germany, [3]Private Dental Practice, Regensburg, Germany

Objectives: Aim of this study was to evaluate the clinical performance of a novel, tooth-colored, self-adhesive bulk-fill restorative (SABF, 3M) compared to a bulk-fill composite (Filtek One, 3M; FOBF) for permanent restorations in class-II cavities. The null-hypothesis was that both materials perform similarly regarding clinical survival and performance.

Methods: In this randomized split-mouth study, 30 patients received one SABF and one FOBF restoration each. Scotchbond Universal (3M) in self-etch mode was used as adhesive for FOBF. SABF was applied without a separate adhesive. Restorations were evaluated by two blinded examiners at baseline (BL), 6-months, 12-months, and 24-months employing FDI criteria. Non-parametric statistical analyses, χ^2-tests (α=0.05), error-rates method and survival-analyses were performed.

Results: 29 out of the initial 30 patients (21-58 years; 21 females) with both restorations under risk were available at the 24-months recall. After 24-months, survival rate was 100% for SABF and 97% for FOBF (one restoration rated score 4 in criterion C12 due to secondary caries). All other restorations revealed clinically acceptable FDI scores (excellent-1; good-2; satisfactory-3) for all criteria at all timepoints. Error-rates method revealed a significant difference between materials in terms of *esthetic properties*, but not regarding *functional* and *biological properties*. Considering *esthetic properties*, both materials yielded clinically acceptable FDI scores (mainly excellent-1 and good-2), with FOBF performing significantly better than SABF in criteria *surface lustre* (A1, p=0) and *color match and translucency* (A3, p=0) at all time points. Over time, *marginal staining* (A2b) increased significantly for both materials ($p \leq 0.001$).

Conclusions: The null hypothesis could not be rejected. Both materials performed similarly regarding clinical survival and performance within 24-months of clinical service. SABF exhibited less favorable, but clinically fully acceptable *esthetic properties* compared to FOBF. The novel, self-adhesive bulk-fill restorative showed promising 2-year-results and may be recommended for clinical use.

0103

Release of Bisphenol a From Dental Polycarbonate Materials

Antonín Tichý[1], Markéta Šimková[2], Josef Schweiger[3], Pavel Bradna[1], Jan-Frederik Güth[4]

[1]Institute of Dental Medicine, First Faculty of Medicine of the Charles University and General University Hospital, Prague, Czechia, [2]Institute of Endocrinology, Prague, Czechia, [3]University Hospital, LMU Munich, Munich, Germany, [4]Center for Dentistry and Oral Medicine (Carolinum), Goethe-University Frankfurt am Main, Frankfurt, Germany

Objectives: Polycarbonates are polymers of bisphenol A (BPA), an endocrine disruptor associated with various pathological conditions. The use of polycarbonates in prosthodontics includes denture base resins, prefabricated temporary crowns and CAD/CAM-fabricated occlusal splints. This study evaluated the release of BPA from dental polycarbonates to methanol and artificial saliva using liquid chromatography/mass spectroscopy (LC-MS).

Methods: Prefabricated polycarbonate crowns (mandibular first premolars, 3M, USA) were scanned using an optical strip-light scanner to prepare their polycarbonate replicas using 3D-printing (Freeformer, Arburg, Germany) or milling from CAD/CAM blocks, Temp Premium Flexible (TPF, Zirkonzahn, Italy) and Tizian Blank Polycarbonat (TBP, Schütz Dental, Germany). Control replicas were milled from polymethyl methacrylate (PMMA) blocks, Temp Basic (Zirkonzahn, Italy). The crowns/replicas were stored at 37°C in methanol or artificial saliva (AS) (n=5), and their eluates were collected after 1, 7, 28 and 84 days. BPA concentrations were measured using a LC-MS/MS method with dansyl chloride derivatization. The amounts of released BPA were expressed in micrograms per gram of material (µg/g).

Results: Significantly more BPA was released to methanol. The highest amounts of BPA were detected after the first day in the eluates of the milled polycarbonates TBP (methanol: 32.2±3.8µg/g, AS: 7.1±0.9µg/g) and TPF (methanol 22.8±7.7µg/g, AS: 0.3±0.03µg/g), followed by 3D-printed replicas (methanol: 11.1±2.3µg/g, AS: 0.1±0.1µg/g) and the 3M prefabricated crowns (methanol: 8.0±1.6µg/g, AS: 0.07±0.02µg/g). Between day 7 and 84, the daily release of BPA to methanol and AS decreased below 2µg/g and 0.6µg/g, respectively. No BPA was released from PMMA to AS, and the cumulative amount released to methanol was 0.2±0.06µg/g.

Conclusions: BPA release from polycarbonates was considerable, especially to methanol, which represents the worst-case scenario of BPA release. The current tolerable daily intake of BPA (4µg/kg bw/day) was not exceeded, but clinicians should remain vigilant, because the biological effects of BPA have not been fully understood yet.

0104

Toothbox: Experimental and Numerical Toolbox for the Development of Dental Composites

Hazem Abouelleil Sayed[1], Gerry Agbobada[2], Pierre-Olivier Bouchard[2], Yoan Bousses[2], Nathalie Brulat-Bouchard[2, 3], Jean-Marc Chenal[4], Pierre Colon[1, 5], Angélique Favre[6], Marta Gallo[4], Rémy Gauthier[1], Brigitte Grosgogeat[1, 7], Christpohe Jeannin[1, 7], Eric Maire[4], Yannick Tillier[2]

[1]Université de Lyon, Université Lyon 1, LMI, UMR CNRS 5615, Faculté d'Odontologie, Lyon, France, [2]CEMEF Centre de Mise En Forme des Matériaux, MINES ParisTech, PSL Research University, UMR CNRS 7635, Sophia Antipolis, France, [3]Service d'Odontologie, CHU de Nice, Hôpital Saint Roch, Nice, France, [4]Université de Lyon, INSA de Lyon, MATEIS UMR CNRS 5510, Villeurbanne, France, [5]Service d'Odontologie, APHP, Hôpital Rotschild, Paris, France, [6]LIP, Villeurbanne, France, [7]Service d'Odontologie, HCL, Lyon, France

Objectives: To exhaustively investigate the properties of commercial dental composites, both experimentally and numerically. This would help manufacturers in defining the best composite formulation to provide commercial materials with optimized physical, thermomechanical, and physicochemical properties.

Methods: An unfilled resin, composed of BisGMA, TEGDMA, UDMA, and EBPADMA, was mixed with different mass fractions of silanated barium glass particles. Three-point bending mechanical tests were applied to the photocured composites to investigate the influence of filler mass fractions, thermal cycling in water (between 5 °C and 55 °C), and samples storage conditions (dry or wet at 37 °C) on the material's elastic properties. Dynamical Mechanical Analyses (DMA) and Differential Scanning Calorimetry (DSC) were performed on composite samples to analyze the influence of experimental parameters (curing temperature, curing time, filler ratio, …) on the polymerization of the organic matrix. The composite polymerization volume shrinkage was measured using *in situ* X-ray micro-tomography volume correlation. A finite element model was then developed, as a potential tool for composites behavior prediction.

Results: A precise protocol for composite samples preparation and photocuring has been defined and validated by comparing the results obtained by different operators in different laboratories. Mechanical experimental results showed a significant influence of thermal aging and storage conditions on the elastic properties of dental composites, with a dependency on filler ratio. DSC and DMA Results showed that the photocuring temperature has an influence on the extent of the matrix reticulation and that filler ratio has insignificant effect on it. The volume correlation analysis showed that the maximum volume shrinkage is reached only after 5 s of photocuring.

Conclusions: Experimental parameters have a great influence on the behavior of commercial dental composites. Early results obtained using a numerical finite element model help to understand the effect of these parameters.

0105

Color Adjustment Potential of Three Universal Composite Resins

Sinem Akgul[1], ceyda gündoğdu[2]

[1]Restorative Dentistry, Gazi University, Faculty of Dentistry, Ankara, Turkey, [2]Restorative Dentistry, Medipol University, Faculty of Dentistry, Istanbul, Turkey

Objectives: Composite resins are commonly used for esthetic rehabilitation and color and contour modifications of teeth and old restorations. The aim of this study was to evaluate the instrumental color adjustment potential (CAP-I) of three commercially available universal composite resins.

Methods: Omnichroma, Estelite Sigma Quick and Filtek Universal Restorative were evaluated in this in vitro study (n=10). Omnichroma was one shade and other materials were selected in shade A2. Single and dual shade specimens were prepared to evaluate CAP of three universal composite resins, For dual specimen preparation, cylindrical cavities with a 6 mm diameter and 2 mm depth prepared on acrylic teeth and restored with restorative materials. Single specimens were produced from composite resins as a replication of the acrylic teeth and unrestored acrylic teeth also used as control group. Instrumental color measurements performed with a spectrophotometer immediately and CIE-Lab coordinates were recorded and then CAP-I of each material was calculated. The data was analyzed with one-way ANNOVA and Tukey's HSD Post hoc test.

Results: When evaluated in terms of color adjustment potential of the composite resins, a significant difference was observed between materials. CAP-I ranged between 0.11-0.23. It was determined that Omnichroma exhibited the highest instrumental color adjustment potential.

Conclusions: Omnichroma was the most pronounced followed by Estelite Sigma Quick and Filtek Universal Restorative. Composite resins with pronounced CAP, improving the esthetic appearance of the restoration, simplifying the shade matching and tolerating for any color mismatch. Universal composite resins with increased color properties may be useful to rehabilitate anterior defects for clinicians.

0106

Effects of Vitamin C on Surface Roughness, Microhardness and Color Stability of Composite Resins

Ayca Sarialioglu Gungor, Ceren Deger

Restorative Dentistry, Bezmialem Vakif University, Istanbul, Turkey

Objectives: The study assessed the changes in color stability, surface roughness and microhardness of three esthetic composite-resin materials after immersed in different effervescent vitamin C tablets.

Methods: Three different types of composite resins were investigated: supra-nano hybrid composite, microfilled hybrid composite, and nanofilled composite resins. Ninety disc-shaped specimens (4-mm diameter, 2-mm thickness) were fabricated using Teflon mold and divided into three groups according to the immersion solution (Redoxon, Sambucol, and distilled water as control) for both composite resins (n=10). The top surface of specimens was ground with 600 grit silicon carbide paper for the standardization. Each group was immersed in the solutions for 24 hours. Surface roughness (Ra, mm) was measured by contact profilometer, while the hardness was measured by Vickers hardness (HV) test. CIE L*a*b* coordinates were recorded by using spectrophotometer and color difference analysis (ΔE) was made using the CIEDE-2000 formula. Data were statistically analyzed using two-way ANOVA and Bonferroni correction test ($p<0.05$).

Results: No significant differences in surface roughness change were recorded according to the immersion solutions for both composite resin groups ($p>0.05$). However, there were significant differences in ΔHV of all composite resins among the control group and the effervescent vitamin C tablets ($p<0.001$). Highest microhardness decrease was obtained in supra-nano hybrid composite specimens when immersed in Redoxon (-8.49±1.62). Statistically significant differences ($p<0.001$) were found between the composite specimens immersed in the solutions in terms of ΔE values. Redoxon appeared to be associated with the most severe alteration in color (4.86±0.63).

Conclusions: Using vitamin C effervescent tablets did not have a significant change in the surface roughness values of the composite resins. However, microhardness values of all composite resin specimens were reduced. Clinically unacceptable discoloration was observed only in micro-filled hybrid composite specimens immersed in Redoxon.

0107

Self-Supervised Learning for Dental Image Analysis

Jose Eduardo Cejudo[1], Philipp Wirth[2], Igor Susmelj[2], Joachim Krois[1, 3], Falk Schwendicke[1, 3]

[1]Department of Oral Diagnostics, Digital Health and Health Services Research, Charité–Universitätsmedizin, Berlin, Germany, [2]Lightly AG, Zürich, Switzerland, [3]Topic Group Dentistry, ITU/WHO Focus Group AI on Health, Geneva, Switzerland

Objectives: A common bottleneck for supervised deep learning in medical and dental image analysis is the availability of expert-annotated data. In this study we assessed the value of self-supervised learning for dental image analysis.

Methods: ResNet-18, a common Convolutional Neural Network architecture, was trained using random initialization versus supervised pretraining on the ImageNet dataset versus self-supervised pretraining using a Momentum Contrast (MoCo) model. Pretraining was done on 3457 unlabeled bitewings radiographs. All models were fine-tuned on 386 expert-annotated radiographs (269 images with caries being segmented pixelwise, 116 without).

Results: Self-supervised pretraining led to significantly higher performance than random initialization (F1-score: 0.435 [0.352, 0.519] vs 0.279 [0.223, 0.336], Negative Predictive Value:0.669 [0.498, 0.839] vs 0.257 [0.016, 0.498], Area-Under-the-Curve: 0.546 [0.506, 0.586] vs 0.497 [0.493, 0.502]) (95% confidence intervals). In contrast, performance was not significantly different between self-supervised pretraining and supervised pretraining on ImageNet.

Conclusions: Self-supervised pretraining may boost the performance of deep learning models for dental image analysis when annotated data are sparse.

0108

Microleakage Assessment of Bioceramic Monoblock Endodontic Obturation

Kostadin Zhekov[1], Vesela Stefanova[1], Zagorchev Plamen[2], Elena Boyadzhieva[1]

[1]Operative Dentistry and Endodontics, Medical University of Plovdiv, Plovdiv, Bulgaria, [2]Medical physics and Biophysics, Medical University of Plovdiv, Plovdiv, Bulgaria

Objectives: The objective of this study is to evaluate the difference of the sealing ability by microleakage assessment of root canal obturations with bioceramic impregnated and standard (non-impregnated) gutta-percha points.

Methods: Single rooted human extracted teeth (n=30) are prepared and obturated using hydraulic condensation technique and bioceramic endodontic sealer BioRoot RCS (Septodont). Samples are divided in two experimental groups (group 1 and group 2, n=12 each) and two control groups – positive control (n=3) and negative control (n=3). For the first experimental group (group 1) impregnated with bioceramic particles gutta percha points (BC Points, FKG) are used, achieving bioceramic monoblock obturation. For group 2 standard gutta-percha points (FKG) are used. Positive control samples are sealed apically with varnish following the endodontic obturation with standard gutta-percha point and bioceramic sealer. For the negative control group root canals are obturated only with standard gutta percha points with no endodontic sealer. Each sample is fixed in a system of Eppendorf tubes and 2% methylene blue dye is delivered with a constant pressure of 1 atm. Microleakage is assessed by spectrophotometry of the receiving solution (TECAN, Sunrise). The results are statistically analyzed using Kruskal-Wallis test and Dunn's Multiple Comparison test.

Results: Results show 0.39% permeability for group 1 and 0.69% permeability for group 2. Statistically significant difference is not found between the two experimental groups, neither with the negative control ($p>0.05$). Statistical differences are observed when comparing the positive control and both experimental groups ($p<0.05$).

Conclusions: Root canals obturation with hydraulic condensation and bioceramic sealer ensure low levels of dye penetration. The bond between the impregnated gutta-percha cones and the bioceramic sealer (bioceramic monoblock) do not enhance the sealing ability of the endodontic obturation assessed by microleakage test.

0109

Filling Ability of Different Obturation Techniques in Curved Canals.

Melis Taner[2], Aysenur Kamaci[1], Necdet Erdilek[2]

[1]Sakarya University Faculty of Dentistry, Sakarya, Turkey, [2]Ege University Faculty of Dentistry, Izmir, Turkey

Objectives: For complete root canal obturation several techniques have been introduced. The aim of the study is to compare filling ability of four different root canal obturation techniqes in severly curved canals.

Methods: Mandibular molar teeth with 30-35^0 curvature and curvature radius between 2-5 mm were distrubuted to the groups equally (n=20). Access cavities opened and root canals were enlarged with Twisted File Adaptive system up to SM3 (35.04). Canals were irrigated with 2 mL 2.5% NaOCl during instrumentation. As a final irrigation 2 mL 5% EDTA, 2 mL 2.5% NaOCl and 2 mL distilled water were used. Passive ultrasonic activation was performed to NaOCl. Root canals filled according to methods below. Group A: Lateral compaction. Group B: Obturation with Soft-Core #35 obturator. Group C: Modified wave compaction using Elements Obturation Unit with 04.35 gutta-percha cone. Group D: Single cone technique with matched gutta-percha cone.

After obturation, access cavities were sealed and the teeth were stored for 2 weeks at an incubator for sealer setting. The teeth were embedded in acrylic resin and sectioned perpendicular to the long axis at levels of 2, 4, 6, and 8 mm from the apex using low-speed saw. Sections were photographed in stereomicroscope under x20 magnification and evaluated by using AutoCAD programme.

Results: No significant differences were obtained between the groups in terms of percentage of voids at any level. Whole groups showed significantly higher gutta-percha and lower sealer filled areas at all levels. In all groups, the area filled with gutta-percha increased from the apical to the coronal section while the area of sealer decreased. Group D showed significantly less percentage of gutta-percha. Group C showed more voids at the apical area and more gutta-percha at the coronal area than other groups, but the difference was statistically insignificant. Group B showed significantly more apical extrusion than all other groups.

Conclusions: Under the conditions of this in vitro study, the single cone technique was not as effective as other techniques. Lateral compaction is an effective technique to fill root canals but has a higher sealer percentage than others, which could effect the long-term prognosis of the treatment. The Soft-Core system showed more incidence of apical extrusion.

0110

Laser Activated Photoacoustic Streaming in Debridement of Round Root Canals

Ivona Bago[1], Lovorka Batelja-Vuletić[2], Antonija Tarle[5], Dragana Gabrić[4], ivica anic[3]

[1]Department of Endodontics and Restorative Dentistry, School of Dental Medicine University of Zagreb, Zagreb, Croatia, [2]Department of Pathology, School of Medicine University of Zagreb, Zagreb, Croatia, [3]Department of Endodontics and Restorative Dentistry, School of Dental Medicine, Zagreb, Croatia, [4]Department of Oral Surgery, School of Dental Medicine University of Zagreb, Zagreb, Croatia, [5]Private Dental Practice, Zagreb, Croatia

Objectives: This study aimed to evaluate the efficacy of a new laser assisted irrigation system, SWEEPS (Shock Wave Enhanced Emission Photo-acoustic Streaming), in debriding pulp tissue from round root canals of freshly extracted premolars.

Methods: Freshly extracted premolars were scanned on CBCT and only those with round root canals were used in the study (n=32). The samples were randomly divided into four experimental groups and the control group: Group 1. Reciproc Blue (RB25/.06) root canal instrumentation and conventional syringe irrigation (CI) of final irrigation protocol (FIP); Group 2. RB25 instrumentation and ultrasonically activated irrigation (UAI) of the FIP; Group 3. RB25 instrumentation and SWEEPS activation of the FIP; Group 4. Only SWEEPS activation of 3% sodium hypochlorite (NaOCl) for 4x30 s without instrumentation. In groups 1, 2 and 3, the total amount of 5 mL of 3% NaOCl was used during instrumentation, and the FIP included:3% NaOCl (30 s), ethylenediaminotetraacetic acid (60 s) and 3% NaOCl (30 s). Eight additional uninstrumented samples served as histological controls. The specimens were demineralized and processed for histological evaluation for measurement of the remaining pulp tissue (RPT) in each canal third. The results were analysed with Mann-Whitney U test (α =0.05).

Results: There was significant decrease of the RPT in all experimental groups ($p<0.05$). In the middle third, instrumentation with UIA and SWEEPS had similar efficacy ($p=0.171$), which was greater than CI group and only SWEEPS without instrumentation ($p<0.05$). In the apical part, SWEEPS with instrumentation was the most efficient ($p=0.002$) in the removal of the RPT, and no difference between UIA and CI groups ($p=0.643$) was reported.

Conclusions: The new SWEEPS technique was more effective than UIA and CI in the removal of RPT in the apical part of round canals after RB25 single file instrumentation. Only SWEEPS without instrumentation was not efficient in root canal debridement.

0112

Antibiofilm Activity of a Brushing Solution in Peri-Implant Biofilm Model

David M. Simões e Martins[1], Leire Virto[1, 2], María Carmen Sánchez[1], Ana Encinas[1], Mariano Sanz[1], David Herrera[1]

[1]ETEP (Etiology and Therapy of Periodontal and Peri-Implant Diseases) Research Group, Complutense University, Madrid, Spain., Madrid, Spain, [2]Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), Madrid, Spain, Madrid, Spain

Objectives: To evaluate the potential antibiofilm effect of a brushing solution in an *in-vitro* peri-implant biofilm model developed on titanium surfaces.

Methods: Titanium discs (Ti-SLA) were pre-treated with a brushing solution (NitrAdine®, test), warm water (negative control) and chlorhexidine and cetylpyridinium chloride (CHX/CPC, positive control). An *in-vitro* validated peri-implant biofilm model (with *Streptococcus oralis, Actinomyces naeslundii, Veillonella parvula, Aggregatibacter Actinomycetemcomitans, Porphyromonas gingivalis* and *Fusobacterium nucleatum)* was developed on the pre-treated surfaces. The discs were treated and re-incubated each 24 h until reach 72 h. Biofilms (24 h, 48 h and 72 h) were analyzed by confocal laser scanning microscopy (CLSM), scanning electronic microscopy and quantitative polymerase chain reaction (qPCR) (viable colony forming units, CFU, per milliliter). A generalized linear model was constructed to determine the effect of the tested products on viable bacterial counts.

Results: Regarding CLSM analyses of biofilm formation and development, Ti-SLA discs pre-treated with test product demonstrated: a) the largest reductions in live/dead cell ratio, being statistically significant between 24-48 h and 24-72 h; and b) a more stable thickness over time, when compared with positive and negative controls.

qPCR analysis showed, after 24 h of re-incubation, lower counts of *S. oralis* and *A. actinomycetemcomitans* on Ti-SLA discs pre-treated with test product, when compared with negative control ($p<0.05$). After 48 h of re-incubation, differences were found between Ti-SLA discs exposed to test solution or negative control, for counts of *S. oralis, A. naeslundii, V. parvula* and *P. gingivalis* ($p<0.05$). After 72 h of re-incubation, test solution showed lower counts for *S. oralis, A. naeslundii, V. parvula, P. gingivalis* and *F. nucleatum*, when compared with negative control ($p<0.05$).

Conclusions: The tested brushing solution can impact the development of *in-vitro* peri-implant biofilms on titanium surfaces, in a biofilm model, by affecting its structure and vitality.

0113

Monopolar Atmospheric Electrical Plasma for Disinfecting Implant Surfaces

Markus Altenburger[1], Michael Bergmann[2], Loic Ledernez[1]

[1]Department of Operative Dentistry and Periodontology, University of Freiburg - Faculty of Medicine, Freiburg, Germany, [2]Department of Microsystems Engineering - IMTEK, University of Freiburg - Faculty of Engineering, Freiburg, Germany

Objectives: Bacterial infection of rough implant surfaces is one main reason for the inflammatory reaction of soft and hard tissues around the dental implant. Conventional treatment attempts using brushes, curettes, powder water jet devices or antibiotics show insufficient results. Also, adverse acute or long-term effects can be observed.

Recent research shows the efficacy of atmospheric electrical plasma in eradicating bacteria. A critical parameter for the success of the treatment with electrical plasma is the delivery of the adequate energy to the biofilm at the site of action, without altering or harming the surrounding tissues. Bipolar electrical setups are often not able to fulfill this task.

Methods: A new electrode setup has been developed to address this problem. One electrode is placed in a gas leading tube connected to a high voltage power source. A second electrode connected to the implant closes the electrical circuit. Thus, the 'guided' plasma is directly ignited on the implant surface protecting the surrounding tissues from accidental contact with the plasma. To evaluate the efficacy and the safety of the system multiple tests were conducted. Artificial biofilms were cultivated on titanium discs in vitro for 14 days. Cultivation, light microscopy and SEM were used to evaluate the absence of microorganisms. For safety analysis temperature measurement took place with and without simulating thermal conduction by surrounding tissues. As second safety parameter electrical discharge was measured with results compliant to EN 60601-1.
Results: This new technique eliminates the biofilm on contaminated titanium implant surfaces very effectively without leaving bacterial remnants on the implant surface. By electrically contacting the implant, the atmospheric pressure plasma is ignited solely on the implant surface and does not harm the surrounding tissues.
Conclusions: Further studies will be conducted to evaluate the efficacy, the safety and the change of the implant surface properties and its effect on cellular adhesion.

0114

Effect of Functionalized Nanoparticles in Biofilms on SLA Titanium Surfaces

Jaime Bueno De Vicente[1], Leire Virto[1], Manuel Toledano-Osorio[2], Elena Figuero[1], Manuel Toledano[2], Antonio-Luis Medina-Castillo[2], Raquel Osorio[2], Mariano Sanz[1], David Herrera[1]
[1]University Complutense of Madrid, Madrid, Spain, [2]University of Granada, Granada, Spain

Objectives: The aim of this study was to assess the antibacterial effect of active polymeric (PPA) nanoparticles (NPs), functionalized or not with calcium, zinc and doxycycline on titanium discs (TiD), in a validated *in vitro* subgingival biofilm model.
Methods: The biofilm model consisted of six bacterial species (*Streptococcus oralis, Actinomyces naeslundii, Veillonela parvula, Fusobacterium nucleatum, Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*). SLA (sandblasted, large grit, acid-etched) TiD were used with the application of NPs with calcium (Ca-NPs), zinc (Zn-NPs), doxycycline (Dox-NPs) and without charge (PPA-NPs), by friction with a micro-brush for 15 seconds. As control, sterile TiDs were used. All discs (n=9 in each group) were incubated under anaerobic conditions during 12, 24, 48 and 72 hours. To analyse the resulting biofilms, scanning electron microscopy (SEM) was used to study the structure of the biofilm, confocal laser scanning microscopy (CLSM) to assess the vitality and thickness, and quantitative polymerase chain reaction (qPCR) to evaluate the bacterial load, expressed as colony forming units (CFU) per mL. General linear models and Bonferroni adjustments were used to evaluate differences among groups.
Results: After 72 hours, PPA-NPs, Ca-NPs, Zn-NPs and Dox-NPs increased the mortality of bacterial cells in the biofilm (70.89%, 80.70%, 70.50% and 85.87%, respectively). Statistically significantly differences were found for dead cells, when compared to the control group (47.21%), for Ca-NPs ($p<0.039$) and PPA-NPs ($p<0.045$). Specifically, Dox-NPs were able to reduce the bacterial load of all studied bacteria, at different time points, with respect to the control group (TiD), except for *Aggregatibacter actinomycetemcomitans.*
Conclusions: Polymeric nanoparticles reduced the vitality and altered the dynamics of biofilm formation, and decreased the bacterial load in the case of Dox-NPs.

0115

Time-Induced Ageing of Titanium Dental Implants

Nagore Arroyo Lamas[1], Unai Ugalde[2], Iciar Arteagoitia[3]
[1]PhD School, University of the Basque Country UPV/EHU, Leioa, Bizkaia, Spain, [2]APERT Research Group, Department of Electronic Technology, University of the Basque Country UPV/EHU, Bilbao, Bizkaia, Spain, [3]Maxillofacial Group, Stomatology Department, BioCruces Health Research Institute, University of the Basque Country UPV/EHU, Leioa, Bizkaia, Spain

Objectives: Titanium is used worldwide as the base material for dental implants due to its excellent biocompatible features. However, the chemical composition of implant surfaces, which plays a crucial role in the osseointegration, is unavoidably contaminated by organic compounds, causing the degradation known as "the ageing of titanium". Thus, this study investigates the surface chemistry of new and 5-year-old commercially available dental implants so as to compare the influence of the time-induced changes on the characteristics of their surfaces.
Methods: X-ray Photoelectron Spectroscopy (XPS) was employed to analyse the chemical composition of the titanium surfaces. First, wide scans were conducted to detect which elements were present on the surface, and then narrow scans were performed around the energy peaks of carbon (C), oxygen (O) and titanium (Ti), to quantify the relative concentration of each element. Both the new and 5-year-old surfaces were subjected to the same characterization process.
Results: XPS analysis showed higher C concentrations on the 5-year-old surfaces than on the new ones; the mean concentration was 31.23±0.56 C at. % (atomic concentration) (95% confidence interval (CI): 26.116-36.050) and 15.4±0.53 C at. % (95% confidence interval (CI): 14.085-16.714), respectively. Specifically, the hydrocarbon concentration was remarkably higher on both surfaces. Along with that, the concentrations of O and Ti were lower on the old surfaces (43.43±1.40 O at. %; 8.4±0.89 Ti at. %) comparing with the new surfaces (57.63±0.15 O at. %; 15.07±0.75 Ti at. %). These outcomes may be related to a reduction of the titanium bioactivity.
Conclusions: There are relevant differences in terms of the chemical composition of the 5-year-old implants with respect to the new ones, specifically in the concentration of carbon compounds, which are associated with the ageing of titanium.

0116

Structured Zirconia Surfaces as Carrier Material for Adult Neural Crest-Derived Stem Cell (NCSC) Transplantation.

Wolf-Dieter Grimm[5, 1], Emre Benlidayi[2], Nikolaj Didenko[1], Alexander Dolgalev[1], Natella Enukashvily[3], Igor Rzhepakovsky[4], Marco A Vukovic[1], Tilman Fritsch[1]

[1]Center for Innovation and Technology Transfer, Stavropol State Medical University, St. Peterburg, Russian Federation, [2]oral Maxilla-Facial Surgery, Cukurova University, Adana, Turkey, [3]Stem Cell Lab, North-Western State Medical University, St. Peterburg, Russian Federation, [4]Institute for Life, North-Caucasian Federal University, Stavropol, Russian Federation, [5]Periodontology, Witten/Herdecke University, Witten, NRW, Germany

Objectives: The neural crest (NC) is a stem cell population, unique to vertebrates, that arises in vertebrate embryos during nervous system formation. Interestingly, NC cells have also been isolated from various adult tissues including dorsal root ganglia, gut, heart, olfactory sheath, hair follicles and craniofacial tissue (Widera et al. 2007). These cells maintain their multipotency as they can be coaxed to differentiate into neuronal and glial cells, smooth muscle cells, melanocytes, bone cells, adipocytes, and chondrocytes. As a result, several groups have proposed their use in applications including treatment for spinal cord injury, deafness, ocular repair or periodontal regeneration (Grimm et al. 2013). However, clinical application is hampered by the need for genetic modification in reprogramming or the limited accessibility of adult tissues where they reside.

Methods: Ceramic implants are new prototype dental implants. SEM was obtained from ceramic implants for observation of the surface morphology. The chemical composition of the surface coating was analyzed with EDX. Four female Stavropol breed sheep have been used for the study (Institutional Animal Ethics Committee approval). NC Ceramic sheets have been placed into mandibular ridges for assessing the osseointegration as described by Duncan (2005). After 12 weeks, block sections were obtained from ceramic NC stem cell sheets. Qualitative histological analysis on non-decalcified sections using Technovit 9100 New with different stains and MicroCT analyses were carried out.

Results: SEM of the surfaces showed the modulation of ceramic surfaces. The results of EDX Analysis of ceramic surfaces will be demonstrated. All ceramic NC stem cell sheets showed signs of osteoconduction and integration at 12 weeks after operation. NC stem cell sheets had large masses of disorganized calcified material which appeared to be resorbed bone tissue. Lack of osteocytes has been illustrated by Tartrate-resistant acidic phosphatase (TRAP).

Conclusions: In summary, bone tissue early integration was significantly strong for the ceramic NC stem cell sheets in our mandibular sheep model 12 weeks after implant insertions.

0117

The Impact of HAMLET, a Human-Milk Protein Complex, on the Ecology of the Oral Microbiome

Navdeep Kaur Brar[1], Roger Junges[1], Anders P. Hakansson[2], Fernanda C. Petersen[1]

[1]Faculty of Dentistry, University of Oslo, Institute of Oral biology, Oslo, Oslo, Norway, [2]Faculty of Medicine, Lund University, Division of Experimental Infection Medicine, Malmo, Sweden

Objectives: Antibiotic treatment not only affects pathogenic bacteria, but also provides the potential to impact the whole human microbiome. To prevent unwanted changes by antibiotics that may favor overgrowth of pathogens, combination therapies with prebiotic agents have received increased attention. Recent studies shows that the protein-lipid complex from human milk, called HAMLET (human alpha-lactalbumin made lethal to tumor cells) exhibits direct bactericidal activity against multiple pathogens and hinders antibiotic-resistance development.

Methods: The aim of this study was to explore HAMLET's potential as a prebiotic to modulate microbiome changes induced by amoxicillin. We utilized an *ex vivo* oral microbiome model with a highly complex microbial diversity that allows growth of uncultivated oral species.

Results: We first exposed preformed biofilms to amoxicillin alone at concentrations ranging from 0.025 µg/ml to 0.1 µg/ml. After 24 hours, none of the amoxicillin concentrations had any significant impact on the total CFUs (colonies forming units) recovered from the microbiomes, while the dry biomass weight increased in the highest concentrations. When exposed to HAMLET at 125 µg/ml and 250 µg/ml, the CFUs decreased significantly at the highest concentration, compared to controls with no treatment. The dry biomass weight increased by 20%. RT-PCR analysis for HAMLET-treated biofilms showed a significant increase in the relative abundance of *Neisseria* and *Streptococcus* at 125 µg/ml. While treatment with 250 µg/ml HAMLET significantly decreased the abundance of both genera. Combination therapy of 0.1 µg/ml amoxicillin and 250 µg/ml HAMLET reduced the CFUs compared to HAMLET alone, and showed nearly a 2-$\log_{10}$ reduction compared to biofilms without treatment.

Conclusions: Our results suggest that HAMLET affects the composition of *ex vivo* oral microbiomes. Additionally, HAMLET has the potential to modify the response of the microbiome to low amoxicillin concentrations. Further, metagenomic studies will be conducted to deepen our understanding of the impact on microbiome composition and resistome development.

0118

Oral Microbiome and Recovery of Renal Function After Kidney Transplantation.

Benedita Sampaio Maia[1, 2], Susana Sampaio[2, 3], Ana Merino-Ribas[4, 5], Joana Campos[2], Manuel Pestana[2, 3], Ricardo Araujo[2]

[1]Faculdade de Medicina Dentária, Universidade do Porto, Porto, Portugal, [2]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal, [3]Nephrology Department, Hospital Universitário de São João, Porto, Portugal, [4]Universitat Autònoma de Barcelona, Barcelona, Spain, [5]Nephrology Department, Hospital Universitari de Girona Doctor Josep Trueta, Girona, Spain

Objectives: The loss of kidney function that characterizes chronic kidney disease (CKD) leads to dramatic changes in body homeostasis, with repercussions in saliva biochemistry and, consequently, in oral microbiome. Kidney transplant is the gold-standard therapy for patients with end-stage kidney disease. Although oral cavity may represent a starting point for pathogens dissemination and complications after kidney transplantation, such as infections and rejection episodes, few studies evaluated the oral microbiome in kidney transplant recipients (KTR). Therefore, we aimed to explore the impact of kidney transplantation on the oral microbiome.

Methods: We conducted a prospective observational pilot study in patients who received a first living donor kidney transplantation (n=6). The microbiome of saliva was analyzed immediately before (maximum 24h before) and three months after transplantation by sequencing of V3-V4 hypervariable regions of 16S rRNA gene using MiSeq Illumina® technology.

Results: After kidney transplantation the renal function recovered significantly (T3 vs. T0, Glomerular Filtration Rate: 75.3±17.6 vs. 6.2±3.7 mL/min/1.73 m^2; Plasma Creatine: 1.0±0.3 vs. 9.0±2.7 mg/dL; Plasma urea: 47.2±13.9 vs. 183.8±30.0 mg/dL, $p<0.001$ for all parameters). The oral microbiome profile before kidney transplant was dominated by Firmicutes, Bacteroidetes, and Proteobacteria. The recovery of the renal function 3 months after kidney transplant was accompanied by a significant increase in the proportion of Fusobacteria and Proteobacteria and a reduction of Firmicutes and Actinobacteria in saliva. Specifically, the lower taxonomic level analysis revealed increases in bacteria belonging to *Escherichia* genus and Bradyrhizobiaceae family as well as reduction in bacteria belonging to *Streptococcus* and *Granulicatella* genera and unclassified Gemellaceae family.

Conclusions: The recovery of renal function after kidney transplantation is accompanied by changes in oral microbiome, in particular associated with an overgrowth of potentially opportunistic pathogens, including those commonly associated with post-transplant infections.

0119

Phenotypic Adaptation of Oral Bacterial Isolates Toward Chlorhexidine-Digluconate and Cetylpyridinium-Chloride

Xiaojun Mao[1], David L. Auer[1], Karl-Anton Hiller[1], Tim Maisch[1], Christiane von Ohle[2], Diana Wolff[2], Cornelia Frese[3], Wolfgang Buchalla[1], Elmar Hellwig[4], Ali Al-Ahmad[4], Fabian Cieplik[1]

[1]University Hospital Regensburg, Regensburg, Germany, [2]University Hospital Tübingen, Tübingen, Germany, [3]University Hospital Heidelberg, Heidelberg, Germany, [4]University Hospital Freiburg, Freiburg, Germany

Objectives: Resistances toward antibiotics have become a major concern for public healthcare. In contrast, studies on development of resistances toward the antiseptics chlorhexidine digluconate (CHX) or cetylpyridinium chloride (CPC) in oral bacteria are scarce, although these antiseptics have been widely used in dentistry for decades. Therefore, the aim of the present study was to investigate phenotypic adaptation of 178 clinical oral bacterial isolates upon repeated exposure to CHX or CPC.

Methods: 113 oral *Streptococcus*-isolates (14 species), 19 oral *Actinomyces*-isolates (3 species) and 20 oral *Rothia*-isolates (14 species) were cultured in Brain-Heart-Infusion-broth, and 26 oral *Veillonella*-isolates (3 species) in Schaedler-broth, and the minimum inhibitory concentrations (MICs) were determined for CHX or CPC. Bacteria from the sub-MIC population were transferred to the next-day-cycle and exposed to CHX or CPC again. This procedure was repeated for 9 times (passages 1-10; 6 biological replicates each). Isolates showing an 8-fold or higher MIC-increase at passage 10 compared to the MIC at passage 1 were regrown in antiseptic-free nutrient broth for 1, 2 and 3 days and re-evaluated in order to assess the stability of the phenotypic adaptation.

Results: In the CHX group, 23 *Streptococcus*-isolates and two *Actinomyces*-isolates showed at least four-fold MIC-increase, and five *Streptococcus* isolates showed an eight-fold MIC-increase as compared to the MIC of the respective parental strain. In CPC group, six *Streptococcus*-isolates and one *Actinomyces*-isolates showed a four-fold MIC-increase as compared to the MIC of the respective parental strain. Culturing the five *Streptococcus*-isolates with eight-fold MIC-increase without selection pressure for up to 3 passages showed unaltered or even further increased (up to 16-fold) MICs.

Conclusions: Oral bacterial isolates show phenotypic adaptation upon repeated exposure to CHX and CPC, which was more pronounced in the CHX group. Further investigations are needed in order to reveal the genetic background behind these phenotypic adaptations.

0120

In-Vitro Activity of Three Propolis on Oral Microorganisms and Biofilm

Sigrun Eick[1], Alexandra Stähli[1], Sandor Nietzsche[2], Egle Milia[3], Anton Sculean[1]

[1]Department of Periodontology, School of Dental Medicine, University of Bern, Bern, Switzerland, [2]Center for Electron Microscopy, Jena University Hospital, Jena, Germany, [3]Department of Medicine, Surgery and Experimental Sciences, University of Sassari, Sassari, Italy

Objectives: Natural products are discussed as alternatives to the commonly used chemicals in antimicrobial therapy. The study was aimed to investigate the antimicrobial activity of propolis originating from two different regions on microbial species being associated with caries, periodontal disease and with Candida infections.

Methods: Commercially available ethanolic extracts of propolis (EEP) originating from South America (Brazilian red propolis, Brazilian green propolis), and Central Europe (European) and as controls a chlorhexidine digluconate solution, an ethanolic solution were used. The minimal inhibitory concentrations (MIC) of propolis and controls against 8 microbial strains were determined before SEM and TEM images visualized the effect of propolis on microorganisms. Then, the activity on three different multi-species biofilms (both formation and existing biofilms) was assessed.

Results: All MIC values of the Brazilian EEPs were low against the tested oral species (<=0.1 mg/ml – 3.13 mg/ml propolis (*Candida albicans*)). The EEP European seemed to be slightly less active than the Brazilians EEPs. The SEM and TEM images suggest an interaction of propolis with the microbial cell wall. EEP European most retarded biofilm formation; after 4 h and 24 h of formation, 1% propolis reduced the total microbial counts (cfu) of the cariogenic biofilm by 2.21 log10 and 5.78 log10 vs. control. Applying 10% propolis of EEP Brazilian green or Brazilian red to already formed biofilms resulted in no (cariogenic biofilm), less than 1 log10 (periodontal biofilm) or less than 2 log10 (Candida biofilm) cfu in biofilms.

Conclusions: The antimicrobial and anti-biofilm activities underline the potential of propolis as an adjunct in oral health care products. Further research is needed to get better knowledge about the anti-inflammatory activity of propolis related to oral diseases.

0121

Inhibition of Immunomediators in Periodontal Ligament-Derived MSCs by 25-Hydroxyvitamin-D_3

Christian Behm, Alice Blufstein, Andreas Moritz, Xiaohui Rausch-Fan, Oleh Andrukhov

University Clinic of Dentistry, Medical University of Vienna, Vienna, Austria

Objectives: Human periodontal ligament-derived mesenchymal stromal cells (hPDL-MSCs) are important in the orchestration of periodontal tissue homeostasis and regeneration. This is mainly achieved by affecting the local immune response via their immunomodulatory abilities, which are activated by inflammatory cytokines, such as interferon (IFN)-γ, tumor necrosis factor (TNF)-α or interleukin (IL)-1β. These cytokines boost the expression of different, mainly immunosuppressive, immunomediators in hPDL-MSCs, such as indoleamine-2,3-dioxygenase 1 (IDO-1), programmed cell death ligand 1/2 (PD-L1/2), cyclooxygenase-2 (PTGS-2) and tumor necrosis factor-inducible gene 6 protein (TSG-6). Studies already demonstrated that the biological active 1,25-diydroxyvitamin-D_3 (1,25-$(OH)_2$-D_3) decreased the expression of different immunomediators in hPDL-MSCs. However, no studies examined the influence of 25-hydroxyvitamin-D_3 (25-OH-D_3), the most abundant 1,25-$(OH)_2$-D_3 precursor in the blood, on the immunomediator expression in hPDL-MSCs. Hence, this study investigated the effect of 25-OH-D_3 on the expression of different immunomediators in cytokine-treated hPDL-MSCs.

Methods: Primary hPDL-MSCs from five different healthy individuals were treated with 100ng/ml IFN-γ or 10ng/ml TNF-α or 5ng/ml IL-1 β in the absence or presence of 25-OH-D_3 (0.1-100nM) for 48 hours. Expression of IDO-1, PD-L1/2, PTGS-2 and TSG-6 were analysed on gene (qPCR) and protein (flow cytometry analysis, ELISA) levels. Additionally, IDO-1 enzymatic activity was determined photometrically.

Results: Expression levels of IDO-1, PD-L1/2, PTGS-2 and TSG-6 were significantly increased by IFN-γ, TNF-α and IL-1β, in the absence of 25-OH-D_3. Adding 25-OH-D_3 caused a concentration-dependent decline of cytokine-induced immunomediator expression levels at the gene and protein level. A significant decrease was observed starting from 10 nM 25-OH-D_3. In the absence of any cytokines, 25-OH-D_3 had no significant influence on immunomediator expression in hPDL-MSCs.

Conclusions: These data show that, beside 1,25-dihydroxyvitamin D_3, 25-OH-D_3 may also inhibit cytokine-boosted immunomediator expression in hPDL-MSCs. This suggests that 25-hydroxyvitamin-D_3 also influences local immune responses and consequently may participate in fine-tuning local inflammatory events in periodontal tissues.

0122

Three-Dimensional Ridge Alterations Following Stepwise Surgical Treatment of Extraction Defects

Daniel Palkovics[1], Csaba Pinter[2], Zsofia Gergely[3], Elod Szasz[3], Balint Molnar[1], Peter Windisch[1, 3]

[1]Department of Periodontology, Semmelweis University, Budapest, Hungary, [2]Empresa de Base Tecnológica Internacional de Canarias, Las Palmas de Gran Canaria, Spain, [3]Widen Private Practise, Budapest, Hungary

Objectives: To present morphological and volumetric hard tissue alterations following stepwise surgical rehabilitation of advanced extraction defects with the application of a cone-beam computed tomography (CBCT) image segmentation and volumetric subtraction analysis.

Methods: 7 surgical sites of 7 partially edentulous patients were treated using a staged approach for surgical rehabilitation. Stage 1: tunneled guided bone regeneration (GBR) approach for alveolar ridge preservation (ARP). Stage 2: vertical and/or horizontal GBR applying a split thickness flap design 6 months following ARP, Stage 3: dental implant placement 6-9 months following GBR. CBCT scans were taken at three timepoints: (i) at baseline, (ii) prior to GBR, (iii) prior to implant placement. CBCT scans were

imported into an open-source radiographic image processing software (3D Slicer). Datasets were reconstructed and three-dimensional virtual models were acquired utilizing a semi-automatic image segmentation method. Superimposition of all three CBCT scans and subsequent volumetric subtraction of reconstructed 3D models were performed to validate volumetric and 3D morphological changes that occurred at each stage of the process (Figure).
Results: Change of hard tissue volume following ARP averaged at 0.61 cm3 ± 0.09 cm3 and 0.67 cm3 ± 0.16 cm3 following GBR (Table). After ARP, the horizontal dimension of the alveolar ridge was maintained and even increased in some cases. Hard tissue formation was visible on denudated root surfaces, in cases where adjacent teeth were involved. After GBR, vertical dimension of the edentulous ridge was restored to ensure prosthetically driven implant placement.
Conclusions: The Digital reconstruction of CBCT datasets and subsequent 3D subtraction analysis may provide a better understanding of the healing mechanisms that occurred during the staged rehabilitation of advanced extraction defects.

0123

Communication Sources Used by Patients Before Teeth Extraction

Modesta Ralyte[1], Milda Vitosyte[1, 2], Linas Zaleckas[1, 2], Alina Puriene[1, 2]
[1]Faculty of Medicine Institute of Odontology, Vilnius University, Vilnius, Lithuania, [2]Vilnius University Hospital Zalgiris Clinic, Vilnius, Lithuania

Objectives: To determine which communication sources (CS) patients use to find the information before the third molars extraction (TME) procedure and patients' need to interact virtually with the doctor.
Methods: 95 patients from Vilnius University Hospital Zalgiris Clinic participated in this study. Prior to the sedation-assisted TME, a questionnaire assessing general demographics and favoured communication sources was administered using a 5-point Likert scales (response frequency 95%). Statistical analysis was performed using the IBM SPSS v.23 statistical package, descriptive statistics, independent sample t-test, Mann-Whitney U, Chi-square, McNemar's tests and linear regression were applied. Statistical significance level $p < 0.05$.
Results: The mean age of respondents was 23.30 (± 6.34), the ratio of men to women was 1:1.7. The main CS were consulting a doctor (68.4%), family members / friends (42.1%) and using social media apps (40.3%). 20-30-year-old patients more frequently (p=0.015) chose social media apps: Facebook (79.3%) and Instagram (66.3%). 30-40-year-old patients preferred (p=0.032)online communication forums (56.1%) and Wikipedia (44.6%). Women more frequently (p<0.001) chose online websites (88.4%) than press (23.1%), while men were more likely to find information about TME on press (87.1%), TV (68.2%) and radio (43.5%) than online websites (24.5%). 80.7% of patients felt the information about TME deficiency online. The majority (63.2%) of patients would like to interact virtually with the doctor performing the procedure, 67.6% would prefer virtual communication before TME during a COVID-19 pandemic. The ability to communicate online would decrease patients' anxiety before TME for 52.6% of the patients.
Conclusions: While doctors' supplied information remains the most important, a significant proportion of patients use various CS prior to the TME procedure. CS should provide more reliable information about teeth extraction as it is a useful channel for patients, especially young generation, seeking support. Doctors could interact with patients online as it reduces patients' anxiety.

0124

Associations Between Anesthesia Type and Anxiety Before Third Molars Extraction

Vakare Jotautaite[1], Austeja Pauliukaite[1], Milda Vitosyte[1, 2], Linas Zaleckas[1, 2]
[1]Institute of Dentistry, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, [2]Vilnius University Hospital Zalgiris Clinic, Vilnius, Lithuania

Objectives: Third molars extraction causes the greatest levels of discomfort among all dental procedures. The aim of this study was to evaluate the association between anesthesia type and anxiety levels before third molars extraction.
Methods: Paper questionnaires were concluded. The demographic information of participants including their age, gender, education was requested to be filled at first. A 10-point Likert scales were used to measure respondents' anxiety before the third molars removal procedure. 207 respondents were divided into two groups depending on the anesthesia type during extraction procedure: intravenous sedation group (n=95) and local anesthesia group (n=112). For the statistical analyses IBM SPSS Statistics 27 version and Microsoft Excel 2007 were used. Descriptive statistics, Mann-Whitney U test and linear regression were used to analyse the data. The significance threshold was set at p<0,05.
Results: Respondents from the local anesthesia group were mostly anxious of possible complications, pain and swelling after the third molars removal procedure (average point scores respectively 6,6; 6; 5,9). Respondents from the intravenous sedation anesthesia group were mostly anxious of possible complications after the procedure, bleeding and cracking sounds during the procedure (average point scores respectively 5,7; 5,7; 5,4). There was a statistically significant difference between the intravenous sedation and local anesthesia group respondent's anxiety: participants from the local anesthesia group were more anxious about a needle stab before the procedure and possible limited mouth opening after a tooth extraction (p<0,05). Their general anxiety was significantly higher than respondents' from the intravenous sedation anesthesia group (p<0,05). Respondents claimed that a leaflet with information about the extraction, sedatives and music could reduce their anxiety before the procedure.
Conclusions: Anxiety levels tend to rise in the presence of local anesthesia before the third molars extraction.

0125

Immature Third Molar Autotransplantation for Replacement of Tooth Row Defects

Miks Lejnieks[1, 2], Ilze Akota[1], Gundega Jakobsone[1]

[1]Riga Stradins University, Riga, Latvia, [2]Baltic Biomaterials Centre of Excellence, Riga, Latvia

Objectives: Autotransplantation of third molars with unformed roots has several biological benefits: preservation and regeneration of bone, providing a functioning tooth, with no need for biomaterials. The aim of the study was to investigate the efficiency of autotransplantation of the immature third molars, performed in the Institute of Stomatology, Riga Stradins University.

Methods: There were 22 patients (7 males and 15 females, mean age 17,9 years, range 14 - 22) with 26 immature third molars autoransplantations. Out of them in one patient tree teeth were transplanted. In two patients two teeth were transplanted. Only first transplanted tooth was included in research. Procedures were performed from 14/08/2019 till 03/08/2020 by the same specialist. Only patients with at least 6-month observation period were included. Three teeth from the maxilla to the maxilla, eight teeth from the maxilla to the mandible, nine teeth from the mandible to the mandible and two from the mandible to the maxilla were transplanted. Gingival pockets and vitality tests for transplanted teeth were assessed clinically. Bone attachment, obliteration of pulp chamber, root length growth and possible appearance of root resorption were evaluated in the periapical radiographs.

Results: An increased gingival pocket of 6-8 mm was observed in two cases. Positive vitality test was assessed in 10 teeth, delayed response- in 6 teeth, no response in 6 teeth. Radiological examination revealed reestablishment of the bone attachment in 20 teeth. Pulp chamber obliteration was observed in 13 teeth. Root continues to grow in length in 6 teeth. Internal root resorption was detected in 1 case.

Conclusions: Autotransplantation of the immature third molars could be considered as effective method for replacement of missing molars. Further development for method using 3D printed replicas and evaluation of long-term results is under way.

0126

Comparing Antimicrobial and Wound Healing Effects of Nano-Silver and Chlorhexidine

Amirhossein Moaddabi[2], Parisa Soltani[1], Sahar Molaei[2], Gianrico Spagnuolo[3]

[1]Isfahan University of Medical Sciences, Isfahan, Iran (the Islamic Republic of), [2]Mazandaran University of Medical Sciences, Sari, Iran (the Islamic Republic of), [3]University of Naples "Federico II", Naples, Italy

Objectives: To compare the antimicrobial and wound healing effects of a mouthwash containing silver nanoparticles with chlorhexidine mouthwash after oral surgery in rabbit models.

Methods: Microbial samples were collected from the oral cavity of 45 rabbit models. After sedation and local anesthesia, a 1-cm long, 1-mm deep wound was created in the lateral border of the tongue on the right side for all rabbits. To control the bleeding, the ends of the wound were sutured. After surgery, digital photographs were obtained from the wounds with standardized settings. The rabbits were then randomly divided into three groups: nano-silver group, chlorhexidine group, and negative control group. For the nano-silver group, a synthetic mouthwash containing 80.38 wt.% water, 9.80 wt.% glycerin, 9.80 wt.% nano-silver suspension with 400 ppm concentration, and 0.02 wt.% sodium saccharine was used. For the chlorhexidine group, 0.2% chlorhexidine solution was used. Four drops of each mouthwash were administered every eight hour for four days using a small syringe. The negative control group did not receive any mouthwash. Microbial samples were collected from all models each day for four days. Colony forming unit (CFU) counts were compared in post-operative and pre-operative samples. In addition, standardized digital photographs were taken each day from the wounds. The area of the wounds was compared post-operatively with the pre-operative values. Data was statistically analyzed using post hoc variance analysis and paired t-test (α=0.05).

Results: Mean CFU counts on post-operative days 3 and 4 were significantly different between the groups, with the nano-silver group showing the lowest values. Additionally, the mean areas of wound were significantly different in the experimental groups, indicating superior wound healing properties of the nano-silver mouthwash compared with chlorhexidine.

Conclusions: Synthetic mouthwash with 9.8 wt.% of nano-silver particles possesses better antimicrobial and wound healing properties compared with 0.2% chlorhexidine.

0127

The Impact of Hypertension on the Oral Health During Pregnancy.

Maria Maia Azevedo[1, 2], Maria de Lurdes Pereira[3, 4], Ana Filipa Ferreira[5], Inês Falcão Pires[5], Carla Ramalho[6, 7], Egija Zaura[1], Benedita Sampaio-Maia[2, 3]

[1]Preventive Dentistry, Academic Center for Dentistry Amsterdam, Amsterdam, Netherlands, [2]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal, [3]Faculdade de Medicina Dentária, Universidade do Porto, Porto, Portugal, [4]EpiUnit- Instituto de Saúde Pública, Universidade do Porto, Porto, Portugal, [5]Unidade de Investigação Cardiovascular - UnIC, Departamento de Cirurgia e Fisiologia, Faculdade de Medicina, Universidade do Porto, Porto, Portugal, [6]Departamento de Ginecologia-Obstetrícia e Pediatria, Faculdade de Medicina, Universidade do Porto, Porto, Portugal, [7]Centro Hospitalar Universitário de São João, Porto, Portugal

Objectives: Pregnancy comprises a panoply of systemic changes that increase the susceptibility for some oral diseases. Several studies suggest a relationship between oral health status and cardiovascular risk factors, such as arterial hypertension. Moreover, the oral hygiene habits of the Portuguese pregnant population have been previously described as ineffective and high levels of dental biofilm have been reported. Therefore, we aimed to assess and correlate the oral health status of healthy and hypertensive pregnant women with their oral hygiene habits.

Methods: Participants were recruited at the Obstetrics Department of a tertiary centre, between 30 and 32 weeks of gestation. Decayed-missing-filled surfaces index (DMFS) and periodontal status (clinical attachment loss-CAL, bleeding on probing-BOP and plaque index-PI) were assessed in ten healthy(H) and seventeen arterial hypertensive pregnant participants(AHT). Oral health hygiene habits during pregnancy were recorded via questionnaires. U-Mann Whitney test, Spearman correlation and Point-Biserial correlation were used for statistical analysis.
Results: Hypertensive pregnant women scored significantly higher in all periodontal parameters when compared to healthy pregnant women (CAL≥4mm: H=2.77±3.96; AHT=21.05±18.57; p=0.007; BOP: H=13.79±15.49, AHT=27.01±11.94, p=0.003; PI: H=57.72±15.65, AHT=86.24±13.32, p<0.01), whereas there was no difference concerning DMFS (p=0.13). No differences were observed between the groups regarding the frequency of toothbrushing, use of complementary mean of oral hygiene or number of dental appointments in the previous year. Toothbrushing frequency was negatively associated with BOP (r=-0.41; p=0.033). Moreover, a negative correlation was observed between the use of a complementary means of oral hygiene, BOP (r=-0.43; p=0.026) and CAL≥4mm (r=-0.40; p=0.039).
Conclusions: The periodontal health of hypertensive women in the 3rd trimester of pregnancy was significantly worse than healthy women. Oral health habits highly impacted periodontal health. It is fundamental to raise awareness on the importance of maintaining good oral health during and after pregnancy in this population of hypertensive women.

0128

Age and Gender-Related Differences in Human Gingival Blood Flow

Barbara Mikecs, Tamás László Nagy, Réka Fazekas, János Vág
Department of Conservative Dentistry, Semmelweis University, Budapest, Hungary

Objectives: The previous studies in gingival microcirculation and wound healing indicated that gender might influence gingival blood flow (GBF) differently in young and old. We aimed to investigate the resting GBF and the vascular reactivity of gingival vessels in females before/after menopause and males in the same age intervals: 20-43 and 50-90.
Methods: Healthy volunteers, without medicatons (n=48) took part in the investigations. They were divided after Engeland (Engeland, 2009) into four age groups: younger men (n=18; mean age:32), older men (n=6; mean age: 62), younger women (n=15; mean age:26), older women (n=9; mean age: 63). GBF was measured with Laser Speckle Contrast Imager (LSCI). After a short (1-2min) baseline measurement, compression was applied on the marginal gingiva with standardized force (100g). After the release of the compression, GBF was monitored for 20 minutes. The systolic and diastolic blood pressure was measured, and the mean blood pressure (MAP) and the vascular resistance (GVR) were calculated. Pearson correlation coefficient (r) was calculated. The group was compared by linear mixed model.
Results: The age positively correlated with the MAP (r=0.47, p<0.001) and the baseline GBF (r=0.42, p<0.01) regardless of gender. No correlation was found between the age and the GVR (r=-0.18, p=0.222). The young male had a significantly higher MAP (97±1.6 mm Hg vs. 90±1.6 mm Hg, p<0.05) and GBF (185±10.4 LSPU vs. 228±8.7 LSPU, p<0.05) than the young female. The max response of GBF negatively correlated with the age (r= -0.43, p<0.01) in both genders. The max decrease GVR after compression was attenuated by age (r=0.41, p<0.01).
Conclusions: The baseline GBF increases with age and is higher in males due to the elevated MAP. The vascular reactivity decreases with age in both genders, but it is not related to the increase in the MAP.

0129

Endothelium-Dependent and Non-Dependent Vasodilation in Human Gingiva

Tamás László Nagy, Barbara Mikecs, János Vág
Department of Conservative Dentistry, Semmelweis University, Budapest, Hungary

Objectives: Post occlusive reactive hyperemia and the blood flow of the mucogingival flap were different between genders. However, the mechanism is unknown. We aimed to compare the endothelium-dependent (evoked by acetylcholine, ACh) and non-dependent (evoked by nitric-oxide, NO) vasodilatation between genders in the human gingiva.
Methods: Gingival blood flow of healthy young (20-30 years, mean age: 24) volunteers (N=21, female=10, male=11) was measured by laser speckle contrast imager (LSCI) and expressed in laser speckle perfusion unit (LSPU). Two wells were constructed with light cured liquid rubber-dam at the gingival sulcus of FDI#12 (test) and #21 (control) teeth. After baseline recording, 3µl 10mg/ml ACh was applied into the test well in the first session and 1mg/ml NO-donor, nitroglycerin was used in the second one. There was at least 1-week break between the two sessions. Physiological saline was applied in the control well in every measurement. The solutions were dropped with Hamilton-syringe. Four 1mm wide regions were evaluated from the gingival margin to the mucogingival line.
Results: Both solutions enhanced blood flow in every region, reaching the maximum level at 2min. The zenith of blood flow level gradually decreased apically for ACh (94±17 LSPU, p<0.01 at coronal; 83±20 LSPU, p<0.05; 69±19 LSPU, p<0.05; 60±17 LPSU, p<0.05 at apical) but not for NO (43±13 LSPU, p<0.01 at coronal;36±14 LSPU, p<0.05; 25±12 LSPU, p<0.05; 26±11 LPSU, p<0.05 at apical). The males had significantly higher blood flow elevation than females in all regions (by 45-55 LSPU, p<0.05) only after ACh but not after NO.
Conclusions: The endothelium-independent vasodilation could spread upstream (apically) in the gingiva without attenuation, contrary to the endothelium-dependent. Young females had more attenuated endothelium-dependent relaxation than males. The distinct mechanisms might explain the differences in flap microcirculation.

0130

Associations Between Sociodemographic Characteristics and Improvement in Oral Health-Related Knowledge

Austeja Pauliukaite[1], Lina Stangvaltaite-Mouhat[2, 3], Jolanta Aleksejuniene[4], Indre Stankeviciene[1], Ruta Bendinskaite[1], Alina Puriene[1]

[1]Institute of Dentistry, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, [2]Department of Clinical Dentistry, Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Norway, [3]Oral Health Centre of Expertise in Eastern Norway, Oslo, Norway, [4]Department of Oral Health Sciences, Faculty of Dentistry, The University of British Columbia, Vancouver, British Columbia, Canada

Objectives: To assess the association between sociodemographic characteristics and increase in oral health-related knowledge.

Methods: 196 Lithuanian adults participated in an online oral health program. The pre-post evaluation study design was used. The baseline questionnaire included demographic characteristics such as education, gender, residence, and age, and oral health-related knowledge. Then participants were invited to read six oral health-related articles in the tailor-maid website. Subsequently, via a second questionnaire we inquired about the number of articles reviewed and assessed participant oral health knowledge. A total of 40 questions inquired about the etiology and prevention of oral diseases. Based on the responses, the total individual oral health-related knowledge score was calculated. The change in oral health knowledge indicated the difference between the baseline (pre-) and the follow-up (post-educational) total knowledge scores. For statistical analyses, the knowledge change score was categorized into two groups with cut-off point median value of '6'. Multivariable binary logistic regression analysis tested associations between sociodemographic characteristics and change in oral health-related knowledge.

Results: The medium age of participants were 54 years (range 18-85 years), 76% (n=149) were females. Age, residence and gender were not significantly related to knowledge change. Lower education (less than 10 years versus 16+ years) negatively associated improvement in oral health-related knowledge (OR 0.3, 95%CI 0.1; 0.7). Participants who read more articles had higher odds for knowledge improvement (OR 1.3 95%CI 1.1; 1.5).

Conclusions: Lower education was negatively and the higher number of articles read was positively associated with the improvement in oral health-related knowledge.

0131

Effects of Psychiatric Drugs on Orofacial Dryness

Alexandra Kovács[1], Krisztina Márton[1], Tamás Demeter[1], Katalin Károlyházy[2]

[1]Department of General Dental Preclinical Practice, Semmelweis University, Budapest, Hungary, [2]Semmelweis University, Budapest, Hungary

Objectives: Our objective was to determine the effects of psychiatric drug-therapies on xerostomia, whole- and minor saliva flow rates in Hungary.

Methods: 361patients (age18-83, 150 male, 211 female) were involved in this study. Experimental group consisted of 188patients and 173 age matched healthy persons served as controls. In the experimental group patients were categorized by the psychiatric drugs they take: SARI, SSRI, SNRI, NASSA, SNRI+NASSA, atypical- and typical antipsychotics, Valproate, Benzodiazepin, Lithium, Tricyclic antidepressants. A questionnaire (16 questions)was designed to determine the subjective presence or absence of oral and extra oral (eye-, skin-,) sicca symptoms. Unstimulated whole saliva flow rate (u-SFR) was determined by the spitting method, left and right palatal (LP,RP) and labial(L)saliva flow rates were measured by the Periotron method. Data were statistically analyzed by the $\chi 2$ and the Student's t-test at a significance level of $p<0.05$.

Results: Among the medicated, 50% of the men and 66% of the women ($p<0.05$) felt xerostomia compared to 31% and 33%in the non-medicated groups. Xerostomia ratios in medicated patients were as follows, in men: SARI: 80% ($p<0.01$), SSRI:37,5%, SNRI:81,8% ($p<0.01$), NASSA:85,7% ($p<0.01$), SNRI+NASSA:100% ($p<0.05$), atypical: 48,9%, typical: 0%, VPA: 40%, BDZ: 53,3%($p<0.01$), lithium: 22,2%, TCA: 50%; in women: SARI: 100% ($p<0.001$), SSRI: 63,5% ($p<0.01$), SNRI: 78,9% ($p<0.001$), NASSA: 64,3% ($p<0.05$), SNRI+NASSA: 75%, atypical: 60,8% ($p<0.001$), typical: 45,5%, VPA: 33,3%, BDZ: 65,6%($p<0.0001$), lithium: 58,8%($p<0.05$), TCA: 60%; compared to non-medicated men (31%) and women (33,3%). Hypo salivation (u-SFR£0.1 ml/min) could only be detected in women TCA group (0,09±0,09 ml/min). Minor saliva flow rates: among medicated men atypical (PS: 1,33,±0,6µl/cm^2/min) and BDZ (PS: 1,29,±0,8µl/cm^2/min) resulted significantly higher flow rate (control: PS:0,59±0,3µl/cm^2/min); among medicated women BDZ (LS: 1,72±2,0µl/cm^2/min) resulted significantly higher flow rate; atypical (LS: 1,01±1,4µl/cm^2/min), typical (LS: 0,31±0,2µl/cm^2/min) and TCA (LS: 0,35±0,7µl/cm^2/min) resulted significantly lower flow rate (controls: PS: 0,46±0,6 and LS: 1,81±1,41µl/cm^2/min). Oral hygiene indexes were significantly higher in experimental groups.

Conclusions: According to our results rate of xerostomia in medicated group was higher than in the control group. Although the different psychiatric drugs resulted different saliva flow rates.

0132

The Prognostic Role of Tumor Budding in Oral Tongue Cancer

Lucrezia Togni[1], Marco Mascitti[1], Giuseppe Troiano[2], Vito Carlo Alberto Caponio[2], Lorenzo Lo Muzio[2], Andrea Santarelli[1]

[1]Marche Polytechnic University, Ancona, Italy, [2]University of Foggia, Foggia, Italy

Objectives: Oral tongue squamous cell carcinoma (OTSCC) represents the most common malignancy of the oral cavity characterized by a short-term survival rate <50%. Despite the improvements in prognostic stratification of the current staging system, its prognosis is poor and unpredictable. This emphasizes the critical need for a predictive model leading the clinical-therapeutic strategies. Accumulating evidence suggested tumor budding (TB) is a reliable prognostic factor in OTSCC. However, a

standardized scoring system is necessary to its assessment. The study aims to evaluate the prognostic role of TB in 210 OTSCC patients surgical treated at Ancona General Hospital between 1990-2015.
Methods: TB was evaluated on hematoxylin and eosin-stained sections in the hotspot area of the tumoral infiltrative front under X200 magnification. TB was scored using two-tier (cutoff: 3buds/x20; 5buds/x20) and three-tier systems (cutoff: <4buds/x20; 5-9buds/x20; ≥10buds/x20) and according to BD-model (DOI cutoff: 4mm; TB cutoff: 5buds/x20) and revised-Grading system (WHO Grading; TB cutoff: 5buds/x20). Univariate and multivariate Cox regression analyses of disease-specific survival (DSS) were performed. A p-values <0.05 was considered as statistically significant.
Results: On multivariate analysis, the two-tier (cutoff: 5buds/x20) and three-tier system resulted independent prognostic factors of worst DSS. High-risk patients had a 2.21 (≥5buds/x20) and 3.08 (≥10buds/x20) times-increased probability of poor DSS compared to low-risk group (<4buds/x20) (p=0.0000). It is significantly increased even for intermediate-risk patients (5-9buds/x20) (HR=1.83; p=0.0021). Furthermore, no statistically differences emerged classifying patients according to BD-model and revised-Grading.
Conclusions: These data confirm the prognostic value of TB in predicting DSS in OTSCC. Classifying patients in two groups using the 5buds cutoff significantly discriminates their survival outcomes. The TB integration in BD-model and revised-Grading does not improve the patient stratification. Since the established role of DOI in pTNM-staging and the poor prognostic value of WHO grading, TB could be implemented as a prognostic marker *per se*.

0133

The Analysis of the Referral Pattern in Oral Medicine

Noemi Coppola, Stefania Balderes, Elena Calabria, Andrea Blasi, Gianrico Spagnuolo, Michele Davide Mignogna, Stefania Leuci
Department of Neurosciences, Reproductive and Odontostomatological Sciences, Oral Medicine Unit, University of Naples Federico II, Naples, Italy

Objectives: The aim of this study was to analyze referral patterns in an oral medicine (OM) unit of Southern Italy and to identify current barriers in the referral process.
Methods: All new OM visits at Federico II University of Naples between July 2020 and January 2021 were identified. Patient demographics, referral source, time between onset and OM consultation, number and types of providers seen before referral, referral diagnoses and visit diagnoses were recorded.
Results: 583 patients were recruited, 292 proceeding from the public health service and 291 from *intramœnia* private practice. 281 were males and 365 females, the mean age was 56,6±16,2 years. Almost all the patients were Caucasian (99,7%). Initial diagnosis, when available, was confirmed 167 (54,1%) times. 47% of the sample had an unclear or no initial diagnosis with an higher percentage in patients proceeding from public health service than in those proceeding from *intramœnia* private practice. On the other hand, patients proceeding from *intramœnia* private practice have been visited by a higher number of clinicians (2,6) than public health patients (1,4) before attending our center. The rates of correct identification for health-care professionals were the following: dentists (54,8%), general doctors (65,4%), maxilla-facial surgeons (50%), ENT (57,1%), dermatologists (33,3%) and rheumatologists (66,7%), others (55,6%). The rates of correct identification of the most frequent (>5) lesions were the following: aphthosis minor (8/10; 80%) burning mouth syndrome (42%), oral carcinoma (36,3%), exfoliatio areata linguae (12,5%), fibroma (27,3%), HPV-related lesions (100%), leucoplakia (86,7%), oral lichen planus (70,1%), osteonecrosis of the jaws (90,9%), persistent idiopathic facial pain (0%).
Conclusions: This is the first study in Italy to assess referral patterns to a specialized OM unit. Most referrals to OM are appropriate, but many are misdiagnosed. Understanding referral process is necessary to improve patient care.

0134

Zoledronic Acid Effect in Different Ages Mice Under Orthodontic Loading.

Ana Zilda N. Bergamo[1], Letícia F. Duffles[1], Renato C. Casarin[2], Léa A. Silva[1], Raquel Silva[1], Paulo Nelson-Filho[1]
[1]Pediatric Clinic, University of São Paulo -School of Dentistry of Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil, [2]Periodontology, University of Campinas- School of Dentistry of Piracicaba, Piracicaba, São Paulo, Brazil

Objectives: Zoledronic acid (ZA), in both pediatric and adult, have been used worldwide. It promotes changes in bone remodeling that can influence orthodontic movement. The aim was to investigate the behavior of bone markers expression and histopathological features in different osteoporotic ages mice under ZA treatment, with or without orthodontic mechanical loading.
Methods: RUNX2, RANK, RANKL, OPG, SOST, MMP13 e SSP1 expression was evaluated by Real-time PCR at 12 hours and 12 days after 0.35N orthodontic force activation. Eighty young and adult mice (C57BL/6J) were distributed into two groups, that received one single dose of ZA, and two control groups received saline solution. Descriptive fluorescence and conventional microscopic analysis of HE-stained sections were done. ANOVA was performed by JMP (α=0.05).
Results: In the young mice ZA down-regulated RANK (p<0.0215), RANKL (p=0.0008), SOST (0.0039), RUNX2 (<0.0001) and SSP1 (p<0.0001). Remarkably, MMP13 (p=0.0352) showed up-regulation. Under orthodontic mechanical loading, up-regulation of mediators expressed by osteoblasts RANK (p=0.032), RANKL (p=0.0273), and by osteocytes SOST (0.026) and SSP1 (p=0.043) were observed. In adult mice, ZA down-regulated SSP1 (p=0.003) and MMP13 (p=0.042), outstanding up-regulated RANK (p=0.0004) and OPG (p=0.0003). Under loading ZA down-regulated RANKL (0.0012), MMP13 (p_{12h}=0.0014; p_{12d}=0.026) and SSP1 (p=0.0045). Microscopic analysis showed a delay in bone remodeling under orthodontic mechanical loading in experimental groups, also cementum resorption, alveolar crest and alveolar bone irregularity, lower count blood vessels and hyaline areas. More expressive alteration in adult mice.

Conclusions: Bone metabolism markers were modulated by zoledronic acid in a different way under orthodontic mechanical loading in young and adult mice. Only adult mice showed a downregulation in the osteoclastogenesis markers under transduction loading. Bone markers mediated by osteocytes were influenced by ZA only in young mice. Microscopic analysis showed in adult mice the most expressive alteration, which could cause many adverse effects in alveolar bone.

0135

The Association Between the Dental and Facial Symmetry in Adolescents

Kristine Lokmane[1], Signe Silinevica[1], Ville Vuollo[2], Gundega Jakobsone[11]
Riga Stradins University, Riga, Latvia, [2]University of Oulu, Oulu, Finland

Objectives: The objective was to investigate the correlation between dental midline shift and facial asymmetry parameters.
Methods: The sample was retrieved from an ongoing growth study at the Riga Stradins University. Seventeen individuals were excluded from the initial sample of 107 individuals for different reasons. The total sample was 90 individuals (47 males and 43 females) with the median age of 15,9 (IQR 15,17 – 16,33). The facial scans were obtained using 3dMD facial scanner. The occlusion was scanned with 3Shape scanner. Facial symmetry was assessed with the surface-based and landmark-based analysis. The differences in the co-lateral position of the molars and canines and the dental midline shift were assessed on the dental scans. Correlations between the occlusal symmetry measurements and the facial symmetry parameters were assessed with the Spearman's correlation. The study was approved by The Ethics Committee (number 6-2/5/1).
Results: There were weak correlations between the dental midline shift and the maximum distance between the mirrored and original face for the mid-face ($r = 0.219$, $p<0.05$), lower midface ($r = 0.311$, $p<0.01$) and lower face ($r = 0.289$, $p<0.01$). Several landmark-based symmetry parameters were weakly correlated with the dental midline shift. The dental arch asymmetry, which was expressed at the canine region, had statistically significant correlations with the midpoint shift for all landmarks, except the upper lip. The shift of the dental midline was weakly associated with the increase of the respective side ex-canthion pogonion angles. The mean Chin Volume Asymmetry index (CVAI) showed that the right side was bigger significantly more often ($p = 0.0007$) in the individuals with the dental midline shift to the right side and vice versa.
Conclusions: The occlusal asymmetry is represented in the face to some extent. The dental midline shift was associated with the dislocation of the midpoints of the facial landmarks and reduced surface symmetry of the chin.

0136

Tension Effect on Human Periodontal Ligament Cells: a Systematic Review

Changyun Sun[1], Mila Janjic Rankovic[1], Matthias Folwaczny[2], Sven Otto[3], Andrea Wichelhaus[1], Uwe Baumert[1]
[1]Department of Orthodontics and Dentofacial Orthopedics, University Hospital, LMU Munich, Munich, Germany, [2]Department of Conservative Dentistry and Periodontology, University Hospital, LMU Munich, Munich, Germany, [3]Department of Oral and Maxillofacial Plastic Surgery, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany

Objectives: To identify all studies in orthodontic tooth movement using an *in vitro* loading tension model applied to human periodontal ligament cells. Secondly, to summarize their findings regarding cell source, force parameters (apparatus, magnitude, frequency, duration) and gene expression and to identify the most significant signaling pathways.
Methods: We followed the PRISMA guideline for systematic reviews. To identify all related studies, an appropriate PubMed search strategy was developed. Selection of the studies was done according to predetermined eligibility criteria. Data of interest (cell source, force and expression related) were extracted into structured tables. Risk of bias in reporting and methodology was assessed with guidelines for *in vitro* studies. Regulated gene sets were analyzed using STRING-DB and GeneAnalytics.
Results: From initially 5,331 identified publications, 137 studies were included with relevant information being extracted and unified. Methodological quality including confounding variables, sample size determination, statistical analysis and optimal time window and reporting quality of justification for the model were identified as the most obvious high risk of bias. Tension application was done either dynamically (103/137) or statically (30/137). Dynamic tension was most frequently applied by commercial systems with magnitude of 10% or 12%. The most common frequencies were 0.1Hz and 0.5Hz for equibiaxial strain and 0.5Hz for uniaxial strain, both for up to 72h. Static tension was applied mostly using flexible-bottom culture dishes (2.5%) or plates (10%) for up to 24h. The most common genes or products were related to osteogenesis (RUNX2, ALPP, BGLAP, TNFRSF11B, COL1A1, SP7, SPP1), osteoclastogenesis and inflammation (IL1B, PTGS2, TNFSF11, PGE2, TNF, CXCL8, IL6) and apoptosis (CASP1, CASP3, CASP5).
Conclusions: We identified the most common force parameters and related gene expressions. Using the pathways and force parameters identified, tension models can be established to investigate the effects of different magnitudes and durations on related biological regulations.

0138

Relationship Between Allergic Rhinitis and Oral Health in Children

Elif Gul Aydin[2], Neslihan Kaya Yilmaz[2], Oner Ozdemir[1]

[1]Medical Faculty, Research / Training Hospital, Sakarya University, Sakarya, Turkey, [2]Faculty of Dentistry, Pediatric Dentistry, Sakarya University, Sakarya, Turkey

Objectives: Allergic rhinitis (AR) is a common disease usually treated with intranasal corticosteroids and antihistaminics. It is well known that these medications cause a low salivary flow rate, and inadequate salivary secretion, which are the known risk factors for oral diseases such as dental caries and gingivitis. On the other hand, the hygiene hypothesis in the etiology of allergic diseases is defined as the leading cause of the increase in the prevalence of allergic diseases. It is also described as one of the risk factors that infections can be protective against the development of allergic diseases. Therefore, this cross-sectional study aimed to compare the oral health status of the children with or without AR.

Methods: A total of 111 children (64 with AR and 47 healthy) between 6-12 years old were included in the study (Ethical commission permission number: E-71522473/050.01.04/489). Patients with AR were enrolled from their follow-up patients of paediatric allergy outpatient clinic. A single examiner evaluated the plaque index (PI), gingival index (GI), decayed, missing, filled teeth (dmft/DMFT) index. A questionnaire recorded the participants' sociodemographic data, tooth brushing habits, oral health conditions, and dietary habits. All statistical analyses were run by commercially available software (IBM SPSS Statistics for Windows, version 22.0, IBM Corp., Armonk, NY, USA).

Results: PI (p:0,27) and DMFT index (0,00) scores were found statistically higher in the individuals with AR, while the GI scores did not differ between groups. There was a significant difference between children with and without AR in the consumption of DMFT / dmft, oral hygiene habits, and sweet snacks.

Conclusions: Our study supports the hygiene hypothesis and shows an inverse relationship between caries and allergic diseases. Further studies are needed to clarify the potential roles of oral health in the etiology of allergic rhinitis.

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Participatory Action Research to Improve Oral Health of Kenyan Children

Alice Grasveld[1, 4], Monique Van der Veen[1], Clarissa Bonifacio[2], Christine Dedding[3]

[1]Preventive Dentistry, ACTA, Amsterdam, Noord-Holland, Netherlands, [2]Pediatric Dentistry, ACTA, Amsterdam, Noord-Holland, Netherlands, [3]Ethics, Law and Medical humanities, Amsterdam UMC, Amsterdam, Noord-Holland, Netherlands, [4]The Healthy Teeth Foundation, The Hague, Zuid-Holland, Netherlands

Background: Caries is the most common childhood disease worldwide. In poor areas in Kenya, such as the remote Mamba village in Kwale County, access to dental care is poor and children often suffer from tooth decay. Toothache is a leading cause of school absenteeism. To raise oral health literacy the dental charity The Healthy Teeth Foundation (THTF) conducted Participatory Action Research (PAR) into oral health amongst children in Mamba village.

Objectives: To co-create a preventive oral health intervention with the community and to evaluate how PAR contributed to the empowerment of all stakeholders.

Methods: Together with the research team and local researchers we assessed the root causes for poor oral health by: participant observation, 15 semi-structured interviews amongst stakeholders, 9 house visits, 5 work visits, and 4 dietary anamneses amongst schoolchildren. In addition we organized 4 focus group discussions to validate and deepen the findings with stakeholders from the community.

Results: Key stakeholders were schoolchildren, their parents, teachers, principal, local dentists, imam, elderly, herbalist and midwife. The main root causes of poor oral health were: poor oral health literacy, strong cultural beliefs surrounding oral health, poor daily care, poor access to water and sanitation, poor diet, poor access to dental care and therefore self-medication and poverty, with school absenteeism as the worst outcome. A supervised handwashing and toothbrushing program serving 1200 schoolchildren was implemented at Mamba primary school and the community made a play about dental care, they tackled the water issues in the village, and organized train-the-trainer workshops for community health workers about oral health.

Conclusions: PAR successfully improved the oral health of children in Mamba Village, Kwale. Throughout the project oral health literacy in the community was improved. In the long run this might reduce tooth decay and reduce school absenteeism.

0140

Effects of Scaling on Biofilm-Epithelial-Cell Interactions

Kiri N. Lang, Anton Sculean, Sigrun Eick, Alexandra Stähli

Department of Periodontology, University of Bern, Bern, Bern, Switzerland

Objectives: To develop a novel in vitro periodontal pocket model for evaluating the effect of root surface instrumentation on biofilm-epithelial-cell interactions.

Methods: An artificial periodontal pocket model was created using impression material. Dentin discs were prepared and incubated for 3.5 days with a biofilm consisting of 12 bacterial strains. Then, the discs were inserted into the pocket model and instrumented for 2 min either with ultrasonics (US) or hand instruments (HI). Subsequently, a glass slide coated with epithelial cells was placed in close vicinity to the discs. After incubation of the pocket model in a 5% CO_2 atmosphere for 6 h residual bacteria of the biofilm as well as bacteria adhering to or invaded into epithelial cells were determined using colony forming unit (cfu) counts and real-time PCR. Further, as a parameter of the proinflammatory cell response interleukin (IL)-8 expression was determined by Elisa assay.

Results: Compared to untreated control HI reduced the cfu counts by 0.63 log10 (not significant) and US by 1.78 log10 (p=0.005) with a significant difference between the treatment modalities favoring US (p=0.048). By trend, lower detection levels of *Tannerella forsythia* were observed in the US group compared to HI. Concerning the interaction with epithelial cells half of the control and the HI samples showed epithelial cells with attaching or invading bacteria while US displayed bacteria only in two out of eight samples. In addition, US resulted in significantly lower IL-8 secretion by epithelial cells compared to the untreated control. Between HI and controls no statistically significant difference in IL-8 secretion was found.
Conclusions: The newly developed model allowed to study biofilm-epithelial cell interaction after root surface instrumentation. Compared to hand curettes ultrasonic instrumentation appeared to be more effective in removing bacterial biofilm and in decreasing the inflammatory response of epithelium to biofilm.

0141

A-PRF+ and EMD in Regenerative Micro-Surgery on Furcation Sites

Luciano Pitzurra, Dimitris Vasdravellis, Sergio Bizzarro, Martijn Rosema, Bruno G. Loos
Department of Periodontology, Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, Netherlands

Objectives: To investigate whether the application of advanced platelet rich fibrin (A-PRF+) in combination with periodontal regenerative microsurgery in furcations involved molars results in higher furcation grade reduction compared to enamel matrix derivative application (EMD) or open flap debridement (OFD).
Methods: A prospective randomized controlled trial was designed to study effects of A-PRF+, EMD or OFD for furcation grade reduction in molars with grade II defect. Patients were randomly allocated in one of three treatment groups: OFD with application of A-PRF+, OFD with application of EMD and OFD alone. The patients were blinded for the treatment received. A minimally invasive microsurgical approach was performed in all groups. Recessions (REC), Pocket Depth (PD), Clinical Attachment Level (CAL) and Furcation Grade (FG) were scored 6 months after the intervention. The quality of healing of the furcation site was scored via Early Wound Healing Index (EHI) on day 3, 1 week, 2 weeks and 6 weeks.
Results: 17 patients (A-PRF+=5, EMD=5, OFD=6) completed the 6 months follow-up. In the A-PRF group, the grade of the treated furcation of three patients out of six improved at the 6 months control, while no improvements were visible in any of the patients in the EMD group and one in the OFD group. The PPD reduction was -0.3±0.5 for the A-PRF+ group, 0.6±1.7 for the EMD group, 2.3±2.2 for the OFD. The CAL gain was -0.5±2 for the A-PRF+ group, 1.5±2 for the EMD group and 2.2±2.6 for the OFD group. Regarding the EHI, we found a worse healing for the A-PRF+ group at each follow-up examination compared to controls.
Conclusions: The use of A-PRF+ was associated with a higher frequency of furcation grade reduction but not improvements in PPD and CAL gain. None of the approaches can guarantee a resolution of a furcation grade II defect at 6 months.

0142

Tooth-Related Factors Affect Pocket Closure in Stage III-IV Periodontitis

Filippo Citterio[3], Burcu Kanmaz[2], Giacomo Baima[3], Federica Romano[3], Mario Aimetti[3], Nurcan Buduneli[1]
[1]Ege University, Izmir, Turkey, [2]Izmir Demokrasi University, Izmir, Turkey, [3]Turin University, Turin, Italy

Objectives: The main goal of periodontal treatment is to arrest the progression of periodontitis and to preserve natural dentition. Persisting deep pockets and bleeding on probing (BOP) following treatment carries a higher risk for disease progression and tooth loss. Pocket closure (PC) has been shown to be influenced by various factors at patient-, tooth- and site-level. The aim of the present retrospective study was to investigate the efficacy of non-surgical periodontal therapy on PC, defined as probing depth (PD) ≤ 4 mm with or without BOP three months after completion of non-surgical periodontal treatment and to identify the factors affecting the probability of PC.
Methods: This retrospective cohort consist of 27 systemically healthy patients with stage III-IV periodontitis with complete medical and dental history, periodontal charts at baseline (T0), and 3-months after non-surgical periodontal treatment (T1), complete intraoral radiographic examination at T0. All patients received quadrant-wise scaling and root planing by the same periodontist. All the sites with baseline PD ≥ 5 mm were included into the present analysis as diseased sites. Multilevel analysis was used to investigate factors at patient-, tooth-, and site-level affecting the likelihood of obtaining pocket closure.
Results: A total of 1997 diseased sites were included in the analysis. PC without considering BOP occurred at 70.2%. When BOP was included in the analysis 58.4% of the sites showed PC. The factors affecting PC were tooth type, mobility, clinical attachment level (CAL) and PD at T0. The odds ratios for each model are presented in Table 1 and Table 2.
Conclusions: Non-surgical periodontal therapy in systemically healthy patients with stage III or IV periodontitis is efficient for pocket closure while the clinical outcomes are significantly influenced by PD, CAL and tooth-related factors.

0143

Long-Term Outcomes of Periodontal Regeneration With Enamel Matrix Derivative (EMD)

Andrea Roccuzzo, Siro De Ry, Niklaus P. Lang, Giovanni E. Salvi, Anton Sculean
Periodontology, University of Bern, School of Dental Medicine, Bern, Switzerland

Objectives: To report the long-term outcomes in periodontal intrabony defects following regenerative surgery with and Enamel Matrix Derivative (EMD).
Methods: Periodontal patients treated with reconstructive surgery with EMD between 1999 and 2012 (follow-up of at least 8 years) were screened (n=548) and invited to participate in a clinical examination. The following clinical parameters were recorded and compared at baseline (at 6-months after non-surgical therapy) (T0), 6 months after surgery (T1) and after at least 8 years follow-up (T2): probing pocket depth (PPD), gingival recession (GR), clinical attachment level (CAL), plaque and bleeding scores.

Tooth survival (0/1), smoking status and frequency of adherence to supportive periodontal therapy (SPT) were also recorded. The primary outcome variable was the CAL change.
Results: 41 patients with a total of 75 treated teeth were available for analysis. Out of these, 68 (tooth survival rate: 90.7%) reached the latest follow-up with a mean observation period of 10.3 years (range: 8.0–21.3). The most frequent reason for tooth loss was recurrence of periodontal disease. Tooth survival curves showed a statistically significant difference between smokers and non-smokers (p=0.028). Mean CAL changed from 8.43 ± 1.86 to 6.47 ± 1.70 (p<0.001) at T1 and 5.91 ± 1.83 (p<0.001) at T2. At T1, a CAL gain of ≥3 mm was measured in 35% of the defects (i.e. 24 of 68), while at T2 it was detected in 51% of cases (i.e. 35 of 68).
Conclusions: The present results have provided evidence that in the great majority of cases, the clinical improvements obtained with EMD, can be maintained on the long-term. However, smoking and tooth type (i.e. maxillary molars) were correlated with an increased risk for tooth loss.

0144

Immunohistochemical Evaluation of Periodontal Regeneration Using a Porous Collagen Scaffold

Jean-Claude Imber[1, 2], Andrea Roccuzzo[1], Alexandra Stähli[1], Anton Sculean[1], Dieter D. Bosshardt[1, 2]
[1]Department of Periodontology, University of Bern, Bern, Bern, Switzerland, [2]Robert K. Schenk Laboratory of Oral Histology, University of Bern, Bern, Switzerland

Objectives: To immunohistochemically evaluate the effect of a porous, volume-stable collagen scaffold (VCMX) on periodontal regeneration.
Methods: Acute two-wall intrabony defects were surgically created in four beagle dogs. The defects were treated with open flap debridement either with VCMX (test) or without (control). After 12 weeks, the dogs were euthanized and specimens processed for paraffin histology. Sections were stained with hematoxylin/eosin. Immunohistochemistry was performed with antibodies against cytokeratins (CKs) for epithelial cells, collagen type I (COL1) and periostin (PER) for periodontal ligament (PDL), bone sialoprotein (BSP) for mineralized tissues, and PCNA for proliferating cells. The number of proliferating cells (proliferative index) and blood vessel number and area were analyzed in the pristine and regenerated PDL.
Results: All defects revealed formation of junctional epithelium, cementum, PDL, and bone with more cementum and bone regeneration in the test group. VCMX remnants were integrated in alveolar bone, PDL, and cementum. No difference in the labeling patterns were observed between test and control sites. BSP labelled both new bone and new cementum. The PDL was heavily labelled for COL1 and PER. 50% of the samples demonstrated no epithelial cell rests of Malassez, whereases the other 50% showed them in both, the pristine and regenerated PDL. Blood vessel area in the new PDL was larger in the test (0.144mm^2±0.03) compared to the control group (0.080mm^2±0.017, p=0.001) suggesting a positive effect of VCMX on angiogenesis. The number of blood vessels was higher in the new PDL (test + control) compared to the pristine one (p=0.012). However, the proliferative index did not show statistically significant differences between the pristine and regenerated PDL (p=0.642), demonstrating that the cell turnover in the regenerated PDL was as high as in the pristine PDL.
Conclusions: The novel VCMX facilitated periodontal regeneration in intrabony defects.

0145

Periodontitis Risk Variants at *SIGLEC5* Impair ERG and MAFB Binding

Ricarda Mueller, Avneesh Chopra, Henrik Dommisch, Arne S. Schäfer
Periodontology, Oral Medicine and Oral Surgery, Charité - Universitätsmedizin Berlin, Berlin, Germany

Objectives: Periodontitis is a common complex inflammatory disease of the oral cavity. It is characterized by inflammation of the oral mucosa and alveolar bone loss. Recently, a genome-wide association study (GWAS) and two GWAS-meta-analyses independently found two associated regions (haplotype blocks) at the inhibitory immune receptor gene *SIGLEC5* to increase the risk for periodontitis. The current study aimed to identify the putative causal variants underlying the associations, to characterize their molecular biological effects and to validate *SIGLEC5* as the target gene.
Methods: We mapped the associated SNPs to DNA elements with predictive features of regulatory functions and screened the associated alleles for transcription factor (TF) binding sites. Antibody electrophoretic mobility shift assays (EMSA) with allele specific probes were used to identify TF binding and to quantifiy allelic effects on binding affinities. Luciferase allele specific reporter assays were used to quantify the effect directions of the associated regulatory elements. We used CRISPR-dCas9 gene activation to validate *SIGLEC5* as target of the association.
Results: EMSA in peripheral blood mononuclear cells (PBMCs) showed that ETS (E-26 transformation-specific) transcription factor related gene (ERG) binds at rs11084095, with almost complete loss of binding at the minor A-allele. Allele-specific reporter genes showed enhancer function of the DNA-sequence at rs11084095, which was abrogated in the background of the minor A-allele. EMSA in B lymphocytes and HeLa cells showed that TF MAF bZIP (MAFB) binds at the common G-allele of rs4284742, whereas the minor A-allele reduced TF binding by 69%. Reporter gene assays showed that the minor rs4284742 A-allele reduced enhancer activity in HeLa cells (p=0.01) and in B lymphocytes (p=0.0005). Using CRISPR-dCas9 gene activation, we showed that the enhancer at rs4284742 strongly activated *SIGLEC5* expression, validating this gene as the target gene of the association.
Conclusions: We conclude that rs11084095 and rs4284742 are putatively causal for the genome-wide significant associations with periodontitis at *SIGLEC5* and abrogate enhancer activity and binding affinity of the TFs ERG and MAFB. Our results imply that *SIGLEC5* has a functional role in endothelial homeostasis and healing of aseptic tissue injuries.

0146

Salivary Macrophage Activation-Related Cytokines in Children With Type1 Diabetes Mellitus

Neslihan Kaya Yilmaz[1, 2], Dogukan Yilmaz[3], Elif Gül Aydin[2], Recep Polat[4], Mervi Gürsoy[1], Ulvi K. Gursoy[1]

[1]Periodontology, Institute of Dentistry, University of Turku, Turku, Finland, [2]Pediatric Dentistry, Faculty of Dentistry, Sakarya University, Sakarya, Turkey, [3]Periodontology, Faculty of Dentistry, Sakarya University, Sakarya, Turkey, [4]Pediatric Endocrinology, Faculty of Medicine, Sakarya University, Sakarya, Turkey

Objectives: Type 1 diabetes mellitus (T1DM) is related to the increased prevalence and severity of periodontitis and also impairs macrophage recruitment and differentiation. The aim of this cross-sectional study was to evaluate the salivary concentration of macrophage activation-related cytokines in children with and without T1DM in relation to periodontal status.

Methods: A total of 151 children (78 with T1DM and 73 systemically healthy) between 3-15 years-old were included in the study (Ethical permission number: E-71522473-050.01.04-15422). Unstimulated salivary samples were collected from all participants before dental and periodontal examinations. Salivary interferon gamma inducible protein-10 (IP-10), monocyte chemoattractant protein (MCP)-1, MCP-2, MCP-3, MCP-4, macrophage-derived chemokine (MDC), macrophage migration inhibitory factor (MIF), monokine induced by IFN-gamma (MIG) and, macrophage inflammatory protein-1 alpha (MIP-1α) concentrations were quantified using the Luminex® xMAP™ technique. All statistical analyses were run by a commercially available software (IBM SPSS Statistics for Windows, version 22.0, IBM Corp., Armonk, NY, USA).

Results: Plaque index (PI%) and bleeding on probing (BOP%) were found significantly higher in individuals with T1DM while healthy group exhibited higher Decayed, Missing, Filled Teeth Index (DMFT) score. T1DM group demonstrated higher concentrations of salivary MCP-1 ($p=0.003$), MCP-3 ($p<0.001$), MIG ($p=0.018$) and, MIP-1α ($p=0.016$) compared to healthy individuals while the concentrations of MCP-2 ($p=0.018$) and, MCP-4 ($p<0,001$) were statistically higher in control group. After adjusting for age, PI%, BOP% and DMFT, significant differences in salivary MCP-1, MCP-2, MCP-4, and MIP-1α concentrations were observed between T1DM and control groups.

Conclusions: In conclusion, our results demonstrate that T1DM disrupts the salivary macrophage activation-related cytokine profile in children. These findings can be an outcome of the impaired systemic immune response in T1DM.

0149

Dentin Regeneration by DMP-1/BMP-2-Plasmid-Modified Nanoparticle-Treated DPSCs on Dentin Scaffolds

Foteini Machla[1], Viktoriya Sokolova[2], Oleg Prymak[2], Kathrin Kostka[2], Benedikt Kruse[2], Varvara Platania[3], Maria Chatzinikolaidou[3, 4], Matthias Epple[2], Athina Bakopoulou[1]

[1]Aristotle University of Thessaloniki, Thessaloniki, Greece, [2]Institute of Inorganic Chemistry and Center for Nanointegration Duisburg-Essen (CeNIDE), University of Duisburg-Essen, Duisburg-Essen, Germany, [3]Materials Science and Technology, University of Crete, Heraklion, Greece, [4]Institute of Electronic Structure and Laser, Foundation for Research and Technology-Hellas, Heraklion, Greece

Objectives: Investigation of the potential of Calcium Phosphate Nanoparticles (CaP NPs), carrying plasmid DNA encoding Dentin Matrix Protein 1 or Bone Morphogenetic Protein 2 (DMP-1/BMP-2-CaP NPs), combined with human Freeze-Dried Dentin Scaffolds (hFDDSs) to promote odontogenic differentiation by Dental Pulp Stem Cells (DPSCs).

Methods: DPSCs were isolated, characterized and seeded on EDTA-treated hFDDSs. CaP NPs were synthesized and characterized [colloidally, by Dynamic Light Scattering; microscopically (SEM) for particle size; and spectroscopically (UV-vis) for functionalization]. Control CaP NPs conjugated with Cy-5 fluorescent dye or carrying the plasmid encoding green fluorescent protein were tested at a range of concentrations (0.5-8μg Ca/ml) for 24h-uptake and 48h-transfection efficiency, respectively (Confocal Microscopy, Flow Cytometry). The effect of CaP NPs on cell viability was assessed by MTT assay. Two most favorable for viability/transfectability concentrations of each DMP-1 or BMP-2-CaP NPs were selected to evaluate cell morphology on hFDDSs (SEM), viability (Live/Dead Staining), and gene expression of odontogenic markers, including DMP-1, BMP-2, DSPP, RunX2, Osterix, MSX-1 and MSX-2 (real-time PCR).

Results: Increasing concentrations of CaP NPs reduced cell viability, while increased uptake and transfection efficiency by DPSCs in a dose- and time-dependent manner. The uptake efficiency reached 51% of the total cell population at the concentration of 8μg/ml, while the transfection efficiency was 37%. Considering the transfectability/viability balance, the concentrations of 1 and 4μg/ml causing reduction of cell viability that did not exceed 45% for the DMP-1 NPs and 38% for the BMP-2 NPs, 72h post-treatment, were further evaluated in DPSC-seeded hFDDSs. Real-time PCR results showed a NP-specific, time-dependent upregulation of odontogenic markers (BMP-2, DMP-1 and DSPP), with downregulation of the transcription factors (Osterix, RunX2, MSX-1, MSX-2). Studies are in progress to evaluate in vitro biomineralization leading to dentin-like tissue formation.

Conclusions: The combination of DMP-1/BMP-2-CaP NPs and DPSCs on hFDDSs presents a promising approach in dentin tissue engineering.

0150

Age-Related Expression of CD34 in Human Dental Pulp Cells

Neshka A. Manchorova-Veleva[1], Margarita L. Guenova[2]

[1]Operative Dentistry and Endodontics, Medical University, Plovdiv, Bulgaria, [2]Laboratory of Haematopathology and Immunology, National Specialized Hospital for Haematological Diseases, Sofia, Bulgaria

Objectives: The expression of the human hematopoietic progenitor cell antigen CD34 is a valuable marker mirroring the vessel and network structures in response to functional needs and dental tissue homeostasis. The aim of the study is to examine and compare the age-related immunohistochemical distribution of CD34 in human dental pulp.

Methods: Ninety freshly extracted intact teeth of healthy individuals were enrolled in the study and arranged in three groups (n=30 in each group) regarding the patients' age: young (14-16 years old), adult (18-40 years old) and senescent (41-78 years old) groups. All teeth were indicated for surgery due to limited jawbone space for eruption (third molars) or periodontal and bone lost (premolars and incisors). Immediately after extraction the molars were fixed overnight in 10% buffered paraformaldehyde and reduced in size by trimming the enamel, superficial coronal dentin and the roots up to 2mm below the CEJ. The coronal dentin-pulp specimens were decalcified in a 3% hydrochloric acid (HCl) for 6 hours and paraffin embedded. Immunohistochemistry using mouse monoclonal antibody CD34 (B-6), Santa Cruz Biotechnology Inc., USA was performed by Leica-Bond Max automated system (Leica Biosystems, Germany). Statistical analysis including Kruskal-Wallis and Mann-Whitney tests were performed ($p<0.05$).

Results: A higher number of immunolabeled sections for CD34 were found in all examined groups without significant difference between the adult and senescent groups compared to the young group ($p>0.05$). In aging pulp, vascularization was not reduced, the density of the vascular network was maintained. Numerous small blood vessels with well-formed walls and CD34 immunopositive endothelial cells were observed throughout the pulp thus revealed the process of vasculogenesis persists into adult life.

Conclusions: Immunohistochemical distribution of CD34+ endothelial cells in all age groups supports the concept of constant angiogenesis in the adult's body, renewal of the vascular network of the dental pulp throughout the life and continuous angio-adaptation.

0151

Canines' Laser Doppler Measured Pulp Blood Perfusion Mean Values

Vesela Stefanova[1], Nikolay Simeonov[2]

[1]Medical University of Plovdiv, Plovdiv, Bulgaria, [2]Medical University of Plovdiv, Plovdiv, Bulgaria

Objectives: Laser Doppler flowmetry is a modern objective non-invasive method for blood flow assessment. The aim of this study is to measure, calculate and compare the mean values of pulp blood perfusion for vital canines.

Methods: The pulp blood perfusion of canines was monitored and measured by laser doppler flowmetry device. Teeth, included in this study, were only vital, with no carious or other lesions, trauma and restorations. The measurements were done for at least three minutes monitoring and recording through hard dental tissues by using needle probes, fixed at the vestibular tooth surface. The pulp perfusion mean values were calculated for every tooth for a period of one minute by the licensed software of the device. The received data was statistically analyzed.

Results: The total number of monitored and measured vital canines was 106. The maxillary canines included into this research were 57 and mandibular canines were 49, left canines were 50 and right were 56. The volunteers' mean age was 24,7 years. The calculated mean value for all canines was 16,2 PU, for lower canines it was 16,6 PU and for upper canines it was 15,8 PU. The mean value for left canines was 14,8 PU, and 17,4 PU for right canines. The mean value for female canines was 17,2 PU and 14,2 PU for male canines. There was no statistically significant difference between mean values of male and female canines neither for maxillary nor for mandibular jaw ($p>0,05$); no statistically significant difference was found between the left and right canines ($p>0,05$).

Conclusions: When objective, precise and noninvasive assessment of dental pulp status of canines is needed in order to take the best decision for optimal clinical treatment or scientific research, the determined laser Doppler blood perfusion mean values of the investigated canines could be taken in consideration.

0154

Prognostic Value of Ultra-High Frequency Ultrasound in Primary SjöGren Syndrome

Rossana Izzetti[1], Giovanni Fulvio[2], Marco Nisi[1], Stefano Gennai[1], Chiara Baldini[2], Filippo Graziani[1]

[1]Department of Surgical, Medical and Molecular Pathology and Critical Care Medicine, University of Pisa, Pisa, Italy, [2]Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

Objectives: Primary Sjögren's Syndrome (pSS) is a chronic autoimmune disease causing progressive impairment of lacrimal and salivary glands due to the development of inflammatory infiltrate in the glandular tissues. The characterization of the glandular infiltrate is extremely relevant as a prognostic factor for the development of lymphoma. In particular, a focus score (FS) ≥3 is considered as a threshold significantly contributing to lymphoma development. The aim of this study is to evaluate the potential role of Ultra-High frequency Ultrasonography (UHFUS) in the identification of patients with a FS ≥3 at higher risk of lymphoma.

Methods: Consecutive patients with suspected pSS were enrolled. All the patients underwent a complete rheumatological diagnostic work-up, UHFUS of minor salivary glands, and surgical biopsy. UHFUS images were evaluated according to the OMERACT scoring system (0= normal glandular parenchyma to 3= diffuse presence of hypoechoic areas in the absence of normal glandular parenchyma, glandular fibrosis).

Results: In total, 168 patients were included. Out of them a diagnosis of pSS was confirmed in 81 patients (48.2%, mean age 55.17±14.34). Seven patients presented a FS ≥3. None of the patients with UHFUS score 0 presented a FS ≥3; only the 3.7% of patients with UHFUS score 1-2 displayed a FS ≥3, whereas the 16.7% of the patients with a UHFUS score 3 had a FS ≥3 ($p<0.05$).
Conclusions: UHFUS score 3 tends to identify patients with a FS ≥3, thus highlighting the high correspondence between the UHFUS scoring and the histology. UHFUS could potentially become an integrating tool in risk assessment and stratification for lymphoma development in pSS patients.

0155

Metabolomics Identify Patterns Indicating Potential Biomarkers in Primary SjöGren's Syndrome

Håvard Hynne[1], Elise M. Sandås[2], Katja P. Elgstøen[2], Helge Rootwelt[2], Hilde Galtung[3], Tor P. Utheim[2, 4], Janicke Liaaen Jensen[1]
[1]Department of Oral Surgery and Oral Medicine, Faculty of Dentistry, University of Oslo, Oslo, Norway, [2]Department of Medical Biochemistry, Oslo University Hospital, Oslo, Norway, [3]Department of Oral Biology, Faculty of Dentistry, University of Oslo, Oslo, Norway, [4]Department of Ophthalmology, Oslo University Hospital, Oslo, Norway

Objectives: In search for salivary biomarkers for primary Sjögren's syndrome (pSS), we investigated the metabolite profile of saliva from two patient groups suffering from dry mouth. A patient group with confirmed pSS and one patient group with sicca symptoms not fulfilling the pSS classification criteria (non-SS) were compared to healthy controls without sicca symptoms.
Methods: The following subjects were included: Ten patients diagnosed with pSS (53.2±13.9 years), fulfilling the American-European Consensus Group classification criteria, ten non-SS patients (51.5±10.6 years), and ten healthy controls (53.7±2.3 years). Participants had no other diseases known to cause sicca symptoms and did not use medications influencing saliva production. All non-SS patients had negative anti-SSA/SSB serum antibodies and negative salivary gland biopsies. Stimulated whole saliva (SWS) was collected according to a standardized predefined protocol and stored at -80 °C. Samples were then analyzed using a validated in-house method for global metabolomics (high performance liquid chromatography – electrospray ionization (+ and -) – high resolution mass spectrometry and a pooled quality control for signal alignment). For data processing and statistical analyzes commercially available software was used.
Results: Mean SWS was 0.7±0.4 mL/min for pSS, 1.0±0.3 mL/min for non-SS, and 1.6±0.9 mL/min for controls. There was a significant difference in salivary secretion between pSS and controls ($p=0.007$), and no significant difference between pSS and non-SS ($p=0.591$). Preliminary Results: employing principal component analysis plots of metabolites showed a distinct separation between pSS and non-SS, and a separation between pSS and controls, indicating different metabolite profiles for pSS vs. non-SS and for pSS vs. controls.
Conclusions: Metabolite patterns for pSS, non-SS and controls showed distinct differences, indicating a unique disease related metabolic profile for pSS. These results may pave the way for future studies identifying salivary markers for pSS.

0156

Factors Influencing the use of Nanomaterials in Dentistry

Victoria Xenaki[1], Mihaela C. Marthinussen[1, 3], Daniela E. Costea[2, 4], Mihaela R. Cimpan[1], Anne N. Åstrøm[1]

[1]Department of Clinical Dentistry, University of Bergen, Bergen, Norway, [2]Department of Pathology, Haukeland University Hospital, Bergen, Norway, [3]Oral Health Centre of Expertise in Western Norway, Bergen, Norway, [4]Department of Clinical Medicine and Center for Cancer Biomarkers CCBio, University of Bergen, Bergen, Norway

Objectives: Nanotechnology has been successfully applied in dentistry to improve dental materials used for prevention and treatment of oral diseases. Many of such dental nanomaterials (DN) have already reached the market and more are expected in the future. At the same time, there is a lack of information regarding the use of DN in Norway. The present study aimed to assesses the prevalence of nanomaterials use for dental patient treatment in Norway and to investigate what socio-demographic and psychological factors covary with the use of DN.

Methods: Electronic questionnaires were distributed to a census of 1792 dentists and dental hygienists employed in the public dental health services in Norway. Bivariate analyses with cross tabulation and chi-square statistics and multiple variable logistic regression with odds ratio (OR) and 95% confidence interval (CI) was conducted in SPSS, Version 25.

Results: 851 participants responded to the survey (47.5% response rate), of them 15.7% were males, 71.0% were dentists and 39.5% belonged to younger age group (22-35 years). The use of DN was confirmed by 63.7% of dentists and 28.7% of dental hygienists. Bivariate analysis revealed more frequent use of DN among participants who received more than moderate amount of information about DN (79.2%) and who felt safe to use such materials (39.9%). Multiple variable logistic regression revealed that participants who were dentists (OR = 2.2, 95% CI 1.3-3.8), who received more than moderate amount of information about DN (OR = 3.0, 95% CI 1.8-5.0), who felt safe to use DN (OR = 2.5, 95% CI 1.5-4.1) and who perceived higher benefits (OR = 2.1, 95% CI 1.3-3.5) and risks (OR = 1.9, 95% CI 1.3-3.0) associated with DN were more likely to use them.

Conclusions: Present findings can be used by policy makers to communicate risks to dental workers and provide balanced information on use of DN.

0157

Infrared Thermography and Face Masks Discomfort Wearing in Clinical Practice.

Antonio Crisante[1], Felice Lorusso[1], Calogero Buggea[1], Luan Mavriqi[2], Antonio Scarano[1]

[1]University of Chieti, Chieti, Italy, [2]University of Albania, Tirana, Albania

Objectives: Individual respiratory protection devices and face masks are essentials for the control of the biological risk of the healthcare workers in dental clinics and hospitals. These devices are currently playing a key role due to the Sars-CoV-2 pandemic also in a non-medical environment in contrast to the large-scale diffusion of the virus. The aim of the present investigation was to evaluate the facial skin temperature and the heat flow generated by N95 masks wearing during the dental surgery clinical practice.

Methods: A total of 20 subjects were recruited and allotted in two groups: the subjects of the group 1 were invited to wear a surgical mask and the group 2 the N95 respirator for 1 h. The Infrared thermography measurements was performed to measure the facial temperature of the perioral region and the perception ratings of the humidity, heat, breathing capacity and discomfort were assessed.

Results: A significant difference of the perioral region temperature and heat was reported between the Group I (surgical mask) and the Group II (N95) ($p < 0.05$). A significant difference of humidity, heat, breathing and discomfort was reported between the Group I (surgical mask) and the Group II (N95) ($p < 0.05$).

Conclusions: The effectiveness of the present investigation highlighted that N95 facemasks are correlated to higher facial skin temperature, increased discomfort and lower wearing adherence compared to the medical surgical masks.

0158

Musculoskeletal Disorders Among French Dentists: a Preliminary Survey

Arthur D'halluin, Bénédicte Enkel, Fabienne Jordana

Dental Faculty, University of Nantes, Nantes, France

Objectives: This survey relates to the study of musculoskeletal disorders (MSDs) for the dental surgeon in order to better understand their distribution in the profession and to identify the aggravating factors in the practice of dental surgery.

Methods: A survey was distributed to dentists in France. A cross-sectional survey design was adopted for this survey. Dentists were invited to complete a survey containing demographic items, extent and location of pain, associated disorders, general health and mental state, feelings, impact on work or family and social life. Standard statistical tests, univariate and multivariate statistics were used.

Results: A survey was distributed to French dentists and 130 responses were collected. Participants were 37.1+/-11.0 years old and included 77.0% women and 23.0% men. 82% of dentists consider themselves to be in good health. 92% have MSDs. The pain is evaluated at 5.31 +/- 2.20 on the Visual Analog Scale (VAS). 78.5% dentists describe associated symptoms such as numbness, tingling, etc. For 87.7% of the dentists questioned, MSDs hamper their work. 36% say that these disturbances have family repercussions and 31% social repercussions.

Conclusions: The objective of this questionnaire is to enrich the data on this occupational health problem in order to obtain a fair and complete forensic recognition of MSDs in dentists in France.

0159

Challenging Encounters in Clinical Dentistry: Investigating Patients' Online Reviews With LIWC

Maria Larsen[1], Gro E. Holde[1, 2], Jan-Are Kolset Johnsen[1]

[1]Institute of Clinical Odontology, University of Tromsø, Tromsø, Norway, [2]The Public Dental Health Service Competence Centre of Northern Norway, Tromsø, Norway

Objectives: In this study, the aim is to investigate the differences in language of dental patients' texts when describing a positive versus a challenging clinical encounter. Such differences may give insight into the patients' emotional and motivational states when writing the reviews.

Methods: 11'765 patient reviews of dental treatments were collected from Legelisten.no. The analysis was conducted using the Linguistic Inquiry and Word Count software on the text in the commentary section. In the analysis, the number of words per review, number of exclamation marks, emotional words, and grammatical tense will be investigated. The reviews with a 4-5-star rating represented a "positive encounter", and the reviews with a 1-2-star rating represented a "challenging encounter". A subsample was drawn from the original sample, consisting of 300 positive encounters and 300 challenging encounters. A non-parametrical Mann-Whitney U test was performed to compare the differences in the distribution of words in each group.

Results: For the positive encounters, the reviews contained fewer words per review, than the reviews describing a challenging encounter ($p<0.001$). The reviews in the challenging encounter group contained a smaller proportion of negative emotional words ($p<0.001$) and a larger proportion of words in future and past tense ($p<0.001$). The reviews describing a positive encounter contained a smaller proportion of words written in present tense ($p<0.001$) and a larger proportion of exclamation marks ($p<0.001$) than the reviews in the challenging encounter group.

Conclusions: Differences in the language of dental patients writing about a challenging encounter versus a positive encounter could provide valuable insights into the emotional and motivational states present at the time of writing.

0160

How Dental Clinicians Identify Dental Anxiety: a Grounded Theory Model

Markus Höglund[2, 1], Inger Wardh[2, 3], Shervin Shahnavaz[4], Carina Berterö[5]

[1]Centre for Orofacial Medicine, Public Dental Service, Linköping, Östergötland, Sweden, [2]Department of Dental Medicine, Karolinska institutet, Stockholm, Sweden, [3]Academic Centre of Geriatric Dentistry, Stockholm, Sweden, [4]Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden, [5]Department of Health, Medicine and Caring Sciences, Linköping University, Linköping, Sweden

Objectives: The vast majority of dental clinicians rely on their experience and intuition to recognise dentally anxious patients. Clinicians that rely on their intuition generally underestimate the patient's level of dental anxiety compared to the patient's self-rating and they may also miss to identify that some patients are dentally anxious. If the dental anxiety is missed, the dental clinician will not be able offer or reefer to evidence based treatment and the patient will continue to suffer. To improve dental clinicians' ability to recognise dental anxiety, there must first be an understanding of how dental clinicians use their intuition. Presently there is no model explaining this process. The aim of this qualitative study was to explore how dental clinicians recognise dental anxiety.

Methods: 11 semi-structured interviews with dental clinicians from the public dental service of Östergötland Sweden was performed. Classical grounded theory was used to construct a model of how dental clinicians recognise dental anxiety.

Results: The core category was identified as observed changes in Actions and Reactions (AR), meaning a marked change in intensity in the AR which the dental clinicians recognise as signs of dental anxiety. The observed change in AR occurs in anticipation of, presence of, or removal of the stressor (treatment). The AR recognised as dental anxiety could be divided into five categories: Sympathetic activation, Patient-reported anxiety, Controlling behaviours, Enduring strategies and Accomplishment. Without an observed change in AR, clinicians will have no point of reference and will not be able to determine with certainty if the patient AR is related to dental anxiety or if this is the patient's normal state of being.

Conclusions: Clinicians identify patients as dentally anxious as they observe the patients' actions and reactions change with proximity to the stressor.

0161

The French National Health Service: Oral Health Education Sessions

Anne-Charlotte Bas, Annabelle Tenenbaum, Sylvie Azogui-Lévy

Dental Public Health, Faculty of Dentistry, Université de Paris, Paris, France

Objectives: Since September 2018, the National Health Service is mandatory for French healthcare students. This is 6 full-time weeks dedicated to health promotion and integrated into university curriculum. Our institution manages 1 year of theoretical courses for 3rd year students and then activities on the field (oral health education sessions: OHES). Every student's group should design their own activities (content and tools). OHES began in October 2019. We present the results of this first year of fieldwork.

Methods: We worked on the student's reports. We selected reports on OHES with a children's audience (65%). Reports contain quiz and narrative sections. We propose quantitative and qualitative analysis according to the part of the report.

Results: 26 working groups (65 students) choose OHES targetting children, from pre-school to high school. The knowledge activities are multiples but quiz and *dinette* come up very often (28 and 22%). Most of the practical activities rely on mouth macromodels (60%) and teeth brushing (24%). Students report progress on communication skills (31%), capacity to adapt their

language to children (60%), developed team's work (36%) and project management (30%). Student's satisfaction focuses on the feeling of « being useful » (26.5%) and being able « to transfer of knowledge to children » (32%). 25% realized the need for dental health information for children.
Conclusions: This first year of OHES shows that dental students can develop and implement health preventive activities with children. It makes them feel legitimate as actors of community dental health.

0162

Inequalities in Tooth Loss and Insurance Schemes in Thailand

Jarassri Srinarupat[1, 2], Takashi Zaitsu[1], Akiko Oshiro[1], Piyada Prasertsom[2], Kornkamol Niyomsilp[2], Yoko Kawaguchi[1], Jun Aida[1, 3]
[1]Oral Health Promotion, Tokyo Medical and Dental University, Tokyo, Japan, [2]Bureau of Dental Health, Department of Health, Ministry of Public Health, Nonthaburi, Thailand, [3]Graduate School of Dentistry,Division for Regional Community Development, Liaison Center for Innovative Dentistry, Tohoku University, Sendai, Japan

Objectives: In Thailand, insurance schemes have different accessibility to dental care services. Dental treatments are covered by public and private dental care facilities depending on type of insurance schemes. We investigate the association of insurance schemes with tooth loss among adults aged 35-44 years old.
Methods: From Thailand's National Oral Health Survey in 2017, data from oral examination and questionnaire answers were analyzed. The number of tooth loss was used as the dependent variable. Insurance schemes were categorized into 4 groups: Universal Coverage Scheme (UCS), Civil Servant Medical Benefit Scheme (CSMBS), Social Security Scheme (SSS), and others (no use, no have, do not know). Poisson regression was applied to estimate the ratio of means on tooth loss with adjustment for covariates, including age, gender, area of residence, educational attainment and income.
Results: Data of 4,534 participants (2,194 males; 2,340 females) were analyzed. The mean age of the participants was 39.6 (SD.=2.87) years old. The median of tooth loss was 1.00. In the univariate models, the ratio of means of those who graduated with moderate education level illustrated a significantly higher chance of tooth loss compared with the highest attainment (RM=1.07 (95%CI, 1.01-1.14)). In relation to insurance, compared to CSMBS, those with UCS and others showed a significantly higher ratio of means 1.11 (95%CI, 1.05-1.17) and 1.33 (95% CI, 1.17-1.52), respectively. All variables (educational attainment, income and insurance) were simultaneously adjusted after each was separately adjusted, a significant association was observed only in the insurance schemes. The ratio of means of participants with UCS insurance and others demonstrated a significantly higher chance of tooth loss compared with CSMBS (RM= 1.13 (95%CI, 1.05-1.22) and 1.37 (95%CI,1.20-1.58), respectively).
Conclusions: Social predictor of tooth loss was insurance schemes. Expanding access to dental care services is required to reduce oral health inequalities in Thailand.

0162.1

The Status of Tooth Loss and Prevalence of Prosthetic Restoration Among Adults in Gaza

Jasmine Elhaj, Waseem B. Mushtaha
Faculty of Dentistry, Al Azhar University, Gaza, Palestine

Objectives: The purpose of this cross-sectional study was to investigate the status of tooth loss and prosthetic restoration among adults in the Gaza Strip, to evaluate the influences of socio-demographic variables on oral health.
Methods: A cross-sectional study was used to assess and evaluate the dentition status and the prosthetic replacement. A stratified random cluster sampling method was used to enroll a total of 388 adults aged 30 to 44, 45 to 59, 60 to >80years in 5 provinces of the Gaza Strip. Adults were randomly selected, examined clinically (teeth not found for any reason were defined as missing teeth), and questioned by interviewers to collect the demographic and socioeconomic backgrounds. The data collection was done in 10 primary healthcare centers. Bivariable associations were investigated among study variables using Pearson Chi-square. The significance level was set to 0.05.
Results: Among 388 subjects, 29.9% had complete dentition, 68.3% had missing teeth and 1.8% were edentulous. The lower right first molar showed the highest percentage of extraction in all age groups. 66% of males and 74% of females had missing teeth. Age, income, and education were associated with teeth replacement by prostheses. Fixed prosthesis was the most commonly used method of replacement (15.5%) than any other type. Educated subjects replaced their missing teeth more than the uneducated people, 41.1% and 27.5% respectively, $X^2 = 5.1$, p= 0.023. Subjects in the middle age (45-49 years) tended to replace the missing teeth more than other considered age groups (42.9%) X^2=12.8, p = 0.002. Participants with higher income tend to replace the missing teeth more than lower-income patients (27.5%) X^2= 16.3, p = 0.001. The majority of people (79.5%) who did not restore their missing teeth said it was due to the financial situation while (4.9%) said they have a busy lifestyle, (3.2%) have dental fear and prefer neglect over going to a dentist, and (4.9%) of people lack dental awareness.
Conclusions: The results showed that 68.3% of the population had at least one missing tooth, 47% of people did not restore their missing teeth. Determinants such as age, income, and education play a significant role in the replacement of missing teeth whereas gender and place of living were not statistically associated with the replacement of missing teeth.

0162.2

Assessment of Anxious Patients' Attitude Towards Oral and Dental Treatments

Alexandra E. Done, Elena Preoteasa, Cristina Teodora Preoteasa
University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania

Objectives: Assessment of patients' attitude towards oral and dental treatments through analysis of the relationship between anxiety and stress.

Methods: During May 2021, a cross-sectional study was carried out on a sample of people aged 18 and over. The data was collected through an online survey. The survey is structured as follows: general data, main reasons for avoiding dental treatments, self-assessment of oral health, and recordings of dental anxiety measured using the dental anxiety scale (DAS) and the index of dental fear and anxiety (IDAF4C).

Results: 132 participants were included, aged between 18 and 83, the majority being females (n=79, 59.4%). Half of the participants experienced dental anxiety, most of them experiencing a medium level. The main reasons for avoiding dental care are the costs of the dental treatments, followed by the lack of urgency. Meanwhile, participants were concerned about dental appointments because of the cost of treatments, painful procedures, and the use of needles and injections. The participants' self-assessment of oral health was negatively linked with age ($r=-0.335$, $p<0.001$) and anxiety level measured by IDAF4C ($r=-0.357$, $p<0,001$) and DAS ($r=-0,271$, $p=0.002$).

Conclusions: Anxiety and stress induced by oral and dental treatments are encountered in all age groups. The study highlights the importance of identifying dental anxiety because anxiety is correlated with the participants' proclivity to avoid dental care and their perception of oral health as being unsatisfactory.

0163

Calibration of Restorations' Evaluation: Feedback From the DECAT Study

Céline Catteau[1, 8], Julia Bosco[15, 9], Nicolas Decerle[2, 10], Franck Decup[15, 9], Kerstin Gritsch[16, 17], François Gueyffier[3, 17], Sophia Houari[15, 9], Sabine Joniot[4, 11], Marc Linez[1, 8], Delphine Maucort-Boulch[3, 18], Catherine Mesgouez-Menez[15, 9], Pierre Millet[5, 12], Paul Monsarrat[4, 11], Michèle Muller-Bolla[6, 13], Patrice Nony[3, 17], Laurent Roche[3, 18], Yann-Loïg Turpin[7, 14], Cyril Villat[16, 17], Brigitte Grosgogeat[16, 17]
[1]Faculty of dentistry, University of Lille, Lille, France, [2]Faculty of dentistry, Clermont Auvergne University, Clermont-Ferrand, France, [3]Université Lyon 1, CNRS, UMR 5558, Laboratoire de biométrie et biologie évolutive, Villeurbanne, France, [4]Faculty of dentistry, Paul Sabatier University, Toulouse, France, [5]Faculty of dentistry, Reims Champagne Ardenne University, Reims, France, [6]Faculty of dentistry, Cote d'Azur University, Nice, France, [7]Faculty of dentistry, University of Rennes, Rennes, France, [8]Centre Hospitalo-Universitaire de Lille, Lille, France, [9]Assistance Publique des Hopitaux de Paris, Paris, France, [10]Centre Hospitalo-Universitaire de Clermont-Ferrand, Clermont-Ferrand, France, [11]Centre Hospitalo-Universitaire de Toulouse, Toulouse, France, [12]Centre Hospitalo-Universitaire de Reims, Reims, France, [13]Centre Hospitalo-Universitaire de Nice, Nice, France, [14]Centre Hospitalo-Universitaire de Rennes, Rennes, France, [15]UFR Odontologie, University of Paris, Paris, France, [16]Faculty of dentistry, Claude Bernard Lyon 1 University, Lyon, France, [17]Hospices civils de Lyon, Lyon, France, [18]Hospices civils de Lyon, Pôle Santé Publique, Service de Biostatistique et bioinformatique, Lyon, France

Objectives: To limit subjectivity in quality assessment of dental restorations, it is advised to use descriptive clinical criteria. Moreover, it is recommended for conducting clinical studies that restoration quality should be assessed by calibrated evaluators. Training-calibration process is most often based on photographs while tactile evaluation remains important. This study aimed to investigate the feasibility of an alternative method using extracted teeth.

Methods: Twelve clinicians of the DEep CAries Treatment study (French randomized controlled trial) group were volunteers to participate. After a training session, they independently evaluated 10 restored extracted permanent teeth arranged in wax models and their respective printed X-ray image. Then, they evaluated 10 photographs of posterior teeth restored with composite resins provided by the e-calib platform. Four FDI criteria have been selected for evaluation: fracture/retention (B5), marginal adaptation (B6), radiographic examination (B9) and recurrence of caries (C12). Each criterion was rated using a 5-grade scale. Criteria were secondly pooled to obtain one binary combined criterion scored either as "failure of the restoration", if at least one of the criteria was scored 4 or 5, or "success". For both processes, inter-examiner reliability among the 12 participants was assessed for each FDI criterion and for the combined criterion using concordance correlation coefficient.

Results: Concordance correlation coefficients and 95% confidence intervals are presented in Table 1. Considering only composite restored extracted teeth, similar reliability was recorded for both criteria B5 (moderate reliability) and B6 (moderate reliability for extracted teeth models and substantial reliability for pictures). For criterion C12, reliability recorded for extracted teeth models was very lower than for pictures (slight *vs* substantial reliability). For combined criterion, inter-examiner reliability was greater using the extracted teeth models than the pictures (substantial *vs* moderate reliability).

Conclusions: In the context of clinical studies, extracted teeth models appear as a promising tool for a complementary approach to pictures and an easier training-calibration process than workshops on patients.

0164

Inhibitory Effects of Various Ions Released from Surface-Active Fillers

Ikram A. Salim[1], Roda Seseogullari Dirihan[1], Satoshi Imazato[2], Arzu Tezvergil-Mutluay[1]

[1]Cariology and Restorative Dentistry, Institute of Dentistry, Turku, Finland, [2]Department of Restorative Dentistry and Endodontology, Osaka University Graduate School of Dentistry, Osaka, Japan

Objectives: Host-derived enzymatic activity at the resin-dentin interface is in part responsible for the limited durability of the restorations. Surface pre-reacted glass-ionomer filler (S-PRG filler) incorporated restorative materials were recently gained attention due to their active ion release properties. The aim of this study was to investigate whether ions released from these fillers could inhibit matrix metalloproteinase (MMP) enzymatic activity in dentin.

Methods: Demineralized dentin beams (lx2x6 mm) were divided into 8 groups (n=10 /group) after baseline dry mass and total MMP activity measurements. Beams were treated with standard solutions containing 100 ppm solutions of boron, fluoride, sodium, silicon, strontium, or 10 ppm aluminum as well as S-PRG solution for 5 min, blot dried and incubated in calcium and zinc containing media at 37°C shaking bath for one week. Untreated demineralized beams served as controls. After incubation period, the loss of dry mass was determined, and aliquots of incubation media were analyzed for (ICTP) using the ICTP RIA kit (UniQ RIA, Orion Diagnostica, Finland) to determine the MMP-mediated collagen degradation via C terminal telopeptide of type I collagen (ICTP). Data were analyzed using one-way ANOVA and Tukey's, α=0.05.

Results: The mean ICTP release in the tested groups was significantly lower compared to control ($p<0.05$). The highest ICTP release occurred in the control group with 56.5 ng ICTP /mg dentin, whereas the fluoride pretreated group showed the lowest ICTP release among all other groups with 15.4 ng ICTP/mg dentin. Strontium, sodium, and S-PRG groups showed a similar amount of ICTP release of 30 ng ICTP /mg dentin.

Conclusions: The result of this work indicated that ions released from fillers have the potential to partly inhibit the endogenous enzymatic activity in demineralized dentin matrices.

0165

Effect of Biomimetic Mineralization on Demineralized Dentin: a SEM/EDS Analysis

Pelin Kukey, Pinar Yilmaz Atali, Ezgi Altuntas, Cafer Turkmen, Betul Kargul

Faculty of Dentistry, Marmara University, Istanbul, Turkey

Objectives: To investigate the effect of Riboflavin as biomimetic mineralization on the chemical composition and crystallinity of demineralized dentin samples in vitro.

Methods: Dentin samples from human teeth were randomly divided into 4 groups: Surfaces treated with Universal bonding agent (Kerr Optibond (Orange, CA, ABD) (Group B), surfaces treated with tri-calcium phosphate (fTCP) (3M ™ Clinpro ™ 5000) followed by 2% Riboflavin (Group CR),) surfaces treated with Universal bonding agent and 2% Riboflavin (Group BR) and control surfaces with no pretreatment (Group C). To assess the morphological changes of the surface was analysed by SEM and quantitative analysis of tissue penetration was performed using energy-dispersive X-ray spectroscopy (EDS). Data was statistically analyzed using Mann Whitney U test for comparison between means at a significance level of 0.05.

Results: The results of microscopic observation of dentin morphology showed that Group CR and Group BR showed a large degree of remineralization on the surface of the dentin treated and the dentinal tubules were generally occluded. The remineralization in fTCP with 2% Riboflavin group was more even than that in bonding with 2% Riboflavin group. The crystals formed during remineralization in dentin have been observed in tri-calcium phosphate and bond with Riboflavin groups. Element mapping revealed a high Ca content within the dentinal tubules. EDS analysis demonstrated Calcium (Ca) content within dentin for Universal Bond group and fTCP with 2% Riboflavin group were 36,8±2,28 and 21,93±3,3 respectively ($p=0.083$). In addition, also Carbon (C) ($p=0.121$) and Oxygen (O) ($p=0.041$) were detected within the precipitates.

Conclusions: Within the limitations of this study, the surface treatment with Riboflavin combined with tri-calcium phosphate resulted in remineralization of demineralized dentin. Further investigations will be necessary on potential innovative bioactive materials of dentine remineralization as minimal intervention is an important concept in clinical restorative dentistry.

0167

Pulpotomy Subsequent to Occlusal Reduction for Prosthesis: Two Case Reports

Stéphane X. Djolé, Yolande Koffi Gnagne, Marie C. Avoaka-Boni

Department of Conservative Dentistry and Endodontics ~ Unité de Formation et de Recherche d'Odontostomatologie, University Félix Houphouët Boigny, Abidjan, Côte d'Ivoire

Objectives: Pulpotomy consists of removing a part of dental pulp in order to a vital pulp therapy. Usually, root canal treatment is performed following excessive occlusal reduction of a tooth for adequate room to restore an edentulous space. These two case reports aimed to examine pulpotomized teeth for the placement of removable partial dentures**.**

Methods: Two patients aged 36 and 58 years were referred to us for root canal treatment prior to crown height reduction (respectively ≠16, ≠17, ≠25 and ≠47). Pulpotomies were carried out in agreement with the practitioner and the patients. The procedure included local anesthesia, occlusal reduction, full pulpotomy according to the standard protocol, and sealing of the pulp chamber with tricalcium silicate-based material (Biodentine™, Septodont, Saint Maur des Fosses, France). At 1 week postoperatively, 3-4 mm deep cavity was prepared with a sufficient Biodentine™ base for the coronal restoration using composite resin (G-ænial® posterior, GC, Tokyo, Japan). The removable partial dentures were placed at another appointment, then clinical and radiographic examinations of pulpotomized teeth were done for up 2 years.

Results: No sensitivity or postoperative pain was reported one week after the procedure. At two years postoperatively, all patients claimed excellent chewing ability with their pulpotomized teeth. The periodontal pain tests were negative, retroalveolar radiographic observations showed normal periodontium, the absence of periapical lesion and root resorption. The teeth were functional and asymptomatic.
Conclusions: Preserving the radicular pulp seems to maintain the proprioceptive and damping abilities of the tooth. So, pulpotomy could be a reliable alternative for the treatment of the teeth whose pulpectomy was indicated in excessive occlusal reduction. Further clinical studies using randomized controlled trials would support this approach.

0168

A Pronounced Apical Translucency with Endodontic Causation? – an Interdisciplinary Case-Report

Sondos Gabris[1], Gordon John[2], Jürgen Becker[2], Michelle A. Ommerborn[1]
[1]Department of Operative Dentistry, Periodontology and Endodontology, Heinrich-Heine-University, Düsseldorf, Germany, [2]Department of Oral Surgery and Central Admittance, Heinrich-Heine-University, Düsseldorf, Germany

Objectives: The present rare interdisciplinary case report presents an incidental finding of asymptomatic apical translucency on a mandibular first molar of a 70-year-old male patient.
Methods: The diagnostic finding was detected through a routine dental examination including a panoramic radiography. The further detailed radiological examination revealed a circular, homogenous, solid translucency with a cross-sectional dimension of approximately 15 mm (Fig. 1). The translucency appears to have contact apical to the mesial root of the tooth 36. The clinical investigation in the field of interest showed neither swelling on palpation nor abnormalities on the teeth except for a delayed pulp sensitivity with CO_2 snow on tooth 36. Following to a thorough examination, the patient obtained a specialized endodontic treatment by using dental operating microscope (Fig. 2) and hereafter he was undergoing to a minimal-invasive surgical intervention.
Results: The pathological diagnosis was hemangioma.
Conclusions: This case illustrates not only a well-documented very rare example of a hemangioma with topographic association to the inferior alveolaris nerve with respect to its combined endodontic and surgical treatment. Moreover, its three years follow-up documentation also presents the importance of a concise preliminary examination in order to reduce possible risks and to achieve the most satisfactory results for the patient.

0169

Regenerative Endodontic Treatment with Blood Clot and PRF: a Clinical Report

Sevgi Arabulan, Ahmet C. Eronat, Rüstü Ö. Öngağ, Nazan Ersin
Department of Pediatric Dentistry, Ege University, Izmir, Turkey

Objectives: Blood clot (BC) and platelet-rich fibrin (PRF) have been used as scaffolds in regenerative endodontic treatment (RET). The aim was to compare the performance of PRF with BC in inducing root development after RET of two traumatized immature necrotic permanent teeth of a patient.
Methods: A 6-year-old boy with trauma to maxillary central incisors applied to pediatric dentistry clinic. Clinical and radiographic examinations revealed both incisors having necrotic pulp without apical periodontitis. In the first appointment both teeth were treated by preparing access cavities and pulp removal. The canal was irrigated with 1.5 % NaOCl. Calcium hydroxide was inserted into the canal for 3 weeks.
At the second appointment, both canals were irrigated with EDTA. Blood was drawn from the patient's forearm for preparation of PRF. After provoking periapical bleeding, the PRF was placed into the canal space of maxillary right central incisor while blood clot alone to the maxillary left central incisor. Mineral trioxide aggregate was placed directly over the PRF and blood clot. Teeth were temporarily restored with glass ionomer cement. Two weeks later, both teeth were permanently restored with composite resin. The patient was followed-up for 28-months, clinically and radiographically.
Results: After 28 months clinical examination revealed no sensitivity to percussion or palpation tests in both teeth. Root elongation, dentinal wall thickening and continued apical closure in both teeth were observed in radiographs. Cone-beam computed tomography images revealed that tooth treated with BC showed narrower root canal in the apical region and tendency for root obliteration compared to tooth treated with PRF.
Conclusions: Both PRF and BC exhibited successful clinical outcomes and continued root canal development in the regenerative endodontic treatment. Nevertheless, PRF may be potentially an ideal scaffold compared to BC in terms of long-term pulp obliteration.

0170

Juvenile Tongue Fibroma: Surgical Management.

Silvia D'agostino[1], Alessandra Bissoli[1], Lucia Caporaso[1], Francesca Iarussi[1], Riccardo Pulcini[2], Marco Dolci[1]
[1]Medical, Oral and Biotechnological Sciences, University G d'Annunzio, Chieti, Italy, [2]Innovative Technologies in Medicine and Dentistry, University G d'Annunzio, Chieti, Italy

Objectives: Juvenifle tongue fibroma (JTF) is a benign tumor which arises in the youth and it is characterised by a hard/dense rosy mass on the tongue surface. JTF occurs when a fibroblasts proliferation is embedded in a collagenous matrix in the exterior tongue area. We report the case of a female 19 years old patient who is now still in follow-up.

Methods: An healthy 19 years old female patient came to our attention telling us about a thick mass on the left tongue dorsum appared two years earlier and now increasing size. The eight-step WHO oral examination was perforemed and an eco color Doppler was requested in oder to planning the escissional biopsy.
Results: The intraoral examination showed a consistent painless pinkish mass in the left dorsal surface of the anterior third of the tongue with an enhancing of the papillae and a dimension of 1.4 x 0.8 cm. The eco color Doppler exam reveals an absence of a significant vascular component, leading the team to exclude the angio-fibroma hypotesis. The escissional biopsy was perfomed by cold blade revealing the lesion reacheed the underlying muscolar level without invading it. The sample was included in specific formalin box and given to the patient in order to perform the hystological analysis at the hospital. The site was sutured with absorbable wire and the patient is now included in a follow-up program waiting for the hystopatological result and with the aim to control tissue healing.
Conclusions: Oral pathology has to become a costant part of dentist's activities. JTF may alter self-perception in young patients, and its surgical management should always be performed both to clarify the lesion's origin and to restore proper structures anatomy.

0171

Semilunar Coronally Advanced Flap Technique for Root Coverage: Case Report

Ismail Marakoglu, Yunus Emre BALABAN
Periodontology, Selcuk University Faculty of Dentistry, Konya, Turkey

Objectives: Gingival recession characterized by apical migration of the gingival margin from the cementoenamel junction and root exposure. Several surgical techniques are reported for the treatment of gingival recession. The goal of the present study was to evaluate the effectiveness with respect to root coverage of a semilunar coronally advanced flap technique for the treatment of Cairo Type 1 gingival recession.
Methods: A 58-year-old systemically healthy female patient presented for routine dental prophylaxis to the Selcuk University Faculty of Dentistry Department of Periodontology. Clinical examination revealed the presence of Cairo Type I buccal recession defects on the left maxillary canine tooth. Presence of adequate width of attached gingiva was noted and his smile analysis low lip line was observed. The width of attached gingiva was 5mm and demonstrated thick biotype. Oral hygiene instructions and education were given to the patient. As the first step of periodontal treatment, calculus removal was performed and the patient was recalled after 4 weeks to commence the mucogingival surgery. Semilunar incision was made extending beyond the mucogingival junction from the mesial papilla to the distal papilla of the tooth with the recession and following the curvature of the receded gingival margin. The flap was held in its new position for 3 minutes with sterile moist gauze. Sutures were not placed. A periodontal dressing was placed on surgical area.
Results: There was no difference observed in the probing depth values, at the baseline and after 1.5 year though the clinical attachment loss reduced from 2.5mm to 0.5mm after 1.5 year. Root coverage of 100% was observed.
Conclusions: Semilunar coronal advanced flap technique is a less traumatic and simple method compared to other methods. Provides satisfactory results for treating type I buccal recession with low lip line defects especially. However, disadvantage of that technique left scar at the semilunar incision site.

0172

Decoronation as Alveolar Ridge Preservation Technique: a Case Report

Alice D'hoore, Dominique Van der Veken, Anne-Céline Vanhecke, Mariano Simón Pedano De Piero, Paul Lambrechts
KU Leuven, Leuven, Belgium

Objectives: To present a case whereby a decoronation technique was used to preserve the alveolar ridge.
Methods: A 15-year-old girl came to the University clinic referred by her own dentist in 2017. The girl was in good health, had no allergies and took no medication. The patient presented a pink spot in tooth 1.2 causing esthetic complaints. After an initial clinical and radiological examination, tooth 1.2 responded sensitive to cold and negative to percussion tests. There was a normal healthy pulp and normal periapical tissues. The diagnosis was an external cervical resorption that probably arose during tooth eruption of the permanent teeth due to pressure of the upper canines on the lateral incisors. According to the Heithersay classification, the external cervical resorption can be classified as class IV. According to Patel's three-dimensional classification, it can be classified as 3Bp. Since the external cervical resorption was too advanced to be treated, a 'watchful waiting' approach was chosen. Further follow-up appointments were conducted in 2018 and 2019 but, due to progression of the lesion, the tooth had to be extracted. After interdisciplinary discussion, the patient opted to decoronate the tooth rather than extract it, so the alveolar ridge could be preserved until the patient reached the age for an implant.
Results: A decoronation technique is often suggested as a treatment option for teeth that have signs of ankylosis. In the treatment options of external cervical resorption, this is often not cited as a possible treatment while this technique can provide preservation of the alveolar ridge.
Conclusions: The case highlights decoronation as a possible treatment option in advanced external cervical resorption cases whereby implant treatment is not yet possible.

0173

Total Restorative Rehabilitation in a Patient with Functional and Esthetic Problems. a Case Report.

Anne-Céline Vanhecke, Marleen Peumans
Restorative Dentistry, KU Leuven, Leuven, Belgium

Objectives: A patient with many old, large unacceptable restorations underwent an orthodontic treatment in combination with orthognatic surgery. At the end of this treatment, stable occlusal contacts were missing and the old restorations needed replacement. The aim of the restorative treatment was to restore function and esthetics in this patient.

Methods: A 60-year-old women was referred to the Department of Restorative Dentistry after orthodontic treatment and orthognatic surgery. A Class II skeletal relationship was corrected. Her teeth showed grayish tetracycline staining. The upper anterior teeth were quite heavily filled and masked with old unacceptable composite veneers. The premolars and molars were also severely damaged and presented large unacceptable worn composite restorations. Function was inadequate due to the presence of unstable contacts on these posterior teeth. Total restorative rehabilitation was required to give the patient a stable occlusion and pleasant smile. Treatment started with exploring all the teeth and treating the caries lesions. One devitalized upper premolar needed to be extracted because no ferrule effect was present. The lower anterior teeth and two molars were restored with direct composite restorations (Essentia, GC). Before starting with the indirect restorative procedures a total wax-up was made by the dental technician. For each tooth, the least invasive indirect ceramic restoration was selected: monolithic zirconium (Zirkonzahn prettau 2) crowns on 11,12,21, 3-unit monolithic zirconium (Zirkonzahn prettau 2) bridges on 23-25 and 44-47, bonded lithium disilicate glass ceramic (Emax) partial crowns on teeth 13, 14, 16, 17, 36 and 48. For each type of restoration, a correct cementation protocol was followed.

Results: The total restorative rehabilitation resulted in an adequate occlusion and articulation. In addition, the esthetic result was very natural. The patient was very satisfied with her new smile.

Conclusions: A full restorative rehabilitation needs a proper treatment planning. Good cooperation and communication with the dental technician play a crucial role in obtaining an optimal and durable result. Yearly follow-up of the patient, with emphasizing good oral hygiene and maintenance of the restorations is absolutely required.

0174

Full Arch Rehabilitation with the Flowable Injection Technique: a Case-Report

Pieter-Jan Swerts, Marleen Peumans
Restorative Dentistry, KU Leuven, Leuven, Belgium

Objectives: Restorative treatment of tooth wear should be as conservative as possible. The aim of this case report is to present a full arch rehabilitation in two patients with moderate generalized tooth wear following the Flowable Injection Technique.

Methods: Two patients with generalized tooth wear presented on the Department of Restorative Dentistry. The first patient was an adolescent with excessive intake of Coca-Cola during several years. His main complaint was shortcoming in esthetics due to the shortening of the anterior teeth. The second patient, a 55-year-old male patient, was a bruxist suffering from erosion from gastric reflux and excessive intake of acidic food. The plan was to restore the worn teeth in both patients with the Flowable Injection Technique. The next protocol was followed: 1. digital impression taken in centric relation, 2. digital wax-up of the worn teeth including an increase in the vertical dimension of occlusion, 3. fabrication of transparent silicone indices on the digital wax-up to transfer the situation of the wax-up intra-orally. After rubberdam isolation, the worn surfaces of the teeth to be restored were roughened by air abrasion (30 mm Al_2O_3), the adhesive system was applied, a highly filled flowable composite was injected throughout the transparent silicone indices and light cured. The worn teeth were restored quadrant by quadrant. The anterior teeth were build-up in the last session with either direct or a combination of direct and indirect restorations.

Results: By working in a digital way, the situation of the wax-up can be copied in an accurate and predictable way in the mouth of the patient, by injecting the flowable composite. Minimal corrections were needed to obtain a stable occlusion. After final finishing and polishing of the restorations, the esthetic result was highly acceptable. One-year follow up showed composite restorations with limited wear and high gloss retention

Conclusions: The Flowable Injection Technique is useful to restore patients with moderate tooth wear in a predictable way with an affordable cost for the patient. This technique is considered as a temporary treatment. Long term follow-up is required to evaluate the long-term stability of these injected composite restorations.

0175

Treatment of Agenesis of Lateral Incisors with Resin-Bonded Ceramic Cantilever Bridges. a Case Report.

Valérie Weemaes, Marleen Peumans
Restorative Dentistry, KU Leuven, Leuven, Belgium

Objectives: Clinical trials show that replacing missing lateral incisors with a resin-bonded cantilever ceramic bridge perform well in the long-term. The objective of this case report is to present the replacement of two missing lateral incisors in an adult patient following a combination of orthodontics and placement of resin-bonded ceramic cantilever bridges.

Methods: A 31-year-old male patient consulted the Department of Restorative Dentistry with agenesis of both maxillary lateral incisors. He complained about the unesthetic appearance as very large diastemata were present between the upper anterior teeth. The treatment plan included: 1. orthodontic closure of the central diastema in order to create sufficient space for replacement of the lateral incisors, 2. replacement of the lateral incisors with 2 resin-bonded zirconia cantilever bridges. This type of restoration was selected because of the minimally invasive character and the relatively low cost compared to tooth replacement with an implant. After orthodontic treatment, an essix retainer was made with replacement of the lateral incisors in composite,

on wich an ovate pontic was formed. After formation of the ponticbed, an intra-enamel preparation was made on the central incisors and an impression was taken. Two weeks later, the monolithic zirconia cantilever bridges were bonded. The inner surface of the zirconia wings was pretreated with airabrasion (30 mm A_2O_3, 1 bar), followed by cementation of the bridges with Panavia V5 (Kuraray Noritake) under rubberdam isolation. Attention was paid that the lateral incisors were discluded during anterior guidance.
Results: The patient was very satisfied with the final esthetic result. Yearly follow-up of the restorations was planned.
Conclusions: Replacing missing lateral incisors with resin-bonded cantilever zirconia bridges is a minimally invasive treatment, easy for the dentist to perform. Attention must be paid to the space available, the quality of the abutment teeth and the occlusion. An accurate bonding protocol must be followed to obtain long-lasting restorations.

0176

Functional and Esthetic Rehabilitation of a Patient. a Case Report.
Ellen Van de Maele, Marleen Peumans
Restorative Dentistry, UZ Leuven, Leuven, Belgium
Objectives: The aim of this case report is to present a functional and esthetic rehabilitation in 59-year-old female patient combining an implant supported prosthesis in combination with full ceramic crowns and veneers on the anterior teeth.
Methods: The patient was referred to the Department of Restorative Dentistry. Her main complaint was shortcoming in esthetics and function in the upper jaw. She has been partially edentulous for about 20 years. The Kennedy class II was restored with a removable cobalt-chrome alloy frame prosthesis. The upper anterior teeth showed discolored, unacceptable composite veneers. Some of these teeth were quite heavily filled. Due to fracture of an abutment tooth of the frame prosthesis the retention of the prosthesis decreased seriously.
The treatment started with exploring the upper anterior teeth (13-24) and home bleaching of the teeth in the lower jaw. Two abutment teeth (23 and 24) of the existing frame needed to be extracted because of absence of ferrule. Two implants were placed (location 16 and 24) in order to transform the Kennedy class II into a Kennedy class III. A rigid anchorage would seriously increase the retention of the new frame. After osseointegration of the implants, two ball abutments were mounted. The remaining upper anterior teeth were restored with a combination of monolithic zirconium crowns (12, 13, 22) and bonded lithium disilicate veneers (11 and 21). Finally, a new partial prosthesis with cobalt-chrome alloy framework replaced the missing teeth. Because of the high smile line, the dental technician provided a tooth-colored resin clasp on the frame.
Results: The patient was very pleased with the esthetic and functional result of combining fixed and removable prosthesis.
Conclusions: Use of implants to support a partial prothesis improves its retention and decreases the movement during function which increases the patient's comfort seriously.

0177

Full-Mouth Rehabilitation in a Young Adult with Severe Amelogenesis Imperfecta: a Case Report
Katrien Blancke, Marleen Peumans
Restorative Dentistry, KULeuven, Leuven, Belgium
Objectives: Amelogenesis imperfecta (AI) refers to a group of genetic disorders that affects enamel development. AI has a serious impact on the self-esteem of the patient. The aim of this AI case report is to present a full-mouth rehabilitation with bonded lithium disilicate glass ceramic restorations.
Methods: A 23-years old male patient, with severe hypoplastic AI asked for an esthetic and functional rehabilitation. Well mineralized hypoplastic enamel was present in a very thin layer and the teeth were brown discolored. Old discolored composite veneers were present on the incisors and the patient had a compromised periodontal health. Orthodontic treatment was needed to correct the occlusal plane, the anterior open bite, the midline shift and the presence of diastemata and rotated premolars. The patient, however, refused to be treated orthodontically because of the high financial cost. Treatment started with a profound periodontal cleaning and oral hygiene instructions. Periodontal surgery was carried out to reduce the pocket depths. At the same time the height of the short clinical crowns in the posterior region was increased. A digital wax-up, made by the dental technician, included an increase in the vertical dimension of occlusion. After evaluation of the mock-up, minimal invasive crown preps were made on 24 teeth. The digital wax-up was transferred intra-orally making use of temporary CAD-CAM crowns. Five months later, the teeth were restored with durable bonded lithium disilicate glass ceramic crowns.
Results: After total rehabilitation with monolithic lithium disilicate glass ceramic crowns, the masticatory function was restored. The problem of increased tooth sensitivity was solved and the restorations allowed to perform a good oral hygiene. The patient was very happy with the final esthetic result. This seriously increased his self-esteem.
Conclusions: Total rehabilitation of a patient with severe hypoplastic AI is complex and requires a structured treatment plan, in which different disciplines are involved. Regular periodontal and restorative follow-up is absolutely required to guarantee the durability and functionality of the total rehabilitation.

0179

Minimal Invasive Treatment of Molar Incisor Hypomineralization: a Case Report

Ezgi Altuntas, Pinar Yilmaz Atali, Cafer Turkmen, Dilek Tağtekin
Restorative Dentistry, Marmara University, Istanbul, Turkey

Objectives: Molar incisor hypo-mineralization (MIH) is an enamel defect that causes opacities in incisor and molar teeth. This case explains combined minimal invasive treatment for MIH.

Methods: A 17-year-old woman attended our clinic for treatment of white opacities on anterior teeth. After the clinical and radiographically examination microabrasion, home bleaching and resin infiltration were planned for the treatment. Tooth Mousse Gel (CPP-ACP, GC) was applied on the lesions for two weeks. Under the rubber dam isolation Opalustre (Ultradent) was applied in 3 consecutive cycles using OpalCups (Ultradent). Fluoride varnish (4% NAF, ProShield, President Dental) was applied for 4 minutes following the microabrasion. Home bleaching gel was applied for 5 weeks with custom made bleaching tray using 16%CP (Opalescence PF, Ultradent). 2 weeks after the bleaching procedure, 15% HCL gel (ICON, DMG) was applied in 5 consecutive cycles, followed by application of ethanol and resin infiltrant (ICON, DMG) onto the white lesions. Polishing was completed with polishing discs (TOR VM). Fluorescence device (FluoreCam, Daraza), DIAGNOdent pen (KaVo), spectrofotometer (EasyShade-V, VITA), intra-oral scanner (BIS-1, BenQ) and D Light (GC) were used for diagnosis, color change, control of lesion size and visibility. Patient were recalled for 7-14 days, 1-3-6 months and scored according to the FDI criteria.

Results: Diagnodent scored initially 04 (tooth #11) and 08 (tooth #12). At end of the treatment scores were changed to 02, 05 respectively. FluoreCam showed the lesions as “improved”. Spectrofotometer showed that the shades of incisor teeth were B3 initially and A1 after bleaching. During recalls, RI applied teeth #11-12 were evaluated and scored as 1.

Conclusions: Following application of remineralization gel, combination of microabrasion, bleaching and resin infiltration may be preferred in reducing lesions and eliminating aesthetic concerns seen in MIH. Intraoral-scanner and D Light improves the visibility of white lesions during the diagnosis and treatment stages.

0180

Reattachment of a Complicated Crown-Root Fracture: 14 Year Follow-up

Esra Can Say[1], Burcu Dikici[1], Gokser Cakar[2]
[1]Restorative Dentistry, Yeditepe University Faculty of Dentistry, Istanbul, Turkey, [2]Periodontology, Altinbas University Faculty of Dentistry, Istanbul, Turkey

Objectives: Complicated crown-root fractures with the involvement of the biological width require immediate attention with a multidisciplinary approach for adequate treatment and successful prognosis. This case report describes the 14-year-follow-up of the reattachment of a complicated crown-root fracture with the involvement of supracrestal attached tissues.

Methods: A 30-year-old female was referred to the Faculty of Dentistry Department of Restorative Dentistry after a traumatic dental injury caused by a car accident. Clinical and radiographical examination revealed a complicated crown-root fracture of the right endodontically treated maxillary central incisor. Upon agreeing on the reattachment of the crown-root fragment to the remnant tooth, informed consent was obtained from the patient. As the fracture was subgingivally located and extended apically to the bone crest, reattachment of the crown-root fragment was performed during an open flap surgery with osseous resection. After isolation and hemorrhage control, a two-step self-etch adhesive (Clearfil SE Bond; Kuraray) was applied on the crown-root fragment and remnant tooth and polymerized. Along with a thin layer of a flowable composite (Clearfil Majesty Flow; Kuraray) a unidirectional pre-impregnated fiber (EverStick C&B; GC) was pressed into the root canal of the remnant tooth and polymerized. Following checking the fit of the fragment, a micro-hybrid composite (G-aenial; GC) was applied to the crown-root fragment, the fragment was placed on the fracture site and the composite was polymerized through the attached tooth. The clinical situation of the reattached tooth was confirmed by clinical assessments at 1 2, 5, 7, and 14 years.

Results: Clinical and radiographical examinations of the reattached tooth after 14 years revealed favorable functional and esthetic outcomes and biomimetic characteristics of the anterior teeth and surrounding periodontal structures.

Conclusions: Reattachment of complicated crown-root fractures with the involvement of supracrestal attached tissues presents a reliable and long-term successful treatment option with the multidisciplinary treatment approach.

0181

Orthodontic Extrusion to Restore an Esthetically Compromised Central Incisor

OLGA GONZÁLEZ CASTRO[2], Nuria Isabel Escribano Mediavilla[1], Maria Victoria Fuentes[1], Laura Ceballos[1]
[1]Nursing and Stomatology Department, Health Sciences Faculty, Rey Juan Carlos University, Madrid, Spain, [2]Esthetics and Endodontics. Health Sciences Faculty, Rey Juan Carlos University, Madrid, Spain

Objectives: To present the management of an endodontically treated central incisor with a compromised esthetic situation due to the presence of a metal-ceramic crown impinging the supracrestal tissue attachment.

Methods: A 41-year-old female patient came to the University clinic concerned about the esthetic appearance of the metal-ceramic crown in tooth 1.1. She had received previous orthodontic treatment and an over-contoured crown impinging the supracrestal tissue attachment. A multidisciplinary management was planned with different treatment phases. In the same visit the old crown and a metal post were removed. The canal root retreatment was performed (Protaper Universal, Dentsply Maillefer) and a thermoplastic obturation (System B, SybronEndo) of the apical third was carried out. Also, a fiberglass post (Relyx Fiber Post, 3M ESPE) was adhesively luted (RelyX Unicem, 3M ESPE). After core build-up with resin composite, a bis-acrylic temporary crown (Protemp 3 Garant, 3M ESPE) was cemented. In the following visit, an acrylic resin temporary crown was definitively luted with a resin modified glass ionomer (Ketac-cem Plus, 3M ESPE). An orthodontic extrusion was performed after a circumferential

supracrestal fiberotomy in order to increase the ferrule and improve the periodontal status. Once the required amount of extrusion was achieved an intermediate tooth stabilization was performed to allow for a complete maturation of bone. The final prosthetic restoration consisted in a monolithic zirconia (Lava TM Plus, 3M ESPE) crown luted with a self-adhesive resin cement (RelyX Unicem 2, 3M ESPE).
Results: After 10-year follow-up, the periodontal tissues were healthy and stable, and the patient was satisfied with the esthetic result. The clinical success of complex cases depends on an adequate planning, a multidisciplinary approach, together a deep knowledge of the available materials.
Conclusions: Orthodontic extrusion is a minimally invasive treatment that allows to manage complex multidisciplinary cases of severely compromised teeth.

0182

A New Device to Distalize Maxillary Molar With High Pseudoelastic Forces.

Carolin Späth
Technische Universität, Muenchen, Germany

Objectives: Distalization of upper molars, followed by maximal anchorage, belong to the most important orthodontic objectives. Various non-compliance devices have been developed to solve this therapeutic problem without the risk of inverse forces, based on skeletal anchoring. These requirements characterise the Longslider, a modification of the Beneslider, by two mini-implants placed along the median palatine suture (Figure 1). The active elements are pseudoelastic NiTi springs with high constant forces up to gf 600 (5,88N) to overcome slider friction and automatic deactivation at the end of the desired distalization extent. The present study explores the sagittal dental movement of the first and second molars within an orthodontic treatment with fixed appliances (vestibular technique).
Methods: 38 patients with symmetric class II malocclusion (24 female, 14 male; age 10,3 to 53,8, mean: 17,4 years) were collected for clinical evaluation. The facial type of 73,7% was moderate retrognathic and of 26,3% orthognathic. Cephalograms were taken before and after orthodontic treatment, all including molar distalization. Two groups were divided refered to the eruption stage of the second molar: Group 1 include patients before his eruption (mean age: 13,2 years) (Figure 1), group 2 after his eruption (mean age: 19,5 years) (Figure 2). The extend of distal movements of the upper molars were evaluated with six linear measurements from distal dental points to the pterygoid vertical (Figure 3); statistical evaluation were carried out with the shapiro-wilk-test.
Results: The intended treatment goal was achieved with a class I occlusion between the first upper and lower molars. Distalization of the first molars could be achieved from 1,0 up to 4,8 mm; the desired translational movement differ with a minor distal tipping of 0,9°, bodiliy translation predominates. Between the two groups no significant differences were evident.
Conclusions: The Longslider is a clinically comfortable, safe and effective non-compliance devise for maxillary molar distalization. The high and constant pseudoelastic forces overcome high frictional forces and seems to optimize translatory tooth movement.

0183

Reimplantation of a Molar with Complex Root Canal Anatomy

Patricia Fernandez Esteban, Laura Bailleul Bermejo, Patricia Terceño Jiménez, Laura Ceballos García
Universidad Rey Juan Carlos, Madrid, Spain

Objectives: To present the management of a molar with complex root canal anatomy and a previous failed root canal treatment by an intentional reimplantation technique.
Methods: A 48-year-old female patient with no relevant medical history complained of pain on percussion and palpation in the tooth 1.7. The mobility and probing were physiological. Radiographically a previous endodontic treatment was detected with an apical radiolucency. Pulpal and periapical diagnosis were previously treated tooth with symptomatic apical periodontitis, respectively. A preoperative CBCT (Carestream, Kodak) was taken to provide a three-dimensional confirmation of the compatibility of the root anatomy with atraumatic extraction. After local anesthesia, a scalpel blade was used to incise the gingival fibrous attachment, with rotation movements and forceps the tooth was extracted and placed on a sterile gauze soaked in sterile saline solution. Visual inspection of the root surface under microscope (Leica, Wetzlar, Germany) was performed and 2-3 mm of the root apex were removed to eliminate possible apical ramification. A root-end preparation was performed with an ultrasonic tip (Endosuccess, Acteon, France) using regularly irrigation with sterile saline solution. The retro-preparation was sealed with TheraCal^{TM} LC (Bisco, Schaumburg, USA). The total extraoral time of the procedure was less than 15 minutes. Flexible splinting (0.4 mm steel wire and resin composite) was done.
Results: The patient was reviewed 2 weeks, 3 and 6 months after the reimplantation. In the 2-week follow-up the splint was removed, and the tooth was asymptomatic and responded normally to percussion. In the 3 and 6-month recalls, periapical bond healing was radiographically confirmed, as well as the absence of other signs or symptoms.
Conclusions: Permanent teeth with a complex root canal anatomy and a previously failed root canal treatment can be successfully treated with an intentional replantation technique restoring the periradicular health.

0184

Zygomatic Implant Penetrated into the Eye Orbit: a Case Report.

Luan Mavriqi[2], Felice Lorusso[1], Calogero Buggea[1], Antonio Scarano[1]

[1]University of Chieti-Pescara, Chieti, Italy, [2]University of Albania, Tirana, Albania

Objectives: Zygomatic implants are currently proposed for severe atrophic maxillary rehabilitations due to a increased long-term success and patients satisfactory level. The aim of the present investigation was to perform a case report of a surgical complication of a zygomatic implant penetration to the orbit.

Methods: A female subject (56-year-old) was visited for pain and swelling at the level of the left orbit after a zygomatic implant procedure. The orbit penetration by the zygomatic implant screw was confirmed by the tomography scan. The patient submitted a surgically removal of the implant and the peri- and post-operative observations were assessed. No signs of neurological complications were reported at the follow-up. The ocular motility and the visual acuity were well maintained while no purulent signs or inflammation were detected in the healing period.

Results: No signs of neurological complications were reported at the follow-up. The ocular motility and the visual acuity were well maintained while no purulent signs or inflammation were detected in the healing period.

Conclusions: The penetration of the eye orbit is a rare complication related to the zygomatic implant procedure that could potentially compromise the eye functions. In the present case report, the implant removal procedure resulted in an uneventful healing phase with the recovery of the sight and movements of the eye.

0185

Gingival Augmentation of an Advanced Recession with Complete Root Exposure

Brunilda Koçi[1], Ervin Koci[2]

[1]Aldent University, Tirana, Albania, [2]Evident Dental Clinic, Tirana, Albania

Objectives: The aim of this clinical case is the treatment of an extremely compromised tooth treated with free gingival graft, root conditioning and apicoectomy.

Methods: A 22-year-old female patient was referred for treatment of tooth number # 14, which presented nonvital, with a deep gingival recession, complete root exposure and no interproximal gingival loss.

The measured defect was 17 mm deep, 4mm wide, 3 mm probing depth in the apical area with BOP and no keratinized tissue present. A laterally stretched (LAST) flap technique with free connective tissue graft was performed. Root surface was polished and treated with EDTA (60 sec). The apex of the root was closed with biodentine.

Results: Root coverage of 88,6 % was achieved one year after the second surgery procedure, with 2 mm keratinized tissue present.

Conclusions: Compromised teeth with complete recession can be successfully treated and maintained with the right multidisciplinary treatment.

0186

Non-Surgical Periodontal Treatment of Amlodipine-Induced Excessive Gingival Growth: a Case Report

Sevilay YegnogluEkc

Periodontology, Ankara University Faculty of Dentistry, Ankara, Turkey

Objectives: Drug-induced gingival enlargement is proportional to the inflammation caused by poor oral hygiene. In this case report, gingival enlargement due to the use of the antihypertensive drug amlodipine, which is a Ca channel blocker derivative, is presented. In the anamnesis of a 56-year-old female patient who applied to our clinic with the complaints of swollen and bleeding gingival enlargement, mobility, pain in chewing and discomfort, it was learned that she had been using 10 mg of amlodipine daily for 6 years due to hypertension. In clinical examination, it was determined that the patient had poor oral hygiene and the presence of gingival growth.

Methods: In the treatment of the patient, it was first replaced with the hypertension drug alternative with the cardiology consultation. The patient was given the first session of dental surface cleaning, polishing, oral hygiene training, and a %0.2 concentration of chlorhexidine mouthwash was prescribed. In the ongoing sessions, root surface straightening procedures were repeated in areas with pockets deeper than 3mm, and oral hygiene motivation was provided. Regression in gingival growth was observed in each session.

Results: With the change of medication, the growth of the gums completely regressed in the 1 st year follow-up after the non-surgical periodontal treatment, no bleeding was observed on probing, and plaque control was achieved. The patient gained a habit of oral hygiene and left our clinic satisfied.

Conclusions: In cases of gingival enlargement due to the use of amlodipine, change of the drug, tooth surface cleaning, root surface straightening, plaque control, oral hygiene motivation and regular patient follow-up play a key role in the treatment of these diseases. It should be kept in mind that plaque control, oral hygiene motivation and regular and frequent patient follow-up are important for the permenant clinical improvement achieved.

0187

Accelerated Extensive Osteonecrosis of the Mandibular Body Under Denosumab Treatment

Lidia Roman[1], Marie-Hélène Vieillard[4], Jean-Michel Maes[2], Jerôme Delattre[3], Fabien Gressier[1], Benjamin Bouet[2], Valentin Rabuel[2], Joel Ferri[2], Laurent Nawrocki[1], Cécile Olejnik[3]

[1]Univ. Lille, CHU Lille, Service d'odontologie, F-59000, Lille, France, [2]CHU Lille, Service de chirurgie maxillofaciale et stomatologie, F-59000, Lille, France, [3]Univ. Lille, Univ. Littoral Côte d'Opale, CHU Lille, Marrow Adiposity and Bone - MABLab ULR4490, F-59000, Lille, France, [4]CHU Lille, Service de rhumatologie, F-59000, Lille, France

Objectives: Medication-related osteonecrosis of the jaws (MRONJ) is a rare complication induced by antiresorptive agents such as bisphosphonates and Denosumab, as well as anti-angiogenics. Invasive dental procedures or chronic apical infections are usually incriminated for the risk of MRONJ.

Methods: A 73-year-old woman consulted for chronic tooth ache. The patient's history includes arterial hypertension, equilibrated diabetes, breast cancer with bone metastases treated with monthly Denosumab (for 2 years) associated with anti-angiogenics. She described the spontaneous loss of three mandibular incisors and belated healing. The first oral exam showed a slightly inflamed but healed mucosa, absence of fistulization and loose, painful mandibular teeth. The intraoral and extraoral palpations of the jaw were painful. The panoramic dental X-ray seemed normal with some bone densification. It was suspected an MRONJ, with interruption of Denosumab treatment within the month. After two months, oral mucosa thickened and mucosal fistulization appeared, followed by discomfort under the chin. Radiographic exams showed a bone densification in the chin area of the jaw with periosteal apposition zones. The MRONJ was confirmed and a switch to bisphosphonates was made. A close clinical surveillance was installed.

Results: The jaw bone complete sequestration happened after one year, with the mandibular body exposed in the mouth, a continuous extra-oral fistulized suppuration treated with antibiotics and the spontaneous loss of all the remaining teeth. The loose jaw bone was extracted under general anaesthesia. Despite the large bone loss, with the lysis of the inferior alveolar canal, especially in the parasymphyseal region, the nerve was not touched, having itself migrated under the dead bone.

Conclusions: We are the first to illustrate such an accelerated evolution of osteonecrosis of the mandibular body under Denosumab, particularly when no dental invasive treatment occurred. This case raises many new questions concerning the MRONJ process and etiologies.

0187.1

Oral Phenotype of Singleton-Merten: a Case Report

Margot C. Riou[2], Muriel de La Dure Molla[1, 2], Stéphane Kerner[1, 2], Sophie Rondeau[3], Adrien Legendre[5], Valérie Cormier-Daire[4], Benjamin P. Fournier[1, 2]

[1]Dental Faculty, Université de Paris, Paris, France, [2]Reference Center of Oral and Dental Rare Diseases, Hôpital Rothschild, Assistance Publique des Hopitaux de Paris, Paris, France, [3]Department of Genetics, Necker Enfants Malades Hospital, Paris Descartes-Sorbonne Paris Cité University, Paris, France, [4]INSERM UMR_S1163: Molecular and Physiopathological basis of osteochondrodysplasia, Institut Imagine, Universite de Paris, Paris, France, [5]Laboratoire de biologie médicale multisites Seqoia – FMG2025, Paris, France

Objectives: Singleton-Merten syndrome is a rare autosomal dominant disorder with blood vessels calcifications, teeth anomalies and bone defects. We aim to describe the oral features of a Singleton-Merten patient, the diagnosis and the treatment he received.

Methods: A 10-years-old patient presented at the pediatric dentistry department for an emergency. After a clinical and radiological examination, he was referred to the Reference Center of Rare Oral and Dental Diseases. In order to investigate the diagnosis, he received a genetic consultation.

Results: We followed him for numerous carious lesions, severe teeth malposition especially in the anterior arch, and oral hygiene deficiency with a 100% plaque index, associated with dental and jawbones anomalies The X-ray did not show any dental agenesis but revealed short roots and a decrease of the alveolar bone height. We observed dysmorphic facial features, fine and space hair, a trigonocephaly with triangular face, discrete hypertelorism, long arched eyebrow and low set-ears. He also presented muscle weakness, cutaneous xerosis and ophthalmologic glaucoma, without intellectual disability. Whole genome sequencing analysis revealed a heterozygous *de novo* variant in *IFIH1* (NM_022168.4) c.2465G>A (p.Arg822Gln) and confirmed a Singleton-Merten syndrome. We carried out the necessary health education and care, a partial denture replaced the hopeless teeth and maintained the vertical dimension.

Conclusions: Few descriptions of oral features occurred in literature between congenital findings and "acquired" pathologies, such as missing teeth. The oral phenotype of these patients remains insufficiently described, while it may contribute to diagnosis. Early diagnosis may prevent delayed teeth loss and improve quality of life.

0188

COVID-19 Risk Perception in a Clinical Dental Education Setting

Mariana Morgado[1], José João Mendes[1, 2], Luís Proença[2, 3]

[1]Clinical Research Unit from CiiEM - Centro de investigação interdisciplinar Egas Moniz, Egas Moniz Higher Education School, Almada, Portugal, [2]Evidence-Based Hub from CiiEM - Centro de investigação interdisciplinar Egas Moniz, Egas Moniz Higher Education School, Almada, Portugal, [3]Quantitative Methods for Health Research from CiiEM - Centro de investigação interdisciplinar Egas Moniz, Egas Moniz Higher Education School, Almada, Portugal

Objectives: This study was aimed to assess COVID-19 perceived risk and its impact on attitudes and management practices of patients exhibiting symptoms compatible with the disease, in a clinical dental education setting.

Methods: A total of 352 participants were enrolled in the study, including senior undergraduate dentistry students (177), fresh graduates (67), dentists/teachers (85) and clinical staff (23), from an university dental clinic located in the southern Lisbon area (Almada, Portugal). The survey, conducted through the application of a self-administered questionnaire, took place from November 2020 to March 2021, during the most striking epidemiological phase of the COVID-19 pandemic in Portugal. Self-perceived risk was assessed through a total numerical score obtained from the individual answers, expressed in a 5-point Likert scale, to a set of COVID-19 risk perception representative questions. For ease of data handling and interpretation the score was converted to a percentage scale. Data were analyzed using descriptive and inferential statistical methodologies.

Results: Results indicate an overall high COVID-19 perceived risk (mean value of 86.3%). No significant differences in risk-perception were found among the four participant groups (p=0.058) as well as when considering a previous infection (p=0.337) or a possible exposition to confirmed or suspected cases (p=0.713). However, a significantly lower risk-perception (p=0.006) was identified for individuals classifying COVID-19 as a moderately dangerous disease when comparing with the ones that classify it as very dangerous (mean values 84.4 vs. 87.2%). Despite this, no significant differences were found when accounting for the attitudes and practices in the management of patients exhibiting SARS-CoV-2 infection-like symptoms, as a function of the disease perceived risk degree (p=0.895).

Conclusions: In face of the results, it is recommended to improve the COVID-19 risk perception by using dedicated measures in the clinical dental education context.

0189

Dental Students' Attitudes Towards Distance Learning During COVID-19 Pandemic

Jovana Kuzmanovic Pficer[1], Miroslav Dragovic[1], Aleksandra Popovac[1], Andja Cirkovic[2], Natasa Milic[2], Biljana Milicic[1]

[1]School of Dental Medicine University of Belgrade, Belgrade, Serbia, [2]Faculty of Medicine University of Belgrade, Belgrade, Serbia

Objectives: The Covid-19 pandemic has led to a change in the way teaching occurs in educational institutions worldwide, which is why distance learning has today become an integral part of education. The aim of the study was assessment of attitudes of dental students about online learning.

Methods: The study was conducted on the first, third, and fifth-year students of the Faculty of Dentistry in Belgrade. The specially designed online questionnaire consisted of 18 items about students' attitudes towards distance learning. Discriminant validity was tested by gender and year of study. Reliability was estimated using Kronbach alpha coefficient (α=0.801).

Results: The research included 190 students, of which 25.3% were male and 74.7% female students, with an average age of 21.1±2 years. 78.7% of students stated that online learning gives them freedom in terms of organization of learning time without a significant difference among years of study (p=0.200). Results show that 54% of first-year, 74.4% of third-year, and 70.7% of fifth-year students completely agree on irreplaceability of clinically gained experience without a statistically significant difference (p=0.145). 66.7% of first-year students highly rated the amount of acquired knowledge, while approximately 30% of third and fifth-year students showed satisfaction, with a statistically significant difference (p=0.020). 55.3% of male students prefer online learning compared to 38.8% of female, while 52% of male and 36.2% of female students would recommend it to other students (p=0.310). 68.4% of respondents think that the best combination for classes is online lectures and clinical practice at the faculty.

Conclusions: The results show that dental students are satisfied with the way online classes are organized and conducted in a pandemic. However, it is important to find ways to compensate for contact with patients and experience in clinical exercises.

0190

Dental Care During COVID-19 Pandemic: Follow-up Survey of Experts' Opinion.

Katarzyna Gurzawska[1, 2], Giulia Brunello[3], Kathrin Becker[4], Jürgen Becker[3], Stefano Sivolella[5], frank Schwarz[6], Björn Klinge[7]

[1]Oral Surgery, University of Birmingham, Institute of Clinical Sciences, College of Medical & Dental Science, Birmingham, United Kingdom, [2]Oral Surgery, Birmingham Community Healthcare NHS Foundation Trust, Birmingham, United Kingdom, [3]Oral Surgery, Universitätsklinikum Düsseldorf, Düsseldorf, Germany, [4]Department of Orthodontics, Universitätsklinikum Düsseldorf, Düsseldorf, Germany, [5]Oral Surgery, University of Padova, Padova, Italy, [6]Oral Surgery, Goethe University Frankfurt, Frankfurt, Germany, [7]Malmo University, Malmo, Sweden

Objectives: The purpose of the present survey is to give an update of European experts' opinion on infection control and prevention in dentistry during second wave of pandemic. The secondary aim was to analyse how experts' opinion changed in the light of the new scientific evidence since the first wave.

Methods: An anonymous online 14-item questionnaire was sent to a total of 27 leading academic experts in Oral (and Maxillofacial) Surgery from different European countries, who had completed a previous survey in April-May 2020. The questionnaire covered the topics of dental setting safety, personal protective equipment (PPE), and patient-related measures to minimise transmission risk. Data collection took place in November-February 2020/21.

Results: 26 experts participated in the follow-up survey. The overall transmission risk in dental settings was scored significantly lower compared to the initial survey (P<0.05), though the risk associated with aerosol generating procedures (AGP) was still considered to be high. Maximum PPE was less frequently recommended for non-AGP (P<0.05), whereas the majority of experts still recommended FFP2/FFP3 masks (80.8%), face shields or goggles (88.5%), gowns (61.5%), and caps (57.7%) for AGP. Most of the experts also found mouth rinse relevant (73.1%) and reported to be using it prior to treatment (76.9%). No uniform opinion was found regarding the relevance of COVID-19 testing of staff and patients.
Conclusions: With the continuation of dental care provision, transmission risk has been scored lower compared to the first wave of pandemic. However, high risk is still assumed for AGP, and maximum PPE remained advised for the respective treatments.

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Virucidal Activity of Oral and Nasal Antiseptics Against SARS-CoV-2: a Systematic Review

Claudia Sillero-Durán, Triana Blanco-Pintos, Alba Regueira-Iglesias, Benjamín Martín-Biedma, Carlos Balsa-Castro, Inmaculada Tomás
Oral Sciences Research Group, Universidade de Santiago de Compostela, FIDIS, Santiago de Compostela, Spain

Objectives: The aim was to analyse the viral-load reductions brought about by the virucidal activity of oral and nasal antiseptics against SARS-CoV-2.
Methods: The article search was conducted using the following electronic databases: PubMed, World Health Organization, Scopus, and Embase. Due to the large number of pre-print publications on COVID-19, a search was also performed of the bioRxiv and MedRxiv databases. Studies that evaluated the virucidal efficacy of any antiseptic against SARS-CoV-2 were eligible for the research (PROSPERO registration number: CRD42020185752).
Results: Thirty-nine articles fulfilled the inclusion criteria. Povidone iodine (PVP-I), chlorhexidine (CHX) and cetylpyridinium chloride (CPC) were the most commonly used antiseptics in the *in vitro* research and PVP-I and CHX in the in *vivo* studies. PVP-I at concentrations of 0.5-1.5% and contact times of 15-60 seconds produced in vitro viral-load reductions ranging from ≥2.5 to >5 log10; for CPC (0.04-0.1%; applications of 20-30 seconds), the reductions were 3 to >5 log10 and for CHX (0.12-0.2%; applications of 30-60 seconds) they were 0.2 to <2 log10. In relation to the *in vivo* studies, although there are findings on the ineffectiveness of PVP-I and CHX, other series revealed that these antiseptics (1% and 0.2% concentrations, respectively) caused viral load reductions of >1 log10 at 60-90 min after their oral application. This virucidal effect seems to be maintained for days with the continued use of these antiseptics.
Conclusions: PVP-I, CPC and CHX have demonstrated *in vitro* virucidal activity against SARS-CoV2, with that of the first two being higher than that of CHX. PVP and CHX appear to demonstrate *in vivo* virucidal activity against this virus, although the evidence is limited and heterogeneous. Further *in vivo* research is therefore essential to corroborate the efficacy of oral and nasal antiseptics in reducing the viral loads of SARS-CoV-2.

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Different Mouthwashes in Sars-CoV2 Prevention: State of the Art

Silvia D'agostino[1], Alessandra Bissoli[1], Lucia Caporaso[1], Francesca Iarussi[1], Riccardo Pulcini[2], Marco Dolci[1]
[1]Medical, Oral and Biotechnological Sciences, University of Chieti Pescara, Chieti, Chieti, Italy, [2]Innovative Technologies in Medicine and Dentistry, University of Chieti, Chieti, CH, Italy

Objectives: Oral antiseptics play a crucial role against infection by lowering the viral load in the oral cavity, and their use is widely applied in standard oral care procedures in the attempt to minimize local infective complications. During pandemic spread, it seemed to be applicable also in lowering Sars-CoV2 viral load and infections, as highlighted in literature, although no evidence is provided on the major efficacy of a specific molecule. The aim of this narrative review was to investigate which compound is the most effective in infection control.
Methods: An electronic search was performed in PubMed database through a cross-match of MeSH (mouthrinses and covid). Abstract availability, free full text, english language, publication between 01/10/2020 and 31/03/2021 were used as inclusion criteria.
Results: As the first research gave 77 results, after the inclusion criteria selection only 19 papers were considered to become part of this review. Chlorhexidine (CHX) together with catechin and quercetin appeared the most suitable one, due to its high affinity with Sars-CoV-2 virus. As suggested by American Dental Hygienists Association (ADHA), Cetylpyridinium chloride (CPC) seemed to produce good results concerning viral load lowering also, although strong clinical and in vitro studies are missing. Mouthrinses with CPC, CHX, Povidone Iodine (PI) were compared with water or cosmetic commercial products in studies with a limited sample population, resulting in the major effectiveness of CPC and PI.
Conclusions: Based on the currently available evidence in literature, many processes could be effective in lowering viral load in aerosol producing procedures, and this seem to be crucial in oral surgery and dental treatments, including oral hygiene. High heterogeneity of procedures described in literature suggest the need of clinical trials to establish common protocols to reduce virus spread and improve caregivers safety.

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Impact of COVID-19 Pandemic on Dental Medication Prescribing in Croatia

Ivana Sutej, Kristina Peroš, Krešimir Bašić

Pharmacology, School of Dental Medicine University in Zagreb, Zagreb, Zagreb, Croatia

Objectives: Everyday clinical work and consequently medication prescribing for dental practitioners have been influenced by the coronavirus disease 2019 (COVID-19) pandemic, mostly due to restriction measures. We investigated the impact of the pandemic on the prescribing pattern of dentists in Croatia.

Methods: Data related to prescription practice used in this research were delivered by the Croatian Health Insurance Institute, a national insurance company for the years of 2019. and 2020. The number of dentists' prescriptions, the cost of medicines in national currency (Croatian Kuna; HRK), and the number of packages prescribed have been included in the analysis.

Results: For the pandemic year 2020. the prescribing practice was higher than in 2019., with antibiotics, analgesics, and antiseptics showing the highest trend in change. The total number of prescriptions increased by 3,5% and the cost increased by 4%. The most dramatic increase was accounted for broad-spectrum antibiotic azithromycin with an increase in prescriptions for 39,3% between years. Amoxicillin with clavulanic acid as the most commonly prescribed medication showed continuation in an increase (+4,1%). Prescribing of ibuprofen and ketoprofen was 9.1% and 11% greater in 2020. respectively. The increase in dispensing antiseptics in total was 24%. A decrease in the pattern was shown for antifungal medications and for antibiotics amoxicillin and cephalexin.

Conclusions: The COVID-19 pandemic has been associated with a marked increase in medication prescribing in Croatia. Restricted access to dental care due to COVID-19 resulted in greatly increased dental antibiotic prescribing, added on a preexisting upward trend. Adaptation of dental care to the COVID-19 era needs to ensure access for all to high-quality urgent dental care and dental prescribing has to remain evidence-based during the pandemic period to ensure rationality and good clinical practice.

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Bonding Effectiveness of new 1/2-Step HEMA-Free Universal Adhesives to Dentin

Mohammed H. Ahmed[1, 2], Benjamin Mercelis[1], Bart Van Meerbeek[1]

[1]Department of Oral Health Sciences, BIOMAT & UZ Leuven (University Hospitals Leuven), KU Leuven (University of Leuven), Leuven, Belgium, [2]Department of Dental Biomaterials, Tanta, Egypt, Tanta University, Tanta, Egypt

Objectives: Despite promoting watersorption and hydrolysis at the adhesive interface, the mono-functional monomer 2-hydroxyethyl methacrylate (HEMA) is still frequently added to universal adhesives (UAs) to prevent phase separation and improve dentin-surface wetting. This study aimed to measure the bonding effectiveness of two 1/2-step HEMA-free UAs.

Methods: The 'immediate' and 'aged' micro-tensile bond strength (μTBS) to flat bur-cut dentin upon 1-week (1w) and 50,000 (TC) thermocycling, respectively, of the 2-step Healbond Max ('HbMax'; Elsodent) and 1-step Healbond MP ('HbMP'; Elsodent) UAs was investigated, with Scotchbond Universal ('SBU'; 3M Oral Care) serving as a reference along with the gold-standard 2-step self-etch (SE) adhesive Clearfil SE Bond 2 ('C-SE2'; Kuraray Noritake) and 3-step etch&rinse (E&R) adhesive OptiBond FL ('Opti-FL'; Kerr), totaling to 5 experimental groups (n=8). All adhesives were applied following the respective manufacturer's instructions, with a split-tooth design in 2/3-E&R and 1/2-SE bonding mode solely employed for the UAs investigated. Data were analyzed using 3-way ANOVA followed by a Tukey post-hoc test ($p<0.05$). Fractographic analysis was carried out using light microscopy followed by SEM ultrastructural analysis of representative fractured specimens.

Results: The adhesives investigated did not significantly differ for μTBS from the references at 1w in E&R mode. SBU_SE recorded significantly the lowest μTBS at 1w. After 50k TC, no significant difference in μTBS was observed between any experimental groups.

Conclusions: The new HbMax and HbMP UAs equally effectively and durably bonded to dentin when employed in E&R or SE bonding mode and as compared with the other reference and gold-standard adhesives investigated.

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Bonding New 2-Step Universal Adhesives to High C-Factor Dentin

Chuliang Tang[1], Mohammed H. Ahmed[1, 2], Chenmin Yao[1, 3], Ben Mercelis[1], Bart Van Meerbeek[1]

[1]Department of Oral Health Sciences, BIOMAT – Biomaterials Research group & UZ Leuven (University Hospitals Leuven), Dentistry, KU Leuven (University of Leuven), Leuven, Belgium, [2]Faculty of Dentistry, Department of Dental Biomaterials, Tanta University, Tanta, Egypt, [3]The State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) & Key Laboratory of Oral Biomedicine Ministry of Education, School & Hospital of Stomatology, Wuhan University, Wuhan, China

Objectives: To determine the bonding effectiveness of experimental 2-step universal adhesives (UAs) to high C-factor class-I cavity-bottom dentin and to assess the potential bond-strength contribution of an additional flowable composite layer.

Methods: Three experimental two-step UA formulations, involving the application of a 10-MDP-based primer followed by a hydrophobic adhesive resin differing only for filler, referred to as BZF-21 (containing bioglass), BZF-29 (silica) and BZF-29_hv (higher silica filler-loading resulting in a higher viscosity), all prepared by GC, along with three representative commercial adhesives, Clearfil SE Bond 2 (C-SE2, Kuraray Noritake), G-Premio Bond (G-PrB, GC) and Optibond FL (Opti-FL, Kerr), were comparatively investigated for their 'immediate' and 'aged' (50k thermo-cycles) micro-tensile bond strength (μTBS), when applied either in etch-and-rinse (E&R) or self-etch (SE) mode, to high C-factor class-I cavity-bottom dentin (n=10; 10 experimental groups). Four additional experimental groups involved the additional application of the flowable composite G-ænial Universal Flo (GC), employed as an intermediate liner in combination with the adhesives BZF-29 and G-PrB, again applied both in E&R or SE mode. Statistical analysis was performed using the Kruskal-Wallis test ($p<0.05$).

Results: All two-bottle universal adhesives performed similarly when compared to the gold standard E&R Opti-FL and SE C-SE2 adhesives and significantly outperformed the one-bottle UA G-PrB. No significant reduction in µTBS upon aging was recorded. The additional flowable composite layer significantly improved G-PrB's µTBS when applied in SE mode.
Conclusions: The experimental 2-step UAs revealed favorable bonding performance in this challenging high C-factor class-I study set-up, comparable to that of the multi-step gold-standard E&R and SE adhesives and superior to that of the 1-step UA investigated, the latter conclusion also previously recorded in similar studies using other 1-step adhesives. An additionally applied flowable composite layer may compensate for the lower bonding effectiveness of 1-step UAs in high C-factor cavity configurations.

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Evaluation of Durability of Bonding Layer in Multi-Step Adhesives

Miki Yamashita, Kyosuke Hirano, Futoshi Fusejima
GC Corporation, Tokyo, Japan

Objectives: In general, multi-step adhesives are reported relatively high bond strength because they have separate bonding step as 2nd or 3rd step with the bonding liquid which forms thick bonding layer and has high mechanical strength. However, most of multi-step adhesives contain HEMA even in such bonding liquid to obtain compatibility with tooth structure, and HEMA is concerned about mechanical durability due to its high hydrophilicity. We have developed a new 2-step self-etch adhesive "G2-BOND Universal" including the bonding liquid, called as 2-BOND with hydrophobic composition and HEMA-free. In this study, mechanical durability for the bonding liquid of G2-BOND Universal and other multi-step adhesive multi-step adhesives were evaluated as bonding layer durability.
Methods: The materials used were G2-BOND Universal (GC, G2B), SE BOND2 (Kuraray, SE2) and OptiBond FL (Kerr, OFL). Flexural strength specimens were prepared in accordance with ISO4049:2019. The specimens were separated in 3 groups for storage, immersed in water at 37 degree C for 1 day(1D), 1 month(1M) and subjected to 20000 thermal cycling (5-55 degree C, 30sec.) (TC20000). Flexural strength wasere measured by using a universal testing machine (AG-IS, Shimadzu Corporation) (n=5). Data were analyzed by Tukey test ($p<0.05$).
Results: Flexural strength of SE2 and OFL were decreased significantly after 1 month immersion in water1M and TC20000 compared with 1 day immersion in water1D. Since thereSE2 and OFL contain HEMA, which may easily uptake water, it might be considered that flexural strength of SE2 and OFL were degraded due to its water sorption. On the other hand, G2B maintained flexural strength both even after 1 month immersion in water1M and TC20000 because G2B is has highly hydrophobic composition as HEMA-free and less water sorption can be expected.
Conclusions: Since G2B is HEMA-free on the bonding liquid, its flexural strength did not decreased by immersing in water for 1 month and after thermal cyclingTC20000thermal cycling. As results, it might be indicated G2-BOND UniversalG2B can be expected to have excellent durability in clinical practice.

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The Effect of Heating on Chemical Polymerized Bonding Material

Masahiro Yumitate[1], Atsushi Mine[1], Mami Higashi[1], Mariko Matsumoto[1], Munechika Takaishi[1], Ryosuke Hagino[1], Bart Van Meerbeek[2], Hirofumi Yatani[1], Shoichi Ishigaki[1]
[1]Osaka University, 1-8 Yamadaoka, Suita-city, Osaka, 565-0871, Japan, [2]KU Leuven, Leuven, Belgium

Objectives: This study evaluated the effect of heating after treatment of the dentin surface with chemically polymerized bonding material (Bondmer lightless, Tokuyama Dental, BL).
Methods: Fifteen human molars were cut at the height of the contour and exposed dentin and then polished with #600 silicon carbide paper. The surface was air-dried for 5 seconds, and the mixed BL was applied to the dentin surface. The experimental groups were divided into four groups according to the surface treatment after applying BL: the P-Cont group, air-blown at 23 °C for 10 s; the HA group, air-blown at 85 °C for 10 s; and the LT group, light irradiation for 10 s. The group that did not undergo any treatment was designated the negative control (N-Cont) group. A resin composite (EstecemII, Tokuyama Dental, Tokyo, Japan) was then built up by 2-mm-thick layers at a time and then light-cured. The specimens were stored in water at 37 °C for 24 hours and then sectioned into beams with a cross-sectional area of 1 mm^2. Micro-tensile bond strength (µTBS) was measured at 24 hours or after storage in water for 1 or 6 months (n=16). The fracture surfaces after the µTBS test were then observed by scanning electron microscopy, and the temperature of the dentin surface at the time of surface treatment was measured with a thermal imaging camera (FLIR, CHINO, Tokyo, Japan).
Results: The µTBS value was significantly higher for the HA (52.0 MPa) and LT groups (45.3 MPa) than for the N-Cont group (27.2 MPa, $p<0.001$). The HA, LT, and N-Cont groups showed a lower rate of interfacial fracture than the P-Cont group, with cohesive fracture within the resin in some specimens.
Conclusions: The bond strength of chemically polymerized bonding materials to dentin was improved by increasing the temperature after applying the bonding materials.

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Long-Term Bond Strength of an Experimental Dual-Curing Universal Adhesive

Michael Barbisch, Céline Zilian, Thomas Koehler, Thorsten Bock
Ivoclar Vivadent, Schaan, Liechtenstein

Objectives: In adhesive cementation of indirect-restorations, clinical success of the luting-system is often correlated with bond-strength. However, initial bond-strength offers little information regarding long-term-stability of the bonded interface. To provide a clinically performing cementation a stable performance of the luting-materials employed in all indicated etching- and curing-modes is paramount. This study evaluates effects of 1 month water-storage on the shear-bond-strength (SBS) between a experimental dual-curing universal-adhesive and a clinically-established dual-curing luting composite. The variables tested for influence on adhesive performance were: etching-mode, curing-mode and tooth-subtrate.

Methods: SBS to bovine incisors was tested according to ISO/TS-29022 (N=5 each group), employing an experimental universal-adhesive (EXDC), 37% phosphoric-acid-gel (Total Etch), Variolink Esthetic DC (VLEDC) and a curing-light (Bluephase Style) (all Ivoclar Vivadent). The adhesives was applied in self-etch and etch&rinse mode and either pre-cured (PC, 10s, Bluephase-Style, 1200mW/cm^2) or not pre-cured (NPC). Fresh composite-rods (Tetric EvoCeram) were sandblasted (100my, Al_2O_3, 1bar), coated with cement, applied to the adhesive-layer, loaded with 500g (15min, room-temperature) and stored in water (24h or 1month, 37°C) before testing. Table 1 shows tested material/mode combinations. Testing employed a ZWICK-ROELL Z010 Universal-Testing-Machine (500N load-cell, 1.0 mm/min.

Results: SBS determined is shown in Table 2. Table 2 shows that SBS-values were stable for at least 1 month of water-storage. Initial SBS-values without pre-curing (NPC) were comparable or higher than with pre-curing (PC). For NPC-groups values for E&R-samples were consistently higher than for SE-samples. For PC-groups SBS increased during storage regardless of etching-mode.

Conclusions: Within the limitations of this investigation, dentin and enamel shear-bond-strength obtained with an experimental dual-curing universal-adhesive and self-cured Variolink Esthetic DC proved tolerant to at least 1month of water-storage, regardless of adhesive's curing- or etching-mode.

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Effect of Dual-Curing Adhesives and Thermocycling on Shear Bond Strength

Thomas Koehler, Michael Barbisch, Christa Lanz, Céline Zilian
Ivoclar Vivadent, Schaan, Liechtenstein

Objectives: The clinical success of indirect restorative treatment is correlated with the bond-strength of bonding-cement-systems. However, initial bond-strength-values offer little information regarding long-term-stability of the bonded interfaces. To ensure clinically reliable cementation long-term-stable performance of the luting materials employed is paramount. This study evaluated the effects of thermocycling on the shear-bond-strength (SBS) to bovine dentin and enamel of a new commercial dual-cure universal-adhesives and an experimental dual-cure adhesive with manufacturers' respective cements. To simulate prolonged aging, initial SBS (24h in water) was compared to SBS after 10'000 thermocycles.

Methods: SBS was tested bovine teeth prepared according to ISO/TS-29022 (N=5). Adhesives were tested with respective manufacturer's cements, combining Scotchbond-Universal-Plus (SBU+, 3M-ESPE) with Relyx-Universal (RXU, 3M-ESPE) and an experimental single-component-adhesive (EXDC, Ivoclar-Vivadent) with Variolink-Esthetic-DC (VLEDC, Ivoclar-Vivadent) according to IFU. Two etch-modes were used: self-etch (SE) and etch&rinse (E&R), all materials were used without light-curing step (SC). Table 1 shows Experimental Groups. Fresh composite-rods were blasted with Al_2O_3 (100my, 1bar), treated with cement and fixed to the adhesive-treated tooth (500g load, 15min, room-temperature). SBS was measured either after 24h (37°C, water, 24h), or thermocycling (10'000 cycles, 5°C-55°C, 30s dwell-time). SBS was determined using a ZWICK-ROELL Z010 Universal-Testing-Machine (500N load-cell, crosshead-speed 1.0 mm/min).

Results: SBS Results are shown in Table 2. Regardless of aging method, values for EXDC/VLEDC were higher for each group compared to SBU+/RXU.

Conclusions: Within the limitations of this investigation the Experimental Adhesive SC exhibited higher SBS values than the Scotchbond Universal Plus. Thermocycling did not affect the SBS of EXDC/VLEDC but it decreased the SBS of SBU+/RXU significantly.

0200

Wettability of Dried Dentin After Biomodification by Collagen Crosslinkers

Kaveh Nikjamal, Arzu Tezvergil-Mutluay
Department of Cariolgy and Restorative Dentistry, Institute of Dentistry, University of Turku, Turku, Southwest Finland, Finland

Objectives: Recently, modification of dentin surfaces using collagen crosslinkers has been introduced to improve the stability of resin-dentin bonds. Moreover, removal of water from dentin is essential for preventing enzymatic degradation of collagen. The aim of this study is to evaluate the wettability of dried demineralized dentin surfaces treated with various concentrations of grape seed extract (GS) or glutaraldehyde (GA).

Methods: Mid-coronal dentin discs (1mm), etched with phosphoric acid for 15s and crosslinked for 60s with GS or GA solutions with 1,2.5,3.5,5,7.5,10,15,20,25,27,30,35 wt% concentrations. The group with no crosslinker pretreatment served as control. Dentin wettability was measured with contact angle measurements using the sessile drop method by Young-Laplace curve fitting mode using an etch and rinse adhesive system (Scotchbond Multi-Purpose, 3M-ESPE). A 3 µl drop of adhesive or primer was dispersed on crosslinked blot-dried dentin and the change in contact angle up to 200s was evaluated. Data were analyzed by one-way ANOVA with the significance level set at 0.05.

Results: Mean contact angle degrees and standard deviation (SD) ranged over time between 37 to 21 (1.7<SD<6.1) for adhesive and 26.3 to 9.5 (1.2<SD<5.5) for primer. No direct correlation between the increasing solution concentration and wettability was observed. Contact angles of adhesive on GS 2.5,10 and GA2.5-5,10 wt% did not significantly increase from the control within 20s ($p>0.05$). Similarly, contact angles of primer on GS2.5,10 and GA3.5 wt% did not indicate any significant difference from untreated dentin ($p>0.05$). The spreading evolution data showed that most of changes occur within the first 20 seconds after the application of drops.
Conclusions: Low-concentration crosslinking of demineralized dentin matrices provides a suitable condition for dry bonding, as they can be wetted successfully within the 20s by an etch and rinse adhesive system. The hydrophobic adhesive layer combined with the dry crosslinked dentin can facilitate more durable dental restorations.

0201

Micro-Tensile Bond Strength of Bulk-Fill Resin Composite to Dentin

Omer Taha Meric, Elif Ozturk Bayazit
Department of Restorative Dentistry, Hacettepe University, Ankara, Turkey

Objectives: The aim of this study was to compare the micro-tensile bond strength (μTBS) of a bulk-fill resin composite cured using two different times to dentin prepared with different surface procedures.
Methods: Twelve freshly extracted noncarious human molar teeth were immersed in acrylic resin and cut 4 mm in height from the highest tubercle crest towards cervical. Half of the dentin surfaces were received 37% phosphoric acid pretreatment for 15 seconds (s), rinsed with water for 20s and gently dried, maintaining wet-bonding. The other half of the samples have not been pre-treated. A universal adhesive was applied to (Single Bond Universal,3M-ESPE) all the dentin surfaces, rubbed for 20s, air-dried for 5s and light-cured for 10s with a LED unit. Tofflemire matrix was adapted on each tooth and a bulk-fill resin composite (Tetric EvoCeram Bulk-Fill, Ivoclar Vivadent) was applied 5 mm in thickness. Totally, four groups were included (n=3 teeth for each group). The tooth-composite blocks were cured either with 20 seconds or 40 seconds with the LED unit. All of the specimens were stored in distilled water for 24 h at 37°C. The samples were sectioned under water cooling to obtain multiple sticks with dimensions of approximately 1.0 mm × 1.0 mm × 10 mm using the non-trimming technique. The μTBS test was performed (n=10 sticks for each group), and the obtained data were statistically analyzed via Mann Whitney U test (SPSS 20.0).
Results: Significant differences were found among the experimental groups ($P < 0.05$). The μTBS values of the acid etch and 40 seconds light curing group (Median±IR=14.8±1.8) were higher than those with the acid etch and 20 seconds light curing group (Median±IR=8±0.8) ($P < 0.05$).
Conclusions: In bulk fill resin composites, the higher the curing time, the higher the bond strength to dentin.

0202

Evaluation of Immediate Bonding Property of Resin Cement

Keita Sato, Kyosuke Hirano, Futoshi Fusejima
GC Corporation, Tokyo, Japan

Objectives: In case of indirect restoration, bonding property of resin cement greatly affects the life of restorations. Especially in immediate bonding property after seating, it can be easily affected by external factors such as saliva exposure, unexpected occlusal forces and stresses when removing excess cement because the resin cement may not sufficiently cured yet. As a result, this may cause initial dropout and be important factor for the life of the restorations. The purpose of this study was to evaluate immediate bonding property of resin cements.
Methods: Three resin cements (G-CEM ONE (GCO, GC) with G-CEM ONE Adhesive Enhancing Primer (AEP, GC), RelyX Universal (RXU, 3M) with Scotchbond Universal Plus (SUP, 3M) and Panavia V5 (PV5, Kuraray) with Panavia V5 Tooth Primer (PTP, Kuraray)) were used in this study. Specimens of bovine anterior tooth dentin were embedded with acrylic resin. Exposed flat dentin surface was finished with 600-grit SiC paper. Bonding area (3.0mm diameter) and cement thickness (0.1mm thickness) were defined by plastic-tape. Stainless-steel rod was adhered to dentin by using each primer and resin cement according to the manufactures' instructions. Bonded specimens were stored in 37°C 100%R.H. for 5 minutes (5min as immediate) or in 37°C distilled water for 24hours (24h). Shear bond strength (SBS) was measured by SHIMADZU AG-IS. [Crosshead-speed 1mm/min, n=5, statistically analyzed (Tukey-Kramer, $p<0.05$)].
Results: GCO+AEP showed statistically higher SBS than the other products in 5min. Even in 24h, GCO+AEP showed the highest SBS among tested products.
Conclusions: In this study, GCO+AEP showed higher immediate bonding property than the other products. It is suggested that this result is brought by touch cure feature, the chemical initiator in AEP which accelerates the chemical cure of the cement from the tooth surface and GCO+AEP may have a high durability for external factors.

0203

Evaluation of Shear Bond Strength of Glass-Ceramic CAD-CAM Materials

Tom Vrombaut[1], Rani D'haese[1], Carlos Eduardo Sabrosa[2], Karen Geber[2], Stefan Vandeweghe[1]

[1]Ghent University, Gent, Belgium, [2]Privat practice for oral rehabilitation and implantology, Rio de Janeiro, Brazil

Objectives: Recently, new reinforced glass-ceramics have been introduced. The aim of this study was to evaluate the shear bond strength (SBS) of 3 different CAD-CAM glass ceramics, luted with their corresponding adhesive cement.

Methods: Disc-like specimens were prepared from 3 different CAD/ CAM blocks (Table 1) and randomly divided into 6 groups (n=10). The surface of the specimens was etched with 5% HF for 20s followed by 5m cleaning in an ultrasonic bath. Stainless-steel rods (4mm diameter) were sandpapered, sandblasted (50µm Al_2O_3), treated with Metal Primer Z (GC) and subsequently cemented under standardized pressure (20g/mm2) onto the etched surface using the corresponding adhesive cement following the manufacturer's instructions or corresponding adhesive treatment (Table 1). SBS was performed in a universal testing machine (Shimadzu) at a crosshead speed of 1mm/min. Half of the samples were tested immediately. The remaining samples were kept in 37° in 100% humidity for 48 hours, followed by thermocycling (5000 cycles), then tested. Failure modes were analyzed using an optical microscope.

Results: SBS values are shown in table 2. Before thermocycling, e.max specimens demonstrated statistically significant higher SBS values (p<0,001). All failures were adhesive. After thermocycling, there was no statistically significant difference in SBS among the materials (p=0.110). Cohesive failures were observed in 50% of the Celtra Duo and 100% of the LiSi Block samples. LiSi Block group demonstrated a significant increase in SBS after thermoclycling (p=0.007), while in e.max group a significant decrease in SBS (p<0.001) was detected. Celtra Duo specimens were not affected by aging (p=0.938).

Conclusions: The results of this study suggest that thermocycling was beneficial to LiSi block. The three CAD/CAM blocks demonstrated comparable results after aging. The type of fracture differed among the materials.

0204

Effect of Primer on Bonding Durability of RMGI to Zirconia

Koji Tanaka, ENZO SHIDA, Daizaburo Mori, Futoshi Fusejima

GC Corporation, Tokyo, Japan

Objectives: The purpose of this study is to evaluate the effect of 10-methacryloyloxydecyl dihydrogen phosphate (MDP) containing primer on the bonding durability of RMGI luting cement, which does not contain MDP, to zirconia.

Methods: FujiCEM Evolve (FCE, GC), FujiCEM2 (FC2, GC) and RelyX Luting Plus Automix (RX, 3M ESPE) were used. Forty Yttria-stabilized-zirconia (Aadva Zr-Block, YSZ, GC) specimens were sandblasted (50µm alumina, 0.15MPa). An additional 40 YSZ-blocks were prepared as jigs for tensile testing. Their surfaces were sandblasting and primed with G-Multi Primer (GC). YSZ specimens were divided into 4 groups. Samples in group I were primed with G-Multi Primer and YSZ-block jig were cemented with FCE. Group II, III and IV samples were cemented with FCE, FC2 or RX without primer, respectively. Each group was subdivided into 2 subgroups (N = 5) according to storage condition. One was stored in water at 37degC for 24 hrs. The other was stored in water for 24hrs and then thermo-cycled (TC, 5-55degC, 30seconds dwell time) for 5000cycles. Tensile bond strength (TBS) test was performed (1mm/min) and statistically analyzed (t-test, p<0.05).

Results: TBSs of FCE were not significantly decreased after thermo-cycling with or without primer. On the other hand, TBSs of FC2 and RX were significantly decreased after thermo-cycling. The rate of decrease in bond strength of Rx was the highest. Cohesive failure of the luting cement was found most frequently in all groups.

Conclusions: FCE has superior bonding durability without the use of primers. For this reason, FCE has clinical advantages due to its ease of use.

0206

Light-Curing Composite Cement Through 3D-Printed and Milled 'Full-Contour' Monolithic Zirconia

Wout Jacobs[1], Bernardo Camargo[1], Evita Willems[1, 2], Stevan Cokic[1], Jef Vleugels[2], Bart Van Meerbeek[1]

[1]Department of Oral Health Sciences, KU Leuven, Leuven, Belgium, [2]Department of Materials Engineering, KU Leuven, Leuven, Belgium

Objectives: To assess light irradiance (LI) delivered by two LED light-curing units (LCUs) and to correlate LI with the polymerization degree of conversion (DC) of a light-curable composite, when light-cured through different thicknesses of one 3D-printed and six milled 'full-contour' zirconia ceramic plates.

Methods: One hundred and forty monolithic zirconia plates (3D-printed by Xjet Carmel 1400C, Xjet; Initial Zirconia HT and UHT, GC; Katana Zirconia HT and UTML, Kuraray Noritake; Lava Plus and Esthetic, 3M Oral Care) with varying thicknesses (0.5, 1.0, 1.5 and 3.0 mm) were prepared, upon which LI delivered through the plates by two LED LCUs (Bluephase G4, Ivoclar Vivadent; Demi Plus, Kerr) was measured using a light spectrometer (Marc Resin Calibrator, BlueLight Analytics). LI was correlated with DC of the composite Clearfil AP-X (Kuraray Noritake), as measured using micro-Raman spectroscopy (Senterra, BrukerOptik) at 5 min, 24 h and 1 week after light-curing.

Results: LI and DC were significantly influenced by zirconia kind and block thickness. No difference in LI and DC was recorded irrespective if the zirconia plates were prepared by additive or subtractive manufacturing. Although DC increased over time for all groups, time did not compensate for the lower DC measured after 5 min.

Conclusions: Although LI is affected by zirconia kind and block thickness, as well as depends on the LCU employed, LI decrease is not directly proportional to DC of the composite light-cured through zirconia. Light-curing composite through uncoloured 3D-

printed zirconia appeared most effective. Light-curing composite through zirconia to reach optimal polymerization requires a proper light-curing protocol with a well-selected LCU.

0207

Usability Properties of Resin Modified Glass Ionomer Cements.

Michelle Carey

SDI Limited, Bayswater, Victoria, Australia

Objectives: This study aims to evaluate and rank the usability of market leading resin modified glass ionomer cement (RMGIC) capsules. The usability factors covered in this study are as follows: activation force (AF), extrusion force (EF), paste viscosity (PV), paste stickiness (PS) and nozzle length.

Methods: Riva Light Cure*(RL), Riva Light Cure HV*(RH), Photac™ Fil Quick Aplicap™† (PF) and Fuji™ II LC‡ (F2) were evaluated in their respective capsules. Activation and extrusion forces were maximum values measured using Instron compression tests (Instron #5942 and #5566 respectively). In both cases, the tests started on detection of the plunger, data was collected from 0-4.5mm each 1.25N at a speed of 150m/min (activation) and 10-13.5mm each 10N at a speed of 45mm/min (extrusion). Paste Viscosity was the maximum value measured by a compression test with a Brookfield CT3 Texture Analyser (TA), measured each 0.1mm from 0 to 4.0mm penetration with a probe (LA39) travelling at 2.00mm/s in an internal diameter 5mm annulus. Paste Stickiness was evaluated from photos taken during a TA compression test using the TA8 Probe at 2.00mm/s. Nozzle length was measured using a vernier calliper. *SDI Limited, †3M, ‡GC Corporation.

Results: The results are reported in Table 2. Statistically significant differences were found in the extrusion forces, paste viscosity and nozzle length. There were no significant differences in activation forces between RL, RH and F2, but PF required an additional tool, reducing convenience. The extrusion force of RH was lowest (best on the criteria above), RL and PF were not significantly different, while F2 was highest. RL and F2 had the longest nozzle length, allowing access to awkward cavities, followed by RH and PF. Stickiness of RH was far lower than the other RMGICs. Each of the RMGICs were ranked across 4 the four handling attributes, and a total for each RMGIC calculated. RH and RL had the highest rank, followed by F2 and PF.

Conclusions: The overall usability of RH and RL compared very favourably to P2 and PF.

0208

Usability Study on Glass Ionomer Cements / Glass Hybrids.

Martin Ong, Paul Farrar

R&D, SDI Limited, Bayswater, Victoria, Australia

Objectives: This study aims to evaluate and rank the usability of market leading Glass Ionomer Cements (GIC) / Glass Hybrids (GH) in their respective capsule systems. The usability factors covered in this study are as follows: Activation Force (AF), Extrusion Force (EF), Paste Viscosity (PV), Paste Stickiness (PS) and Nozzle Design (ND).

Methods: Riva Self Cure/ SDI Limited (SC), Riva Self Cure HV/ SDI Limited (HV), Ketac™ Universal Aplicap™/ 3M (KU) and Equia Forte ® HT/ GC Corp (HT) were evaluated in their respective capsule delivery systems. Activation Force of a capsule (Plunger) was determined via the maximum value measured by a compression test (INSTRON #5942). Force values were recorded every 1.25 N over a travel distance of 4.5 mm at a travel speed of 150 mm/min. Extrusion Force of a capsule was determined via the maximum value measured by a compression test (INSTRON #5566). Force values were recorded every 10 N between the travel distance of 10 – 13.5 mm at a travel speed of 45 mm/min. Paste Viscosity of an extruded cement was determined via the maximum value measured by a compression test (Brookfield CT3 Texture Analyser). Force values were recorded every 0.1 mm over a travel distance of 4.0 mm with a probe (LA39) speed of 2.00 mm/s. Paste Stickiness of an extruded cement was evaluated via photos taken during a compression test (Brookfield CT3 Texture Analyser). Probe (TA8) was used at a travel speed of 2.00 mm/s. Nozzle design was evaluated based on the nozzle length measured vertically from the nozzle base to tip.

Results: Results indicate that: HV and SC performed best on Activation Force. HV performed best on Extrusion Force. HV and KU performed best on Paste Stickiness. SC and HT performed best on Nozzle Design. Refer to tables for further description of usability factors, detailed results, and evaluation of results.

Conclusions: Within this study, the results indicates that HV has the highest overall usability score (14), with SC being a close runner-up (13). This was achieved through a combination of a user-friendly capsule design and smart product design.

0209

Impact of Cold Atmospheric-Pressure-Plasma on Shear-Bond-Strength in Two-Piece-Abutments

Carolin-Isabel Görgen[1], Moataz Bayadse[1], Katharina Hegner[2], Doris Vollmer[2], Stefan Wentaschek[1]

[1]Department of Prosthetic Dentistry, University Medical Centre, Mainz, Germany, [2]Max-Planck-Institute for Polymer Research, Mainz, Germany

Objectives: The usage of CAD/CAM-manufactured single implant restorations is a common method in prosthetic dentistry. Several benefits lead to an adhesive connection of individualized ceramic crowns and prefabricated titanium bonding bases. However, the bonding has to resist permanent thermal, chemical and mechanical stress in the oral cavity. The durability of the bonding is a weak point and especially dependent on sufficient surface pretreatment. Cold atmospheric-pressure-plasma (CAP) is a conventional method for improving surface characteristics in car industry and space engineering. The aim of this study was to evaluate the impact of CAP on shear-bond-strength in two-piece-abutments.

Methods: Two different pretreatment protocols were tested in this study. Ten CAD/CAM-manufactured and sintered crowns of zirconia and ten prefabricated titanium bonding bases were divided into 2 groups (n=5): (A) as specified by the manufacturer (sandblasting (Al_2O_3, 50µm, 1bar) + adhesive primer), (B) sandblasting + CAP + adhesive primer. Optimal pretreatment with CAP (piezobrush PZ2, relyon plasma GmbH, Regensburg, Germany) was evaluated in pretrial studies by analyzing water contact angles on polished surfaces after different duration of exposure. The wetting properties suggested a minimal CAP application time of 15 seconds. After pretreating the interfaces, the pieces were glued together under standardized pressure by using Hybrid-Abutment-Cement by VOCO (VOCO GmbH, Cuxhaven, Germany). The specimens were thermocycled for artificial aging and then shear-bond-strength (SBS) was measured.
Results: The shear-bond-strength in two-piece-abutments pretreated with CAP was higher than in those without. Mean values differed significantly (Mann-Whitney-Test, p=0.008) from 601.6±86.3N (Group A) to 755.0±37.2N (Group B).
Conclusions: The study showed that additional usage of CAP for surface pretreatment in two-piece-abutments leads to a significant increase of shear-bond-strength by about 25%. Thus, CAP can be recommended as pretreatment procedure in this special field of indication. Further investigations by using different ceramic materials and luting cements are intended.

0210

Conditioning of Titanium Surface with Cold Atmospheric Pressure Plasma

Moataz Bayadse[1], Carolin-Isabel Görgen[1], Katharina Hegner[2], Doris Vollmer[2], Stefan Wentaschek[1]
[1]Department of Prosthetic Dentistry and Materials, Mainz, Rheinland-Pfalz, Germany, [2]Max Planck Institute for Polymer Research, Mainz, Rheinland-Pfalz, Germany

Objectives: Adhesive bonds are widely used in dentistry. Several treatment options depend on this technology. The surfaces of adhesively bonded materials are pretreated differently in order to increase the bond and their long-term stability. Plasma technology is also used in engineering for surface-conditioning. Plasma exposure increases the surface energy which leads to the formation of a reactive layer. Cold-atmospheric-pressure-plasma (CAP) affects the surface without changing the structure and is intended to improve surface wettability. This can be measured via the contact angles (CA) of a liquid drop. The aim of this study was to evaluate the influence of different exposure times of a CAP-treatment on surface wettability of a titanium-aluminum-vanadium-alloy (Ti6Al4V).
Methods: Titanium-alloy samples were polished in a standardized manner and their roughness (Ra, Rz) was measured (Perthometer, Mahr, Germany). 15 samples were plasma treated for 5, 10, 20, and 30 seconds, respectively. 15 samples in the control group were not plasma treated. Standardized plasma treatment (Piezobrush PZ3, reylon Plasma, Germany) was performed in a custom frame.CA were measured with water (3µl) using a goniometer (Krüss Advance 1.9.2.2, Germany). Statistical analysis was performed using ANOVA.
Results: The roughness parameters Ra (0.45 µm ± 0.12 µm) and Rz (3.5 µm ± 0.8 µm) does not show a significant variation between the samples. CA on the titanium surface is reduced by approx. 70% after 5 seconds of plasma treatment. The mean CA-values are presented in the table. The groups differed significantly ($p<0.001$).
Conclusions: Hydrophilicity of the surface further increased with prolonged plasma treatment. This could be due to a homogenization of the chemical properties. Through this the reliability of an adhesive connection could increase, for example when bonding hybrid restorations.

0210.1

Gelatinolytic Activity in Dentin Powder and at Adhesive-Dentin Interfaces

Xin Li[1], Jennifer Vandooren[2], Jorge Perdigao[3], Kirsten L. Van Landuyt[1], Bart Van Meerbeek[1]
[1]KU Leuven (University of Leuven), Department of Oral Health Sciences, BIOMAT & UZ Leuven (University Hospitals Leuven), Dentistry, Leuven, Belgium, [2]KU Leuven, Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Immunobiology, Leuven, Belgium, [3]University of Minnesota, Department of Restorative Sciences, Minneapolis, Minnesota, United States

Objectives: To investigate MMP-2/9 gelatinolytic activity in dentin treated with two gold-standard adhesives. Additionally, the effects of incubation and temperature change during light-curing of dental adhesives on MMP-2/9 activity were assessed.
Methods: The gelatinase levels and gelatinolytic activity in dentin powder and at the interface with dentin upon treatment with the three-step etch-and-rinse adhesive OptiBond FL ('OFL'; Kerr) and two-step self-etch adhesive Clearfil SE Bond 2 ('CSE2'; Kuraray Noritake) were evaluated using gelatin and *in situ* zymography, respectively. Recombinant human full-length MMP-2 (rhMMP-2, R&D Systems) and MMP-9 (produced in house) were activated and incubated (37°C) to measure 24-h and 1-w enzymatic activity using a fluorogenic DQ-gelatin assay. The temperature increase was recorded during light-curing of the adhesives using the LED light-curing unit Bluephase 20i (Ivoclar Vivadent) in 'HIGH' and 'TURBO' modes (10 s). The influence of this temperature change was also evaluated (fluorogenic DQ-gelatin assay).
Results: Gelatin zymography disclosed the presence of potentially active MMP-2/9 in dentin powder etched with phosphoric acid immediately and, although weaker, still after 1-w incubation. No gelatinases were detected in dentin powder treated with OFL or CSE2. *In situ* zymography revealed wide variation from very mild to very intensive interfacial fluorescence. The fluorogenic DQ-gelatin assay revealed a significant decrease in MMP-2/9 activity after 24-h (18.2/33.3%) to almost 0 after 1-w incubation (one-way ANOVA: $p<0.05$). Light-curing temperature increased up to 52-58°C and 57-60°C for OFL/CSE2 in high and turbo mode, respectively. MMP-2/9 gelatinolytic activity decreased significantly to 32.2/15.3% at 60°C and 11.8/9.9% at 70°C (one-way ANOVA: $p<0.05$).

Conclusions: While phosphoric-acid etching activated MMPs in dentin, the gold-standard adhesives generated no obvious MMP activation. *In situ* zymography produced inconsistent interfacial gelatinolytic activity. In solution, MMP-2/9 activity decreased to almost fully inactive after 1-w. Heat also decreased MMP-2/9 activity, though at temperatures slightly higher as induced by light-curing.

0210.2

Does Chloramine-T Affect Bonding of Universal Adhesives to Dentin?

Mariko Matsumoto[1, 2], Hidehiko Sano[2], Bart Van Meerbeek[1]

[1]Department of Oral Health Sciences, BIOMAT & UZ Leuven (University Hospitals Leuven), Dentistry, KU Leuven (University of Leuven), Leuven, Belgium, [2]Department of Restorative Dentistry, Hokkaido University Graduate School of Dental Medicine, Sapporo, Japan

Objectives: To determine the effect of tooth-storage solution, 0.5% aqueous chloramine-T (chl-T) versus distilled water (di), on the shear bond strength (SBS) to dentin of the two universal adhesives Tokuyama Universal Bond (TUB; Tokuyama) and Scotchbond Universal (SBU; 3M Oral Care).

Methods: Fifty caries-free human molars were randomly assigned to five experimental groups: TUB_di: teeth stored in di with TUB bonded to dentin; TUB_di_rub: idem with TUB actively rubbed onto dentin; TUB_chl-T: teeth stored in chl-T with TUB bonded to dentin; SBU_di; SBU_chl-T. The embedded teeth with P400 SiC-paper ground/exposed dentin were immersed in 37°C water for 1 h, upon which each adhesive was applied strictly following the manufacturer's instructions. Each tooth was next mounted into an Ultradent jig with the 3-mm diameter hole of the double-sided tape perfectly aligned to the center of the jig hole. Composite (Estelite Universal Flow, Tokuyama) was next added (3-mm height) and light-cured for 20 s (Bluephase 20i, Ivoclar Vivadent: 1200 mW/cm^2). Upon storage in 100% humidity at 37°C for 1 h, the bonded specimens were immersed in di at 37°C for 1 wk prior to being subjected to SBS. The data were analyzed by one-way ANOVA and Scheffé's post-hoc test at α=0.05 (SPSS statistics ver. 24, IBM). Failure analysis was conducted by scanning electron microscopy.

Results: SBS of SBU to dentin was significantly higher than that of TUB. Although SBS upon prior tooth storage in chl-T was lower than when stored in di for both TUB and SBU, this difference was not significant. Most fracture modes were recorded as 'adhesive interfacial failures' for TUB versus mostly 'mixed failures' for SBU.

Conclusions: Tooth storage in 0.5% aquous chloramine-T did not significantly affect bonding effectiveness of the two universal adhesives investigated to dentin.

0210.3

Influence of Different Enamel Pretreatments on Microtensile Bond Strength of Fissure Sealant

Dafina Doberdoli, Hrvoje Juric

Pediatric Dentistry and Prevention, University of Zagreb School of Dentistry, Zagreb, Croatia

Objectives: The aim of this *in vitro* study was to evaluate the microtensile bond strength of a resin-based pit and fissure sealant to human enamel after pretreated with a different laser pulse length of an Er: YAG laser (2940 nm) with or without additional acid etching.

Methods: Forty-two freshly extracted molars were randomized into the following six groups based on pretreatment choice: Grup1: Super Short Pulse Mode (SSP)50 µs, Group 2: Quantum Square Pulse Mode (QSP), Group 3: Medium Short Pulse Mode (MSP) 140 µs, Group 4: SSP+ acid Etch, Group 5: QSP+ acid Etch, Group 6: MSP+ acid Etch. After application of sealant teeth were prepared for the microtensile bond strength test. Microtesnsile bond strength was assessed and compared among the test groups. Two ways analyses of variance and the Games Howell method were used for statistical analyses.

Results: The lowest microtensile bond strength was registered in SSP group (24.79 MPa), followed by QSP + acid ECTH (27.92 MPa) and SSP + acid ECTH (28.16 MPa). The highest bond strength was registered in the group MSP +acid ECTH (36.09 MPa), and in the groups MSP and QSP the same bond strength was achieved (32.42 and 32.03 MPa, respectively). There were statistically significant differences between SSP, MSP, and QSP groups, despite the fact that etching is not a statistically significant factor there is its statistically significant influence in the interaction with the studied groups.

Conclusions: Under the conditions of this present in vitro study, additional acid etching did not significantly increase microtensile bond strength, and laser alone can be used for enamel pretreatment before composite fissure sealant placement.

0210.4

Microleakage Study of Three Different Adhesive-Resin Composite Combinations

Edoardo Ferrari Cagidiaco[1], Guenda Zucca[2], Marco Ferrari[3]

[1]Department of Prosthodontic and Dental Materials, Siena University, Siena, Italy, [2]Department of Prosthodontic and Dental Materials, Siena University, Siena, Italy, [3]Department of Prosthodontic and Dental Materials, Siena University, Siena, Italy

Objectives: The aim was to evaluate the influence of several adhesive systems on the microleakage of direct composite restorations with one proximal margin located below the cement-enamel junction (CEJ) and the other above in the enamel.

Methods: Standardized MOD cavities with proximal margins located 1mm below CEJ were prepared in 30 human molars and randomly divided into 3 groups. Three adhesive systems were combined with an injectable resin composite (G-aenial Universal Injectable). An incremental technique was followed to restore the cavities. In Group 1 G2-BOND Universal (GC), in Group 2 Clearfil SE Bond 2 (Kuraray) and in Group 3 OptiBond FL (Kerr) were used. Sample teeth were tested for microleakage scoring the amount of tracer (Silver nitrate) along the marginal interfaces in 5 degrees.

Results: At the dentin interfaces Group 1 scored 1,3, Group 2 2,3 and Group 3 1,3. At the enamel site Group 1 scored 0, Group 2 0,2 and Group 3 0,1. Differences in leakage at dentin and enamel adhesive interfaces were also evaluated for statistical significance ($P < 0.05$). At the dentin interface, statistically significant differences emerged in microleakage scores between Group 2 and the other two groups whilst no difference was found at the enamel interface.
Conclusions: The G2-BOND Universal showed the best marginal seal, similarly to OptiBond FL in both dentin and enamel interfaces. The enamel interfaces showed an extremely good seal whilst the dentin interfaces always showed leakage.

0211

Soft-Tissue Cell Response to Novel Functionally Structured Piezoelectric-Ceramic Composites

Beatriz Fernandes[1], Joana Marques[1], Mariana B. da Cruz[1], Laura Tiainen[2], Michael Gasik[3], Óscar Carvalho[2], António Mata[4, 5], Filipe Silva[2], João Caramês[6]
[1]Oral Biology and Biochemistry Research Group – UICOB, Faculdade de Medicina Dentária,Universidade de Lisboa, Lisbon, Portugal, [2]Center for Microelectromechanical Systems (CMEMS), Mechanical Engineering Department, University of Minho, Guimarães, Portugal, [3]Department of Chemical and Metallurgical Engineering, Aalto University, Espoo, Finland, [4]Oral Biology and Biochemistry Research Group – LIBPhys-FTC UID/FIS/04559/2013, Faculdade de Medicina Dentária,Universidade de Lisboa, Lisbon, Portugal, [5]CEMDBE - Cochrane Portugal, Faculdade de Medicina Dentária, Universidade de Lisboa, Lisbon, Lisbon, Portugal, [6]Implant & Tissue Regeneration Group - UICOB; LIBPhys - FCT UID/FIS/04559/2013, Faculdade de Medicina Dentária,Universidade de Lisboa, Lisbon, Portugal

Objectives: Barium titanate ($BaTiO_3$) piezoelectric ceramic is commonly employed to promote bone regeneration once can mimic the stress-generated potentials of natural bone. However, it is not yet known what are the effects on soft tissue. The aim of this study is to evaluate piezoelectric effect of barium titanate functionalized zirconia implant surfaces on human gingival fibroblasts behavior.
Methods: Composite discs with 5 wt.% $BaTiO_3$ in yttria stabilized zirconia (YSZ) were prepared through press-and-sintering technique (n=15). Contact poling was carried out in silicon oil bath under DC 2 kV/mm electric field at 130 °C for 30 min followed with field cooling. Reference samples of YSZ were processed parallel to the composites. Human gingival fibroblasts were cultured on discs for 7 days by previously described methods. Cell adhesion and morphology were observed at 1 day of culture using back-scattered electrons/scanning electron microscopy (BSE/SEM) and fluorescence microscopy. The average area occupied by each cell at 1 day of culture was calculated using the ImageJ software. Cell viability was evaluated at 1, 3 and 7 days using a commercial resazurin-based method. Results were presented as mean ± standard deviation (SD). Group comparisons were tested using ANOVA (Tukey's post-hoc) using appropriate statistical software and significance was set at $p<0.05$.
Results: Cell viability increased over time for all groups. However, no statistically significant differences in cell viability were observed between groups. The proliferation ratio decreased by 5-7% from 3 to 7 days of culture, with the exception of non-poled composite group which increased by 6% (1.22±1.21). Average cell area was significantly higher in YSZ group ($2.58x10^9±9.39x10^8 nm^2$) comparing to other groups, which showed similar results.
Conclusions: Despite the potentiating effects on bone regeneration, the piezoelectric effect of barium titanate-ceramic composites does not appear to influence cell viability and proliferation of fibroblasts when compared to the reference zirconia surface.

0212

Tracking Testing of Pulp Response of Indirectly Restored Depredated Vital Molars

Mariela Tsanova[1], Neshka A. Manchorova-Veleva[1], Plamen Zagorchev[2]
[1]Medical University, Faculty of Dental Medicine, Plovdiv, Bulgaria, [2]Medical University, Faculty of Pharmacy, Plovdiv, Bulgaria

Objectives: The aim of the present study is to evaluate whether the pulp vitality is preserved and fully recovered after indirect restoration for a period of 1 year by means of Electric Pulp Testing (EPT).
Methods: The pulp vitality of 32 molars with deep and extensive caries lesions was rated. For this purpose EPT was performed by means of electric tester (Scorpion Dental Optima). All of the teeth were isolated, dried and the intensity of the submitted stimuli is then increased. Tracking tests are made after cementation of zirconia restoration (Katana™ Zirconia, Kuraray Noritake Dental Inc.) and on the 6th, 9th and 12th month. Bonferroni's Multiple Comparison Test was used for statistical analysis of the results.
Results: The results from electrical pulp testing shows that there is statistical significance between the pre-operative values and those at 6th, 9th and 12th month and between those at 6th and 12th month. After excluding the main reason for tooth decay and achieving full airtight restoration, the results stabilized and we observe a constant decrease of values at 6th, 9th and 12th month in comparison to the control group.
Conclusions: The analysis of the gathered data shows a long-term result in aesthetic and function, as well as not only preserved pulp vitality of the restored teeth, but also a continuous resistant healing process. Favorable conditions for that is the sparing preparation, the excellent adhesion of the restoring material to the treated tooth tissues and the high biological potential of zirconia ceramic.

0213

Polymeric Doped Nanoparticles Induce Apatite Formation at Radicular Dentin.

María T Osorio[1], Manuel Toledano[1], Fátima S. Aguilera[1], Estrella Osorio[1], Manuel Toledano-Osorio[1], Germaine Escames[2], Antonio-Luis Medina-Castillo[3], Modesto T. López-López[4], Marta Vallecillo Rivas[1], Esther Muñoz-Soto[1], Raquel Osorio[1]

[1]Faculty of Dentistry, University of Granada, Granada, Spain, [2]Department of Physiology, Biomedical Research Center, Faculty of Medicine, University of Granada, Granada, Spain, [3]NanoMyP, Nanomateriales y Polimeros S.L., Spin-Off company of the University of Granada, Granada, Spain, [4]Faculty of Science, Applied Physics Department, University of Granada, Granada, Spain

Objectives: To investigate the effect of polymeric nanoparticles (NPs) doped with melatonin in hydroxyapatite formation at radicular dentin, after endodontic treatment.

Methods: 18 teeth were subjected to root canal treatments. Undoped-NPs (U-NPs) and ML-doped NPs (ML-NPs) were applied before the sealing cement. A control group without NPs was included. Teeth were sectioned exposing the interface between dentin and the sealing cement. Dentin surfaces were then studied by nano-hardness measurements, X-ray diffraction and transmission electron microscopy through selected area diffraction and bright-field imaging, after 24 h and 6 m of storage. ANOVA and Student-Newman-Keuls ($p<0.05$) were used for comparisons.

Results: Means and standard deviations of nanohardness (GPa) of dentin are displayed in the table. Letters indicate differences between NPs and numbers between storage time-points. Cervical dentin treated with undoped NPs maintained its nano-hardness values after 6 m of storage, but it decreased at apical dentin. When ML-NPs were used, nano-hardness was similar over time, regardless the dentin location. Root dentin treated with ML-NPs produced, in general, high crystallinity of new minerals, with thicker crystals than those produced in the rest of the groups. After 6 m, crystals became organized in randomly oriented polyhedral, square polygonal block-like apatite or drop-like apatite polycrystalline lattices when ML-NPs were used.

Conclusions: New polycrystalline formations encountered in dentin treated with ML-NPs produced structural dentin stability and high mechanical performance at the root. Supported by Ministry of Economy and Competitiveness and European RegionalDevelopment Fund [MAT2017-85999P MINECO/AEI/FEDER/UE].

0214

Zinc-Doped Polymeric Membranes Improve Osteoblastic Proliferation and Differentiation.

Manuel Toledano[1], Manuel Toledano-Osorio[1], Francisco Javier Manzano-Moreno[2, 3], Antonio-Luis Medina-Castillo[4], Victor Javier Costela-Ruiz[5], Raquel Osorio[1], Concepcion Ruiz[5, 3]

[1]Faculty of Dentistry, University of Granada, Granada, Spain, [2]Biomedical Group (BIO277), Faculty of Dentistry, University of Granada, Granada, Spain, [3]Instituto Investigacion Biosanitaria, ibs, Granada, Spain, [4]NanoMyP, Nanomateriales y Polimeros S.L., Spin-Off company of the University of Granada, Granada, Spain, [5]Biomedical Group (BIO277), Department of Nursing, University of Granada, Granada, Spain

Objectives: To evaluate the capacity of zinc-doped nanostructured polymeric membranes to improve the proliferation and differentiation of osteoblastic cells.

Methods: Nanostructured membranes were produced by electrospinning and functionalized with SiO_2-NPs (NanoMyP, Granada, Spain). They were then doped with zinc by immersion in a $ZnCl_2$ solution. Three study groups were stablished: 1) Undoped membranes (HOOC-M), 2) SiO_2-NPs functionalized membranes (HOOC-Si-M), 3) SiO_2-NPs functionalized membranes and doped with zinc (Zn-HOOC-Si-M). They were subjected to MG63 osteoblast-like cells culturing (ATCC, Manassas, VA, USA). Proliferation was assessed by MTT-assay and differentiation by evaluating the alkaline phosphatase (AP) activity by a spectrophotometric method. Three membranes of each group were subjected to each test and both tests were performed in triplicate. Mean comparisons were conducted by one-way ANOVA and Tukey tests ($p < 0.05$).

Results: The results are stated in the Table and are expressed as means and standard deviations. In the case of the MTT assay the results are expressed as absorbance; the AP activity is expressed as International Units (IU) of AP per mg of total proteins. Different letters indicate statistically significant differences between groups. The osteoblasts cultured on the membranes doped functionalized with SiO_2-NPs and doped with Zn showed a higher proliferation. This augmentation was of 15% in the HOOC-Si-M group and of 40% in the Zn-HOOC-Si-M. In the case of the AP activity, an increase was also found in the HOOC-Si-M and Zn-HOOC-Si-M group of 20% and 60%, respectively, when compared with the control.

Conclusions: The functionalization of the polymeric membranes with SiO2-NPs and Zn enhances the proliferation and differentiation of osteoblastic cells. Supported by Ministry of Economy and Competitiveness and European Regional Development Fund [MAT2017-85999P MINECO/AEI/FEDER/UE].

0215

Hyaluronic Acid Affects Hydrophilicity of Scaffolds and Stem Cell Differentiation

Aashild Johansen[1], Øyvind Goksøyr[1], Mohammed A. Yassin[1], Tove Kivijärvi[2], Anna Finne Wistrand[2], Kamal B. Mustafa[1]

[1]Department of Clinical Dentistry, University of Bergen, Bergen, Norway, [2]KTH Royal Institute of Technolog, Stockholm, Sweden

Objectives: Synthetic degradable polymers such as poly(L-lactide) and poly(trimethylene carbonate) and their copolymers have been investigated for use as scaffolding materials in bone tissue engineering applications. To overcome their limitations, including inherent hydrophobicity and lack of recognition sites for cells, functionalizing them with natural polymers are of interest. Hyaluronic acid (HA) is a glycosaminoglycan distributed naturally in the human body possessing great hydrophilic properties. Besides being a structural element of the extracellular matrix, HA plays an important role in cell proliferation, migration and wound healing. The present study was aimed to investigate the effect of HA on the hydrophilicity of poly(L-lactide-co-trimethylene

carbonate) poly(LLA-co-TMC) scaffolds. Furthermore, attachment and osteogenic differentiation of human adipose tissue-derived stem cells (hASCs) cultured on the polymer scaffolds was evaluated.
Methods: Stem cells derived from adipose tissue of patients during routine surgical procedures were seeded onto poly(LLA-co-TMC) scaffolds coated with three different concentrations of HA (0,1%, 0,25% and 0,5%). Non-coated scaffolds were used as controls. Contact angle measurements were performed after exposing the scaffolds for hASCs and culture medium. Furthermore, viability and attachment of hASCs were assessed in vitro using live/dead stain and scanning electron microscopy (SEM). The mRNA expression of 3 osteogenic markers was determined by quantitative real-time reverse transcriptase-polymerized chain reaction analysis (qRT-PCR).
Results: The hydrophilicity of the polymer scaffolds was affected by adding HA. Stem cells derived from adipose tissues were spread and entrapped in the pores of the scaffolds. The qRT-PCR analyses demonstrated a greater expression of the osteogenic markers Collagen I, RUNX2 and Osteopontin by the cells grown on the scaffolds coated with HA.
Conclusions: Our results indicate that HA improved the hydrophilicity of poly(LLA-co-TMC) scaffolds and promoted the attachment and osteogenic differentiation of mesenchymal stem cells.

0216

Biodegradation Pattern of Collagen Membranes for Guide Bone Regeneration
Marta Vallecillo Rivas, Manuel Toledano-Osorio, Cristina Vallecillo, Manuel Toledano, Raquel Osorio
University of Granada, Granada, Spain
Objectives: To assess the effect of different storage media in the degradation ratio of five different collagen membranes; Biocollagen, Heart, Evolution X-fine, CopiOs and Parasorb Resodont.
Methods: Membranes samples (*n=30*) were submitted to different degradation tests: 1) hydrolytic degradation in phosphate buffer solution (PBS)(*n=15*) and 2) bacterial collagenase from *Clostridium histolyticum* solution (CH)(*n=15*). Immersion periods up to 50 d were tested. At each time point, thickness (Th) measurements were performed with a digital caliper. ANOVA and Student-Newman-Keuls were used for comparisons ($p<0.05$). Differences between time-points within the same membranes and solutions were assessed by pair-wise comparisons ($p<0.001$).
Results: Mean thickness (mm) and standard deviations are presented in the Table. Significant differences between membranes are shown with low-case letters and with capital letters for differences between degradation tests, within the same membrane. All membranes exceeded 50 d immersion in PBS. Evolution X-fine attained the highest resistance to CH, exceeding 50 d of immersion. None other membranes exceeded more than 7 d in CH.
Conclusions: The bacterial collagenase solution performed as the most aggressive test. Evolution X-fine has the best resistance to degradation.

0217

Differential Biodegradation Pattern of Collagen Matrices for Soft Tissue Augmentation.
Cristina Vallecillo, Manuel Toledano-Osorio, Marta Vallecillo Rivas, Manuel Toledano, Raquel Osorio
University of Granada, Faculty of Dentistry, Granada, Spain
Objectives: To quantify the effect of storage media in the degradation ratio of three different collagen matrices.
Methods: Pieces of 10x10mm^2 of Fibro-Gide (FG), Mucograft (MG) and Mucoderm (MD) were submitted to three different degradation tests: 1) enzyme resistance -0.25% porcine trypsin solution (T)-, 2) bacterial collagenase resistance -*Clostridium histolyticum* solution (CH)-, and 3) hydrolytic degradation -phosphate buffer solution (PBS)-, during different immersion periods up to 50 d. Three specimens of each matrix type were employed for each test (N=27). At each time point, thickness measurements were performed with a digital caliper. ANOVA and Student-Newman-Keuls were used for comparisons ($p<0.05$). Differences between time-points within the same membrane and solution were performed by pair-wise comparisons ($p<0.001$).
Results: Mean thickness (mm) and standard deviations are in Table 1. Significant differences between matrices are shown with low-case letters and with capital letters for differences between degradation tests, within the same membrane. FG attained the highest resistance to all degradation challenges followed by MG. MG was completely reabsorbed at 48 h in CH and 28 d in PBS. MD was completely degraded at 48 h in CH and 14 d in PBS. FG did not exceed the 14 d storage in CH.
Conclusions: The bacterial collagenase from CH performed as the most aggressive degradation solution. FG was the best resisting the several degradation tests.

0218

Preliminary in Vitro Toxicity Testing of Zinc Oxide Nanoparticles Incorporated Into a Tissue Conditioner
Razia Adam[1], Germana Lyimo[1], Annette Olivier[2]
[1]Restorative Dentistry, University of the Western Cape, Cape Town, South Africa, [2]Oral and Dental Research Laboratory, University of the Western Cape, Cape Town, South Africa
Objectives: Tissue conditioners are routinely used to treat denture stomatitis. However, their use remain problematic. The addition of nanoparticles to tissue conditioners has been explored as a possible therapy. However, there is concern about the safety. This study aimed to evaluate the cytotoxicity of FITT™ modified with green synthesized zinc oxide nanoparticles (ZnOnps).
Methods: ZnOnps were synthesized as described by Lyimo (2020). The ZnOnps were added to the FITT™(Kerr Dental) liquid in 0.5%, 1%, 2%, 20%, 25% and 30% (vol/vol) and manually homogenized into disk-shaped specimens (n=6). The discs were placed in 96 well plates containing Dulbecco's Modified Eagles Medium (DMEM) and Human Oral fibroblast cell line (van Wyk et al, 1994).

The medium was supplemented with 10% fetal bovine serum, penicillin (10.000U/ml) and streptomycin (10.000 µg/ml) (Biochrom Ltd). Experiments performed in triplicate. Cell viability was determined after 24, 48, 72 hours of incubation using the methylthiazol tetrazolium assay. Absorbance was measured at wavelength 540 nm to determine the number of viable cells.
Results: Following exposure of fibroblasts to the ZnOnps, the mean optical density (OD) values increased to 0.5% (OD=0.483), 1% (OD=0.494), and 2% (OD=0.521) when compared to the control over 24 hours (OD=0.385). Similar results were seen over 48 and 72 hours. Discs modified with 20% was the only group to exhibit a reduction in mean OD values over 48(OD=0.219) and 72 hours (OD=0.23).
Conclusions: Within the limitations of this study, tissue conditioner discs modified with 0.5%, 1%, 2%, 25% and 30% ZnOnps resulted in a proliferation of cells and therefore exhibited no signs of cell toxicity. The modification with 20%ZnOnps warrants further investigation to determine the cause in a reduction of cell viability.

0219

PEG-Modified-Curcumin Functionalized Calcium-Phosphate: Sustained Drug Delivery System for Osteosarcoma Treatment.

Mingjie Wang[1], Chunfeng Xu[1], Menghong Li[1], Dong Xu[2], Chang Du[2]
[1]Section of Restorative and Reconstructive Oral Care, Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, Netherlands, [2]Department of Biomedical Engineering, School of Materials Science and Engineering, South China University of Technology, Guangzhou, China

Objectives: To develop a biocompatible biomimetic-calcium-phosphate-coating to achieve a local-sustained drug delivery for osteosarcoma treatment.
Methods: PEG-modified-curcumin (mCur) was incorporated into biomimetic calcium phosphate coating (BioCaP). The release kinetics of mCur from mCur-incorporated BioCaP (mCurCaP) was determined in phosphate buffered solution at pH 7.4 and pH 6.5 (simulating tumor environment pH) every week, and up to 6 weeks. The biocompatibility and anticancer effects of BioCaP and mCurCaP were assessed in the MC3T3-E1 pre-osteoblasts and MG63 osteosarcoma cells (ATCC, USA) through the indirect method according to ISO10993-5. Briefly, 200 mg BioCaP/mCurCaP were soaked in 1 ml growth medium for 72 h. The pre-osteoblast and osteosarcoma cells were treated with the aforementioned BioCaP and mCurCaP extracts, and the cell viability was measured by AlamarBlue after 48 h cell culture.
Results: mCurCaP achieved a sustained release in pH 6.5 and pH 7.4, and significantly higher mCur release was observed at pH6.5 (76.4±4.9%; mean±SEM) than at pH7.4 (28.5±1.1%) in 6 weeks (Mann-Whitney U, n=3, $p<0.05$). Pre-osteoblast cell viability was not inhibited by mCurCaP extracts. Comparing to the osteosarcoma cell viability that was treated with BioCaP extract, osteosarcoma cell viability significantly decreased to 64±6% (independent sample t-test, $n=6$, $p < 0.05$) when cells were treated with mCurCaP soaked extracts for 48 h.
Conclusions: mCur could be incorporated into BioCaP, and sustained mCur release could be achieved within at least 6 weeks, while a higher mCur release in simulated tumor environment pH. The mCurCaP extracts were biocompatible to pre-osteoblasts but significantly inhibited osteosarcoma cell viability, suggesting that mCurCaP is biocompatible and promising for post-surgical local application in order to prevent tumor recurrence and avoid systemic side effects.

0220

Bibliometric Analysis of the Scientific Research in Zirconia Implants Rehabilitations.

Felice Lorusso[1], Calogero Buggea[1], Luan Mavriqi[2], Antonio Scarano[1]
[1]University of Chieti-Pescara, Chieti, Italy, [2]University of Albania University, Tirana, Albania

Objectives: The zirconia implants for oral rehabilitation has been proposed as metal-free material for dental implant due to high biological, aesthetic, and physical properties. The present systematic investigation evaluates the bibliometric output of zirconia dental implant for clinical oral rehabilitations.
Methods: The initial screening was performed by Scopus and Web of Science electronic databases using a comprehensive search strategy. The procedure has been conducted independently by paired reviewers that screened the articles, performed the papers selection and collected the study data. The inclusion criteria were restricted to clinical research only. Data on article title, co-authors, number of citations received, journal details, publication year, country and institution involved, funding, study design, marginal bone loss, survival rate, failure, follow-up, and the author's bibliometric data were collected and evaluated.
Results: A total of 29 clinical papers were published between 2008 and 2020, while 19 articles (65.5%) were published in the last five years and ten papers published before 2015. The highest number of studies was in 2015 (n = 6, 20.6%) followed by 2013 and 2017 (n = 4, 13.7% for each). All papers included were prospective studies, while the most common was cohort study (n = 12, 41.4%), followed by case series (n = 9, 31%), and RCT (n = 5, 17%). According to the hierarchy of evidence levels (Is) [77,78], the available evidence supporting the use of zirconia implants is 17% level II, 41.4% EL IV, and the remaining EL VI.
Conclusions: The present review highlighted that zirconia implants scientific production have been more prominent in the last ten years. Zirconia implant could represent a valuable option for oral rehabilitation with high stability of the marginal bone loss and a survival rate comparable to titanium dental implants. Moreover, the papers with open access policies have produced a significant influence on the amount of the total citation with a large heterogeneity about the topic/total Citations% ratio and authors' H-index.

0221

Bacterial Adhesion on Laser and Milled Micro-Texturized Zirconia Implant Surfaces

Neusa Marina da Silva[1], Mariana B. da Cruz[1], Joana Marques[1], Óscar Carvalho[2], Sara Madeira[2], Filipe Silva[2], João Caramês[3], António Mata[4, 5]

[1]Grupo de Investigação em Biologia Oral e Bioquímica - Unidade de Investigação em Ciências Orais e Biomédicas (UICOB), Universidade de Lisboa, Faculdade de Medicina Dentária, Lisboa, Portugal, [2]Center for Microelectromechanical Systems (CMEMS), Mechanical Engineering Department, University of Minho, Guimarães, Braga, Portugal, [3]Implant & Tissue Regeneration Group - Unidade de Investigação em Ciências Orais e Biomédicas (UICOB); LIBPhys-FTC UID/FIS/04559/2013, Universidade de Lisboa, Faculdade de Medicina Dentária, Lisboa, Lisboa, Portugal, [4]Grupo de Investigação em Biologia Oral e Bioquímica - Unidade de Investigação em Ciências Orais e Biomédicas (UICOB), LIBPhys-FTC UID/FIS/04559/2013, Universidade de Lisboa, Faculdade de Medicina Dentária, Lisboa, Lisboa, Portugal, [5]Centro de Estudos de Medicina Dentária Baseada na Evidência (CEMDBE) - COCHRANE Portugal, Universidade de Lisboa, Faculdade de Medicina Dentária, Lisboa, Lisboa, Portugal

Objectives: Implant surface modification has been widely used to increase osseointegration and more recently to reduce bacteria adhesion and biofilm formation. The aim of this pilot in vitro study was to evaluate and compare the initial adhesion of *Streptococcus oralis* on Zirconia implant surface micro-texturized by laser machining and conventional milling.

Methods: A total of 12 Zirconia discs with 8 mm in diameter and 2 mm in thickness were prepared using the pressing and sintering technique. Ultra-high resolution Field Emission Gun Scanning Electron Microscopy (FEG-SEM) substrate micrographs and roughness using contact profilometry were obtained for the tested discs before bacterial culture. Discs were randomly distributed into two groups according to the micro-texturing technique performed: conventional milling (Conventional) and Nd: YAG laser machining (Laser). *Streptococcus oralis* CECT 907T strain were seeded on the discs at exponential phase and cultured at 37°C in anaerobic condition. Biomass was evaluated after 1 and 5 hours of incubation with 0.1% violet crystal (v / v), using a direct reading of the absorbance units (AU) at 595 nm. Viability was determined after 1 hour of culture with a bacterial viability assay kit (SYTO / Propidium Iodide), using Confocal Laser Scanning Microscopy (CLSM). The CLSM images were analyzed by FIJI software and the viability results expressed as a mean percentage. Appropriate statistical software was used to perform the analyses and the significance was set at p <0,05.

Results: Biomass increased over time in both groups. The comparison of biomass between the groups did not reveal statistically significant differences (p>0,05). Values of bacterial viability based on CLSM image analysis were similar in the Laser group compared to the conventional group

Conclusions: Streptococcus oralis adhered to all zirconia textured samples after 1h and 5h of incubation. The type of texturization (Laser or milling) did not appear to affect the initial colonization by Streptococcus oralis.

0222

Translucency and Mechanical Properties of Monolithic Zirconia Ceramics

Stevan Cokic[1], Line Etiennot[1], Jef Vleugels[2], Bart Van Meerbeek[1], Fei Zhang[1, 2]

[1]Department of Oral Health Sciences, BIOMAT – Biomaterials Research group & UZ Leuven (University Hospitals Leuven), Dentistry, KU Leuven (University of Leuven), Leuven, Belgium, [2]Department of Materials Engineering (MTM), KU Leuven (University of Leuven), Leuven, Belgium

Objectives: To correlate phase composition and microstructure with optical and mechanical properties of representative commercially available dental zirconia for monolithic restorations.

Methods: Three zirconia ceramics stabilized by different amounts of yttria were studied. One 3Y-TZP (3M Lava Plus, 3M Oral Care), one 4Y-PSZ (Katana ML, Kuraray Noritake) and one 5Y-PSZ (Initial UHT, GC) zirconia grade were sintered according to the manufacturer's instructions and mirror-polished. The chemical composition was determined using X-ray fluorescence (XRF), the phase composition characterized using X-ray diffraction (XRD), while the grain size was measured using scanning electron microscopy (SEM). Translucency Parameter (TP) and Contrast Ratio (CR) were measured with a spectrophotometer (n=10/group). Indentation fracture toughness (n=10), Vickers hardness (n=10) and biaxial strength (n=20) of sintered specimens were assessed. Statistical analysis involved one-way ANOVA with post-hoc Tukey's HSD test and Pearson correlation test (α=0.05).

Results: 3M Lava Plus showed lower amount of Y_2O_3 in the tetragonal phase (≈2.7 mol%) which resulted in a lower amount cubic phase (22 vol%) compared to the Katana ML (≈2.3 mol% and 44 vol%, respectively) and GC-UHT (3.5 ≈ mol% and 68 vol%, respectively). TP of GC-UHT (37.2) was the highest compared to Katana ML (27.7) and Lava Plus (24.6). Although there were no statistical differences in Vickers hardness between materials, 3M Lava PLUS showed the highest fracture toughness values (3.7±0.1 MPa $m^{1/2}$). On the other hand, biaxial flexural strength and mechanical reliability was the highest for Katana ML (928±87 MPa and 10.82, respectively) followed by 3M Lava PLUS (773±107 MPa and 8.09, respectively) and GC-UHT (680±163 MPa and 4.69, respectively).

Conclusions: Even though the improvement in the last generation of Y-TZP could be seen, future research still needs to focus on the improvement of both optical properties and mechanical reliability of zirconia ceramics.

0223

Evaluation of Acid Resistance for Novel Lithium Disilicate Glass-Ceramic Block

Toshihiko Azuma, Shigenori Akiyama, Futoshi Fusejima
R&D, GC Corporation, Tokyo, Japan

Objectives: Recently, lithium silicate glass-ceramic restorations, especially fabricated by CAD/CAM technology, have become popular. As dental restorative materials are used for long term in oral condition, chemical durability is an important property for aesthetics and physical properties. We have developed novel lithium disilicate glass-ceramic block for CAD/CAM, which doesn't require crystallization firing after milling. The aim of this study is to evaluate the acid resistance for novel lithium disilicate glass-ceramic block compared with existing lithium silicate glass-ceramic blocks.

Methods: Three test groups were prepared, 1) LiSi block (GC, LS), 2) e.max CAD (Ivoclar Vivadent, EM), 3) CELTRA DUO (Dentsply-Sirona, CD). Flat specimens were cut out from the blocks, and these samples were polished by #1000 SiC paper. After that, EM was heat-treated for crystallization following the manufacturer's instruction. Chemical solubility test was conducted (n=5) according to ISO 6872 (2018). One-way ANOVA and Tukey test was used to assess the significant difference ($p<0.05$). To analyse crystal structure, each fresh specimen was immersed (5N NaOHaq, 60°C, 5d). SEM (SU-70, HITACHI) observation was carried out, and relative crystalline surface area (RCSA, n=5) was calculated by image analysis software (Image J, NIH).

Results: Amount of dissolution for each specimen is shown below table1. LS was the significantly lowest in these samples. Figure1 shows LS has fine crystal precipitation and high density (RCSA was 72.4±1.8%). In general, glass matrix is easier to dissolve than crystal. Therefore, it is assumed that LS indicates high acid resistance than other specimens.

Conclusions: Due to high density and fine crystal, acid resistance of LS is greater than other samples. It is considered that LS is useful for dental restorative material over the long term.

0224

Clinical Evaluation of Thermo Viscous Bulk-Fill Composite in Class II Cavities

Ali I. Abdalla
Faculty of Dentistry, Restorative Dentistry, Tanta Univerity, Tanta, Gharabia, Egypt

Objectives: To evaluate the clinical evaluation of thermo viscous composite, preheated composite and normal composite in class II cavities.

Methods: Forty Patients with Class II cavities were participated and provided written informed consent to participate in the study. For each patient at least 3 restorations were made. The teeth were randomly divided into three groups (n=40 each). The prepared cavities will be restored with adhesive {Futurabond U} and composites according to manufacturer's instructions as follow: Group 1: Viscalor bulk. Group 2: Grandio SO bulk fill with preheating. Group 3: Grandio SO bulk fill without preheating. All restorations were placed in bulk technique and Viscalor was preheated and placed using Viscalor Dispenser as recommended by the manufacture. Each restoration was evaluated clinically immediately after finishing and polishing, after 6 months and after one year using modified USPHS Criteria.

Results: All restorations could be evaluated after one year. No loss of restoration was reported for any restorative technique. With respect to color match, one Viscalor and one Grandio SO without preheating showed slight color change {Bravo score}. For anatomic form, marginal adaptation, cavosurface marginal discoloration all restorations shoed Alfa score. No secondary caries was reported at any of the restorations after one year.

Conclusions: There were no significant differences between the three restorative techniques after one year ($P>.05$). Restorations did not exhibit postoperative sensitivity after one year.

0225

Five-Year Clinical Performance of a Universal Adhesive

Laura Ceballos García[1], Maria Victoria Fuentes[1], Isabel Giráldez[1], Bruno Baracco[1], Jorge Perdigão[2]
[1]Idibo Research Group, Rey Juan Carlos University, Alcorcón, Spain, Spain, [2]Division of Operative Dentistry, University of Minnesota, Minneapolis, Minnesota, United States

Objectives: To evaluate the effect of the application of an additional hydrophobic bonding resin on the clinical performance of a universal adhesive applied in etch-and-rinse (ER) or self-etch adhesive (SE) strategy in non-carious cervical lesions (NCCLs) after 5 years.

Methods: Scotchbond Universal Adhesive (3M Oral Care) was applied in 134 NCCLs of 39 subjects using different adhesive approaches; (1) 3-step ER; (2) 2-step ER; (3) 2-step SE; and (4) 1-step SE. An extra layer of a hydrophobic bonding resin was applied for groups 3-step ER and 2-step SE. All lesions were filled with Filtek Supreme XTE resin composite (3M Oral Care). Restorations were evaluated at baseline and 5 years later using the modified USPHS criteria. Mann-Whitney U and Wilcoxon tests were performed and the survival rates (retention/fracture) were analyzed using Kaplan-Meier and Log-rank tests ($p<0.05$).

Results: After 5 years the recall rate was 74.4%. The cumulative survival rate was 96.9% for 3-ER, 96.8% for 2-ER, 71.4% for 2-SE and 81.3% for 1-SE strategies. Log-rank-test was statistically significant ($p=0.006$). Retention rates were 100% for both ER groups, 75% for 2-SE and 81.3% for 1-SE. At 5 years, 2- and 1-SE approaches showed similar retention rates, being lower than 3- and 2-ER. A significant decrease in retention rate was detected for 2-SE ($p=0.007$) and 1-SE ($p=0.014$) groups between baseline and 5 years. Also, 2-ER and 1-SE strategies showed a significant deterioration in marginal discoloration after 5 years ($p=0.004$).

Conclusions: The addition of a hydrophobic bonding resin to the recommended application of Scotchbond Universal adhesive did not improve its clinical performance in NCCLs after 5 years. Higher retention rates were found when this adhesive was applied in ER-mode.

0226

Clinical Evaluation of Digital - Conventional Smile Design with Ceramic Veneers

Mohamed Hussein[1], Ahmed s. el kady[2], Mahmoud M. Alsharqawy[2]

[1]Assistant lecturer, Alexandria, Egypt, [2]professor, Alexandria, Egypt

Objectives: Randomized clinical study (18 months) to evaluate the difference between using Digital smile design versus conventional mock-up technique for closure of maxillary median diastema with ceramic veneers. One of the main esthetic problems affecting the dimension of the smile is the presence of Maxillary median diastema (MMD). Conventional wax-up smile design is powerfully suggested before such treatment. Digital smile design (DSD) seems to be a useful diagnostic tool for dentogingival alterations. Recently, computer-aided design and computer-aided milling technology (CAD/CAM) have been integrated to facilitate designing and fabricating accurate esthetic restorations.

Methods: Forty patients with median diastema were allocated for this study. Restorations with ceramic veneers were done using two different smile design techniques. The test group (n=20) used DSD. Control group (n=20) used conventional wax-up technique. Patients of both groups back to the clinic for a clinical follow-up examination at intervals of immediate postoperative and at 1, 3, 6, 12, and 18 months according to the Esthetic evaluation form.

Results: Data were collected and entered into the computer using the SPSS program for statistical analysis (version 21). It was found that within the follow up intervals up to 18 months Digital smile design had a better enhancement among the esthetic parameters with 94.44 % than conventional smile design with 88.89 % without significant difference found between them ($P \geq 0.05$).

Conclusions: Digital smile design was superior in terms of occlusal and phonetic analysis while concerning dentofacial analysis both techniques are nearly equal.

0227

Instant Whitening Effect of a New Chewing Gum

Pier Francesco Porciani, Simone Grandini

Dental School, University of Siena, Siena, Italy

Objectives: To evaluate the optical instant whitening effect of one new chewing gum versus one control (placebo) chewing gum.

Methods: 100 subjects joined the trial and were randomly and equally assigned to the two groups (test and control). A personal silicon mask was made for each participant to allocate the tip of the Vita® Easyshade Advance 4.0 device which was used to score the tooth color with CIELAB system to calculate two whitening indexes ΔWIO and ΔWI_D. The volunteers in the test and control groups chewed one piece (2g) of sugar-free chewing gum, one added with 200 ppm of E132 plus 1.7mg of spirulina, or one identical without color (Perfetti Van Melle S.p.A., Lainate, Italy). The tooth color was scored before the assumption of one piece of the gum assigned and after 2'30" of its mastication. The differences of ΔWIO and ΔWI_D between baseline and after mastication were analyzed with the paired T-Test, those between the two gums with the unpaired T-Test. The study satisfies the ethical principles stated in the Declaration of Helsinki.

Results: All subjects (mean age 32.6±11.4 years, 54 females and 46 males) completed the trial. None reported problems linked to the assumption of chewing gums. In the test group, the WIO and WI_D differences pre-post treatment were respectively 1.71±3.13 ($p<0.001$) and 1.00±1.66 ($p<0.001$). In the control group, the WIO and WI_D differences pre-post treatment were respectively 0.03±3.75 (p=NS) and 0.20±2.12 (p=NS). Between the two groups, it was reported a statistical difference either for ΔWI_D ($p<0.05$) and ΔWIO ($p<0.05$). See Table.

Conclusions: The observations reported in this study validate the optical instant whitening effect of the test chewing gum after 2'30" of mastication showing a statistically significant increase in instant optical whitening perception as approximated by the ΔWIO and ΔWI_D indexes.

0229

Long-Term Antimicrobial Effects of 4-META/MMA-TBB Resin Containing Antibacterial Agents

Mao Yamamoto[1], Masanao Inokoshi[1], Muneaki Tamura[2], Kumiko Yoshihara[3], Shunsuke Minakuchi[1]

[1]Gerodontology and Oral Regabilitation, Tokyo Medical and Dental University, Yushima, Tokyo, Japan, [2]Microbiology, Nihon Univeristy, Kanda Surugadai, Tokyo, Japan, [3]Health and Medical Research institute, National Institute of Advanced Industrial Science and Technology, Takamatsu, Kagawa, Japan

Objectives: We aimed to assess the long-term antimicrobial effects of a 4-META/MMA-TBB resin (Bondfill SB Plus, Sun Medical) containing antibacterial agents.

Methods: We employed two antibacterial agents, benzalkonium chloride (BAC) and cetylpyridinium chloride (CPC), and prepared 4-META/MMA-TBB resins which contain either 5.0wt% BAC (BAC5.0wt%) or 5.0wt% CPC (CPC5.0wt%). Disk-shaped specimens (10-mm diameter; 2-mm thickness) were prepared and assessed the long-term antimicrobial effect using agar diffusion test (n=6/group) up to 24 weeks. Three oral microorganisms (*Streptococcus mutans, Streptococcus sobrinus,* and *Actinomyces naeslundii*) were inoculated in BHI agar medium, and the specimens were allowed to put on the agar. The growth inhibition zone was confirmed after aerobically cultured at 37 ° C for 24 hours. Twenty-four hours prior to each measurement of the inhibition zone, 4-META / MMA-TBB resin specimens were placed on new agar plates. The medium was changed every week until the third week, and then every two weeks thereafter. The obtained data were subjected to statistical analysis using two-factor repeated measures ANOVA followed by multiple comparison testing using Shaffer modified Bonferroni correction method. For two-factor repeated measures ANOVA, independent factors "type of resin", and "time", as well as the mutual interaction "type of resin" × "time" were included. The total evaluation period was 24 weeks.

Results: Two-factor repeated measures ANOVA revealed that significant differences in inhibition zone of BAC5.0wt% and CPC5.0wt% for all microorganisms, as well as time and mutual interaction "type of resin" × "time". Inhibition zone of BAC5.0wt% was larger than that of CPC5.0wt% up to 4th week. However, the inhibition zone of BAC5.0wt% substantially decreased after 4th week. Finally, inhibition zone of BAC5.0wt% was comparable to that of CPC5.0wt%.
Conclusions: The growth of oral microorganisms was inhibited by 4-META/MMA-TBB resin containing antimicrobial agents (BAC or CPC).

0230

Evaluation of Microbial Contamination of Resin Composites Inside Their Dispensers

Claudia Mazzitelli[1], Andrei C. Ionescu[2], Uros Josic[1], Eugenio Brambilla[2], Annalisa Mazzoni[1], Lorenzo Breschi[1]
[1]University of Bologna, Bologna, Italy, [2]University of Milan, Milan, Italy

Objectives: The use of the same spatula for resin composite handling during restorative procedures is doubtfully. This study aimed at evaluating the microbial contamination inside the dispensing syringes of different types of resin-based composites (RBCs).
Methods: Nanohybrid RBCs differing in matrix chemistry and fillers were used for the studym [FiltekTM Supreme XTE (3MTM); Venus Pearl (Kulzer GmbH); Admira Fusion x-tra (Voco)] to evaluate the microbial viability on their surfaces with/out light-curing. Standardized Streptococcus Mutans, Candida Albicans, Lactobacillus Rhamnosus, or mixt plaque inocula were exposed on uncrured RBCs in an *in vitro* model reproducing clinical procedures. Half of the RBC specimens were cured after exposure. Microbial viability was evaluated with an MTT-based test. Data were analyzed with three-way ANOVA and Tukey's tests ($p<0.05$).
Results: The use of one spatula during dispensing procedures showed microbial contamination of all RBCs after one hour of storage. The used strain and RBCs type influenced the results ($p<0.05$), but photocuring RBCs did not reduce microbial contamination ($p>0.05$).
Conclusions: Microbial species' viability on uncured RBCs poses a non-negligible risk of cross-infection. The use of two spatulas or preliminary dispensed material on a separate pad are clinically recommended to reduce contamination risk during RBCs handling.

0231

Evaluation of Gloss Retention of Resin Composite After Toothbrush Wear Test

Satoshi Jin, Futoshi Fusejima
GC Corporation, Itabashi-ku, Tokyo-to, Japan

Objectives: Resin composite restorations have been widely used in dental treatments. This method is one of the simple and aesthetic treatment, but daily brushing leads to loss of surface gloss and aesthetics. Recently, GC has developed a new resin composite using nano-filler technology. In this study, we evaluated gloss retention property of various composite by toothbrush wear test.
Methods: G-aenial A'CHORD (GC), Tetric Prime (Ivoclar Vivadent), Filtek Universal (3M), ceram X Spectra ST (Dentsply Sirona), Omnichroma (Tokuyama Dental), Grandio SO (Voco), and Clearfil Majesty ES-II (Kuraray Noritake Dental) were examined in this study. The surface of cured specimens was polished with diamond paste (dia-polisher paste, GC) to gloss of more than 90%. Then, it was brushed by toothbrush (Prospec, GC) with toothpaste (White & White, Lion)/water 1:2 weight ratio. After 1,200 brush cycles, gloss of surface was measured by glossmeter (Nihondenshoku) and results were analyzed by one-way ANOVA ($p < 0.05$).
Results: The gloss of G-aenial A'CHORD was maintained at 50% after brushing 1,200 cycles. On the other hand, the gloss of other specimens decreased to 30% or less than 10%. This result indicates that G-aenial A'CHORD has high gloss retention property.
Conclusions: G-aenial A'CHORD shows the highest gloss retention property and can be expected to maintain long-term aesthetics.

0232

Shear-Bond Strength and Optical Properties of Short Fiber-Reinforced CAD/CAM Composite

Enas Mangoush[1], Lippo Lassila[1], Pekka Vallittu[1, 2], Sufyan Garoushi[1]
[1]Department of Biomaterials Sciences and Turku Clinical Biomaterial Center., University of Turku, Turku, South-West Finland, Finland, [2]City of Turku Welfare Division, Oral Health Care., Turku, Finland

Objectives: The aim of this study was to assess the shear-bond strength (SBS) of resin-luting cement to experimental short fiber-reinforced CAD/CAM composite (SFRC) compared to conventional CAD/CAM, 3D printed, and laboratory composites. Moreover, translucency parameter (TP) and light transmission of different thicknesses of the composite were evaluated.
Methods: Five groups of composite discs were prepared (SFRC, Cerasmart 270, GC Temp PRINT, Pro3dure GR-17, Gradia Plus) and divided into subgroups (n=8/group) according to surface treatment protocol (hydrofluoric acid and air-particle abrasion). SBS test was performed using the universal testing machine until failure, failure modes were visually analysed. TP value of each composite was calculated at various thicknesses (1, 2, and 3 mm) over white and black backgrounds by spectrophotometry to assess the CIELAB values of the specimens (n=5/group). Curing-light transmission through composite at various thicknesses (1, 2, and 3 mm) was quantified by MARC calibrator. SEM was used to examine the CAD/CAM composites after surface treatment. The data were analyzed using two-way ANOVA ($p=0.05$) followed by Tukey HSD test.
Results: Material type and surface treatment has a significant effect on SBS. Laboratory composite (Gradia Plus) showed the highest SBS values (22.4 MPa) among tested composites. Regarding CAD/CAM blocks, SFRC showed higher SBS (13.4 MPa) than Cerasmart 270 (10.4 MPa), however, the difference was not statistically significant ($p>0.05$). Cerasmart 270 exhibited higher TP values (19.8, 11, 5) than SFRC (14.5, 5.2, 1.6) at different thicknesses.

Conclusions: Air-particle abrasion of all composites was more effective in enhancing SBS than acid etching. Experimental SFRC CAD/CAM composite showed higher SBS than Cerasmart 270. For all composites, TP and light transmission decreased as thickness increased.

0233

Polymerization Kinetics of Composites with Two Types of Bioactive Glass

Matej Par[1], Katica Prskalo[1], Tobias T. Tauböck[2], Danijela Marovic[1], Thomas Attin[2], Zrinka Tarle[1]
[1]School of Dental Medicine, University of Zagreb, Zagreb, Croatia, [2]Center for Dental Medicine, University of Zurich, Zürich, Switzerland

Objectives: To investigate the effect of two types of bioactive glass (BG) on polymerization kinetics of experimental resin composites.

Methods: Resin composites were based on a photo-curable Bis-GMA/TEGDMA (60:40 in wt%) resin system. The composites with a total filler load of 70 wt% were functionalized with varying amounts (5, 10, 20, and 40 wt%) of either BG 45S5 or a low-sodium fluoride-containing BG. Uncured composites were cast in Teflon molds (n= 6, diameter=6 mm, height=2 mm) on top of a diamond ATR crystal and light-cured using 1,100 mW/cm^2 for 20 s. Real-time changes in degree of conversion (DC) occurring during light-curing and 5 min post-cure were monitored at the data collection rate of 2 s^{-1} using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). From the time-dependent DC curves, the first derivative was calculated to represent reaction rate.

Results: At the end of the 5-min observation period, DC values for the composites functionalized with BG 45S5 ranged from 59.2–66.5%, and were significantly reduced by increasing BG amounts. In contrast, the 5-min DC values for the low-sodium fluoride-containing BG (64.7–67.0%) were not significantly affected by BG amounts. The polymerization rate for BG 45S5 (6.3–12.5 %/s) decreased as a function of higher BG amounts. The composites functionalized with low-sodium fluoride-containing BG had comparatively higher polymerization rates (12.7–14.3 %/s) and showed a statistically significant reduction of polymerization rate only for the highest BG amount (40 wt%). Maximum polymerization rate occurred within 0.8–1.4 s from the start of light-curing and represented a less discriminative parameter due to high data scattering.

Conclusions: The 5-min DC values and polymerization rate were significantly diminished by the addition of BG 45S5, while the addition of a low-sodium fluoride-containing BG showed a minor effect on kinetic parameters.

0234

Fluorescence in Dental Composite Resins

Javier Tapia Guadix[1], Cristina Parise Gré[2], Laetitia Lavoix[2]
[1]Bio-Emulation, Madrid, Spain, [2]GCE, Leuven, Belgium

Objectives: Since human teeth exhibit a natural fluorescence, the latter has become a key feature in composite resins when highly aesthetics restoration is desired. UV lights have changed in the last years and it remains unclear if the fluorescence of composites matches the fluorescence of natural teeth under different UV lights. This study evaluated the fluorescence of different composite resins using natural tooth as reference under two different light conditions.

Methods: Five composites, G-ænial A'CHORD (GC), Tetric Prime (Ivoclar Vivadent), Filtek Universal (3M), Ceram.x Spectra STHV (Dentsply) and Estelite Asteria (Tokuyama), all in shade A2, were used to prepare veneers of tooth 1.1, with 1 mm thickness. Veneers were provisionally placed in a patient using glycerin gel. Each veneer was photographed twice using a digital camera with macro lens. Lighting was provided by two different devices for independent UV (365nm) and near-UV (405nm) illumination on each picture respectively. Images were developed using Camera Raw software (Adobe). Exposure was calibrated referencing tooth 2.1 with the sample measurement tool in L*a*b* color mode, for a Luminosity L* value of 45. The intensity of the composite's fluorescence in relation to natural teeth was then quantified by measuring the luminosity value (L*) with the sample measurement tool inside Camera Raw.

Results: The intensity of the fluorescence varied from almost unnoticeable to very stark difference. With the exception of G-ænial A'CHORD, the samples over-reacted to near-UV excitation, creating an exaggerated emission.

Conclusions: These results show the fluorescence response varies among different composite resins. G-ænial A'CHORD was the only material presenting a uniform response.

0235

Color Stability – Application of Synthetic Dyes

Michael Westphal, Thorsten Bock, Benjamin Gebhardt, Yasmin Semi, Christine Kegele
Ivoclar Vivadent AG, Schaan, Liechtenstein

Objectives: To investigate uptake of synthetic dyes into filling-composites, the color-change ΔE to that effected by coffee-staining was compared. Overall-motivation is to improve standardization of color-stability-measurements over currently used natural-products.

Methods: Color-stability of Tetric-Prime (A3, Ivoclar-Vivadent, Z00B3Y, ø=20mm, h=2.5mm, P1000-surface) was tested. To effect maximum contrast to the filling-composite's inherent shade, the following blue dyes were used:
Toluidine-Blue (CAS_92-31-9, cationic dye) Brilliant-Blue (CAS_3844-45-9, anionic dye). Dyes were applied as an 0.1-wt%-solution in deionized (DI)-water and 85/15-vol%-water/ethanol. To test for staining in lipophilic environment, Oil-Blue-N (CAS_2646-15-3, charge-neutral) as an 0.1-wt%-solution in glyceryl-trioctanoate (CAS_ 538-23-8) was tested. DI-water and coffee (Nestle-Gold, DI-water) served as references. The CIE-values (ISO_11664-4:2008, CIE_S_014-4/E) were determined using a Minolta-CM-3700d-

spectrometer before(0h) and after 2h, 5h and 24h immersion in staining-solutions(37°C). Further measurements were performed after 24h and brushing for 10 sec (Signal-toothpaste, regular brush-hardness) to determine absorption (durable material-penetration) and surface-adsorption of dye.
Results: Cationic dyes stain significantly stronger than anionic dyes and strongest in 15%-ethanol.
Anionic dyes show higher adsorption than cationic dyes and stain least in 15%-ethanol. Lipophilic dyes behave similar as hydrophilic dyes. Coffee was closest simulated in terms of ΔE by Brilliant-Blue in alcohol.
Conclusions: Within the limits of the investigation, color-measurements after immersion in synthetic dyes show that cationic dyes (Toluidine-Blue) exhibit the strongest discoloration. Influence of medium exists since alcoholic-solution displayed highest discoloration-rates. Anionic dyes (Brilliant-Blue) led to lower staining and in alcoholic-solution a lower dye-uptake-rate was found. Anionic dye shows a much higher adsorption rate than cationic dye. The presumed reason is that cationic molecules bind better to the alkaline glass-fillers commonly used. Lastly, lipophilic (Oil-Blue-N) dyes behave the same as hydrophilic dyes.

0236

Material-Dependency of Temperature Changes During Light-Curing in Bulk-Fill Composites

Maria Inês Martins[1], António Delgado[1, 2], Mário C. Polido[1], Luís Proença[1], Rui Igreja[3], Ana M. Azul[1]
[1]Centro de Investigação Interdisciplinar Egas Moniz (CiiEM), Almada, Portugal, [2]Biomaterials & Tissue Engineering, Eastman Dental Institute, UCL, London, United Kingdom, [3]Materials Science, FCT, Universidade Nova de Lisboa, Almada, Portugal

Objectives: To assess the temperature rise during light-curing, of two different bulk-fill composites with different compositions and recommendations, taking into account the remaining dentin floor thickness (RDT).
Methods: Two commercial bulk-fill application resin composites were used (Filtek Bulk-Fill, 3M ESPE; SonicFill, Kerr Dental Corporation). Six experimental groups (n=10) were assigned considering the material used (FB or SF) and the remaining dentin thickness (0, 1 or 2mm). Open occlusal Black Class I cavities were simulated in a clay mould apparatus, equipped with a K-type thermocouple running to the bottom of the pulpal floor. Human coronal dentin discs were sectioned in a water-cooled hard-tissue microtome originating 1mm or 2mm thick discs. These were placed inside the mould, over which composites were layered, according to manufacturer recommendations. In the control group (0mm), composite was layered without substrate. Composite specimens were covered with an acetate sheet and light-cured at zero distance, using a high-intensity LED curing unit operating at 1470 mW/cm^2, 430-480 nm (Elipar Deepcure-S, 3M ESPE). Results were analyzed using a two-way ANOVA and Tukey's HSD for post-hoc analysis, at a significance level of 5%.
Results: Temperature rise during light-curing was registered with all experimental groups, although it reached higher values with lower RDT. Temperature variation (DT) during light-curing was significantly higher for FB when compared to SF with all RDT groups (p<0.05). A temperature drop was seen with FB_0, whilst a rise was registered with SF_0, during bulk-fill placement of the composites. The DT peaked at 11.5±1.6°C for FB_0, while it peaked at 5.4°C for SF_0. The lowest temperature was registered with SF_3 (0.9±0.2°C).
Conclusions: Considering material variations, RDT seems to be a significant factor to ensure protection of the pulp complex, and its vitality, during light curing. Temperature rise with contemporary materials is a relevant phenomenon that should be considered by clinicians.

0237

Effect of Effervescent Multivitamin on Surface Roughness of Microhybrid Composites

Bengü Doğu, Seda Özmen, Selinsu Öztürk, Pinar Yilmaz Atali, Bilge Tarçin
Restorative Dentistry, Marmara University Faculty of Dentistry, Istanbul, Turkey

Objectives: The consumption of effervescent tablet vitamins increased during the Covid-19 pandemic. The aim of this *in vitro* study was to evaluate the effect of an effervescent tablet (containing vitamin C, D, and Zinc) on the surface roughness of microhybrid composites.
Methods: A total of 50 disc shaped (8x2mm) composite samples with shade A2 were prepared in 5 different groups (n=10): Triofill Microhybrid (Dentac), Estelite Posterior (Tokuyama), Geanial Anterior (GC), Charisma Opal (Kulzer), Beautifil II (Shofu). Samples were polymerized with a light-curing unit (Valo Cordless, Ultradent) in accordance with the manufacturers' guidelines. Finishing and polishing procedures were performed using Finishing Discs (Bisco). Over a period of 30 days, the control samples were kept in distilled water and the experimental samples were immersed in 200 ml water with 1 effervescent tablet for 2 min a day with 24 h intervals between the immersion cycles. The pH of the vitamin solution was measured using strips (OF, China) and found to be as 3.0. Surface roughness measurements (Ra) were recorded initially and at the end of 30 days (Mitutoyo SJ-201P). The data were evaluated using the Wald chi-square test. The significance level was set at p <0.05.
Results: Composite type had statistically significant effect on Ra values (p<0.001). The mean Ra values of Triofill (0.362) and Beautifil II (0.404) were significantly higher than Estelite Posterior (0.222), Geanial (0.227), and Charisma Opal (0.222) (p<0.001). The highest change in Ra was detected in Beautifil II. Ra values of Triofill and Beautifil II samples were above the clinically acceptable threshold (0.2).
Conclusions: The multivitamin tablet with a pH of 3.0 is thought to have a different effect on the surface roughness depending on the content of the tested microhybrid composites. Material selection is important for patients who regularly use multivitamin tablets.

0238

Effect of Effervescent Multivitamin on Color Stability of Nanohybrid Composites

Ayse Senol, Ezgi Acar, Pinar Yilmaz Atali, Bilge Tarçin
Restorative Dentistry, Marmara University Faculty of Dentistry, Istanbul, Turkey

Objectives: The aim of this study was to evaluate the effect of effervescent multivitamin tablets, whose use has increased with the covid-19 pandemic, on discoloration of two different nanohybrid composites.
Methods: 40 disc-shaped (8x2 mm) composite samples of 2 different nanohybrid composite (Ceram.X one Universal, Dentsply; Triofill Nanohybrid, Dentac) were prepared using silicon molds (n=10) and polymerized with a LED curing device (1000 mW/cm2; VALO Cordless LED, Ultradent) for 20 s. Following polishing with Finishing Discs (Bisco) samples were immersed in distilled water for 24 h and initial color measurements were performed with a spectrophotometer (Vita Easyshade V). Control group samples (n=20) were kept in refreshed distilled water and the experimental group samples were immersed in solutions containing 1 effervescent tablet and 200 ml water for 2 min every 24 hours for 30 days. In all groups, color measurements were repeated following immersion. All color measurements were performed on a gray background according to CIE (L*a*b*) and ΔE values were calculated. Data were analyzed using two-way analysis of variance with the significance level set at $p<0.05$.
Results: The highest mean ΔE value was obtained with the Triofill Nanohybrid in the control group. It was followed by Ceram.X one and Triofill Nanohybrid in the experimental group and Ceram.X one in the control group. Composite type and solution interaction had statistically significant effect on ΔE values ($p = 0.015$). The average ΔE values of Ceram.X one and Triofill Nanohybrid were 1.20 and 7.65 in distilled water solution, and 4.20 and 3.57 in vitamin solution.
Conclusions: The color changes in Ceram.X one and Triofil Nanohybrid composites immersed in multivitamin solution were above the clinically noticeable level (ΔE <3.3). Within the limitations of this study, it can be concluded that the use of effervescent multivitamin tablets have unfavorable effects on color stability.

0239

Tubular Occlusion and Sealing After Polymeric Nanoparticles Application at Radicular Dentin

Raquel Toledano[1], Manuel Toledano[1], Fátima S. Aguilera[1], Estrella Osorio[1], Manuel Toledano-Osorio[1], Germaine Escames[2], Antonio-Luis Medina-Castillo[3], Christopher D. Lynch[4], Esther Muñoz-Soto[1], Raquel Osorio[1]
[1]Faculty of Dentistry, University of Granada, Granada, Spain, [2]Faculty of Medicine. Department of Physiology, Biomedical Research Center. CIBERFES, Ibs., University of Granada, Granada, Spain, [3]NanoMyP. Spin-Off. University of Granada., Granada, Spain, [4]Restorative Dentistry, University Dental School & Hospital/ University College Cork, Cork, Ireland

Objectives: To investigate the effect of novel polymeric nanoparticles (NPs) doped or not with melatonin (ML) in dentin tubular occlusion, after endodontic treatment.
Methods: The effect of undoped NPs and ML-doped NPs (ML-NPs) was tested in radicular dentin, at 24 h and 6 m. A control group without NPs was included. Endodontic treatments were performed and NPs were applied before the sealant cement application. Three groups were established: 1) Control, 2) NPs and 3) ML-NPs. Ten specimens were included in each group. Sealing ability was evaluated trough a fluid filtration test and tubules were morphologically evaluated by scanning electron microscopy (SEM) and atomic force microscopy (AFM). ANOVA and Student-Newman-Keuls ($p<0.05$) were used for comparisons.
Results: Means and standard deviations of fluid filtration values ($\mu l\ min^{-1}$) are displayed in the table. Distinct letters indicate differences between NPs and numbers between storage time-points. Both NPs produced an efficient and durable sealing, different from the control group. Tubular occlusion was observed in ML-NPs group by AFM and SEM analyses. Undoped-NPs produced closure of some tubules and porosities at the expense of a relative mineral amorphization. After ML-NPs application, tubules appeared completely blocked with intratubular mineral formations.
Conclusions: NPs improved sealing and tubular occlusion after endodontic treatment.

0240

Novel Pastes Containing Zinc-Doped Polymeric Nanoparticles for Dentin Hypersensitivity

Raquel Osorio[1], Manuel Toledano-Osorio[1], Estrella Osorio[1], Antonio-Luis Medina-Castillo[2], Manuel Toledano[1]
[1]Faculty of Dentistry, University of Granada, GRANADA, Granada, Spain, [2]2 NanoMyP®, Nanomateriales y Polímeros S.L., Spin-Off company ugr, GRANADA, Granada, Spain

Objectives: To assess the ability of dentifrices containing zinc doped polymeric nanoparticles (NPs) to enduringly occlude the dentinal tubules, reinforcing dentin mechanical properties.
Methods: Dentin surfaces were treated with EDTA to expose dentinal tubules and were brushed with (1) distilled water (DW), (2) with pastes containing 1% of zinc-doped NPs (ZnNPs) or (3) Sensodyne®. Topographical (atomic force and scanning electron microscopies) and nanomechanical analysis were assessed on treated dentin surfaces (n=3) after artificial saliva immersion, and after citric acid challenge. ANOVA and Student-Newman-Keuls ($p<0.05$) were used.
Results: Means and standard deviations of complex modulus (GPa) of dentin at intertubular and intratubular spaces are displayed in the table. Groups with distinct letters within each dentin type are significantly different. Tested pastes produced efficient tubule occlusion (100%) after just one application and reinforced mechanical properties of intertubular dentin. After citric acid challenge, just the pastes containing zinc-doped NPs were able to maintain tubular occlusion (100%), as specimens treated with Sensodyne® have 30% of tubules opened. Mechanical properties were maintained at dentin treated with Zn-doped NPs, but in the case of specimens treated with Sensodyne® complex modulus values were reduced.

Conclusions: Pastes with zinc-doped NPs were able to produce acid resistant tubular occlusion and increased mechanical properties of dentin. Supported by Ministry of Economy and Competitiveness and European Regional Development Fund [MAT2017-85999P MINECO/AEI/FEDER/UE].

0240.1

Three-Body Wear of Modern Universal Restorative Materials Against Zirconia

Jan Schuessler, Ulrich Lohbauer
Dental Clinic 1, University of Erlangen-Nuremberg, Erlangen, Germany

Objectives: To evaluate the three-body wear of three universal direct restorative materials opposed to zirconia (ZrO_2) antagonist wheels in an ACTA wear machine.

Methods: Specimens (n=10) suitable for the compartments of the ACTA wear machine were prepared from three direct composite resins (Gaenial A-CHORD, GC / Filtek Universal, 3M / Tetric Prime, Ivoclar). After storage (28 days, distilled water, 37 °C) 200,000 wear cycles were performed against an antagonist wheel (Y-TZP, Prettau, Zirkonzahn, d=20mm, h=6mm, F=15N, f=1Hz, 15% slip) in millet seed suspension as third medium in the ACTA wear machine. Mean abrasion depth and the roughness (Ra) were analysed in a 3D optical non-contact profilometer (CT100, CyberTechnologies, Eching-Dietersheim, Germany) equipped with a confocal white-light sensor CHR-600 (CyberTechnologies (z-res.=0.02µm)). Statistical analysis was performed using non-parametric statistics (Kruskal-Wallis and Mann-Whitney-U test; $p<.05$).

Results: The results showed significant difference of the mean vertical loss among the materials ($p<.001$). Gaenial A-CHORD (12.74 ± 5.85 µm) showed the statistically best wear resistance among the materials tested. Tetric Prime (17.01 ± 4.86 µm) and Filtek Universal (17.14 ± 7.89 µm) performed equally. The ZrO_2 wheels had no signs of abrasion.

Conclusions: After 200,000 wear cycles, all materials showed very low material loss. Gaenial A'CHORD was the most wear resistant material tested. Based on this study results, the use of the universal direct composites under investigation, especially in the load bearing, occlusal molar region can be recommended.

0240.2

Surface and Handling Characteristics of Universal Restorative Resin Composites

Eija Säilynoja[1, 2], Sufyan Garoushi[1], Roosa Prinssi[2], Pekka Vallittu[1], Lippo Lassila[1]
[1]University of Turku, Turku, Finland, [2]Stick Tech Ltd., Turku, Finland

Objectives: Surface and handling properties have been particularly important in driving the development of restorative resin composites. In this work three modern universal resin composites were evaluated in terms of surface polishability, wear and handling properties. The selected composites were G-aenial A'CHORD, Tetric Prime and Filtek Universal**.** Surface gloss, wear, optical translucency and viscosity were used to evaluate the overall performance of the A2 shade composites.

Methods: The gloss was measured after a clinically relevant polishing process. The measurement was performed with Glossmeter (ZGM 1110) at 60° incidence angle. A 2-body wear test was conducted using a dual-axis chewing simulator (SD Mechatronik). The viscosity and translucency parameter (TP) at different thicknesses were measured using rheometer (HAAKE RheoStress) and spectrophotometer (CM-700d), respectively. Data were statistically analyzed with analysis of variance ANOVA ($p = 0.05$).

Results: Differences were observed between the tested resin composites. The G-aenial A'CHORD and Filtek Universal had less wear (33 & 29 µm) and higher gloss (89 & 91 GU) values than those of Tetric Prime (50 µm & 82 GU). In addition, TP values of G-aenial A'CHORD and Filtek Universal were lower than Tetric Prime at all thicknesses. The viscosity of G-aenial A'CHORD was significantly lower ($p<0.05$) than other tested resin composites.

Conclusions: G-aenial A'CHORD showed improved surface and optical characteristics as well as non-sticky consistency in handling. These clinically relevant findings might indicate good performance of G-aenial A'CHORD as universal restorative material.

0240.3

The Physical Properties of Zeolite Incorporated Silver-Reinforced Glass Ionomer Cement

Spencer Lang[2], Jessica Hao[1, 2], Francis Mante[2], Kresimir Pavelic[3], Fusun Ozer[2]
[1]Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Department of Restorative Dentistry, University of Pennsylvania School of Dental Medicine, Philadelphia, Pennsylvania, United States, [3]Juraj Dobrila University of Pula, Pula, Croatia

Objectives: Zeolite-clinoptilolite is able to promote antibacterial properties of dental materials in the long-term when incorporated with inorganic cations. This project examined the physical properties of zeolite when added to commercially available Ag-reinforced Glass Ionomer Cement (GIC) (SDI Limited, Australia).

Methods: Samples were prepared by incorporating 0 %-control, 0.5 %, 1 %, 2 %, or 4 % wt of zeolite-clinoptilolite (Panaceo International GmbH, Austria) into GIC. Four physical property tests were conducted: Vickers surface hardness, water sorption (W_{sp}), water solubility (W_{sl}), and flexural strength. Vickers hardness readings of 10x2mm disks (n=10) were taken before and after immersing the disks in 37°C dH_2O for 1 week. For the W_{sp} /W_{sl} tests, 10x2mm disks (n=12) were immersed in 37°C dH_2O. The values for W_{sp} /W_{sl} were calculated based on the average values of the weights. For the flexural test, 25x5x2 mm bars (n=10) were subjected to a 3-point bending test until the sample bar fractured. The data from all four tests were further analyzed via t-test at $\alpha = 0.05$.

Results: The results from the surface hardness, W_{sp}, and flexural strength tests suggested that adding 0.5-4 % wt of zeolite to Ag-reinforced GIC does not diminish its physical properties ($p > 0.05$). However, the W_{sl} Results showed that higher concentrations of zeolite, such as 1, 2, and 4 %, had a statistically significant increase in water solubility ($p < 0.05$) compared to the control.

Conclusions: Although up to 4 % wt zeolite can be incorporated into GIC, further research is needed to determine the impact that higher W_{sl} values may have on the physical integrity of the material. Finally, based on the promising results of the physical tests, Ag ion-release and antimicrobial tests should be conducted to understand all aspects of the material.

0240.4

Effect of Heating Upon Optical Properties of a Nanofilled Composite Resin

Corina M. Prodan, Cristina Gasparik, Diana Dudea
Prosthetic Dentistry and Dental Materials, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Cluj, Romania

Objectives: To evaluate the color changes generated by repeated heating of a universal nanofilled composite resin.
Methods: A total of 42 disks (n=14) (diameter 10 mm, thickness 1 mm) were fabricated from the universal nanocomposite-resin, Omnichroma (Tokuyama), after exposure of the respective syringes at 1, 5, and 10 cycles of heating at 45°C. An additional group of 14 samples was made as control, without previous heating. Specimens were polymerized using Woodpecker Led. H Orto curing lamp on both sides and immersed (24 h) in distilled water. The color coordinates (lightness L* and color coordinates a* and b* have been measured with a spectrophotometer (SpectroShade (MHT), with the samples placed on white, black, and grey backgrounds respectively. Differences in the color parameters ΔL^*, Δa^*, Δb^*, as well as color difference DE00 and difference in translucency parameter ΔTP have been calculated between control and samples heated with different thermal regimens.
Results: DE00 varied between 1.57- 1.26, ΔL^* ranged between 1.38-1.00, Δa^* -0.61- -0.09, whiles Δb^* varied between 0.07- -0.15. Mean TP varied between 23,92 - 25,74, with the highest values for the composite subjected to 10 cycles of heating.
Conclusions: A variation of the color coordinates has been found as a result of the composite heating, which exceeded the perceptibility threshold, but not the acceptability one. When the cycles of heating are increased in number, the variation decrease, and the color coordinates seem to be closer to the nonheated control.

0240.5

Splint Design Affects Stress Distribution at Posterior-Mandibular Teeth With Reduced Supporting Periodontal Tissues

Erkin Gulbey[1], Sema Belli[2], Gurcan Eskitascioglu[3], Damla E. Yapici[1], Sema Hakki[1]
[1]Department of Periodontology, Selcuk University, Konya, Turkey, [2]Department of Endodontics, Selcuk University, Konya, Turkey, [3]Private practice, Ankara, Turkey

Objectives: The objective of this three dimensional-finite element analysis (3D-FEA) was to evaluate the effect of different splint designs and materials on the stress distribution around mandibular posterior region with 30 % horizontal bone loss.
Methods: Six different test models in order to simulate splinting scenarios for mandibular 2nd premolar and 1st and 2nd molars were created. The teeth were considered as positioned in a jaw with the horizontal defect. Micro CT was used during the creation of the models. Following conditions were then simulated: 1) fiber-reinforced composite splint-buccal (FRCS-b), 2) fiber-reinforced composite splint-lingual (FRCS-l), 3)fiber-reinforced composite splint-occlusal (FRCS-o), 4)composite splint-buccal (CS-b), 5)composite splint-lingual (CS-l), 6) composite splint-occlusal (CS-o). Chewing forces were simulated during loading and stress accumulations and distributions on the teeth and surrounding tissues were examined by using von Mises and maximum principal stress criteria.
Results: In posterior splint models with horizontal defect, when vertical forces were applied on the cancellous bone, lower stress values were observed around 37 and 36 in occlusal splint groups while stresses were more in the cancellous bone in the mesial 37 of the FRCS-o group. In the lingual splint groups (FRCS-l, CS-l) stresses were high at distal of 37. Stresses were low around tooth no.36 in FRCS-b, FRCS-l, CS-b, CS-l groups and tooth no.35 in resin splint groups (FRCS-o, CS-o). Under vertical forces on the cortical bone, higher stress values were measured in the resin splint groups (FRCS-l, CS-l) at 37 distal and around 35. In the resin splint groups (FRCS-o, CS-o) in occlusal surface loaded models, lower stress values were measured around the 37th and 35th while higher stress values were measured around the 36th tooth. The highest stress values were measured around 36 mesial and 35 in the resin splint groups applied to the buccal and lingual surfaces. The lowest stress values measured in these regions were measured in the FRCS-o group.
Conclusions: Although 3D-FEA may not mimic the dynamic nature of oral environment, the findings of this study demonstrated that occlusal splints look safer for second molar under vertical loading. Occlusal splints have also the advantage of providing better oral hygiene for the patient with reduced periodontal supportive tissues (Selcuk University Scientific Research Coordination-BAP).

0240.6

Mechanical Properties, Water Sorption and Solubility of Universal Resin Composites

Ersilia González[1], Maria Victoria Fuentes[1], Laura Ceballos[1], Jorge Perdigao[2]
[1]IDIBO Research Group, Nursing and Stomatology Department, Rey Juan Carlos University, Alcorcón, Madrid, Spain, [2]Department of Restorative Sciences, Division of Operative Dentistry, University of Minnesota, Minneapolis, Minnesota, United States

Objectives: To evaluate the elastic modulus, flexural strength, water sorption and solubility of several universal resin composites.
Methods: Five universal resin composites: G-ænial A'Chord (GA) (GC), Essentia Universal (EU) (GC), Omnichroma (O) (Tokuyama), Tetric Prime (TP) (Ivoclar Vivadent), Filtek Universal (FU) (3M Oral Care), were evaluated and compared with a nanofilled resin composite used as control, Filtek XTE Supreme (FS) (3M Oral Care). Flexural strength (MPa) and elastic modulus (GPa) were determined according to ISO 4049. Bar shaped-specimens (25x2x2mm, n=10) were prepared and light-cured with a LED SmartLite Pro (Dentsply Sirona) applying five overlapping points for 20 s. Samples were subjected to three-point bending test using an Instron 3345 after 24h water storage at 37°C. Five disc-shaped samples (15x1mm) were also prepared, light-cured using the same

protocol, and submitted to a water sorption and solubility test, according to ISO 4049. Data were analyzed using one-way ANOVA and Tukey post-hoc test ($p<0.05$).
Results: FU showed the highest flexural strength (130.62), followed by FS (108.48) and AC (81.76), TP (81.66), EU (71.99) and O (69.71) without statistical differences between them. Modulus of elasticity of FU (8.15) and FS (8.58) were statistically similar, but higher than those of the other resin composites. Regarding sorption, TP, O and FU showed lower values than FS. For solubility, AC, EU and O resulted in higher values than FS. TP and FU did not show differences in solubility compared with FS.
Conclusions: Overall, universal resin composites showed lower mechanical properties than the nanofilled composite FS, with the exception of FU. Water sorption was similar for all universal composites, while FU resulted in lower solubility than EU and AC and similar to FS.

0241

Occlusal Caries Detection *in Vivo* and on 3D Digital Models

Panagiotis Ntovas[1], Stavroula Michou[3, 2], Azam Bakhshandeh[3], Kim Ekstrand[3], Christoph Vannahme[2], Christos Rahiotis[1], Afrodite Kakaboura[1]
[1]Operative Dentistry, University of Athens, Athens, Greece, [2]Optics Developer and Research, 3Shape, Copenhangen, Denmark, [3]Odontology, University of Copenhagen, Copenhagen, Denmark

Objectives: To evaluate the diagnostic accuracy of visual caries detection performed on 3D digital models and compare it to *in vivo* visual examination.
Methods: Fifty-two molars and premolars scheduled for extraction were included in this study. One to three independent examination sites were defined on the occlusal surface of each tooth and were clinically inspected using ICDAS criteria. Afterwards, the examined teeth were scanned intraorally with TRIOS 4 intraoral scanner (3Shape TRIOS A/S, Denmark) using white light to obtain 3D color models, followed by illumination with light 415nm wavelength. The latter excites fluorescence by the dental tissues that were mapped on the 3D models. On the same visit, the teeth were extracted and stored until histological assessment could be conducted by an independent examiner. The examination sites were annotated on the 3D models and after six months, the principal examiner visually inspected and scored all examination sites on the high-resolution 3D models visualized with specific software (3Shape A/S, Denmark) using ICDAS criteria. Caries scoring was done first on the color models and thereafter on the models with the fluorescence texture.
Results: At the examined histological levels, all methods showed comparable diagnostic performance, with no significant differences between the areas under the ROC curves ($Az>0.65$, $p>0.05$). All methods showed similar diagnostic accuracy ranging from sufficient to good at more initial caries lesion stages (ACC > 59), and from very good to excellent at more extensive caries stages (ACC > 77). The intra-examiner reliability was perfect for all methods (kappa > 0.80).
Conclusions: Visual inspection of 3D digital color models or models with a fluorescence overlay can help detect occlusal caries lesions with accuracy equivalent to direct clinical visual inspection. Caries detection on 3D models has the potential to be utilized for teledentistry purposes.

0242

Validation of a New Feasible Approach for Sleep Bruxism Diagnosis

Michael Franken[1], Nicole P. Walentek[1], Nora Bergmann[1], Andreas Gotter[3], Ralf Schäfer[2], Michelle Alicia Ommerborn[1]
[1]Department of Operative Dentistry, Periodontology and Endodontology, Heinrich-Heine-University, Düsseldorf, Germany, [2]Clinical Institute of Psychosomatic Medicine and Psychotherapy, Heinrich-Heine-University, Düsseldorf, Germany, [3]Gofficient UG, Aachen, Germany

Objectives: A new diagnostic method for quantification of sleep bruxism (SB) activity has been developed, aiming to combine good validity parameters with a convenient handling. The purpose of the present double-blind controlled-clinical trial was to verify the new method by using the current gold standard as a benchmark in an appropriate sample size and to quantify the subjective handling properties.
Methods: Overall 45 subjects (10 SB and 35 non-SB subjects) participated in this investigation. For SB diagnosis, each participant received a two-night ambulatory polysomnographic recording (PSG) including audio-video recordings. The new diagnostic method consists of a 0.5 mm narrow diagnostic plate (DIABRUX) that was manufactured in a vacuum-press similar to a hard plate. After identification of SB and non SB subjects, each subject had to wear the DIABRUX for five consecutive nights and the occurring abrasion on the surface was automatically calculated by a specifically for this purpose developed software in pixels thus resulting in a sum score, viz. the pixel score.
Results: The comparison between the SB and non-SB group estimated a mean pixel score of 1,306.25 (SD 913.22) for the SB group and a mean pixel score of 380.65 (SD 483.29) for the non-SB group (3.4 times higher in SB) ($p<0.001$). When calculating the receiver-operator characteristic (ROC) curve, a cut-off value for the pixel score of 506.65 was detected as most appropriate. At this cut-off value, sensitivity of the new diagnostic method amounted to 100% and specificity to 80%. The positive and negative predictive value accounted for 59% and 100%. Regarding the handling properties of the DIABRUX, wear comfort and practicability (for each Median=8) were found to be advantageous.
Conclusions: The new diagnostic method appears to be a valid and user-friendly tool that can be used for preventive dentistry and for the acquisition of larger sample sizes within sophisticated study designs.

0243

CBCT Compared to Ultra-high Resolution Photon-Counting CT for Dental Imaging

Stefan Sawall[2], Sinan Sen[1], Holger Gehrig[1], Ti-Sun Kim[1], Christopher Lux[1], heinz-peter schlemmer[2], Sarah Heinze[1], Joscha Maier[2], Marc Kachelriess[2], Maurice Ruetters[1]

[1]Heidelberg University Hospital, Heidelberg, Germany, [2]German Cancer Research Center (DKFZ), Heidelberg, Germany

Objectives: Volumetric imaging of dental structures usually requires a spatial resolution that cannot be achieved by conventional clinical computed tomography (CT) systems but is available in dedicated cone-beam CT systems (CBCT). Novel clinical photon-counting (PC) CTs offer a spatial resolution of about 200 µm and might allow for acquisitions close to conventional dental CBCTs with the added benefits of increased soft tissue contrast and high scan speeds. Herein, the capabilities of these new system in comparison to state-of-the-art dental CBCTs shall be evaluated.

Methods: Acquisitions of 5 cadaveric heads were performed in an experimental CT system (SOMATOM CounT, Siemens Healthineers, Germany) containing an ultra-high resolution PC detector (0.25 mm pixel size) as well as in a dental CBCT scanner (Veraview X800, Morita, Japan). Acquisitions were performed using a tube voltage of 120 kV and varying dose levels between 4 mGy and 50 mGy ($CTDI_{16cm}$) in case of PCCT and using 102 kV in case of the CBCT and a dose of 1397 mGycm2. Image reconstruction was performed with an isotropic voxel size of 0.125 mm in case of CBCT and 0.134 mm in case of PCCT. Quality of delineation of gingiva, hard tissues, periodontal space as well as apical osteolysis were used as figures of merit for comparison of both technologies and were assessed by two readers using a five-point rating scale (1=excellent, 2=good, 3=moderate, 4=poor, 5=not visible).

Results: Apical osteolysis, periodontal space as well as enamel, dentine, pulp-chamber and gingiva identified in CBCT were found in all PCCT reconstructions. Both devices showed excellent image quality of all structures assessed. A reduction of dose to 4 mGy still allowed for distinct identification of osteolytic foci, enamel, dentine, pulp-chamber, periodontal space and gingiva in PCCT acquisitions.

Conclusions: Ultra-high resolution PCCT is able to reliably identify different diagnostic entities and structures relevant for dental diagnostics similar to conventional dental CBCT.

0246

Segmentation of Dental Restorations on Panoramic Radiographs Using Deep Learning

Csaba Rohrer[1], Joachim Krois[1, 2], Jonas A. Rodrigues[1, 3], Falk Schwendicke[1, 2]

[1]Department of Oral Diagnostics, Digital Health and Health Services Research, Charité–Universitätsmedizin, Berlin, Berlin, Germany, [2]ITU/WHO Focus Group on AI for Health, Topic Group Dental Diagnostics and Digital Dentistry, Geneva, Switzerland, [3]Department of Surgery and Orthopedics, UFRGS, School of Dentistry, Porto Alegre, Brazil

Objectives: Deep Convolutional Neural Networks such as U-Net have been widely used for medical image segmentation. Dental restorations are prominent features of dental radiographs. Applying U-Net on the full panoramic image is challenging, as the shape, size and frequency of different restoration types vary significantly. It was hypothesized that models trained on smaller equally spaced rectangular image crops (tiles) of the full panoramic would outperform models trained on the full panoramic scan.

Methods: The dataset consisted of 1781 panoramic radiographs. Fillings, crowns, root canal treatments, and implants were segmented pixelwise by dental experts. The radiographs were cropped into different number of tiles for training. We used U-Net architecture pretrained on ImageNet. The data set was randomly split into a training (70%), validation (20%), and test set (10%), for fine-tuning, model selection and evaluation, respectively. The Dice loss function with adaptive learning rate, early stopping and data augmentation was used for model training.

Results: Training with an increased number of tiles improved the model performance and reduced the time of model convergence. The F1-score for the full panoramic image was 0.68, compared to 0.80, 0.90 and 0.94 for 6, 10 and 20 tiles, respectively. For root canals treatments, which are small, cone-shaped features that appear less frequent on the radiographs, the performance improvement was considerable.

Conclusions: Semantic segmentation models trained on panoramic radiographs are biased towards the more frequent and extended classes, hence, reducing the accuracy of the classifier. Training on tiles of panoramic radiographs and pooling the individual results thereafter, improved classification performance and reduced time to model convergence for segmenting dental restorations.

0247

Anatomical Tooth Relationships for Determining Age Using a Semi-Automated Approach

Paulina Varas-Quintana[1], Angela Aneiros-Ardao[1], Nicolás Vila-Blanco[2], Carlos Balsa-Castro[1], Maria José Carreira[2], Inmaculada Tomás[1]

[1]Oral Sciences Research Group, Universidade de Santiago de Compostela, FIDIS, Santiago de Compostela, Spain, [2]Centro Singular de Investigación en Tecnoloxías Intelixentes, Universidade de Santiago de Compostela, FIDIS, Santiago de Compostela, Spain

Objectives: This study evaluated the predictive potential of a method for estimating the chronological age of children and adolescents based on measuring the height and width of their mandibular teeth.

Methods: We analysed 650 orthopantomographies of Spanish Caucasians (330 boys, 320 girls) aged 5 to 16, obtained from the School of Medicine and Dentistry of the Universidade de Santiago de Compostela (Galicia, Spain). The heights/widths of teeth numbers 47, 46, 45, 44, 34, 35, 36 and 37 were measured. The variable height/width ratio of tooth 47 had the greatest Spearman correlation with chronological age (rho ≥0.914), and was therefore used to predict chronological age by applying a simple linear regression and evaluating the model obtained using the coefficient of determination (R^2). The actual and predicted chronological ages were compared to obtain the error and absolute error values.

Results: The R^2 value was good in both models, with the independent variable "R47" explaining 86 and 84% of "chronological age" in the boys and girls, respectively. The height/width ratio of tooth 47 produced a mean and median error of 8.924^{-17} and -0.029 years for the boys and 5.770^{-17} and -0.010 years for the girls. The mean and median absolute error was 0.944 and 0.810 years for the boys and 0.971 and 0.892 years for the girls. The 6-9 and 15-16 age groups had the lowest and highest errors, respectively, in both sexes.

Conclusions: The height/width ratio of tooth 47 produced an average error of less than one year when estimating the chronological age of subjects aged up to 16. This degree of optimal precision is comparable to that of other age-estimation procedures for children and adolescents, but has the added value of greater ease when obtaining measurements than approaches like Cameriere's.

0247.2

A New Method to Improve Reproducibility of Tooth Color Assessment

Caterina Perra, Pier Francesco Porciani, Simone Grandini

University of Siena, Siena, Italy

Objectives: Multiple instrumental measures of tooth color can be affected by the difficulty to hold the same position when determining them even using a modern portable digital spectrophotometer. To reduce this bias, it was developed an individual silicon mask to place the instrument reading tip always in the same position and angle on the surface of the tooth thanks to a precisely fitting hole.

Methods: Two tests were planned to evaluate the effect of a customized silicon mask on the variability of multiple measurements of color coordinates (L, a, b) and correlated whitening indexes (WIO, WI_D). It was examined the intra-operator variability in the values given by a portable digital spectrophotometer (Vita Easyshade Advance 4®) on natural teeth of at least four different Vita® shades with or without the mask at the same time (test 1) or with the mask before and after 2.5 minutes (test 2).

Results: In test 1, the application of this personal silicon mask slightly modified the real color of the tooth but significantly reduced the variability of the measures of color coordinates and the WIO and WI_D indexes versus the same measures obtained without the mask (Levene's test <0.01 for the pool of data from different Vita® shades). Moreover, in test 2, there were no significant differences between WIO or WI_D measured at different times.

Conclusions: This preliminary study suggests that the use of an individual silicone mask improves the precision of a portable digital spectrophotometer device for multiple measurements of L, a, b and WIO, WI_D. Regardless of the real color of the tooth, this method could be useful especially for studies focused on the evaluation of optical whitening treatments, scoring the differences between two measures at different times.

0247.3

Comparative Evaluation of Dental Plaque Indication Methods

Vladimir Grosser[1], Larisa Sarap[2], Irina Nikolskaya[1]

[1]Therapeutic Dentistry, Pirogov Russian National Research Medical University, Moscow, Russian Federation, [2]Pediatric Dentistry, Altai State Medical University, Barnaul, Russian Federation

Objectives: Dental plaque (DP) indication is considered among the most effective methods of motivating oral hygiene. This study compares two methods of indication: Qscan plus (Q+) device and disclosing tablets (DT).

Methods: Q+ is based on quantitative light-induced fluorescence (QLF) technology which allows visualising porphyrin-containing DP associated with pathogenic anaerobic bacteria. DT are erythrosine-based. 60 patients aged 5-21 years were examined and divided into two groups: 30 aged 17-21 (1st group) and 30 aged 5-7 (2nd group). The study procedure included examining and photographing 6 anterior teeth of the upper and lower jaw using Q+ (both groups) and DT (1st group), questioning (1st group) and creating two "maps" of DP distribution. One shows pathogenic DP visible in Q+; the other shows DP disclosed with tablets.

Results: The area of DP detected by DT is significantly larger than the area of fluorescent plaque seen in Q+. In the 1st group, the largest accumulations of DP porphyrins were found in the lower jaw anterior teeth interdental spaces (31-33 and 41-43) and on the canines (13, 23, 33, 43). In the 2nd group, pathogenic DP was mostly detected in the lower jaw anterior teeth interdental spaces (73-83) and on the upper and lower jaw anterior teeth cervical areas. 86.67% of the participants would prefer to use the Q+ as a personal plaque indicator.

Conclusions: Based on the modeling of dental plaque distribution maps indicated by different methods, it was concluded that pathogenic dental plaque covers a smaller area than plaque generally. The study participants favored dental plaque optical indication by using the QScan Plus device over the more traditional staining method.

0247.4

Effective Dose of Cone Beam Computed Tomography: a Systematic Review

George Kalfountzos, Anastasia Mitsea
Oral Diagnosis and Radiology, NKUA, Athens, Greece

Objectives: To perform a systematic literature review concerning dosimetry of dentomaxillofacial CBCT imaging with emphasis on exposure protocols and factors affecting dose levels.

Methods: Studies published from 2007 to 2020, identified to MEDLINE®, Cochrane Library, Scopus and Trip Medical Database, using MeSH and free-text terms. The systematic review performed in compliance with PRISMA. Included original studies, used anthropomorphic dosimetry phantoms and dosimeters, utilized only tissue weights from the 2007 ICRP recommendations for calculating effective dose, including pertinent information regarding the scanner used, FOV size, exposure technique, phantom type and dosimeter used, published in English language. Studies without detailed description of exposure protocols and measurement Methods: were excluded. A data extraction sheet created to record effective doses, exposure protocols and measurement methods. Study screening and data extraction carried out independently by two observers. Statistical analysis performed with STATISTICA 12.5 software.

Results: From the 1834 articles initially retrieved, 1735 discarded (as they didn't meet the inclusion criteria) and only 24 used since they met the inclusion criteria. Meta-analysis was not possible to be performed. Reported adult effective doses ranged from 4 to 461 μSv for small FOVs. Child effective doses ranged from 5-582 μSv for small FOVs, 7-769 for medium FOVs and 8-488 for large FOVs. In most cases an anthropomorphic phantom representing the skull of an adult male was used. Only in 4 cases pediatric phantoms corresponding to patients aged 10 years were used.

Conclusions: The most significant statistical correlation was found between the effective dose and the FOV and particularly the height of the FOV. So far, a generally accepted measurement protocol has not been adopted. A protocol with a minimum consensus on measurement methods is recommended. The most reliable dosimetry method is still based in anthropomorphic phantoms. The review discloses the need for a future study on effective doses related to various voxel sizes.

0248

Surface Wear and Cyclic Fatigue Resistance of Novel NiTi Instruments

Merima Balic[1], Dubravka Milovanović[3], Ivona Bago[1], Gianluca Plotino[2], ivica anic[1]
[1]School of Dental Medicine, University of Zagreb, Zagreb, Croatia, [2]Sapienza University of Rome, Rome, Italy, [3]Institute for General and Physical Chemistry, Belgrade, Serbia

Objectives: The aim of this study was to evaluate surface wear and cyclic fatigue resistance of reciprocating (Reciproc Blue, WaveOne Gold) and rotary (XP-endo Shaper, TruNatomy) instrumentation systems, after use in curved canals at body temperature.

Methods: Twenty new NiTi instruments (n=5/each group): Reciproc Blue, WaveOne Gold, XP-endo Shaper (XPS) and TruNatomy Prime (TRN) were used twice for the instrumentation of simulated curved canals (40°) in plastic resin blocks. The instrumentation was performed according to manufacturer's instructions for each system at body temperature using 5 mL of 2.5% NaOCl for each canal. The surface of each instrument was evaluated before instrumentation, and after the first and second use in curved canals by using noncontact 3D optical surface profilometer (Zygo NewView™ 7100). Three roughness parameters were evaluated: the average roughness (Ra), root mean square roughness (Rq), and peak to valley average height (Rz). After two uses, the tested instruments were submitted to cyclic fatigue (CF) test to evaluate the time to failure. The obtained data were analyzed with one-way analysis of variance ANOVA and post-hoc tests (α =0.05).

Results: The TruNatomy instruments showed no significant difference in roughness compared to the reciprocating files, irrespective of the evaluation stage (p=1.0). The XPS instruments showed significantly higher values of roughness compared to the TruNatomy, in every stage, especially after the second use ($P < .008$). The reciprocating instruments had significantly higher CF resistance compared to rotary instruments ($P < .05$). Reciproc Blue showed the highest CF resistance, with 188,4 sec time to failure. XPS had the lowest CF resistance (55 sec), with no significant difference compared to TruNatomy (67 sec).

Conclusions: The TruNatomy and reciprocating instruments showed minimal surface wear, after two uses in curved root canals. The Xp-endo Shaper exhibited significantly higher surface wear. Reciprocating instruments exhibited superior cyclic fatigue resistance compared to novel rotary instruments.

0249

Efficacy of 5-Aminolevulinic Acid and red LED Against Endodontic *Enterococcus Faecalis*

Teocrito Carlesi[1], Morena Petrini[1], Domenico Tripodi[1], Federico Vecchiet[2], Giovanna Iezzi[1], Adriano Piattelli[1, 3], Simonetta D'Ercole[1]
[1]Medical, Oral and Biotechnological Sciences, University of Chieti, Chieti, Ch, Italy, [2]Unit of Dentistry, S.S. Annunziata Hospital, Chieti, Chieti, Italy, [3]Casa di Cura Villa Serena del Dott. L. Petruzzi, Fondazione Villaserena per la Ricerca, Città S. Angelo, Pescara, Italy

Objectives: Photodynamic antibacterial therapy represented one method of disinfection in endodontics. *Enterococcus faecalis* is considered a multi-resistant specie and a possible cause of failure of endodontic treatment. The aim of this work was to evaluate the effect of the association of a novel gel containing 5% 5-aminolevulinic acid with a red LED irradiation against *E. faecalis*.
Methods: Test 1: The ALAD gel (Aladent, Alphastrumenti, Italy) was added to *E. faecalis* broth culture at the final concentration of 50% and 10% v/v. The suspensions were dark incubated at 37 °C for 1 h. The suspensions 10% ALAD were irradiated with 7 and 20 min LED device; the suspension 50% ALAD was irradiated for 7 min.
Test 2: *E. faecalis* broth culture was incubated for 25 min with ALAD 10%, 25%, 50%v/v and irradiated for 5 min. The number of CFUs was determined seeding on agar plates the samples exposed and unexposed to LED light. The cells viability was evaluated with a BacLight LIVE/DEAD Viability Kit (Molecular Probes, Invitrogen, USA).
Results: 10% ALAD, 1 h of incubation promoted a significant reduction of CFUs, but the association 50% ALAD + LED 7 min produced a total inactivation and an evident killing effect on *E. faecalis* cells, characterized by the presence of 95% of dead cells. By decreasing the time of ALAD incubation to 25 min, 25% and 50%v/v concentrations with 5 min of LED exerted a significant reduction of *E. faecalis,* with no significant differences.
Conclusions: The *in vitro* tests demonstrated that ALAD gel with LED irradiation exerts a potent antibacterial activity against *E. faecalis.* The effective bacteria-killing and cells reduction of this photodynamic protocol encourages the use on endodontics. Clinically, the ALAD gel is introduced into the canal with an endodontic needle and activated by an endodontic tip of the dedicated lamp.

0250

Irrigant Activation in Complex Root Canal Systems: a Comparative µCT-Study

Lukas Keim[1], Matthias Widbiller[1], Ralf Schlichting[2], Birgit Striegl[3], Karl-Anton Hiller[1], Rebecca Jungbauer[4], Wolfgang Buchalla[1], Kerstin M. Galler[1]
[1]Department of Conservative Dentistry and Periodontology, University Hospital Regensburg, Regensburg, Germany, [2]Private Practice, Passau, Germany, [3]Department of Biomedical Engineering, Ostbayerische Technische Hochschule (OTH), Regensburg, Germany, [4]Department of Orthodontics, University Hospital Regensburg, Regensburg, Germany

Objectives: The aim of this study was to develop a standardized model system to investigate endodontic irrigation and activation techniques and to assess the efficiency of different activation methods in regard to removal of hard tissue debris in complex root canal systems.
Methods: The mesial roots of 45 extracted mandibular molars were scanned by µCT and distributed randomly into three groups (n=15) of irrigant activation: sonic activation by EDDY® (VDW, Munich, Germany), laser activation by AutoSWEEPS® (shock-wave enhanced emission photoacoustic streaming, FOTONA, Ljubljana, Slovenia) and conventional needle irrigation as a control. Tooth roots were fixed in individual 3D-printed holders to facilitate root canal enlargement to size 30.07 under constant irrigation with sodium hypochlorite (5.25%). To enable standardized quantification of remaining debris, artificial $BaSO_4$-containing dentine powder was compacted into the isthmus and lateral canals, followed by a second µCT-scan. Final irrigation was performed using EDTA (17%) and sodium hypochlorite in combination with the respective activation method. The volume of remaining artificial debris was quantified after a third scan. Data were analysed nonparametrically by Mann-Whitney *U*-tests at an α=0.05 level of significance.
Results: The newly developed model enabled a high level of standardisation in terms of irrigation volume and duration such that only the mode of activation influenced the cleanliness of the canal system. Activation of the irrigant proved to be significantly more effective than conventional needle irrigation regarding the removal of debris, which persisted particularly in the apical third of the root canal in the control group. The reduction of artificial debris was similar for sonic- and laser-activated irrigation without statistical differences in the respective root segments.
Conclusions: The newly developed model system for testing irrigation procedures proved to enable high standardization of experimental parameters. Efficiency of irrigation was significantly enhanced with laser- and sonic-based activation, especially in the apical third.

0251

Effect of Ultrasonic Activation on Porosity of Bioceramics Apical Plugs

Saulius Drukteinis[1], Goda Bilvinaite[1], Paulius Tusas[1], Hagay Shemesh[2]
[1]Institute of Dentistry Faculty of Medicine, Vilnius University, Vilnius, Lithuania, [2]Academic Centre for Dentistry Amsterdam, Amsterdam, Netherlands

Objectives: The present study evaluated the effect of ultrasonic activation on the porosity of BioRoot RCS/modified single cone and MTA Flow root canals fillings used as plugs in the apically perforated moderately curved roots.
Methods: Fifty-six mesial root canals of mandibular first molars were enlarged with ProTaper NEXT X1-X5 rotary instruments 2mm beyond the apical foramen and randomly divided into four experimental groups according to the material and technique used for root canal obturation (n=14): BioRoot RCS/single cone (BR/SC), BioRoot RCS/single cone with ultrasonic activation (BR/SC-UG),

MTA Flow (MF) and MTA Flow with ultrasonic activation (MF-UG). The ultrasonic tip was placed 2mm short of the working length inside the canal after the injection of the flowable cement and activated for 10 seconds. The specimens were scanned before and after root canal obturation with a micro-computed tomography scanner at an isotropic resolution of 9.9µm. The differences between groups in the apical 5mm of root canal fillings were analyzed using the Kruskal-Wallis and Mann-Whitney tests with the significance level set at p <0.05.

Results: None of the obturation technique provided a void-free root canal filling in the apical 5mm. Considerably higher percentages of open and closed pores were observed in the MF and MF-UG groups (p <0.05), with the highest porosity being in the MF-UG fillings. No significant differences were observed only between BR/SC and BR/SC-UG groups, where the quantity of open and closed pores within the obturated root canals remained similar (p >0.05) regardless of the lower mean porosity determined in the BR/SC group's fillings.

Conclusions: The direct ultrasonic activation had no considerable impact on the porosity distribution of BR/SC fillings, while MF fillings demonstrated significantly higher overall porosity after ultrasonic activation.

0252

Clinical Symptoms and OHRQoL Four Years After Primary Endodontic Treatment

Isabell Johnsen, Asgeir Bårdsen, Sivakami Rethnam Haug

Department of Clinical Dentisty, Bergen, Norway

Objectives: Clinical signs and symptoms are seldom reported in outcome studies. The aims of this study were to investigate (1) the presence of clinical signs and symptoms and (2) patient's perceived oral health-related quality of life (OHRQoL) four years after primary non-surgical root canal treatment (RCT).

Methods: 234 patients (257 endodontically treated teeth) who were previously included in a quality assurance study was offered a 4-year recall appointment at University of Bergen, Bergen, Norway. All patients received a thorough clinical examination and filled out the Oral Health Impact Profile 14 (OHIP-14) questionnaire to assess OHRQoL. Persistent pain after RCT was registered on a numerical rating scale (NRS) from 0 to 10 where 0 is 'no pain' and 10 is 'worse possible pain'. Response to individual items in OHIP-14 questionnaire were registered on a Likert scale. Individual domain score was derived by summing up responses of the two items within a particular domain. SPSS Version 25 (IBM Corp, Armonk, NY) was used for statistical analysis.

Results: A total of 149 patients (160 teeth) attended the 4-year (range 4-6year) recall appointment. Clinical signs and symptoms were registered in 18 (11.3%) teeth. The most frequent symptom was tenderness to percussion (n=10) followed by tenderness to palpations (n=7) and pain (n=4; NRS score of 1,5,6 and 8). Sinus tract was present in three teeth and two teeth presented with endodontic pocket to the apex. Several teeth presented with more than one symptom. Patients with clinical signs and symptoms had a significantly poorer OHRQoL in four domains, which were physical pain, psychological discomfort, physical disability, and psychological disability (p<0.05).

Conclusions: Four years after primary RCT, 11.3% of teeth presented with clinical symptoms and signs of infection. Patients with clinical signs and symptoms had a significantly poorer OHRQoL.

0253

Does Endodontic Treatment Improve Healing of Endo Periodontal Lesions: Systematic Review.

Kadiatou Sy, Etienne Deveaux, Kevimy Agossa

University of Lille, Inserm, CHU Lille, U1008—Controlled Drug Delivery Systems and Biomaterials, Lille, France

Objectives: Several studies have shown that the presence of untreated endodontic infections impairs healing after periodontal therapy. However, it is unclear whether endodontic treatments can mitigate this risk factor or even improve periodontal healing. Endo-periodontal lesions (EPL) are clinical situations in which both periodontal and pulpal pathologies are concomitant. The aim of this systematic review was to assess the effect of endodontic therapy (ETH) on EPL healing outcomes.

Methods: Two reviewers conducted independent searches of the Medline/Pubmed and ScienceDirect databases up to and including December 2020. Studies reporting at least periodontal pocket depth (PPD) before/after ETH alone or combined with periodontal therapy for EPL were included. Studies on periodontal regenerative/reconstructive procedures were excluded.

Results: Five randomized controlled trials (RCT) and two observational studies published between 2014 and 2020 were selected from among 431 citations. Overall, ETH alone or combined with periodontal debridement (SRP; scaling and root planing) resulted in an improvement in periodontal pocket depth (PPD) and clinical attachment levels (CAL) ranging from (i) PPD: –0.91 to –2.22 mm and CAL: +0.60 to +2.17 for ETH alone, (ii) PPD: –1.39 to –2.21 mm and CAL: +1.32 to +2.56 mm for ETH + non-surgical SRP, and (iii) PPD: –2.15 to –3.31 mm for ETH + surgical SRP. Three RCT reported heterogeneous results (two studies were supportive and one was not) with respect to the adjunctive benefit of using temporary intracanal medications (ICM) on periodontal outcomes. Two RCT failed to show any effect of immediate or delayed SRP with respect to ETH.

Conclusions: The very low-certainty evidence suggests that endodontic therapy (alone or combined with SRP) may have a positive effect on PPD reduction and CAL gain in EPL. The adjunctive effect of intracanal medication should be further investigated.

0253.1

Agreement Between Teacher and Student in Preclinical Endodontic Treatment Assessments

Patricia Terceño, Virginia Belliard Tiol, Bruno Baracco, Dayana María Da Silva Gonçalves, Nuria Isabel Escribano Mediavilla, Laura Ceballos García, Maria Victoria Fuentes Fuentes

Stomatology, URJC, Madrid, Madrid, Spain

Objectives: To determine the agreement amongst faculty member assessments and student self-assessments of preclinical endodontic treatments using a rubric.

Methods: Data were collected from 44 fourth-year dental students at preclinical endodontic practices at Rey Juan Carlos University in Spain during the 2019-2020 academic year. Students performed root canal treatments in hand-held extracted human molars (six root canal treatments per student) (n=198). For the evaluation of each treatment, a rubric with several categories (radiographic evaluation, access preparation, shaping procedure, obturation) was used. The overall score was calculated on a ten-point scale by adding scores of each weighted category (10% radiographic evaluation and 30% each for the rest). After the self-assessment of the students, three previously calibrated and blinded teachers performed the evaluation of the root canal treatments after random assignment. Quadratic weighted Kappa and ICC were calculated (α=0.05).

Results: Agreement between teachers and students was moderate for radiographic assessment (0.56) and for access preparation (0.49), and substantial for shaping procedure (0.62) and for obturation (0.67). ICC between numerical global scores was moderate (0.58).

Conclusions: The agreement in overall scores between teacher assessments and student self-assessments of endodontic preclinical treatments was moderate. Overall, student scores were lower than those of teachers.

0253.2

Pulp Exposures Management Preferences Among Spanish Dentists.

Laura Bailleul[1], Maria Victoria Fuentes[1], Sophie Doméjean[2], Laura Ceballos[1]

[1]Rey Juan Carlos University, Alcorcón, Madrid, Spain, [2]Clermont Auvergne University, Clermont-Ferrand, France

Objectives: The objective was to study the attitude and management of extremely deep caries lesions and pulp exposures in permanent teeth, with open or closed apices, among Spanish dentists that perform Operative Dentistry treatments.

Methods: A web-based open and anonymous survey, approved by the ethics board of the Rey Juan Carlos University (#0212202023920), was elaborated using Microsoft Forms (Microsoft Office 365) and distributed among dentists in Spain. The questionnaire was electronically administrated through social media with the collaboration of scientific societies, organisations, and dental councils. The survey was available for 25 days and, afterwards, results were descriptively analysed.

Results: 538 responses from dentists that perform operative treatments of caries lesions were received. Pulp exposures in vital and asymptomatic teeth were mainly treated by direct pulp capping, in open and closed apices (80 and 81%, respectively). This percentage decreased to 57% when the tooth presented reversible pulpitis, regardless of the apex status. If an irreversible pulpitis was diagnosed in an immature tooth, 52% decided to carry out the endodontic treatment rising to 90% if the apex was closed. Although 53% of the dentists considered pulpotomy an alternative to endodontic treatment for pulp exposures or irreversible pulpitis cases, only 26% routinely perform it. Regarding the clinical procedure, a dry cotton was preferred to obtain hemostasia (40%) and Biodentine was the biomaterial of election (55.6%), followed by MTA (23%) and calcium hydroxide (10.5%). Partial and total pulpotomies were chosen as a minimally invasive evidence-based treatment that allows maintaining pulp vitality.

Conclusions: Spanish dentists reported significant variation in the management of pulp exposures in extremely deep caries lesions. The decision was mainly influenced by the clinical symptomatology and not by the apex status. Pulpotomy was considered a valid alternative to endodontic treatment and bioceramic materials are preferred over calcium hydroxide.

0254

Oral Cleaning Habits and Quality of Life in the Elderly

Inês C. Santos[1, 2], Kateryna Rudysh[3], Fabrícia Martins[3], Luís Proença[1], Ana C. Manso[1, 3], Mário C. Polido[1, 3], José João Mendes[1, 3], Helena Canhão[2]

[1]Clinical Research, CiiEM, Caparica, Portugal, [2]EpiDoC Unit - CEDOC, Nova Medical School, Lisbon, Portugal, [3]Clinical Research Unit, Egas Moniz University, Caparica, Portugal

Objectives: This exploratory study aims to characterize oral cleaning habits in the elderly and evaluate their impact on quality of life.

Methods: The study, approved by a state-recognized Ethical Committee, included 80 elderly who attended a university dental clinic in Portugal over a 3-month period. Inclusion criteria were age 65+ years, being non-institutionalized and having signed informed consent. Participants were distributed into four groups according to their age (years): 65-70, 71-75, 76-80 and 81+. Information was gathered through a questionnaire about oral cleaning habits (toothbrushing frequency, use of interdental cleaning aids (ICA) and mouthrinses) and oral health-related quality of life (OHRQoL) was obtained through Geriatric Oral Health Assessment Index (GOHAI). It was considered having "good oral cleaning habits", brushing twice or more a day and using ICA or mouthrinses. OHRQoL was classified according to GOHAI scores as "high" (34-36), "moderate" (30-33) or "low" (<30). Data were analyzed through descriptive and inferential statistical methodologies. A significance level of 5% (p=0.05) was considered.

Results: The majority of participants brush their teeth twice or more a day (76.3%). Also, the majority use mouthrinses (65.0%), except for the age group 76-80, where 46.2% use them. Concerning the use of ICA, the majority (61.3%) do not use them. However, in the age group 76-80, the majority of the participants use them (53.8%). According to our criteria, 63.7% of all participants have

good cleaning habits. The distribution according to their OHRQoL score was the following: 36.3% (high), 32.5% (moderate), and 31.3% (low). No significant association was found between the OHRQoL and age (p=0.260) or the oral cleaning habits (p=0.967).
Conclusions: Overall, participants exhibit good oral cleaning habits, with the majority brushing their teeth twice a day or more often. The majority do not use any ICA but uses mouthrinses. OHRQoL was not found to be related to oral cleaning habits.

0256

Primary Stability of a Self-Tapping and Round Apex Dental Implant.

Alessandro Cipollina[1], Margherita Tumedei[2], Adriano Piattelli[2], Antonello Falco[3], Francesco de angelis[2], Felice Lorusso[4], Mariastella Di Carmine[1], Giovanna Iezzi[2]

[1]Private practice, Loreto Aprutino, Italy, [2]Department of Medical, Oral and Biotechnological Sciences, University of Chieti, Chieti, European Region, Italy, [3]private practice, Viale Gabriele D'Annunzio, 73, 65127, Italy, [4]Department of Innovative Technologies in Medicine and Dentistry, University of Chieti, Chieti, Italy

Objectives: The dental implant primary stability and micromovement absence represent critical factor for dental implant osseointegration. The aim of the present in vitro investigation was to simulate the bone response on different polyurethane densities the effect of self-tapping threads and round apex implant geometry.
Methods: A total of 40 implants were positioned in D1, D2, D3 and D4 polyurethane block densities following a calibrated drilling protocol. The Insertion, removal Torque and resonance frequency analysis (RFA) means were calculated.
Results: All experimental conditions showed insertion torque values >30 Ncm. A significant higher insertion torque, removal and RFA was present in D1 polyurethane. Similar evidences were evidenced for D3 and D4.
Conclusions: The effectiveness of the present study suggested a valuable clinical advantage for self-tapping threads and round apex implant using, such as in case of reduced bone density in the posterior maxilla.

0257

Primary Stability of NanoShort, Short and Standard Dental Implants.

Adriano Piattelli[1, 2], Luca Comuzzi[3], Margherita Tumedei[1], Camillo D'Arcangelo[1], Giovanna Iezzi[1]

[1]Department of Medical, Oral and Biotechnological Sciences, University "G. D'Annunzio", Chieti, Italy, [2]Casa di Cura Villa Serena del Dott. L. Petruzzi, Città Sant'Angelo, Italy, [3]Private Practice, San Vendemmiano (TV), Italy

Objectives: Aim of the present study was to compare the insertion torque (IT), the pull-out values (RT) and the resonance frequency analysis (RFA) values of Nanoshort (3 mm long), Short (7 mm long) and tandard length implants (10 mm long) inserted in polyurethane solid rigid blocks and sheets of different thicknesses and densities.
Methods: The simulation has been performed comparing the IT, the pull-out values, and the RFA of Nanoshort, Short and Standard dental implants (AoN implants S.r.l., Grisignano di Zocco, Vicenza, Italy) positioned in polyurethane foam solid rigid blocks with densities of 10 and 20 Per Cubic Feet (PCF) and sheets of 1 mm, 2 mm and 3 mm thicknesses and of 20 and 30 PCF densities.
Results: The IT measurements for Short implants ranged between 12.57 Ncm in 20 PCF of 1 mm to 39.2 Ncm in 3mm 30 PCF. The higher the polyurethane density and the thicker the additional cortical sheets, the higher the IT and pull-out for all types of implants ($p<0.05$). Increased IT values in Short implants when inserted in 30 PCF of 3 mm, lower values for 4 mm diameter Nanoshort implants when inserted in 20 of 1 mm cortical (4-6 Ncm) In these latter cases, however, the primary stability appeared to be sufficient ($p<0.05$). In 3 mm sheets, the IT values for Nanoshort implants were good (15-20 Ncm). No significant difference were detected about IT and RT within the implants tested on 10 PCF (without cortical layer) and 1mm Lamina 20PCF/30PCF ($p<0.05$).
Conclusions: The NanoShort and Short implants showed a good level of primary stability in artificial bone in all experimental situations, the Nanoshort even with very low ISQ values, and could represent a useful tool to avoid regenerative procedures in atrophic jaws.

0258

Primary Stability in D3 Synthetic Bone of Different Implant Macrogeometries

Federico Vecchiet[1], Stefano Fanali[2], Margherita Tumedei[2], Pamela Pignatelli[2], Francesco Inchingolo[3], Adriano Piattelli[2, 4], Giovanna Iezzi[2]

[1]"SS. Annunziata" Hospital, Chieti, CH, Italy, [2]Department of Medical, Oral and Biotechnological Sciences, University "G. D'Annunzio" of Chieti-Pescara, Chieti, CH, Italy, [3]Department of Interdisciplinary Medicine, University of Bari "Aldo Moro", Bari, Bari, Italy, [4]Fondazione "Villa Serena" per la Ricerca, Città Sant'Angelo, PE, Italy

Objectives: The aim of this research was to study, the primary implant stability on polyurethane foam sheets, wide-threaded design of implant compared to narrow-threaded implant.
Methods: Two implant designs (FMD, Rome, Italy)were positioned on polyurethane block in vitro: The wide-threaded implant, and the narrow-threaded implant. Moreover, for each group two size were considered: 3.8mm x 12mm and 4.8mm x 12 mm. The insertion torque (IT) values, the removal strength (RT), and the Periotest analysis were evaluate.
Results: A significant higher IT and RT was recorded for wide-threaded implant ($p<0.01$) if compared to narrow-threaded implant. A higher stability of wide-threaded implant was evidenced by Periotest measurements ($p<0.01$).
Conclusions: The wide-threaded implant design showed a higher primary stability on alveolar cancellous synthetic bone in vitro and are suggested for a clinical application in poor density bone ridge.

0259

Accuracy of Guided Implant Surgery Using Desktop 3-D Printed Guides

Rani D'haese[1], Tom Vrombaut[1], Geert Hommez[1], Hugo De Bruyn[2, 1], Stefan Vandeweghe[1]

[1]Ghent University, Ghent, Belgium, [2]Radboud University Medical Hospital, Nijmegen, Netherlands

Objectives: The increasing popularity of desktop 3D printers, makes guided surgery more accessible. The aim of this in-vitro study was to evaluate the accuracy of single tooth guided implant surgery, by means of a 3D printed tooth supported guide.

Methods: Fifteen implants were virtually planned to replace a missing first mandibular molar, using planning software for guided implant surgery (Exoplan®, Exocad GmbH, Darmstadt, Germany). A tooth supported guide was designed and manufactured using a desktop 3D printer (D40, Rapidshape GmbH, Heimsheim, Germany). The implants were placed fully guided in the casts and a digital impression was taken to register their position in the cast. This scan was compared with the virtual position in the implant planning software, using metrology software (Geomagic Qualify X, Geomagic Research Triangle Park, NC, USA).

Results: The mean angular deviation was 2.63° (SD 1.69°; range 0.38° - 5.99°), the mean coronal deviation, 0.52 mm (SD 0.25; range 0.09 mm – 1.07 mm) and the mean apical deviation 0.90 mm (SD 0.47; range 0.14 mm – 1.74 mm). The absolute apical mean deviation in bucco-lingual direction (x-axis) was 0.70mm (SD 0.42, 0.12 mm – 1.65 mm) ($p<0.001$), in the mesio-distal direction (y-axis) 0.34mm (SD 0.26; range 0.01 mm – 0.80 mm) (0.650) and in the vertical direction (z-axis) 0.32mm (SD 0.27; range 0.02 mm - 1.00 mm) ($p=0.010$).

Conclusions: Based on this in vitro study, an in-house planning followed by a desktop 3-D printing of a tooth-supported guide yields an acceptable accuracy for single implant placement which opens possibility for future clinical implementation. Further investigations are required to confirm this in a clinical setting.

0260

The Microscopical Effectiveness of Dental Implants Retrieved for Different Causes

Margherita Tumedei[1], Adriano Piattelli[1], Marco degidi[2], carlo mangano[3], Giovanna Iezzi[1]

[1]Department of Medical, Oral and Biotechnological Sciences, Università di Chieti, Chieti, Italy, Italy, [2]Private practice, Bologna, Italy, [3]Private Practice, Como, Italy

Objectives: For decades, the histologic evidence about osseointegration and the bone-implant interface has been discussed in the literature. In this review, the effectiveness of dental implants retrieved for different causes was evaluated.

Methods: a literature search was performed in databases for papers about implants retrieved from humans published by the Implant Retrieval Center of the University of Chieti-Pescara, Italy.

Results: Sixty-eight articles were selected into categories based on topics. The data indicated high level of bone-to- implant contact, lamellar bone close to the surface, roughness related to an increased bone response, organized and remodeled bone after loading, and peri-implant interfaces subjected to a continuous dynamic function. The best bone tissue response seems to be present around implants with a moderately rough surface. The peri-implant bone appeared to have a higher level of organization with the passing of time, as well as many remodeling areas. Well-organized, mineralized, remodeled lamellar bone has been found around human retrieved implants even after a very long functional loading history. BIC percentages varied greatly, from the low 30s to the high 90s.

Conclusions: Even when the BIC was quite low, the implants remained stable and able to support the loading of dental implants in the long-term. It is very important to study well-integrated human retrieved dental implants with surrounding bone tissue.

0261

Evaluation of Implant Wettability with Blood vs Autologous Platelet Liquid

Felice Lorusso[1], Calogero Buggea[1], Luan Mavriqi[2], ANTONIO SCARANO[1]

[1]University of Chieti, Chieti, Italy, [2]University of Albania, Tirana, Albania

Objectives: The physicochemical characteristics of the titanium surfaces, such as wettability, protein binding, cell adhesion and proliferation are tightly related to the osteointegration process of the dental implant in the host bone. The aim of this investigation was to evaluate the wettability of two titanium surfaces, sandblasted and double acid etched (group S/E) and sandblasted (group S), using blood and *Autologous Platelet Liquid*.

Methods: The surface ultrastructure and roughness were evaluated by scanning electron microscopy (SEM) and atomic force microscopy (AFM). The wettability was measured by the static contact angle (CA) assessed with the sessile drop technique. The fibrin clot derived by blood vs APL was also evaluated by SEM observation.

Results: The fibrin clot structure that develops from blood and APL, knowing that a greater clot, firmly attached to implant can facilitate cell migration to the implant interface. Both of titanium surfaces showed a hydrophobic characteristic, regardless of the wetting liquid used: S surface showed higher CA values, for both the wetting fluids used. Lower CA values on S/E surface are attributable to the different surface energy, which depends on different surface topography (the S surfaces was rougher) and on chemical composition.

Conclusions: The clot obtained from whole blood differs from APL clot due to different cellular composition and fibrin density. Structural differences was present between blood and APL but it is not possible to attest one's superiority on another clot because other factors such as stability to contraction, elasticity and resistance to fibrinolysis should be investigated.

0263

Periodontopathogens on the Implant Temporary Crowns- a Pilot Study

Aleksandra Popovac, Jovana Kuzmanovic Pficer, Jelena Milašin, Nadja Nikolić, Branka Trifković, Miodrag Šćepanović, Aleksandra Milić Lemić

University of Belgrade, School of Dental Medicine, Belgrade, Serbia

Objectives: Periimplant disease is the result of an imbalance between host response and bacterial load. This infection is attributed to the gram-anaerobic microflora, most of them also associated with periodontal disease as *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Fusobacterium sp., Prevotella intermedia, Aggregatibacter actinomycetemcomitans, Staphylococcus aureus,* and *Candida albicans*. The present study aimed to identify the presence of the main types of periodontopathogen microorganisms attributed to the periimplant disease on temporary crowns of the implant-supported restorations.

Methods: Biological materials were collected from the abutment surfaces and implant supported temporary restorations. Bacterial DNA was extracted using sodium hydroxide based chemical protocol and the presence of specific bacteria was evaluated by means of polymerase chain reaction (PCR), using previously designed species-specific primers. The temporary crowns on temporary abutments were used for conditioning and contouring the emergence profile on peri-implant soft tissue. Provisionals were made from composite materials and were used for 3-6 months before the insertion of the final restoration.

Results: In the analysed biomaterial from 13 temporary crowns, *Fusobacterium spp* dominated (in 84.6% samples), followed by *Porphyromonas gingivalis* (in 61.5% samples). *Aggregatibacter actinomycetemcomitans* and *Tannerella forsythia* were found in 38.5% samples and rarely present were *Treponema denticola* and *Prevotella intermedia* (in 7.7% samples). In most cases (63.6%), *Fusobacterium spp* were identified together with *Porphyromonas gingivalis.* Samples mostly contained 4 or 5 periodontopathogens (together in 69.5% cases), and in 7.7% of cases all examined pathogens were identified.

Conclusions: The results revealed the presence of periodontopathogens on analysed surfaces. Precise identification of different types of periodontopathogens in bacterial colonization of implant abutments and gingival surfaces of the crowns is important for early detection of the infection and consequently for the prevention and proper treatment of periimplant disease. Further research is ongoing with more samples and microorganisms quantification.

0264

Evaluation of an Antibacterial Internal Implant Coating by Voc's Analysis

Francesca Postiglione[1], Felice Lorusso[1], Calogero Buggea[1], Luan Mavriqi[2], Antonio Scarano[1]

[1]University of Chieti, Chieti, Italy, [2]University of Albania, Tirana, Albania

Objectives: The precision of the implant-abutment prosthetic junction is a critical point for the biofilm prevention and bacteria proliferation due to early peri-implant diseases. The aim of the present study was to investigate the efficacy of a novel antibacterial coating of implant internal chamber by real time volatile organic compounds (VOCs) biosensor.

Methods: A total of 20 subjects and 40 internal connection dental implants were evaluated in the present study. A total of 20 implant was provided by antibacterial internal coating (Test) and 20 screws presented a regular connection without the treatment (Control). The VOCs measurements were measured at the baseline (T_0) after the cover unit removal, after 7 days (T_1) and at 14 days (T_2).

Results: No significant difference were reported at T_0 (baseline), while the Test and Control groups showed a VOCs max peak mean respectively of 2.15±0.71 ppm and 2.21±0.69 ppm ($p>0.05$). At T_1 and T_2 as significant difference between the Test and Control Groups was detected ($p<0.01$). At T_2 the Test max peak was 2.29±0.73 ppm and the Control was 3.65 ±0.91 ppm ($p<0.01$).

Conclusions: The antibacterial internal coating revealed a useful capacity to significantly reduce the VOCs activity at the level of the implant internal chamber and could be useful to prevent early peri-implant disease.

0266

Laser Microtexturing of Titanium Surfaces for Reduced Oral Biofilm Formation.

Andrei C. Ionescu, Eugenio Brambilla

Biomedical, Surgical, and Dental Sciences, University of Milan, Milan, Italy

Objectives: Surface micro- and nanotexturing is an effective way to improve osseointegration of dental implant titanium surfaces. When finely tuned, it can also be a novel way to modulate biofilm formation and possibly help reduce peri-implantitis. This study aimed to evaluate surface characteristics and biofilm formation on two laser microtextured titanium surfaces, comparing them with machined, polished, and grit-blasted surfaces.

Methods: Laser microtexturing was performed as either a series of 20 µm-wide pits, distanced 30 µm, or parallel 20 µm-wide grooves of variable depth (10-30 µm). Machined, polished, and grit-blasted surfaces were obtained conventionally. Surfaces were characterized using 3D SEM imaging, surface roughness, surface free energy, and energy-dispersive X-ray spectroscopy (EDS). An oral microcosm biofilm model was developed on titanium disks' surfaces (n=21/group) using a continuous-flow bioreactor for 48 h. Adherent, viable biomass was quantitatively evaluated (MTT test), surfaces were analyzed using confocal laser scanning microscopy, scanning electron microscopy, and EDS.

Results: Laser-treated surfaces displayed peculiar topographies with similar surface roughness to grit-blasted surfaces. Machined surfaces showed lubricant contamination. Grit-blasting deposited alumina and silica remnants, while laser treating deposited a TiO2 rich layer between ablation spots/grooves. Laser-treated surfaces showed the lowest biofilm formation, not significantly different from machined and polished surfaces, while grit-blasted surfaces showed three times higher biofilm formation.

Microorganisms preferably colonized the edges of the laser-created pits and grooves, with very little or no biofilm formation observed inside. Both rough features and the TiO2 rich layer on the edges may explain this behavior.
Conclusions: Laser microtexturing of titanium surfaces created surface microtopographies and also influenced surface chemical composition. Both microtextures were equally effective in reducing biofilm formation and should be furtherly investigated for their capacity of preventing peri-implantitis in vivo.

0267

Micro-CT and Histomorphometrical Study of Graphene-Doped/Poly-Methyl-Methacrylate Implants in Rabbits Bone

Roberto Duraccio[1], Felice Lorusso[1], Fabio Di Carlo[1], Calogero Buggea[1], Luan Mavriqi[2], Antonio Scarano[1]
[1]University of Chieti, Chieti, Italy, [2]University of Albania, Tirana, Albania

Objectives: The graphene-doping processes has been reported as a useful application for improving the mechanical behaviour and the biological properties of metal free biomaterials and methacrylate polymers for medical devices. The aim of the present investigation was to perform a comparative evaluation of the Graphene doped Poly(methyl methacrylate) (GD-PMMA) implants osseointegration compared to PMMA implants on rabbits bone model.
Methods: a total of eighteen adult New Zealand white male rabbits were treated in the present in vivo investigation for a total of 36 dental implant positioned into the articular femoral knee joint: 16 GD-PMMA fixtures (Test) and 16 PMMA fixtures (Control). The animals were euthanized at different timepoints after 15, 30 and 60 days and the retrieved biopsies were evaluated by Micro-CT and histomorphometry measurement.
Results: Histologically, all implants positioned were healed and well-integrated into the bone site and the surface appeared in tight contact with cortical bone along the upper threads, while the lower threads were in contact with either newly formed bone or with marrow spaces. The histomorphometry and Micro-CT assessment reported that the Test and Control implants were well osseointegrated and the bone was in direct contact with wide portions of the implant surfaces, including the space in the medullary canal.
Conclusions: The effectiveness of the present in vivo investigation reported that GD-PMMA surfaces are able to improve the osseointegration in rabbit bone. Further in vitro and vivo animal studies are necessary to evaluate of graphene-doping processes to increase the dental implant properties for a clinical application.

0268

Narrow Implants Display Negligible Marginal Bone Loss in T2DM Patients

Hakan Bilhan[2], Anton Friedmann[2], Mehmet Selim Yildiz[1], Angelina Bespalova[2], Daniel Diehl[2]
[1]Periodontology, Altinbas University, Istanbul, Turkey, [2]Periodontology, Witten/Herdecke University, Witten, Germany

Objectives: Type 2 diabetes mellitus (T2DM) constitutes a group of metabolic disorders, which is characterized by immoderate serum glucose levels, leading to increased risk of biologic complications in dental implant therapy following exacerbated inflammation. Narrow diameter implants (NDI) were developed for placement in diminished ridge dimensions, making it possible to circumvent extensive lateral augmentation procedures. In order to avoid unpredictable wound healing complications in T2DM patients, NDI may represent a feasible alternative for patients with decreased alveolar bone width. This pilot study aimed at comparing the marginal bone loss at NDI in T2DM and normo-glycemic patients over a 4-year period.
Methods: One to two narrow-diameter tissue level implants were placed in the posterior maxilla or mandible of 16 T2DM patients with HbA1C>6.5% and 16 normo-glycemic patients (HbA1C< 6.0), respectively. After a three-month integration period implants were loaded. Digital periapical radiographs were taken and analyzed at baseline (T0), after 12 (T1) and 48 months (T2). Marginal bone level (MBL) was assessed in silico by means of a perpendicular line connecting the most coronal point of the bone crest in contact with the implant to a fixed reference. The delta values were calculated as $MBL=MBL_{T1\,/\,T2} - MBL_{T0.}$
Results: Separate from three patient dropouts for unrelated reasons, the overall implant survival rate after 48 months resulted in 100%. The radiographically determined marginal bone loss between groups revealed no significant differences.
Conclusions: The study reveals radiographically stable crestal bone condition at NDIs used in the posterior areas from all participating patients. Accounting for the relatively long observation period, no biological or technical complications occurred regardless of the underlying metabolic condition. Thus, specifically the T2DM patient may benefit from the treatment with narrow diameter implants making complex surgical augmentative interventions redundant.

0269

Preload and Friction of Carbon-Coated Screws in Implant-Abutment Connections

Kawe Sagheb[1], Keyvan Sagheb[2], Stefan Wentaschek[1]
[1]Department of Prosthodontics, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany, [2]Department of Oral and Maxillofacial Surgery, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany

Objectives: The preload in an implant-abutment-screw arises when the tightening-torque leads to elongation of the screw. Preload depends on friction and the applied tightening-torque. For steel screws, it is presumed that the major part of tightening-torque is used to overcome friction while less than 10% is transferred to preload. For this reason and because titanium alloys have greater friction than steel, some manufacturers use carbon-coated screws to reduce friction and thereby achieve higher preload at the same torque. To calculate preload for a specific tightening-torque the coefficient of friction (COF) is needed. The aim of this in vitro study was to measure preload and to calculate COF for an implant-abutment complex with a coated screw.

Methods: A custom load assembly was used to measure the preload after applying tightening-torque. COF was then calculated using these measurements. Preload was measured for 25 units of unused titanium-implants, abutments and carbon-coated screws (Replace-Select-Tapered implants, Temporary Abutments, Abutment Screw Nobel Replace, Nobel Biocare AG, Zürich, Switzerland). A tightening-torque of 25 Ncm was applied three times to each screw.
Results: For the coated screws, there is an apparent and significant decrease of preload and increase of COF after each repetition. The table below depicts the results for preload and COF.
Conclusions: Even with coated implant-abutment-screws, the COF is high (COF steel: 0.08-0.16). Repeated tightening lead to decrease of preload and increase of COF. These effects can be attributed to increasing friction by cumulative micro-damages in the surfaces. The results suggest that it can be advantageous to use a new screw for the final insertion of the denture.

0270

The 30-Year Experience of the Implant Retrieval Center: Histological and Histomorphometrical Effectiveness of the Dental Implant Loading on the Peri-Implant Soft and Hard Tissues.

Michele Di Cosola[1], Margherita Tumedei[2], Adriano Piattelli[2], Marco degidi[3], carlo mangano[4], Giovanna Iezzi[2]
[1]private practice, Bari, Italy, [2]Department of Medical, Oral and Biotechnological Sciences, università di chieti, Chieti, Italy, [3]Private Practice, Bologna, Italy, [4]Private Practice, Como, Italy

Objectives: The aim of the present review was to assess the histological and histomorphometrical data from the paper published by our Laboratory on peri-implant bone in dental implants in different loading conditions.
Methods: The papers published in different implant loading conditions, in dental implants retrieved from humans, and in the Hard Tissues Research Center of the University of Chieti-Pescara, Italy, were screened on MEDLINE/PubMed, Embase, Scopus, and other electronic databases until 31 December 2018. Only articles that reported the histological and histomorphometrical values of the Bone-Implant Contact (BIC) were selected.
Results: The system selection provided a total of 155 papers. The manuscripts included for the narrative review were 57. These papers provided histological and histomorphometrical data.
Conclusions: The bone remodeling around dental implants was found to be a dynamic process; loading changed the microstructure of the peri-implant bone; and implants were found to provide a successful function, over several decades, with different range of degrees of BIC in vivo (varying from a little more than 30% to a little more than 90%). Loaded implants presented a 10%–12% higher BIC values when compared to submerged, unloaded implants, and rougher surfaces had, on average, about a 10% higher BIC than machined surfaces.

0271

Hybrid Screwmentable Implant-Supported Restorations: Systematic Review and Case Series

Katia Sarafidou, Georgia Vasileiadi, Marilena Kalliopi Louvrou, Eirini Moldovani, Petros Koidis, Maria Kokoti, Athina Bakopoulou
AUTH, Thessaloniki, Greece

Objectives: Traditionally, implant-supported restorations are either cement- or screw-retained. Lately, a hybrid design that combines advantages of both previous categories has been adopted by clinicians. Such prostheses consist of a suprastructure with a screw access channel, which is permanently cemented on a customized abutment or a prefabricated base; thus, the restoration is being screwed as one-piece into the implant body. Aim of the present systematic review was to identify data on hybrid screwmentable prostheses and evaluate their long-term performance. A series of clinical cases is also presented.
Methods: The search strategy followed the guidelines of the preferred reporting items for systematic reviews and meta-analyses (PRISMA). The electronic search was conducted by using the PUBMED, Cochrane and Google Scholar databases and was supplemented by a hand search by two independent reviewers. Inclusion criteria were studies on the hybrid type of prosthesis with publication dates from 2004 to April 2020.
Results: The search concluded in 494 records. After screening 24 were finally included in the review. These presented significant heterogeneity concerning the manufacturing process and the materials used. Some authors opted to cement the supraconstruction directly onto the abutment (two-piece restoration), while others machined a mesostructure (abutment), which was bonded onto a titanium-insert (three-piece). Zirconia, lithium disilicate and titanium alloy constitute the materials of choice for the fabrication of such prostheses. The aesthetics, mechanical and biological properties of screwmentable restorations appeared satisfying. Data on the survival and success rates are limited. Debonding and restrictions connected with the screw access channel were reported as disadvantages. These can be avoided by technique optimization, as described in the case series.
Conclusions: The hybrid screwmentable design is a promising alternative for implant-supported restorations by providing passive fit, retrievability, and excess cement control. Further studies are required to evaluate long-term clinical behavior.

0272.1

Stress Distribution of One-Piece and Two-Piece Mini-Implant Overdentures (Various Attachments)

Trang N. Bui[1], Manabu Kanazawa[3], Natsuko Murakami[2], Noriyuki Wakabayashi[2], Awutsadaporn Katheng[1], Sai Tun Naing[1], Sahaprom Namano[1], Maiko Iwaki[4], Shunsuke Minakuchi[1]

[1]Gerodontology and Oral Rehabilitation, Tokyo Medical and Dental University, Tokyo, Japan, [2]Removable Partial Prosthodontics, Tokyo Medical and Dental University, Tokyo, Japan, [3]Oral Digital Process, Tokyo Medical and Dental University, Tokyo, Japan, [4]General Dentistry, Tokyo Medical and Dental University, Tokyo, Japan

Objectives: Mini-implants have been reported with lower mechanical strength and increased risks of implant fractures despite numerous recognizable advantages. The optimal mini-implant attachment system with the one-piece or two-piece concept regarding stress distribution remains unclear. This study aims to investigate and compare the stress distribution within implants themselves of one-piece and two-piece mini-implant overdentures with 3 different attachment systems (Ball, Locator, and Magnetic) using 3D finite element (3D FE) analysis. The result of this study may assist clinicians in choosing the most beneficial implant attachment system design for mini-implant overdenture.

Methods: Six 3D-FE models simulating a segment of the mandible incorporating one-piece or two-piece mini-implant having Ball, Locator, or Magnetic attachment system and overdentures were created using Rhinoceros 7. Vertical and oblique loading (45° to the longitudinal axis of the implant) of 100N was applied onto dentures. Stress distribution within implants was analyzed using ANSYS 19. Von Mises stress was chosen since it is accepted as metal materials' fracture criterion based on elastic mechanics.

Results: Though one-piece mini-implants tended to show lower maximum stress compare with two-piece ones (average 12.11%), there was no statistically significant difference. The stress value in mini-implants under oblique loading was approximately 1.419 times that under vertical loading. Maximum von Mises stress was found at the neck of implants, which surrounded by cortical bone in all models and during both loading conditions. Magnet attachment systems experienced the greatest stress among the three designs.

Conclusions: Within the limitations of this study, the following Conclusions: might be drawn: There is a trend that one-piece mini-implants exhibited lower levels of implant maximum equivalent stress comparing to two-piece mini-implants. The oblique force was considered more unfavorable for implant stress distribution. Mini-implants overdenture with Locator and Ball attachment systems demonstrated lower stress within implants than Magnet attachment system under vertical and oblique loading conditions.

0272.2

Bone Dimensions of Oligodontia Patients: Case-Control Study

Nicolas Dupré, Orianne Gondel, Rufino Felizardo, Maria Carra, Benjamin P. Fournier, Stéphane Kerner

Université de Paris, Paris, France

Objectives: Oligodontia (OD) is a rare developmental dental anomaly characterized by agenesis of at least six permanent teeth. Affected patients require functional and esthetic rehabilitation. Placement of dental implants can be difficult due to the alveolar bone morphology. The purpose of this retrospective case-control study was to determine whether the bone volume of the maxilla and mandible are altered in OD patients compared to controls.

Methods: CBCTs of 53 adult OD patients (40 maxillary, 32 mandibular) were analyzed and compared with those of 82 (51 maxillary, 31 mandibular) age- and sex-matched controls. Alveolar bone height and thickness at 3, 6, 9 and 12mm depth were evaluated at every dental site (third molars excluded).

Results: For OD patients, the maxillary areas with permanent teeth demonstrated decreased bone height for incisive-canine teeth (17,1±3,0mm vs 19,2±3,9mm, $p<0.001$). Bone thickness was significantly reduced in all areas. In edentulous sites, height was reduced in the incisive-canine area (15,0±4,1mm vs 19,4±4,4mm, $p<0.001$) and bone thickness was significantly decreased in the premolar and molar area. In mandibular areas with permanent teeth, a decrease in bone height was observed for OD patients for both incisive-canine (28,5±4,1mm vs 32,3±3,2mm, $p<0.001$) and premolar areas (29,9±3,1mm vs 27,8±4,0mm, $p<0.001$). Bone thickness was significantly reduced in the incisive-canine area at 3 ($p<0.001$), 6 ($p<0.01$), and 12 mm ($p<0.001$) depth. In edentulous sites, every depth revealed a significant decrease in thickness. Within the OD group, maxillary and mandibular bones were significantly thicker when permanent or temporary teeth were persistent in all areas.

Conclusions: Contrasted with controls, OD patients appeared to have reduced bone volume. Since the absence of teeth in OD patients may lead to this volume deficit of the maxilla and the mandible, it may be beneficial to keep temporary teeth functional to limit alveolar bone resorption.

0272.3

Cellular Adhesion of Gingival Keratinocytes Treated with Platelet Rich Fibrin (PRF) on Titanium Surfaces- a Scanning Electron Microscope Study

Dareen M. Fteita

Department of Periodontology, Institute of Dentistry, Turku, Finland

Objectives: The soft tissue integration between the abutments of dental implants and the surrounding tissues is clinically central for the implant survival rate.

Methods: The growth media used to culture the Human gingival keratinocytes (HMK) were: elutes of titanium-PRF (T-PRF), leukocyte and platelet rich fibrin (L-PRF), and mammalian cell culture medium (SFM-X). The titanium disks used were titanium grade 4 (Ti4), titanium grade 5 (Ti5), and HA disks. The cell cultures to determine the proliferation rate and cell adhesion were performed at 37 °C in a CO2 incubator for 6 h and 24 h. For the scanning electron microscopy (SEM) imaging, the cells on different

disks surface were chemically fixation with 5% glutaraldehyde, followed by a chemical dehydration in graded ethanol series (50%, 70%, and 98%) and allowed for overnight air-drying. The mounted disks on SEM specimen metal stubs were carbon-coated for 150 seconds. The LEO 1530 Gemini SEM (Carl Zeiss, Oberkochen, Germany) used to visualize the topographical features of the specimen.
Results: As a result, after 6 h incubation, further cell adhesion and spread had been observed on the Ti4 and Ti5 disks, in comparison to the HA group. However, the application of PRF enhanced the cell spread only on HA disks.
onclusions: To conclude, epithelial cell adhesion and spread were titanium surface type dependent and PRF has a regulatory role in the attachment.

0272.4

Increased Peri-Implant Keratinised Mucosa Width by Xenogenic Dermal Matrix (Mucoderm®). a Case Series

Xinda LI, Peter Windisch, Attila Horváth
Department of Periodontology, Faculty of Dentistry, Semmelweis University, Budapest, Hungary

Objectives: An inadequate amount of peri-implant keratinised mucosa (PIKM) often imperils peri-implant health. If left untreated, peri-implant mucositis or peri-implantitis may occur. Therefore, sufficient width of keratinised mucosa around dental implants is considered one of the critical factors for the long-term success of dental implants. The gold standard of augmentation of PIKM is the use of autograft. However, the harvest of autogenous gingival grafts is associated with pain/discomfort and donor site morbidity. To overcome the inherent drawbacks of this surgical harvesting, alongside the limited volume, we aimed to evaluate a novel surgical technique with xenogenic dermal matrix (XDM) to increase PIKM.
Methods: Twelve patients with adequate peri-implant hard tissue but lack of PIKM-width (PIKM-W) were enrolled and signed an informed consent form. A split-thickness flap was gently prepared from the mucogingival junction towards the vestibule and sutured apically to the periosteum to create a non-moving periosteal bed. The XDM (mucoderm®; Botiss, Germany) was then rehydrated, precisely trimmed and positioned onto the periosteal site with its coronal edge tucked under the mucosa margin. Immobilisation of the XDM was then achieved with a deep periosteal anchoring sling and superficial monofilament mattress sutures. No gingival dressing was utilised, but chemical and professional mechanical plaque control instructions were given. The width of the PIKM was measured at baseline, 6 and 12 months later.
Results: All patients completed the study (4 females, 8 males; 55.9±14.4 years; non-smokers) with 21 implants (Straumann, Switzerland). Neither allergy, suppuration, rejection, ulceration, nor significant pain/discomfort were recorded. An adequate amount of PIKM, as well as a deep vestibule, was accomplished after one year. Mean PIKM-W gained statistical highly significance from 0.42±0.47 mm to 3.17± 1.21 mm at 6 months and 2.36±1.34mm at 12 months ($p<0.001$).
Conclusions: The investigated XDM may serve as a suitable substitute to autogenous soft tissue grafts for the establishment of ideal PIKM-width and deep vestibule. However, these positive results must be tested in well-designed randomised clinical trials to draw a firm clinical conclusion.

0273

***in-Silico* Selection of the Best 16S RRNA Gene Primers for Detecting Oral Bacteria and Archaea**

Alba Regueira-Iglesias[1], Lara Vázquez-González[2], Nicolás Vila-Blanco[2], Carlos Balsa-Castro[1], Maria José Carreira[2], Inmaculada Tomás[1]
[1]Oral Sciences Research Group, Universidade de Santiago de Compostela, FIDIS, Santiago de Compostela, Spain, [2]Centro Singular de Investigación en Tecnoloxías Intelixentes, Universidade de Santiago de Compostela, FIDIS, Santiago de Compostela, Spain

Objectives: The objectives of the present study were: 1) To analyse the coverage of 16S rRNA gene primers *in silico* using oral-specific databases containing 16S rRNA gene sequences from bacteria and archaea found in the oral cavity; and 2) To describe the best primer pairs for each domain.
Methods: A total of 369 distinct, individual primers were identified from oral microbiome studies and other ecosystems. These were evaluated against a previously published oral bacteria 16S rRNA sequence database, which was modified by our group, and a self-created oral-archaea database. Each database contained the genomic variants detected for all the species included. Primers with a species coverage (SC) ≥75.00% were selected for the analyses, and the two databases were used to examine 4638 primer pairs. These base pairs were then grouped into three categories according to their mean amplicon length: 100-300, 301-600 and > 600 bps.
Results: In relation to the three amplicon-length categories, the best bacteria-specific pairs targeted the 3-4, 4-7 and 3-7 16S rRNA gene regions, with SC of 97.14-98.83%; the optimum archaea-specific primer pairs amplified regions 5-6, 3-5 and 3-6, with an estimated SC of 95.88%. Finally, the best pairs for detecting the two domains targeted regions 4-5, 3-5 and 5-9, with SC values of 94.54-95.71% and 96.91-99.48% for bacteria and archaea, respectively.
Conclusions: The primer pairs with the best coverage for detecting oral bacteria were: KP_F048-OP_R043 (primer pair position for Escherichia coli J01859.1: 342-529); KP_F051-OP_R030 (514-1079); and KP_F048-OP_R030 (342-1079). The optimum pairs for detecting oral archaea were: OP_F066-KP_R013 (784-undefined); KP_F020-KP_R013 (518-undefined); and OP_F114-KP_R013 (340-undefined), while for the two domains jointly, they were: KP_F020-KP_R032 (518-801); OP_F114-KP_R031 (340-801); and OP_F066-OP_R121 (784-1405). The primer pairs with the best coverage identified herein are not among those described most widely in the oral microbiome literature.

0274

Redundancy of 16S RRNA Genes in Genomes from Oral Bacteria and Archaea

Alba Regueira-Iglesias[1], Lara Vázquez-González[2], Triana Blanco-Pintos[1], Carlos Balsa-Castro[1], Maria José Carreira[2], Inmaculada Tomás[1]

[1]Oral Sciences Research Group, Universidade de Santiago de Compostela, FIDIS, Santiago de Compostela, Spain, [2]Centro Singular de Investigación en Tecnoloxías Intelixentes, Universidade de Santiago de Compostela, FIDIS, Santiago de Compostela, Spain

Objectives: This investigation evaluated the number of 16S rRNA genes present in the complete genomes of bacteria and archaea inhabiting the human mouth.

Methods: We analysed 710 complete genomes (518 bacteria, 192 archaea) taken from the NCBI nucleotide database and extracted their 16S rRNA genes using Edgar's algorithm. The number of 16S rRNA genes/genome and repeated sequences/genome (variants) were calculated at the strain level. The same data were generated for the higher taxonomy levels and the mean number of 16S rRNA genes was obtained using the NumPy and pandas modules from Python.

Results: The oral bacteria genomes had a mean of 4.5 intragenomic 16S rRNA genes, an average of 2.6 of which were variants. Of 507 bacterial strains analysed, 126 had four genes/genome, 91 had six, and 83 had five; conversely, 30 strains had one and 73 ≥7. The maximum was 11 genes/genome in five Bacillus anthracis strains. More than half the bacteria strains had either one or two gene variants/genome. The oral archaea genomes had a mean of 1.96 intragenomic 16S rRNA genes, with an average of 1.43 variants. Of the 177 archaeal strains analysed, 80 had one gene/genome, 58 had three, 33 had two and seven had ≥4. The maximum was five genes/genome in Methanococcus maripaludis and Sulfolobus acidocaldarius. Most archaeal strains had either one or two gene variants/genome.

Conclusions: The number of intragenomic 16S rRNA genes in the oral bacteria ranged from one to 11, with four or more being most common. The number of genes/genome in the oral archaea was between one and five, with more than half of the strains having at least two. Intragenomic 16S rRNA gene redundancy must be considered to ensure the accurate interpretation of the data on microbial diversity.

0275

***Candida Albicans* Adherence Control by Alginate / Xylitol Containing Toothpaste**

Tatiana V. Kupets

Scientific and medical programs, 1. WDS Laboratory, Moscow, Russian Federation

Objectives: In *Candida albicans* (*CA*) high risk groups (impaired salivary gland function, diabetes mellitus, etc.), antifungal prophylaxis reduces incidence and severity of infections by preventing colonization and cytotoxicity of *CA*. The study aimed to evaluate the antiadhesive properties of toothpaste containing xylitol / potassium alginate (AXT) compared to placebo (PT) relative to *CA* on the human musculoskeletal fibroblasts (HMF) model

Methods: AXT or PT toothpastes containing identical basic ingredients were used as 10% solution in Eagle medium. The tested sample additionally included Potassium alginate and Xylitol. HMF were cultivated in Leighton tubes in Eagle medium for 24 hours at 37°C, until a confluent monolayer was formed. 1.8 ml of toothpaste solutions and 0.2 ml of *CA* 10^8 CFU/ml were added to incubate for 2 hours at 37°C. HMF were washed, fixed with 96% ethanol, stained and studied microscopically. Adhesion index was measured by calculating the average number of *CA* cells attached to one eukaryotic cell (EC); percentage of monolayer cells affected was calculated.

Results: Incubation of *CA* with HMF for 2 hours resulted in complete destruction of the monolayer. Adhesion index was 12-14 CFU per 1 EC. Incubation of fibroblasts with 0.04% toothpastes did not lead to any destruction after a 2-hour exposure. When PT was used, fibroblast monolayer degraded 90% due to *CA* cytotoxicity. The remaining fibroblasts (10%) were infected with the test strain. Adhesion index was 12-14 CFU per 1 EC.

When AXT was used, *CA* cytotoxic effect on HMF was no more than 10%. Adhesion index was 1-2 CFU per 1 EC. The percentage of affected cells was 2%.

Conclusions: AXT lowers the intensity of HMF destruction in presence of *CA* by 90%, and helps significantly lower microbial adhesion (7-10 times) compared to placebo. Alginate and xylitol in toothpastes is promising in clinical practice when increased risk of candidiasis is detected.

0276

Impact of the Antifungal Fluconazole on Streptococcal Growth and Oral Microbial Biofilms

Louise M. Dornelas-Figueira[1], Antônio P. Ricomini-Filho[3], Altair A. Del Bel Cury[1], Fernanda C. Petersen[2]

[1]Department of Prosthodontics and Periodontology, University of Campinas, Piracicaba, São Paulo, Brazil, [2]Department of Oral Biology, University of Oslo, Oslo, Oslo, Norway, [3]Department of Physiological Sciences, University of Campinas, Piracicaba, Brazil

Objectives: Antifungals have the potential to disturb the balance of microbial communities in the gut. While this effect is mainly attributed to the direct effect on fungal populations in the gut and possible host cytotoxic effects, less is known about its potential anti-bacterial effect or the impact on the microbiota in other body sites. The objective of this study was to evaluate the effect of fluconazole on streptococcal growth and oral biofilms.

Methods: The *Streptococcus* strains, *S. mitis* MI048 and *S. mutans* SM120, had a Pldh-luc reporter for growth assessment in planktonic cultures. For the oral biofilms, we used an *ex vivo* model enabling the growth of a highly complex microbial community. Fluconazole was assessed at different concentrations: salivary (2,6 µg/mL), ½ peak plasma (2,2 µg/mL), peak plasma (4,4 µg/mL), 2x peak plasma (8,8 µg/mL), and at a mouth rinse concentration (2 mg/mL).

Results: The highest fluconazole concentrations resulted in increased viable counts of *streptococci* and total microorganisms in the oral microbiome biofilm model, despite a tendency for reduced biomass and similar pH values by the end of the experiment. In the planktonic assay, the highest fluconazole concentrations reduced and delayed the growth of *S. mitis*, while no significant effect was observed for *S. mutans* growing under similar conditions, or in *S. mitis* grown as biofilms.
Conclusions: Although fluconazole is used as an antifungal agent, our results suggest that it may have an antibacterial effect against oral streptococci and impact the development of oral microbial biofilms. The findings are of relevance, given the importance of microbial homeostasis for human health, together with current challenges related to the increase in antibiotic resistance and the need for new strategies to fight infections.

0277

Cervicofacial Cellulitis of Dental Origin: Clinical and Bacteriological Features

Amira Besbes[1, 2], Eya Moussaoui[3, 5], Amani Medini[3], Olfa Bouallegue[4], Lamia Oualha[3, 5]
[1]Microbiology/Immunology Department, Faculty of Dental Medicine, University of Monastir, Monastir, Tunisia, [2]Monastir Academic Dental Clinic, Monastir, Tunisia, [3]Dental Medicine Department, Sahloul Teaching Hospital, Sousse, Tunisia, [4]Microbiology Department, Sahloul Academic Hospital, Sousse, Tunisia, [5]Oral Medicine and Oral Surgery Department, Faculty of Dental Medicine, University of Monastir, Monastir, Tunisia

Objectives: to describe the clinical and bacteriological profile of odontogenic cervico-facial cellulitis in a Tunisian university hospital.
Methods: This cross-sectional study was conducted on patients hospitalized for cervicofacial cellulitis of dental origin in the department of maxillofacial and plastic surgery during the period from January 2014 to December 2019. Patients hospitalized for odontogenic cervicofacial cellulitis and for whom microbiological analyses were performed, were included in the study. Data were collected from patients' records in Sahloul Academic Hospital, Sousse, Tunisia. Identification was carried out according to the conventional bacteriological methods. Data were collected and analyzed in the SPSS 2018 (trial version).
Results: Totally, 272 patients were hospitalized for cellulitis of dental origin. Bacteriological analysis was done for only 18.3 % of them. Thirty two percent of this group were aged between 30 and 40 years old. There was a male predominance (70%). Patients were admitted mainly for circumscribed cellulitis. Only 2% of them were affected by diffuse forms. The study allowed to identify 79 strains; 49% of them were oral S*treptococci* and 44% were obligately anaerobes. *Enterobacteria* represented 5% of the isolated bacteria. *Streptococcus constellatus* and *Streptococcus intermedius* represented respectively 54% *and* 23% of the isolated oral *Streptococci*. Several antibiotics were prescribed according to the clinical conditions and/or the bacteriological analysis. Amoxicillin/clavulanic acid was the most prescribed molecule as a 1st line antibiotic. It was prescribed separately in 70% of the cases. Second-line antibiotic treatment was prescribed for 19 patients. Amoxicillin/clavulanic acid associated with another molecule was prescribed as a 2nd line antibiotherapy in 79% of the cases.
Conclusions: Management of dental origin's cellulitis must be based on the topographic form, the associated general signs and the patient's condition. Bacteriological analyses should be done for immunosuppressed patients and/or in case of therapeutic failure in order to adjust the antibiotherapy prescription patterns.

0278

Blood and Saliva Contamination on Protective Eyewear During Dental Treatment

Nora Bergmann, Isabell Lindörfer, Michelle Alicia Ommerborn
Department of Operative Dentistry, Periodontology and Endodontology, Heinrich-Heine-University, Duesseldorf, Germany

Objectives: Dental treatments are associated with the appearance of potentially infective aerosols, saliva and blood splashes. Protective eyewear is therefore an important part of personal protective equipment in the dental practice. The aim of the present study was to investigate the quantitative blood and saliva contamination of protective eyewear during different dental treatments using forensic techniques. Furthermore, the efficacy of disinfection was analysed.
Methods: N=53 standardized protective eyewear shields worn during different dental treatments were analysed. Detection of blood contamination was performed by application of luminol solution onto the surface of safety shields, resulting in chemiluminescence. To visualize saliva contamination, a special forensic testpaper was used. After standardized disinfection of the protective eyewear, further analysis was conducted using the same techniques. Statistical analysis was performed using SPSS.
Results: Onto 60.4% of protective eyewear surfaces, macroscopically detectable contamination was found after dental treatment. Blood contamination (median 330.00 pixel) was observed on all shields after dental treatment using luminol as detection agent. After professional tooth cleaning highest amount of blood contamination was detected (median 1,087.00 pixel). Overall, the statistical differences between different dental treatments tend to be significant (p=0.054). Significant differences of saliva contamination was observed between the different measurements (p<0.001). In general, the amount of saliva contamination was low. After disinfection, no saliva contamination was detected, but a small amount of blood contamination (0.02%) could still be found.
Conclusions: After dental treatment protective eyewear shows a contamination with saliva and blood. Seemingly macroscopically clean protective eyewear contains up to 12% surface contamination with blood. Disinfection seems to be effective against saliva and blood contamination. As standard for infection prevention in the dental practice the use of protective eyewear during each treatment and disinfection of protective eyewear after each patient is necessary.

0278.1

Breath Parameters in Relation to Tongue Microbiota in a Healthy Population

Marije M. Kaan[1], Bob T. Rosier[2], Bernd W. Brandt[1], Wim Crielaard[1], Bart J. F. Keijser[1, 3], Egija Zaura[1]

[1]Preventive Dentistry, Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, Netherlands, [2]Health and Genomics, Center for Advanced Research in Public Health, FISABIO Foundation, Valencia, Spain, [3]Microbiology and Systems Biology, TNO, Zeist, Netherlands

Objectives: Halitosis has a worldwide prevalence of up to 30% and has an enormous impact on social and psychological well-being. Halitosis results from volatile suffer compound (VSC) production by the oral microbiota and is affected by different factors. Clinical studies describing these factors in healthy populations are limited. The aim of this study was to describe breath and tongue microbiota parameters in relation to clinical measurements and oral hygiene habits in a healthy population.

Methods: Systemically and periodontally healthy adults were clinically examined. Tongue coating thickness and colour were visually assessed and BOMP and plaque indices were scored. Oral hygiene habits were assessed using a questionnaire. Breath parameters were assessed by measuring VSC levels using a Halimeter and by organoleptic scoring. Microbial samples were collected from the posterior part of the tongue and bacterial composition was assessed using 16S rDNA amplicon sequencing (Illumina).

Results: 264 individuals (mean age ± SD: 22.59 yo ± 2.6; 45% females) were included. Tongue microbiota significantly differed by gender (F=1.78, p=0.042), tongue discoloration (F=4.02, p=0.0001), tongue coating thickness (F=3.17, p=0.0001), plaque index (F=2.04, p=0.0176), tongue brushing frequency (F=2.62, p=0.0005), and VSC levels (F=1.68, p=0.0194) but not with organoleptic score (F=1.03, p=0.3844) and BOMP (F=1.71, p=0.0500). Subjects with relatively high VSC levels contained significantly more *Actinomyces*, but less *Neisseria* compared to subjects with relatively low VSC levels. No relationship between tongue cleaning behaviour and tongue coating or discoloration was found.

Conclusions: There is a relationship between tongue microbiota and breath parameters in a healthy population. The influence of individual risk factors for developing halitosis should be further researched to clarify the role of the oral microbiota.

0278.2

Drip Flow Biofilm Reactor for Dynamically Profiling Periodontal Multispecies Communities

Justien Ghesquière[1], Kenneth Simoens[1], Erin Koos[1], Nico Boon[2], Wim Teughels[3], Kristel Bernaerts[1]

[1]Chemical Engineering, KU Leuven, Leuven, Belgium, [2]Biotechnology, UGent, Gent, Belgium, [3]Periodontology & Oral Microbiology, KU Leuven, Leuven, Belgium

Objectives: The onset of periodontitis is related to a complex interplay between bacterial interactions in the oral community and its environment, inducing a state of microbial imbalance. Revealing largely unknown mechanisms behind dysbiosis initiation benefits from *in vitro* model systems which allow in-depth biofilm profiling under dynamically controlled conditions. In this study, live confocal imaging and metabolic profiling of a 5-species periodontal biofilm in a drip flow reactor are established. Aerobic flow conditions mimic the oral cavity and return spatiotemporal information.

Methods: The drip flow biofilm reactor is inoculated with *S. gordonii*-GFPmut3* (*Sg*), *S. oralis*-GFPmut3* (*So*), *S. sanguinis*-pVMCherry (*Ss*), *F. nucleatum* (*Fn*), and *P. gingivalis*-SNAP26 (*Pg*) for an anaerobic recirculation start-up phase of 8 hours, followed by an aerobic feeding phase. Bacterial composition derived from confocal images and qPCR, metabolites and structural biofilm features are monitored each day.

Results: Over 72 hours, the biofilm reaches a compositional and metabolic steady state. Quantification based on 28 confocal images after 72 hours shows a mean thickness of 39.0±6.5µm, a substratum coverage of 70.9±6.1% and a roughness coefficient of 0.018±0.006 based on three biological replicates. Depth profiles illustrate the dominance of the GFP-labeled organisms, being *Sg* and *So*, over the entire biofilm depth (67.8±2.6%), followed by *Ss* (23.6±0.8%) and *Pg* (8.6±0.46%) and this is confirmed by qPCR measurements. Glucose from the medium is fully consumed, pyruvate is partially consumed, and ornithine, lactic acid, acetic acid and formic acid are the observed products.

Conclusions: The drip flow setup shows reproducible growth of a 5-species periodontal biofilm with relative abundancies similar to those found in the oral cavity. This promising model will be used to investigate interactions between key bacteria in multispecies oral communities.

0279

Human Osteoblasts Proliferative Response to a Novel Photodynamic Therapy Protocol

tania v. Pierfelice[1], Emira D'Amico[1], Morena Petrini[1], Natalia Di Pietro[1, 2], Domitilla Mandatori[1, 2], Valeria Schiavone[1, 2], Letizia Pelusi[1, 2], Camillo D'Arcangelo[1], Assunta Pandolfi[1, 2], Adriano Piattelli[1, 3], Giovanna Iezzi[1]

[1]Medical, Oral and Biotechnologies Science, University of Chieti, Chieti, Chieti, Italy, [2]Center for Advanced Studies and Technology-CAST, University of Chieti, Chieti, Italy, [3]Fondazione Casa di cura Villa serena, Città Sant'angelo, Italy

Objectives: The success of photodynamic therapy mediated by the topical application of 5-aminolevulinic acid and light irradiation (ALA-PDT) in the treatment of neoplastic tissues encouraged the production of a novel gel, specially formulated for oral applications. We have shown the effectiveness of ALA-PDT for inactivating, *in vitro*, gram positive and negative bacteria. Despite the proven strong antibacterial and antifungal effect, which makes it an ideal product for the treatment of periodontitis and peri-implantitis, we have no information regarding its potential effects on bone tissue. The aim of the present work was to evaluate, in *vitro*, the human osteoblast proliferation in response to ALA-PDT.

Methods: Osteoblasts, explanted from human mandible bone fragments, were plated ($6x10^3$ cells/well) and treated after 24h with different concentrations (10%, 50%, 100% v/v) of ALADENT (5-aminolevulinic acid at 5%, commercialized as ALADENT —-

Alphastrumenti -) for 45 minutes. Subsequently, the cultures were irradiated with 7 minutes of 630 nm LED at an intensity of 380mW/cm^2 (ALA-PDT), compared to either unexposed cells (CTRL) and cells exposed to LED alone (LED). The proliferation study was carried out by CellTiter 96-assay (Promega) at 48h and 72h after the treatments. Statistical analysis was performed by T-test with a statistical significance established at $p<0.05$.
Results: Following 48h of incubation, a significant increased cell proliferation (+22%) was observed only in cells subjected to ALA-PDT (100% v/v) respect to the osteoblast cultures either unexposed or exposed to LED alone. At 72h cells treated with LED alone, and those exposed to ALA-PDT with any concentrations (10%, 50%, 100% v/v) showed a significant increased proliferation, respect to unexposed control. In particular 100% ALA-PDT demonstrated an increase as 257% of cell proliferation.
Conclusions: Although further studies are needed, our data, for the first time, shed light on a new potential mechanism of 5-aminolevulinic acid action on osteoblast proliferation process.

0280

Analysis of Young and Old Human Dentin Using Raman Spectroscopy

Ekaterina G. Karteva[1], Neshka Manchorova[1], Teodora Karteva[1], Zhelyazko Damyanov[2]
[1]Operative Dentistry and Endodontics, Medical University - Plovdiv, Plovdiv, Plovdiv, Bulgaria, [2]Institute of Mineralogy and Crystallography, Bulgarian Academy of Sciences, Sofia, Bulgaria

Objectives: The objective of this study was to evaluate the potential of Raman spectroscopy in the investigation of the crystallographic structure of human dentin. The method determines the vibrational modes of the molecules within a material, by analyzing the interaction of light with its chemical bonds. This provides information on its chemical structure and phase. Furthermore, our aim was to look into the composition changes that might occur in crown dentin with aging.
Methods: The samples used were taken from crown dentin of intact extracted human teeth. They were ultrasonically cleaned, stored in a 0.2% thymol solution at 4 degrees Celsius for up to 3 months. The “young dentin” samples (G1) were taken from impacted third molars in patients aged 20-30, “old dentin” samples (G2) were extracted premolars of patients aged 40-65. Each sample was embedded in epoxy resin and polished with a silicone carbide paper of 1200 grit size. Raman spectroscopy was performed using a Horiba Jobin-Yvon T64000 triple-grating spectrometer.
Results: The v1(PO4) stretching corresponds to hydroxyapatite and is the strongest observed peak – near 960 cm-1. The v2(PO4) stretching (at around 432 cm-1) is more visible in G1, and scattered in G2. The v4(PO4) stretching at 591 cm-1 is clear in G1, but fragmented in G2, and with a decreased intensity (and observed at 585 cm-1). The peak observed at 1003 cm-1 is assigned to octacalcium phosphate. It has a higher intensity in G2.
Conclusions: The changes in the $\nu_4(PO_4)$ stretching in G2 could be an indication of the transition of α-carbonated hydroxyapatite into b-carbonated hydroxyapatite. The detected presence of octacalcium phosphate, as well as the less intensity of the peaks is a sign of an increased concentration of mineral substances with a lesser calcium content. This, in turn, could lead to a decreased toughness in the old dentin group.

0281

Micro- and Nano-Scale Observations of Fluorosis Enamel

Abdallah MOUHIBI[1], Naji Kharouf[2], Youri ANTZ[2], Hamdi jamal[3], Jihed ZGHAL[4], Abderrahman Andoh[1], Olivier Etienne[2, 5]
[1]Fixed Prosthetic, University Hassan II, Casablanca, Morocco, [2]INSERM U 1121, Strasbourg, France, [3]ICube laboratory, UMR 7357, Strasbourg, France, [4]LEME, Paris, France, [5]Prosthodontics dept, University of Strasbourg, Strasbourg, France

Objectives: This study aims to characterize mild and severe fluorosis enamel, using SEM and AFM observations at different depth of preparation and following different etching treatments.
Methods: Sixty permanent incisors were collected and divided into three different groups (n=15 sound teeth, n=27 mild fluorosis, n=26 severe fluorosis). In each group, one representative maxillary incisor was chosen and its buccal face was partially prepared to a controlled depth of 0.4 mm using a calibrated bur. Then, the buccal face was divided into four areas: one kept as control (Ctrl) and the three others prepared with different etching treatments (H3PO4 30 s (OPA30), H3PO4 90 s (OPA90), H3PO4 30 s + NaOCl 30 s (OPA30SH)). Subsequently, the surfaces were observed with SEM and AFM. Roughness Average (Ra) measurements were obtained from AFM observations.
Results: The SEM observations revealed etching patterns of various types. In the four superficial enamel areas, type 3 and 4 were observed in both fluorosis teeth. The prepared enamel areas in the mild fluorosis tooth showed patterns of type 2 after all three etching treatments. However, the demineralization of the interprismatic substance was more noticeable in OPA30-zone and OPA30-SH. In the severe fluorosis tooth, type 3 pattern was observed in the OPA90-zone, whereas OPA30SH-zone and OPA30-zone showed type 2 pattern with the slight dissolution of the interprismatic substance. According to AFM, the highest Ra values were measured in the prepared areas, after the OPA30 treatments. Means of 2.45µm (SD 0.48) and 2.64µm (SD 0.73) were measured for mild and severe fluorosis tooth respectively. The lowest Ra values were measured in the superficial areas treated with OPA30SH (O.11µm (0.04) and 0.24 (0.03) for mild and severe fluorosis). All Ra values were higher in the fluorosis teeth compared to the sound tooth, whatever the depth.
Conclusions: Within the limits of our study, as observed with SEM and AFM, the different etching patterns of the superficial surfaces did not offer micro-interlocking favorable aspects, whereas the deepest surfaces (0.4 mm) showed favorable enamel etching patterns. According to the Ra measurements and among all tested etching treatments, the conventional H3PO4 30 sec can be recommended as it showed the highest roughness.

0282

Vertical Incision is a Factor of Postoperative Bleeding on Warfarin

Kaori Ueda, Kazumasa Kubota, Masanao Inokoshi, Eijiro Yamaga, Shunsuke Minakuchi
Gerodontology and Oral Rehabilitation, Tokyo Medical and Dental University, Bunkyo, Tokyo, Japan

Objectives: This study aimed to evaluate the relationship between the incidence of postoperative bleeding after tooth extraction and causes of postoperative bleeding in elderly patients on warfarin.
Methods: This study was a retrospective study including the elderly patients aged over 65-years old on warfarin, who underwent single or multiple teeth extractions. Exclusion criteria were as followed: patients did not take warfarin at the day of extraction, and PT-INR was 3.0 or higher before extraction. Patients were unable to follow instructions due to neuropathy. Mandibular impacted wisdom teeth extractions were also excluded. The primary endpoint was the occurrence of postoperative bleeding, defined as oozing or bleeding for 24 hours to 7 days after tooth extraction. The incidence of postoperative bleeding was also calculated for cases in which longitudinal incisions were intentionally made during tooth extraction or in which longitudinal incisions were made due to gingival tears. Fisher's exact test was used to analyze between the incidence of longitudinal incisions and postoperative bleeding.
Results: There were 12 patients with both vertical incision and postoperative bleeding, 23 patients with no vertical incision and postoperative bleeding, 3 patients with vertical incision and no postoperative bleeding, and 164 patients with neither vertical incision nor postoperative bleeding. Fisher's exact test showed a significant difference, showing that $p=0.0015(p<0.01)$ postoperative bleeding was significantly higher when there was a vertical incision. There were not significant difference between PT-INR under 3.0 and postoperative bleeding.
Conclusions: This retrospective study revealed that the longitudinal incision has an impact on the occurrence of postoperative bleeding in the patients on warfarin. When performing extractions, surgeons should be mindful of the impact of longitudinal incisions on postoperative bleeding.

0282.1

Oral Mucosa Wound Healing: a Comparative Clinical Study

Ngoc Estelle E. Nguyen[1], Robert Durand[2], Clarice Nishio[2], Julien Strippoli[3], Stéphane Kerner[4], Benjamin P. Fournier[1]
[1]Oral Biology, Université de Paris, Paris, France, [2]Faculty of Dental Medicine, Université de Montreal, Montreal, Quebec, Canada, [3]Private Practice, Tassin-De-La-Demi-Lune, France, [4]Department of Periodontology, Université de Paris, Paris, France

Objectives: During wound healing, oral mucosa shows minimal scar formation compared to skin tissues. However, the healing process of the overall oral mucosa has only been visually evaluated. The objectives of this clinical comparative study were to quantitatively analyze oral mucosa healing and compare cicatrization between keratinized gingiva and non-keratinized mucosa.
Methods: Fourteen (14) patients received 26 interdental incisions as part of an orthodontic treatment combined with piezocision. Incisions were made in both keratinized gingiva and non-keratinized mucosa. Wounds were evaluated at five time points [RD1] (D0, D7, D21, D60, D150[RD2]) using scar length and surface evaluation and via the Mucosal Scarring Index (MSI). Follow-up photographs were assessed quantitatively using a computer software (ImageJ®).
Results: Significant reduction in scar length until D21 ($p<10^{-3}$) and scar surface area until D60 ($p<10^{-3}$) occurred in both keratinized gingiva and non-keratinized mucosa. These two variables showed no significant differences, so D21 and D60 represented healing endpoints. Visual evaluations of the wounds showed significant improvements of MSI scores at D21 ($p<10^{-3}$). No visual score improvements were noticed at later assessments. At D150[RD1] , mandibular non-keratinized mucosa wounds tended to heal with minimal scar formation (66%, $p<0.05$), resulting in an MSI score of 2.64 (SD ±2.81). In the keratinized gingiva, the maxillary posterior region healed with no visible scars ([0-3] = 57%, $p<10^{-3}$) ; while the anterior region healed with more scars ([3,5-10] = 61%, $p<10^{-3}$).
Conclusions: Scar assessments suggest that overall, oral mucosa heals with minimal scar formation (may below 3[RD1]). Upon further analysis, MSI scores showed that non-keratinized mucosa tends to scar more than keratinized gingiva.

0283

Characterization of the 4NQO rat Model for Oral Squamous Cell Carcinoma

Jolien Van den Bosch[1], Karen Libberecht[1, 2], Céline Erens[1], Leentje Rasking[1], Pascal Gervois[1], Ivo Lambrichts[1], Esther Wolfs[1]
[1]Biomed, Hasselt university, Diepenbeek, Belgium, [2]laboratory of neurobiology, center for brain and disease research, VIB, KU Leuven, Leuven, Belgium

Objectives: Head and neck cancer is often associated with a bad prognosis. Current treatments including chemotherapy, radiotherapy and surgery leave the patient with severe discomfort such as speaking difficulties, missing facial parts, etc. Therefore, the development of new and targeted therapies is highly necessary. For future studies, a carcinogen-based rat model for head and neck cancer was established based on the administration of 4-nitroquinoline-1-oxide (4NQO).
Methods: To optimize the model and to gain insight in the development of tumors over time, 4NQO was applied in Wistar rats. Two different exposure methods were used: application directly on the surface of the tongue (3 times a week, 5 mg/mL 4NQO in propylene glycol), and administration of 4NQO through the drinking water (at libitum, 0.1 mg/mL). Every 4 weeks, 3 rats of both groups were sacrificed and histological sections of the tongue were examined. Tumour progression and histopathological changes of the epithelia were observed by microscopic analysis of Masson’s trichrome staining. Tumour characterization was evaluated by immunohistochemistry. Human malignancy markers and cell proliferation markers such as Ki-67, P63 and MAGE-3 are used to optimize the animal model and to decide on the better application mode.

Results: In both application groups, epithelial changes are observed leading to the formation of tumors as shown by the different staining methods used in this study.
Conclusions: In conclusion, we successfully optimized the 4NQO rat model for head and neck cancer in our lab. The drinking water administration method will be used for future studies.

0283.1

Oral Exercises and Hygiene Interventions Improves OHRQoL in Parkinson's Disease

Sara Baram[1], Merete Karlsborg[2], Esben B. Øzhayat[1], Merete Bakke[1]
[1]Department of Odontology, University of Copenhagen, Copenhagen, Denmark, [2]Department of Neurology, Bispebjerg University Hospital, Copenhagen, Denmark

Objectives: Parkinson's disease (PD) is associated with reduced oral health and impaired orofacial function, which may be diminished by systematic oral interventions (Baram et al. 2020). However, the subjective experience of orofacial symptoms and their impact on the oral quality of life (OHRQoL) as well as and the effect of improvements are not fully investigated. The aim of this study is to investigate the effect of interventions for improvement of oral hygiene and function on the self-reported orofacial function and OHRQoL.
Methods: 29 patients with moderate to advanced PD (followed in neurological department in a university hospital) participated in a randomized controlled study with delayed intervention. The patients were instructed in a standardized home exercise program for the jaw and masticatory muscles consisting of training of jaw opening (JawTrainer), and lip and chewing exercises (Ulmer Oral Screen and Proxident Fluoride Gum). Furthermore, they were instructed in an individualized oral hygiene program. The effect on self-reported orofacial function and OHRQoL was measured after 2 and 4 months using the Nordic Orofacial Test – Screening (NOT-S), the oral health impact profile (OHIP-14), self-reported drooling score and subjective mastication ability. Non-parametric analyses were performed on the outcome parameters, as the data were not normally distributed.
Results: Self-reported oral health and function before the intervention were significantly correlated to the severity and duration of PD. After instruction 83-89% of the patients reported performing the exercises 4-5 days per week. After 2 months of interventions the NOT-S and drooling score were significantly improved and after 4 months, also the OHIP-14.
Conclusions: The orofacial symptoms affected the OHRQoL in the patients. The interventions improved the self-reported orofacial function and OHRQoL. In addition, it was possible to implement such simple interventions in the allied multidisciplinary healthcare surrounding the PD patient, but the long-term affect is uncertain.

0283.2

A Systematic Review of Mandibular Advancement Device Side Effects

Mirian Dang[1], Sandra Chan[2]
[1]University of Toronto, Toronto, Ontario, Canada, [2]Eastman Dental Hospital, London, United Kingdom

Objectives: To assess the impact of time on mandibular advancement devices (MAD) and to investigate the long-term skeletal and dental side effects of MAD.
Methods: Six online databases were searched electronically including the Web of Science, Medline, PubMed, Scopus, and Cochrane Central Register of Controlled Trials (CENTRAL). Potential eligible randomized controlled trials were searched without restrictions in publication date or language. Descriptive cross-sectional studies, case-series, case reports, conference abstracts and opinion-based articles were excluded. Studies that were included involved patients with at least three years of wearing MAD for obstructive sleep apnea and the assessment of skeletal and/or dental side effects. For each parameter included in the studies, the 95 percent confidence interval was calculated between follow-up and baseline. A meta-regression analysis was completed.
Results: One thousand one hundred and twenty papers were identified from the electronic database search. Duplicate records were then excluded and the abstracts and titles of 432 were screened. A total of 25 studies were included in this systematic review which had a follow-up of 3 to 10 years. An increase in the anterior face height, ANB (A point, nasion, B point), and lower incisor inclination were reported as side effects. And a decrease in the inclination of upper incisors, overbite and overjet were also identified as side effects. All the outcomes had a moderate quality of evidence according to the Grading of Recommendations Assessment, Development and Evaluation. For all parameters, the duration of therapy influenced the side effects ($P < 0.05$).
Conclusions: Skeletal and dental side effects were evident in the use of MAD therapy and were influenced by the therapy duration. The skeletal side effects were less clinically relevant compared to the dental side effects. Obstructive sleep apnea patients require continuous monitoring as the side effects may progress.

0283.3

Embryonic Markers SOX2-OCT3/4: Immunohistochemical Expression in Oral Leukoplakia/Cancer

Dimitrios Andreadis[1], Vasilis Zisis[1], Elefthrios Anagnostou[1], Penelope Anastasiadou[1], Konstantinos Vahtsevanos[2], Anastasia Fotiadou[3], Nikolaos Kechagias[4], Athanasios Poulopoulos[1]

[1]Dept Oral Medicine/Pathology, School of Dentistry, Aristotle University, Thessaloniki, Greece, [2]Clinic of Oral and Maxillofacial Surgery, "G.Papanikolaou" Hospital, School of Dentistry, Aristotle University, Thessaloniki, Greece, [3]Histology Lab, Thessaloniki, Greece, [4]Clinic of Oral and Maxillofacial Surgery, "Ag. Loukas" Hospital, Thessaloniki, Greece

Objectives: Cancer stem cells (CSCs) have a critical role in carcinogenesis and tumor growth. Embryonic stem cell markers as transcription factor SOX-2 and Octamer-binding-transcription-factor (OCT3/4) have been used to investigate the cancer stem cells. This study focus on the immunohistochemical detection of SOX-2 and OCT3/4 expression in oral leukoplakias and oral squamous cell carcinomas (OSCCs).

Methods: Immunohistochemical detection of SOX-2 and Oct3/4 in tissue-sections from paraffin embedded samples of 21 cases of oral squamous cell carcinomas (OSCC) and 30 cases of potentially malignant lesions (Leukoplakias), compared to normal oral epithelium. The samples were retrieved from the archives of the Department of Oral Medicine/Pathology, School of Dentistry, Aristotle University of Thessaloniki, Greece during the period 2009-2019. Statistical analysis was performed through SPSS Pearson Chi-square and the significance level was set at 0.05 (p=0,05).

Results: The nuclear staining of OCT3/4 was present in only 3/21 OSCC (with poor-moderate grade of differentiation). The nuclear staining of SOX-2 was observed in the basal and parabasal layers of the normal epithelium. Overall, the expression of SOX-2 was significantly increased in OSCCs compared to oral leukoplakias (p-value=0.005), In contrast, the expression of SOX-2 was not statistically significantly altered among the different grades of leukoplakias (p-value=0.152) and of OSCCs (p-value=1.000).

Conclusions: The characteristic altered expression of embryonic markers SOX-2/OCT3/4 in oral potentially malignant (leukoplakias) and OSCC lesions suggest the presence of CSCs-phenotype in oral epithelial dysplastic transformation and tumorigenesis. The clinical application of SOX-2/OCT3/4 as prognostic factors requires the experimental evaluation in larger number of samples.

0283.4

DARK1 Hypermethylation in OSCC and Oral Leukoplakia

Athanasios Poulopoulos[1], Apostolos Chatzivasileiou[1], Petros Papadopoulos[1], Eirini Louizou[5], Aikaterini Tsirtsaki[5], Konstantinos Vahtsevanos[2], Antonios Tsekos[4], Anastasia Fotiadou[3], Dimitrios Andreadis[1]

[1]Dept Oral Medicine/Pathology, School of Dentistry, Aristotle University, Thessaloniki, Greece, [2]Clinic of Oral and Maxillofacial Surgery, "G.Papanikolaou" Hospital, School of Dentistry, Aristotle University, Thessaloniki, Greece, [3]Histology Lab, Thessaloniki, Greece, [4]Clinic of Oral and Maxillofacial Surgery, "Ag. Loukas" Hospital, Thessaloniki, Greece, [5]Molecular Biology Lab, Bioiatriki SA, Thessaloniki, Greece

Objectives: Epigenetic changes (including hypermethylation) are heritable modifications in gene expression without alterations of the DNA sequences. They occur more frequently than gene mutations and are potentially reversible. Epigenetics may play a key role in the initiation and progression of oral squamous cell carcinoma (OSCC) and DNA-hypermethylation have been implicated as an effective mechanism of tumor suppressor gene inactivation. DAPK1(9q21.33) is a tumor suppresssor gene, associated with hypermethylation in different types of cancer including OSCC.

Methods: Evaluation DNA-hypermethylation of DAPK in samples taken from the laboratory of Oral Medicine of the Dentistry School, Aristotle University of Thessaloniki and Ag. Loukas Hospital of Thessaloniki, Greece: 60 samples of oral leukoplakia-OL, subdivided into: hyperkeratosis/no dysplasia, mild, moderated and severe dysplasia), 40 samples of OSCC subdivided into well-differentiated, moderately differentiated and poorly differentiated as well as normal oral tissues. DAPK hypermethylation was evaluated using bisulfate conversion and Methylation specific PCR-MSP techniques.

Results: DAPK1 hypermethylation was associated with OSCC but not with OL. Significant difference in DAPK1 hypemethylation was found between OSCC and normal tissues ($p<0.001$) and between OSCC and OL ($p<0.0001$). There was no difference in DAPK1 hypermethylation between various grades of OSCC. 39% of samples of OL-mild dysplasia expressed DAPK 1hypermethylation. Significant difference in DAPK1 hypermethylation was found between OL-mild dysplasia and OL- severe dysplasia (p=0.032). No significant difference in DAPK1 hypermethylation was found between OL and normal tissues (p=0.685).

Conclusions: Since epigenetic changes occur early in carcinogenesis and are potentially reversible, they could be used as bio-markers, even as therapeutic targets in human cancer. These results indicate that DAPK1 is epigenetically inactivated by hypermethylation in most cases of OSCC but not in OL and this could be associated with initiation, progression and prognosis of OSCC.

0284

Oral Health Related Quality of Life During Treatment with Braces

Greta Petrusyte, Olga Svediene, Vilija Berlin

Vilnius University, Vilnius, Lithuania

Objectives: Dental and occlusal anomalies are quite common problems in today's society. Frequently these disorders are treated with braces. Due to uncomfortable construction, constant presence in the oral cavity, braces can affect physical, social and psychological condition of the patient. The aim of this study was to evaluate patients' oral health related quality of life during orthodontic treatment with braces.

Methods: An original Oral Health Impact Profile questionnaire was modified. 30 questions were divided into groups: 1. functional limitations, 2. physical pain, 3. psychological discomfort, 4, physical disability, 5. psychological disability, 6. social disability and handicap. Study was approved by the ethics committee. Questionnaire survey included 340 orthodontic patients from Lithuanian University of Health Sciences and a private practice. Response rate was 94,4 % (238 woman and 83 men).
Results: The study found that patients most often (47,2%) suffered from physical pain: 82,9 % of them felt toothache, 66,4 % found it uncomfortable to eat food, 53,3 % had painful aching in mouth. 42,5 % experienced functional limitations: 93,1% had food catching in their braces and 62,6% indicated that appearance was affected. 33,7 % indicated physical disability: 81% avoided to eat some foods, 46,4% were unable to brush their teeth properly. 31,5% indicated psychological discomfort (37,4% of patients felt unhappy due to dental problems). 12,7% experienced psychological disability and in rare cases (1,3%) felt social disability and handicap.
Conclusions: Treatment with braces affect oral health related quality of life of orthodontic patients. However, only less than every second patient experienced some kind of discomfort related to treatment with braces. Toothache, difficulty eating and food catching were the most common complaints.

0285

OHRQoL: Influence of Different Factors During Orthodontic Treatment with Braces

Greta Petrusyte, Olga Svediene, Vilija Berlin
Vilnius University, Vilnius, Lithuania

Objectives: Treatment with braces affect oral health related quality of life (OHRQoL) of orthodontic patients. Active treatment consists of three stages. Earlier studies showed that only less than every second patient experienced some kind of discomfort, and complaints differ from patient to patient. Therefore, the aim of this study was to evaluate the influence of patient's gender, age and treatment stage to the OHRQoL during orthodontic treatment with braces.
Methods: An original Oral Health Impact Profile questionnaire was modified and study was approved by the ethics committee. Participants included 340 orthodontic patients from Lithuanian University of Health Sciences and a private practice. Response rate was 94,4%: 238 women and 83 men (age 11-43 years). Patients were divided into following age groups: a) younger than 18 (117 patients), b) 18-25 (148), and c) 26 and older (56). According to treatment stage, patients were divided into 1st, 2nd and 3rd groups, 107 patients in each.
Results: The study found that complaints related to functional limitations were the most prevalent complaints in women (45,8%) and physical pain - in men group (36,4%). Women indicated to experience more difficulties compared to men in 26 out of 30 questions, and difference in 14 of them was statistically significant ($p<0,05$). In all age groups, patients referred difficulties associated with physical pain (42,7%, 49,3% and 50,6% respectively in a, b and c groups). Patients older than 25 years indicated to experience more difficulties in chewing, felt less happy and less relaxed compared to younger patients ($p<0.05$). Most of the difficulties were experienced during the 3rd treatment stage. However, toothache occurred during all the treatment with no differences between stages($p>0,05$).
Conclusions: During orthodontic treatment with braces OHRQoL was compromised the most in the 3rd stage of treatment, women and in patients older than 25 years.

0286

Thermocycling Induces Severe Material Wear in Orthodontic Sealants

Daniel Diehl[1], Ella A. Naumova[2], Wolfgang H. Arnold[2]
[1]Pharmacology, Witten/Herdecke University, Witten, Germany, [2]Witten/Herdecke University, Witten, Germany

Objectives: White Spot lesions around brackets constitute a frequent side effect during orthodontic therapy. Among fluoride administration, sealing of the smooth surfaces around brackets is a widely used preventive measure to counter this issue. This investigation aimed to compare different sealing agents in terms of surface roughness and retention during thermal stress.
Methods: Six different materials were investigated, three fluoride varnishes (*Bifluorid 12, Fluor Protector*, *Protecto CaF_2 Nano*) and three composite-based surface sealants (*Pro Seal*, *Ortho Solo*, *Control Seal*). Each product was administered to 10 bovine incisors according to the manufacturer's protocol and surface roughness (Ra) measurements were performed by means of focus variation. Then, three consecutive interventions in the thermal cycler (5°C - 55°C, 1000 cycles) were carried out. After each intervention, Ra was measured to evaluate the roughness alterations compared to an untreated control group. Statistical analysis of mean Ra values and control group comparisons were performed using nonparametric tests. Material retention was evaluated visually under 10x magnification.
Results: The composite sealants exhibited a statistically significant increase of Ra directly after administration ($p \leq 0.0003$), followed by a significant reduction in relation to the thermal stress intervention ($p \leq 0.0187$). This reduction led to significantly lower Ra values than the control group ($p \leq 0.0147$). However, two materials revealed notable material dysfunction. On the other hand, fluoride varnishes exhibited a heterogenous surface roughness of which one presented with significantly higher Ra than the control group ($p=0.0005$). Furthermore, all fluoride varnishes were subject to substantial loss of function.
Conclusions: Composite materials outperformed the fluoride varnishes in terms of robustness towards thermal stress. However, thermally induced stress led to substantial wear in all but one sealant. Future studies should examine the influence of mechanical stress to improve translatability to the clinical performance and investigate the in vivo durability of orthodontic surface sealants.

0287

Bisphenol a Release from Orthodontic Composite Resins

Raoul Bationo[3, 1], Lydie Beugré-Kouassi[1], Jean-Bertin Beugré[1], Fabienne Jordana[2]

[1]Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire, [2]Dental Faculty, University of Nantes, Nantes, France, [3]Bogodogo Hospital, Ouagadougou, Burkina Faso

Objectives: The purpose of this study was to evaluate and to quantify the release of bisphenol A from orthodontic composite resins after immersion in various media.

Methods: Resin samples (Blugloo, Grengloo, Transbond XT, Transbond LR) were cured for 20 seconds and immersed in distilled water, RPMI 1640 culture medium and ethanol for 24 hours. The eluates were extracted using solid phase extraction (SPE Bond Elut-NH_2) and then analyzed by gas phase chromatography and mass spectrometry with a quantification limit of 0.01 µg/mL.

Results: The lowest amount (0.19 µg/mL) of BPA released was observed in distilled water and the highest (1.75 µg/mL) in RPMI 1640 culture medium (table).

Conclusions: Orthodontic resins can release bisphenol A according to the immersion medium.

0288

Genotype-Phenotype Correlation in Patients with Primary Failure of Eruption

Cristina Grippaudo[1, 3], Concetta Cafiero[1], Agnese Re[1], Isabella D'Apolito[1], Pietro Chiurazzi[2, 4]

[1]Head and Neck - Dental Institute, Università Cattolica del Sacro Cuore, Rome, Italy, [2]Medical Genetics Institute, Università Cattolica del Sacro Cuore, Rome, Italy, [3]Dental Institute, Fondazione Policlinico Gemelli IRCCS, Rome, Italy, [4]Medical Genetics Institute, Fondazione Policlinico Gemelli IRCCS, Rome, Italy

Objectives: Primary failure of eruption (PFE) is characterized by non-syndromic eruption failure of permanent molars, which fail in their eruptive process partially or completely. This condition is different from mechanical eruption failure (MFE) due to a mechanical obstacle, and ankylosis. Recent studies have shown that PFE is due to heterozygous variants in the *PTH1R* gene, encoding the PTH receptor. The objective of this study was to identify clinic and radiographic signs of PFE using the diagnostic rubric of Rhoads et al. (2013) and comparing the phenotypic characteristics between patients with and without *PTH1R* variants.

Methods: Forty-four patients with eruption disorders were collected at the Dental Clinic of Università Cattolica del Sacro Cuore in Rome. Clinical examination and panoramic X-rays were employed to assess the dental phenotype and the localization of eruption problems. Clinical signs were evaluated according to the diagnostic rubric and patients' DNA was sequenced to identify *PTH1R* variants. Potential pathogenicity of variants was evaluated using multiple genetic databases providing information on variant frequency and predicting functional effects on the PTH receptor.

Results: Mutational analysis of the *PTH1R* coding sequence revealed 14 different variants in 30 patients and 8 first-degree relatives (9 exonic and 5 intronic). Patients with variants classified as pathogenic (class 5), likely pathogenic (class 4) and some variants of uncertain significance (class 3) had the typical phenotype reported in the literature. Patients without *PTH1R* variants or with (likely) benign variants (class 1-2) had an atypical phenotype often involving only one tooth or a single arch.

Conclusions: Our results suggest that clinical and radiographic exams are sufficient to suggest the presence of *PTH1R* variants but genetic analysis is important to confirm the diagnosis and predict the prognosis.

0289

Genetic Associations of Tooth Agenesis in Central European Sample Group

Sema Safi[2], Leonie Weinhold[3], Nikolaos Daratsianos[2], Julia Fazaal[4], Matthias Schmid[3], Andreas Jäger[2], Matthias Weider[1], Elisabeth Mangold[4], Kerstin Ludwig[4], Lina Gölz[1]

[1]Department of Orthodontics and Orofacial Orthopedics, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany, [2]Department of Orthodontics, University Hospital of Bonn, Bonn, Germany, [3]Department of Medical Biometry, Informatics and Epidemiology, University Hospital of Bonn, Bonn, Germany, [4]Institute of Human Genetics, University of Bonn, Bonn, Germany

Objectives: While more than a hundred candidate genes have been suggested to be involved in tooth development in animal models, only a few of them have been confirmed as risk genes for tooth agenesis (TA) in humans. To date, the largest genome-wide association study was performed in an Icelandic population (Jonsson et al. 2018). However, data derived from Central Europeans is lacking. We aimed to evaluate and fine-map genetic associations of the Icelandic population in a Central European sample group.

Methods: Our study group consisted of 55 patients with TA of either maxillary lateral incisors (N=28), canines (N=9), premolars (N=21), molars (N=3) or mandibular incisors (N=12), canines (N=4), premolars (N=37) or molars (N=6); the control group without TA comprised 138 individuals. Genotyping was performed on an Illumina Global Screen Array. Genotype quality control was done using Plink-1.9 and R. SNPs were removed if one of the following conditions was true: significant difference of missing rate (cases vs. controls), call rate<0.95; deviation of Hardy-Weinberg equilibrium (P<1x10-6), minor allele frequency<0.05 (computed separately in cases and controls). Per marker allele dosages were tested for association with oligodontia under a frequentist additive logistic regression model. Analysis was run using SNPTEST.

Results: At marker level, 267'619 variants remained in analysis after QC. After imputation and post-imputation QC, 6'339'646 SNPs were included in final analysis. From this genome-wide data set, we extracted information on TA risk variants detected in the Icelandic population (for TA and for selective TAs). Of the eleven variants detected previously, five variants were available in our dataset. We could confirm that rs35956082-A is significantly associated with agenesis of maxillary lateral incisors (P=0.015), but also with maxillary (P=0.017) and mandibular premolars (P=0.004) in our Central European study group.

Conclusions: Our results contribute to a deeper understanding of genetic architecture of tooth development and disease.

0290

Impact of Myeloid HIF-1α on Orthodontic Tooth Movement in Mice

Christian Kirschneck[1], Nadine Straßmair[1], Jonathan Jantsch[2], Peter Proff[1], Agnes Schröder[1]

[1]Department of Orthodontics, University of Regensburg, Regensburg, Germany, [2]Institute for Medical Microbiology and Hygiene, University of Regensburg, Regensburg, Germany

Objectives: The transcription factor hypoxia inducible factor 1α (HIF1α) is stabilized by a lack of oxygen and controls the adaptation of tissue to hypoxic conditions through targeted expression of genes. During orthodontic tooth movement, forces are exerted on the tooth to be moved and tension and pressure zones are created in the periodontal ligament. In addition to periodontal ligament fibroblasts, which comprise the main cell population in the periodontal ligament, immune cells such as macrophages are also exposed to these mechanical loads. While HIF1α in periodontal ligament fibroblasts can be stabilized not only by a reduced oxygen levels, but also by mechanical compression, pressure application alone is not sufficient to stabilize HIF1α in macrophages as previously shown. The present study was carried out to investigate the role of myeloid HIF1α during orthodontic tooth movement.
Methods: In 20 wild-type mice (WT) and 20 HIF1α-floxed LysMCRE mice without HIF1α expression in myeloid cells (Hif1αΔmyel) an elastic rubber band was inserted at the left upper jaw side between the 1st and 2nd molars and left in situ for 7 days to induce experimental orthodontic tooth movement. The right jaw side served as control (split-mouth design). µCT was performed to determine periodontal bone loss, tooth movement and bone density. RNA was isolated from the periodontal ligament at the control side and the orthodontically treated side and the expression of genes involved in bone remodeling was examined.
Results: The extent of tooth movement was increased in Hif1αΔmyel mice. This could be due to a lower bone density of the Hif1αΔmyel mice. The deletion of myeloid Hif1α was associated with an increased expression of Ctsk and Acp5, while expression of both Rankl and its decoy receptor Opg were increased.
Conclusions: HIF1α from myeloid cells appears to play a regulatory role in orthodontic tooth movement.

0291

Three-Dimensional Analysis of Soft Tissue Changes After Orthognathic Surgery

Roberto Rongo, Immacolata Trotta, Rosaria Bucci, Alessandra Seguino, Ambra Michelotti, Vincenzo D'Antò

Department of Neurosciences, Reproductive Sciences and Oral Sciences, University of Naples, Naples, Italy

Objectives: The aim of this retrospective study is the evaluation of soft tissues changes by 3-D facial scans after orthognathic surgery.
Methods: A total of 59 patients (30 F, 29 M) were recruited in this study (mean age 27.7±7.2). Among these 10 were skeletal Class II patients (mean age of 23.1±4.1), and 49 were skeletal Class III patients (mean age of 26.2±8.2). All patients underwent orthognathic surgery and had a 3D photo taken at two time points: at T0, preoperative and at T1, between three months and one year after the surgery; the images were registered and analyzed using 3dMDVultus Software. A total of 23 soft landmarks were identified and 25 parameters were constructed for the analysis of soft tissues. The paired t-test was used to compare the results of the group at T0 and T1. The level of significance was set at $p < 0.05$.
Results: In the Class II sample, statistically significant changes were shown by the angles LSNLI (-1.0°±1.3°), SSNSL (-1.3°±1.6°), SLNPG (-0.7°±0.7°), the submental angle (12.9°±13.2°) and the UPPER LIP THICKNESS (-1.4mm±1.8mm). In the Class III sample statistically significant changes were shown by the angles LSNLI (0.9°±1.8°), LINSN (0.8°±1.9°), LINPG (-0.4°±0.9), SSNSL (2.0°±1.7°), SNNSL (1.4°±1.9°), SNNSS (-0.7°±1.0°), UPPER LIP POSITION (-1.6°±1.7°) that highlight the sagittal correction of the malocclusion and a decrease is observed of LOWER LIP HEIGHT (-1.7mm±2.8mm). The profile became more convex (FACIAL CONVEXITY -2.1°±3.5°). The nose width presents a statistically significant increase (Nose Width 2.7°±2.3°).
Conclusions: In Class II patients there is a greater effect on the mandibular level characterized by the advancement of the SL point with opening of the SUBMENTAL ANGLE. In Class III patients there is a normalization of sagittal disharmony, with an increase in facial convexity.

0292

Association Between Aggressive Apical Root Resorption and Intermaxillary Elastics

Paula Iber, Cristina Teixeira, Raquel Senen Carramolino, Alejandro Iglesias-Linares

Universidad Complutense de Madrid, Madrid, Spain

Objectives: To determine whether orthodontic treatment with fixed orthodontic appliances and intermaxillary elastics is associated with a higher prevalence of aggressive external root resorption (>5mm).
Methods: A retrospective analytical observational case-control association analysis was conducted with 240 patients treated with orthodontic appliances with available radiographic, diagnostic, and clinical records from the UCM database, who were eligible for participation in the present study and met the inclusion criteria. Roots were measured from before and after treatment on panoramic orthopantomographic. The use of elastics during orthodontic treatment was registered, making difference between the use of class II or III elastics and anterior elastics. For the statistical analysis, a descriptive analysis of the data was performed. In addition, the $\chi 2$ test was applied. The interaction of the use of elastics with the severe resorption parameter was established by backward conditional binary logistic regression. The risk of EARR was calculated by means of the Odds Ratio with a 95% confidence interval taking a value of $p < 0.05$ as statistically significant (SPSS 25.0 IBM Spain).
Results: Nearly the entire sample used class elastics (97.9%) and only 5 patients did not use them. And only 62 patients used anterior elastics during treatment (25.8%). Based on the sample analyzed, the results of the EARR measurement were classified

into two groups: with severe root resorption (>5mm) and without severe resorption (<5mm). Of the 240 patients, 71 had severe resorption (29.5%), compared to 169 who did not (70.5%).
Practically all patients used class elastics during the treatment; 97.2% of those with EARR and 98.2% of those without EARR. Anterior elastics were used by 25.4% of the patients who developed EARR and by 26% of those who did not suffer severe EARR. Therefore, the results obtained do not show a statistically significant association ($p>0.05$) between the appearance of severe EARR and the use of elastics.
Conclusions: In this sample, the use of class II elastics, class III elastics and anterior elastics during the orthodontic treatment was not directly associated with a higher prevalence of severe EARR.

0293

Color Changes of Esthetic Elastomeric Ligatures Due to Exogenic Pigmentation

Vytautas Sabataitis, Vilija Berlin
Orthodontics, Vilnius University Hospital Zalgiris Clinic, Lithuania, Vilnius, Lithuania

Objectives: To evaluate and compare *in vitro* color change due to staining solutions of the most frequently used aesthetic orthodontic elastic ligatures offered by different companies in Lithuanian market.
Methods: Fourteen different types ligatures were immersed for 72 hours in four different solutions: distilled water (control), coffee, black tee, and wine. Ligatures' color change was measured with spectrophotometer and evaluated by the Commission Internationale d'Éclairage color system and National Bureau of Standards units
Results: Coffee was the most staining solution. Color change of all ligatures were from 4,32 (appreciable) to 24,71 (change to another color). Statistically significant and the smallest color change was observed in these ligatures: "Tooth" (GC), "Tooth" (America Orthodontics) and "Clear" (3M) with "Pearl" (American Orthodontics). The biggest color change was observed in "Clear" (American Orthodontics), "Clear" (GC), "Clear" (Ormco) and "Tooth" (Ormco) ligatures after the immersion. In group of all "Clear" ligatures only elastic ligatures by 3M showed statistically significant smallest colour alteration ($p<0.001$).
Conclusions: All tested elastic orthodontic ligatures change their color after immersion in staining solutions and the color alteration is perceptible by human eye. Statistically significant color change between different types of ligatures was found.

0294

Dental Plaque Retention and Fixed Orthodontic Devices: a Literature Review

Laurence E. Lupi-Pegurier[1, 2], Heloise Pagetti[1, 2], Carole Charavet[3, 2]
[1]Oral Public Health, Cote d'Azur university, Nice, PACA, France, [2]Orthodontics and dentofacial orthopedics, CHU de Nice, Nice, PACA, France, [3]Orthodontics &dentofacial orthopedics, Cote d'Azur University, Nice, PACA, France

Objectives: Orthodontic treatment with fixed appliances is known to be associated with a risk of dental plaque accumulation leading to both demineralization of enamel and periodontal diseases. The aim of this literature review was to investigate the influence of different designs and types of orthodontic devices such as brackets (conventional, self-ligating, metal or ceramic) and ligatures (elastomeric or metallic) on the aggregation of dental plaque.
Methods: Comprehensive searches of 3 databases (PubMed, GoogleScholar and The Cochrane Library) were completed until december 2020 to identify all peer-reviewed publications possibly relevant to the review. Title and abstract screening was performed to select articles for full text retrieval, according to inclusion and exclusion criterias. The reference lists of included studies were hand searched. Following the selection of all studies and the extraction of pertinent data, the risk of bias and the confidence in the observed results were appraised.
Results: Out of 3712 studies, 31 were finally selected. First of all, no statistically significant difference appeared between self-ligating and conventional brackets in term of dental plaque accumulation. Similarly, although metal brackets seemed to retain the dental biofilm less than the ceramic ones, no significant difference was noted between these two types of brackets. Finally, elastomeric ligatures were clearly more prone to retain dental plaque, probably because of aging of the surface.
Conclusions: Within the limitations of this study, the design and type of orthodontic devices seem to have limited influence on the dental plaque accumulation. Maintaining an optimum level of oral hygiene by the patients remains the most important.

0295

General Pain Sensitivity and Dental Pain Perception in Sleep Bruxers.

Michelle A. Ommerborn[1], Adem Özbek[1], Maike Grunwald[1], Nicole P. Walentek[1, 2], Michael Franken[1], Ralf Schäfer[2]
[1]Department of Operative Dentistry Periodontology, and Endodontology, Heinrich-Heine-University, Düsseldorf, Germany, [2]Clinical Institute of Psychosomatic Medicine and Psychotherapy, Heinrich-Heine-University, Düsseldorf, Germany

Objectives: Little is known with respect to the general pain sensitivity in sleep bruxism (SB) subjects. Therefore, the present study was conducted to compare the individual general pain sensitivity as well as the dental pain perception of SB subjects with that of non-SB subjects.
Methods: This study was designed as blinded, controlled clinical trial and included 104 participants. The diagnosis of SB was based on the clinical criteria of the American Academy of Sleep Medicine, which revealed 53 SB subjects and 51 non-SB subjects. The study conception included two measurement periods. At the first measurement period, a thorough dental examination as well as the verification of probable SB was performed by one trained dentist. During the second measurement period, the cold-pressor-test, electrical pulp testing (EPT) and thermal pulp testing with CO_2-snow were executed by another trained dentist being blind to the SB diagnosis. The statistical analysis included the subjective general-tooth sensitivity (numeric analogue scale), general-pain

threshold, general-pain tolerance, general-subjective pain intensity (visual analogue scale, VAS), EPT-units, CO_2-application time, CO_2-pain duration, and subjective CO_2-pain intensity (VAS) by using one-factorial MANOVA.
Results: The one-factorial MANOVA with SB diagnosis as the independent variable revealed a significant difference between SB and non-SB subjects with $F(8,91) = 2.61$, $p=0.01$. The post-hoc univariate ANOVAs showed statistically significant differences between both groups regarding the subjective general-tooth sensitivity ($p<0.01$), general-pain tolerance ($p=0.02$), and a statistical trend for subjective CO_2-pain intensity ($p=0.07$).
Conclusions: As derived from the present results, concerning most of the objective variables related to general pain sensitivity and tooth related pain perception, SB subjects seem to react and feel similar to non-SB subjects. However, as SB subjects subjectively perceive their teeth to be more sensitive and the subjective pain intensity resulting from CO_2-testing more intense in combination with a reduced general-pain tolerance, data might point to a somatosensory amplification.

0296

Back and Neck Pain: Comparing Acute and Chronic Painful TMD

Jack Botros[1, 2], Ana M. Velly[1, 2], Mervyn Gornitsky[1, 2]
[1]Faculty of Dentistry, McGill University, Montreal, Quebec, Canada, [2]Dental Department, Jewish General Hospital, Montreal, Quebec, Canada

Objectives: To compare the occurrence of back pain (BP) and neck pain (NP) between acute and chronic painful temporomandibular disorder (TMD).
Methods: Acute (≤ 3 months) and chronic (> 3 months) painful TMD participants were recruited from four hospitals/clinics in Montreal and Ottawa, Canada in accordance with the Diagnostic Criteria of TMD. The presence of BP and NP was assessed using a self-reported checklist. Patient Health Questionnaire-8 and Generalized Anxiety Disorder-7 were used to self-report depression and anxiety symptoms, respectively. Univariate and multivariable logistic regression analyses were employed to assess the likelihood of BP and NP in chronic relative to acute painful TMD.
Results: This study enrolled 487 adults with painful TMD: acute (n = 118, 24.22%) and chronic (n = 369, 75.77%). Relative to acute painful TMD, the chronic group had almost twice the odds of reporting BP or NP (odds ratio (OR) = 1.84, 95% confidence intervals (CI) = 1.21 - 2.78). Reporting both was not associated with chronic painful TMD (OR = 0.93, 95% CI = 0.46 - 1.88). Participants with chronic painful TMD had twice the likelihood to report NP compared to those with acute (OR = 2.17, 95% CI = 1.27 - 3.71). BP was not associated with chronic painful TMD (OR = 0.96, 95% CI = 0.57 - 1.64). All analyses were adjusted for age, sex, anxiety, and depression.
Conclusions: The association of NP with chronic painful TMD suggests that regional comorbidity such as NP is implicated in the process of painful TMD chronification.

0297

ULF-TENS Application Reduces Discomfort and Pain During Hygiene Session

Luca Guerrini, Eleonora Ortu, Roberta De Felice, Antonella Barone, Davide Pietropaoli, Annalisa Monaco
Life, Health and Environmental Sciences, University of L'Aquila, Sulmona, L'Aquila, Italy

Objectives: Periodontal non-surgical therapy, consisting in scaling and root-planing, has a key role in maintaining and improving oral health and beyond. However, some patients perceive discomfort and pain during hygiene sessions. Ultra low frequency transcutaneous electric nervous stimulation (ULF-TENS) reduces acute and chronic pain perception. From these basis, we hypotized to use ULF-TENS to reduce discomfort during oral hygiene.
Methods: Twenty patients, (10M/10F), aged between 30 and 75yr who need oral hygiene, were randomly allocated to crossover controlled split mouth study. In particular, patients were randomly allocated to either 1) undergo 2 quadrants dental hygiene with shame ULF-TENS application, then 10' washout, and then undergo the remaining 2 quadrants dental hygiene with ULF-TENS application; or 2) undergo 2 quadrants dental hygiene with ULF-TENS application, then 10' washout, and then undergo the remaining 2 quadrants dental hygiene with shame ULF-TENS application. Maximum voluntary opening (MVO), pain perceived with or without ULF-TENS were evaluated using a validated VAS scale. Comfort was also checked. Data normality was tested by Shapiro-Wilk test, then t-test was used. Bonferroni correction was applied as appropriated.
Results: ULF-TENS enhanced MVO during dental hygiene session (w/o ULF-TENS: 35±5.63 vs w/ ULF-TENS 41±6.2; $p<0.001$). Self-perceived pain was reduced when ULF-TENS was applied (w/o ULF-TENS 2.5±1.85 vs w/ ULF-TENS 0±0.85; $p<0.001$). ULF-TENS application enhanced dental hygiene comfort (w/o ULF-TENS6.5±1.38; w/ ULF-TENS10±0.44; $p <0.001$).
Conclusions: These data demonstrate a positive role of TENS in potentially uncomfortable treatments, offering relief even for scaling and root-planing related pain. Further studies are needed in order to test TENS potential to improve patients' compliance and reduce dental fear.

0298

Periodontal Disease in Association with Female Sex Hormones.

Angeliki Davari, Antonia Sotiropoulou, Xanthippi Dereka

Department of Periodontology, School of Dentistry, National and Kapodistrian University of Athens, Athens, Attiki, Greece

Objectives: This narrative review aims to a) depict the clinical signs and symptoms of the periodontal tissues that are related to changes in female steroid sex hormones (FSSH) during puberty, pregnancy and menopause, and b) present the available knowledge on periodontal therapeutic management of female patients.

Methods: Published data on the clinical changes in periodontal tissues of female patient during the life cycle and the therapeutic approaches were reviewed.

Results: Although gingival inflammation is a response to bacterial plaque accumulation, it is widely known that estrogen and progesterone have direct and indirect effect on cellular, vascular, microbial and immunological level in the periodontal tissues. The clinical signs and symptoms may be exaggerated during puberty, menstrual cycle, pregnancy, peri-menopause and post-menopause. Clinical evidence shows increased gingival inflammation without an increase in plaque levels during puberty, significant gingival inflammation and bleeding due to imbalance in sex hormones in menstruation, remarkable gingival alterations such as increased gingival pockets, bleeding, hyperplasia during pregnancy, and that the severity of the existing periodontal disease may be affected during menopause and post-menopause. Polycysting ovary Syndrome (PCOS), oral contraceptives intake and hormonal replacement therapy (HRT) may also have an impact on the periodontal status. Periodontal health in female patients is of great importance. Oral hygiene instructions and reinforcement during their life is essential. The periodontal therapeutic approach further includes issues concerning the ideal time period for treatment during menstrual cycle, the proper management of pregnant woman and menopausal woman with osteoporosis, and the adjustment of therapy for women under HRT and women with PCOS.

Conclusions: FSSH may modify periodontal health. Female patients may present with periodontal and systemic conditions that may alter the periodontal therapy. Meticulous medical history is necessary. The appropriate diagnosis and therapeutic approach and the frequent professional intervention are crucial for periodontal health stability.

0300

L-PRF Membranes from Patients on Anticoagulants Differ From Controls

Anna Ockerman[1], Amber Hendrickx[1], Wouter Willekens[5], Heleen Fehervary[5], Julie Vastmans[5], Wim Coucke[2], Peter Verhamme[3], Constantinus Politis[6], Marc Quirynen[7], Reinhilde Jacobs[6, 4]

[1]Oral & Maxillofacial Surgery - Imaging & Pathology, KU Leuven, Leuven, Belgium, [2]Certified Freelance Statistician, Heverlee, Belgium, [3]Cardiovascular Sciences, University Hospitals Leuven, Leuven, Belgium, [4]Dental Medicine, Karolinska Institutet, Stockholm, Sweden, [5]Biomechanics, KU Leuven, Leuven, Belgium, [6]Oral & Maxillofacial Surgery - Imaging & Pathology, UZ Leuven, Leuven, Belgium, [7]Oral Health Sciences, KU Leuven, Leuven, Belgium

Objectives: As antithrombotics interfere with blood coagulation they may influence the generation and properties of leukocyte- and platelet-rich fibrin (L-PRF) membranes. Therefore, we evaluated L-PRF properties between membranes originating from patients on anticoagulants or antithrombotics and controls.

Methods: We performed tensile tests and cell counts to compare the mechanical properties (elastic or E-modulus, ultimate tensile strength and stretch at rupture) and cellular content (platelets and leukocytes) of L-PRF membranes.

Results: A total of 35 patients donated blood for the tensile test: 13 controls, 12 on anticoagulants, and 10 on antiplatelets. Tensile results (E-moduli data) showed stiffening behavior amongst the three groups. Compared to the control membranes, the anticoagulant membranes were weaker (ultimate tensile strength 0.57±0.24 MPa versus 0.76±0.25 MPa, difference of 0.33 MPa, 95% CI [0.23;0.63], P=0.03) and could not be stretched as far (1.8±0.3 versus 2.0±0.3 times their initial length, difference of 0.2, 95% CI [0.05;0.41], P=0.01) (Figure 1). The cell counting was performed on the samples of 55 patients: 23 controls, 16 on anticoagulants, and 16 on antiplatelets. The rate of platelets was similar (±50%) in the three groups. The rate of leukocytes was lower in the anticoagulant group compared to the controls (69±10% versus 78±8%, difference of 9%, 95% CI [0.3;17.6], P=0.04), and mainly driven by a lower rate of lymphocytes (difference of ±12%, 95%CI [3.3%; 19.8%], P=0.04). There were no differences between the antiplatelet and control groups for the aforementioned variables.

Conclusions: Our results indicate that L-PRF membranes originating from patients on an anticoagulant therapy are weaker, rupture quicker while stretched, and contain a lower rate of leukocytes than L-PRF membranes of patients not taking these drugs. The clinical relevance of these findings should be further investigated.

0300.1

Patient-Reported Morbidity Following Different Soft Tissue Augmentation Procedures

Aliye Akcali[1], Pinar Meric[2], Nejat Nizam[2]

[1]Department of Periodontology, Faculty of Dentistry, Dokuz Eylul University, Izmir, Turkey, [2]Department of Periodontology, Faculty of Dentistry, Ege University, Izmir, Turkey

Objectives: The aim of this study was to evaluate patient-reported pain perception following different soft tissue augmentation procedures.

Methods: A total of 89 patients (Mean age±S.D.: 35.73±10.26; Gender (F/M): 67/22) were included in the present study. Soft tissue augmentation modalities performed were: connective tissue graft-micro surgery (n=15), connective tissue graft-macro surgery (n=15), free gingival graft-macro surgery (n=15), free gingival graft-micro surgery (n=15), free gingival graft-cyanoacrylate (n=15), free connective tissue graft (n=6), pediculated connective tissue graft (n=8). Pain perception were measured through visual

analog scale (VAS) following different soft tissue augmentation surgeries (starting from first day including seventh day). Operative time was recorded as well. The data obtained were subjected to Kruskal-Wallis, Friedman and Mann-Whitney U tests and Spearman correlation analysis.
Results: Post-operative complication such as bleeding was not observed in any patient. VAS scores of both recipient and donor sites were decreased in all groups during the first postoperative week. Patient-reported pain perception showed statistically significant differences between the study groups at all time points ($p<0.05$). Length of the surgeries were different among the procedures. Free gingival graft-cyanoacrylate took least time (Mean time±S.D.: 26.87±2.13min.), whereas operative time for connective tissue graft-micro was maximum (Mean time±S.D.: 72.67±11.65min.) ($p<0.05$). Operative time was correlated with pain-perception at first postoperative day for both recipient and donor sites ($p<0.05$).
Conclusions: Pain perception based on a VAS score was influenced by the modality of soft tissue management. Determining the patient-reported pain outcomes might help clinicians for better patient communication and anxiety control as well as provision of patient-oriented approaches.

0301

Accuracy of Combined Salivary Biomarkers for the Diagnosis of Periodontitis: a Meta-Analysis

Triana Blanco-Pintos, Alba Regueira-Iglesias, Angela Aneiros-Ardao, Carlos Balsa-Castro, Maria Mercedes González-Peteiro, Inmaculada Tomás
Oral Sciences Research Group, Universidade de Santiago de Compostela, FIDIS, Santiago de Compostela, Spain

Objectives: This study conducted a meta-analysis of the diagnostic accuracy of combinations of salivary biomarkers for detecting periodontitis in systemically healthy subjects.
Methods: Studies were eligible for the analysis if they: examined combinations of molecular biomarkers detected in the saliva of individuals with clinically diagnosed periodontitis; and provided a binary classification table (or sensitivity and specificity values and group sample sizes). Six electronic databases were searched, and meta-analyses were performed using the hierarchical summary receiver operating characteristic, which adjusts the classification data using random-effects logistic regression (PROSPERO registration number: CRD42020175021).
Results: Meta-analyses were possible for five of the 16 potential biomarker combinations identified. The combinations IL1beta and IL6 and IL6 and MMP8 were associated with sensitivity and specificity estimates of 82% and 84%, and 84% and 82%, respectively; the combination of IL1beta and MMP8 had estimated sensitivity and specificity values of 79% and 83%. The three combinations of IL1beta, IL6 and MMP8 were determined to have a sensitivity of 81% and a specificity of 84%. The four combinations of IL1beta, IL6, MIP1alpha and MMP8 had estimated sensitivity and specificity values of 78%.
Conclusions: IL1beta and IL6, IL6 and MMP8, and IL1beta and MMP8 were the most investigated combinations for the diagnosis of periodontitis. Their combined use has excellent potential for detecting the disease, while their potential is good for detecting non-periodontitis status. This diagnostic capacity is not improved by incorporating other biomarkers. The use of these two salivary biomarker combinations appears to improve their performance in diagnosing periodontitis compared to their use individually, although more evidence is needed to corroborate these observations due to the limited number of published studies and the heterogeneity of the results.

0302

RCT on Supragingival Plaque Removing Efficacy of Low-Abrasive Air-Polishing System

Michael Wolgin[1], Alexandra Frankenhauser[1], Natalia Shakavets[2], Klaus-Dieter Bastendorf[3], Adrian Lussi[4], Andrej M. Kielbassa[1]
[1]Department of Operative Dentistry, Periodontology, and Endodontology, Danube Private University, Krems an der Donau, Austria, [2]Department of Pediatric Dentistry, Faculty of Dentistry, Belarusian State Medical University, Minsk, Belarus, [3]Private Practice Dr. Strafela-Bastendorf, Eislingen, Germany, [4]Department of Restorative, Preventive and Paediatric Dentistry, School of Dental Medicine, University of Bern, Bern, Switzerland

Objectives: While air polishing with different highly abrasive powders has been proved efficient for sub- and supragingival application, few studies concerning the quality of supragingival biofilm removal using the low abrasive Erythritol powder (EP) exist. The aim of the present study was to compare the efficacy of supragingival air polishing using EP in comparison with the traditional rubber cup method.
Methods: A sample of 32 young adults, suspending oral hygiene measures for 48 hours, were enrolled in the present double-blind clinical study. Using a split-mouth design, tooth polishing was conducted with either rotating rubber cups and prophylaxis paste or air polishing. While 16 subjects received air polishing in the second and fourth quadrants, for the other half of participants the reverse sequence was applied. Detection of biofilm was assessed using the modified Quigley-Hein index (QHI) before and after treatments. To determine the differences of QHI sum scores achieved prior to polishing, immediately after the polishing procedure and 24 hours later, statistical significance and power output data were assessed using a two-way analysis of variance (ANOVA), followed by a Tukey's honestly significant difference (HSD) to test multiple pairwise comparisons.
Results: QHI scores corresponded with significantly better performance of air polishing, both immediately ($P< .001$) and 24 hours later ($P < .001$) in comparison with the conventional rubber cup method ($P < .001$).
Conclusions: Supragingival biofilm removal by means of air polishing combined with low abrasive Erythritol seems to be more efficacious than the traditional polishing method.

0303

Individualization of Brushing-Vibrating Plaque Control by Lamellar Full-Mouth Device Uniqe

Tomas Lang[1], Michael Keiner[2], Karl W. Weich[1], Peter Gaengler[3]

[1]ORMED Institute for Oral Medicine at the University of Witten/Herdecke, Witten, Germany, [2]BLBR GmbH, Grünwald, Germany, [3]Conservative Dentistry, Faculty of Health, University of Witten-Herdecke, Witten, Germany

Objectives: Biophysical lamellar brushing actions demonstrated effective plaque control in clinically validated robot testing at coronal and occlusal planimetrical fields/tooth (PPI, oPPI; Gaengler et al. 2021). Aim was to test(i) individual full-mouth lamellar pieces, compare(ii) with Philips Sonicare and assess(iii) different brushing time with same robot programme using clinically validated plaque simulation.

Methods: Serial oral hygiene lamellar toothbrush Uniqe (BLBR 202001, Grünwald, Germany) is offered with 3 mouthpieces S, M, L. Robot brushed replicated human KaVo teeth in anatomic position coated with plaque simulation (Pepin et al. 2020), occlusal force 7.5 N, vibration 120Hz, manual movements transversally, vertically, sagitally, 60s, foam Nanosaar BLB031-34 (BLBR, Grünwald, Germany) - 7 cycles per mouthpiece. Control PhilipsSonicare DiamondClean (SensitiveHead, Drachten, Netherlands) brushed with special robot programme, 120s according to recommendations. Uniqe mouthpiece M brushed teeth with foam for 30s, 45s, 60s and 120s. Computer-assisted plaque assessment at coronal fields - 4 sites/tooth with 4 risk areas (next to gumline, in-between) - revealed plaque removal in percentage per field/area. Data underwent statistical analysis (independent two-sample t-test).

Results: Foam-filled mouthpieces executed combined brushing-vibrating plaque removal action with chewing motions and manual motions in consecutive transversal, vertical and sagittal directions. UniqeM as best fitting device brushes, consequently, at hidden areas highly significantly better ($p<0.01$) than UniqeL.

Uniqe MOA demonstrates equality in total plaque removal in comparison to Philips, with highly significantly better results ($p<0.001$) at lingual areas and - in contrast - harmonic means around 4 sites of all single teeth. Optimal plaque removal was achieved with 60s (95% smooth surfaces, 67% next to gumline, 50% in-between) with 30s results not acceptable, 45s results sub-optimal.

Conclusions: Best fitting mouthpiece, optimal brushing time and novel foam are crucial to elicit the unique brushing-vibrating lamellar Mechanism of Action MOA.

Optimal plaque control in clinically validated robot testing constitutes clinical testing in RCTs.

0305

Impact of Periodontitis and Erectile Dysfunction on Adverse Cardiovascular Event

Francisco Mesa[1], Miguel Arrabal-Polo[2], Ricardo Muñoz[1], Miguel Arrabal-Martin[2], Maria Amada Martin-Amat[1], Alejandro Rodríguez[1], Antonio Magan-Fernandez[1], Manuel Bravo[3]

[1]Periodontology, University of Granada, Granada, Granada, Spain, [2]Urology, University of Granada, Granada, Granada, Spain, [3]Preventive Dentistry and Epidemiology, University of Granada, Granada, Granada, Spain

Objectives: Our aim was to determine if the presence of periodontitis and erectile dysfunction has any effect on the incidence of major adverse cardiovascular events (MACEs).

Methods: A prospective study was performed on male patients that attended the Urology service. Erectile dysfunction was diagnosed according to the International Index of Erectile Function. Sociodemographic data were gathered and a complete periodontal examination was performed (pocket probing depth, clinical attachment loss, gingival bleeding index, plaque index and number of teeth) in each patient, and periodontitis was diagnosed according to the criteria by Lopez et al. The number of MACEs having occurred during the follow-up time as well as the number of the ones previous to follow-up were registered. Bivariate analyses, as well as a multivariate analysis, with general linear model using a Poisson-distribution, were performed, adjusting for age and previous diagnosis of cardiovascular disease.

Results: Information from 158 patients were collected over a maximum follow-up period of 6 years (mean follow-up = 4.2 years). A greater number of MACEs occurred in the group that presented periodontitis and erectile dysfunction (p=0.038) and also in the patients with previous diagnosis of cardiovascular disease, as expected. After adjusting by age and previous cardiovascular disease in the multivariate analysis, the annual MACE-event rate was estimated to be 3.7 times higher compared to the comparator group (p=0.049). Other periodontal clinical variables together with erectile dysfunction supported in tendency the results but were not statistically significant.

Conclusions: Patients jointly diagnosed with periodontitis and erectile dysfunction have a higher risk of suffering a major adverse cardiovascular event.

0305.1

Mechanical behaviour and tensile strength of the Autologous Platelet Gel (APG) membranes activated by autologous thrombin and calcium chloride.

Calogero Buggea[1], Felice Lorusso[1], Luan Mavriqi[2], ANTONIO SCARANO[1]

[1]University of Chieta-Pescara, Chieti, Italy, [2]University of Albania, Tirana, Albania

Objectives: The autologous platelet concentrates has been proposed to improve the healing of the soft and hard tissues with several applications in medicine and dentistry. The aim of the present investigation was to evaluate in vitro in a standardized environment the mechanical behaviour of autologous platelet gel (APG) membranes activated by autologous thrombin and calcium chloride.

Methods: A total of 20 APG membranes were used in the present experiment: 10 samples activated by autologous thrombin and calcium chloride (Group I) and 10 specimens were not activated (Group II). The tensile resistance and the elasticity modulus (Young) were measured through a universal static loading test (Lloyd 30 K, Lloyd Instruments Ltd., Segensworth, UK).
Results: Group I (activated) showed a tensile strength of 373.5 ± 14.3 MPa and the Group II reported a significant lower mean of 360.5 ± 16.3 MPa ($p<0.05$). The Young's modulus was 145.3 ± 10.4 MPa for Group I and 140.3 ± 15.3 MPa for Group II ($p>0.05$).
Conclusions: The results of the present in vitro mechanical investigation showed that the APG is provided by useful mechanical proprieties and the activation with autologous thrombin and calcium chloride seems to produce no influence in mechanical proprieties.

0306

Cytotoxic Effect of Hyaluronic Acid vs. Chlorhexidine Gels in Vitro

Joana F. Marques[3], Ana F. Marques[3], Neusa Marina da Silva[3], Mariana B. da Cruz[3], António Mata[1, 2]
[1]GIBBO -LIBPhys-FCT UID/FIS/04559/2013, Faculdade de Medicina Dentária da Universidade de Lisboa, Lisboa, Portugal, [2]CEMDBE - Cochrane Portugal, Universidade de Lisboa, Lisboa, Portugal, [3]Oral Biology and Biochemistry Research group - UICOB, Faculdade de Medicina Dentária da Universidade de Lisboa, Lisboa, Portugal

Objectives: Chlorhexidine gels have been recommended to treat oral ulcers. However, recent hyaluronate formulas have been introduced with the allegation of lower cytotoxic effects. This work aims to compare the in vitro cytotoxicity of topical hyaluronic acid-based aphthae treatment gels in comparison to a chlorhexidine gel in human gingival fibroblasts.
Methods: Human gingival fibroblasts were seeded in 48-well plates and grown for 24 hours according to previously described and tested procedures. Two topical gels used in oral mucosa ulcers (BAFT - Bexident AFT, BG – Bexident Gums) were tested. Five different dilutions (75%, 50%, 25%, 12,5% and 6,25%) were applied on cells for 15 minutes or 4 hours (n=8). Cell viability was evaluated at 1 and 2 days after exposure, using a resazurin-based assay and results were converted to % of the negative control and presented as mean±standard deviation. Cell morphology was assessed through phase-contrast microscopy (PCM) and alterations were graded according to ISO 10993-5:2009(E). Comparisons between groups were made through paired t-test, statistical significance was defined at $p<0,05$.
Results: After 15 min exposition a mean decrease of <20% in viability was observed for BAFT while for BG a decrease of >80% was observed for all dilutions. After 4h of exposure, a concentration-dependent reduction of viability for BAFT was observed with higher values (20.66±0.92% to 97.96±1.71%) comparing to BG (ranging from 18.95±1.44% to 19.37±1.75%) ($p<0.05$). The decrease in cellular viability was most significant for BG for all times and concentrations tested, rendering it the product with the highest cytotoxicity. PCM image analysis revealed moderate to severe alterations in cells subjected to gel BG for all times, while for gel BAFT only slight to mild changes were observed.
Conclusions: Of the tested products with application in oral ulcer treatment, the chlorhexidine-based gel produced higher cytotoxicity profiles when compared to the one based on hyaluronic acid.

0307

Cytotoxicity of Commercial Hyaluronate-Based Aphthae Treatment Gels

Ana F. Marques[1], Neusa Marina da Silva[1], Mariana B. da Cruz[1], Joana Marques[1], António Mata[3, 2]
[1]Oral Biology and Biochemistry Research group - UICOB, Faculdade de Medicina Dentária, Lisboa, Portugal, [2]CEMDBE- Cochrane Portugal, Universidade de Lisboa, Lisboa, Portugal, [3]GIBBO LIBPhys-FTC UID/FIS/04559/2013, Faculdade de Medicina Dentária da Universidade de Lisboa, Lisboa, Portugal

Objectives: To evaluate the in vitro cytotoxic effects of topical hyaluronic acid-based aphthae treatment gels on human gingival fibroblasts.
Methods: Human gingival fibroblasts were seeded in 48-well plates and grown for 24 hours according to previously described and tested procedures. Four different hyaluronate-based gels (A - Bexident®AFT, B - GUM®AftaClear , C - GENGIGEL®, D - Aloclair®Plus) were tested. Five different gel/culture medium concentrations of each gel were incubated with cells for 15 minutes or 4 hours (n=8). Negative (medium) and positive (DMSO) controls were used. Cell viability was evaluated at 1 and 2 days after exposure, using a resazurin-based assay and results were converted to % of the negative control. Cell morphology was assessed through phase-contrast microscopy (PCM) and alterations were graded according to ISO10993-5:2009(E). Comparisons between groups were made through ANOVA with Tukey post-hoc tests, statistical significance was defined at $p<0,05$.
Results: In 15-minute evaluation Gels A and B presented higher viability than the other groups ($p<0.05$) with no cytotoxic effects. Gel C presented intermediate results for lower concentrations and was cytotoxic for 75% dilution. When exposed for 4h, all gels presented cytotoxicity for the 75% dilution. At lower concentrations (50%, 25% and 12.5%) gels A, B and C respectively, recovered viability up to 70%. Gel D had a significant cytotoxic effect at all timepoints and concentrations which was significantly lower than all other groups ($p<0.05$). PCM images confirmed viability results moderate to severe alterations in cells subjected to Gel D for all times, while for gels A-C only slight to mild changes were observed.
Conclusions: Of the tested products, Bexident®AFT and GENGIGEL® showed low to no cytotoxicity. GUM®AftaClear had intermediate cytotoxicity and Aloclair®, containing aloe barbadensis extract, showed a significantly higher cytotoxic potential in gingival fibroblasts comparing to the other tested gels.

0308

Development of a Liposomal Dry Mouth Product for Long-Term Relief

Julie Frigaard[1], Janicke Liaaen Jensen[1], Hilde Galtung[2], Marianne Hiorth[3]

[1]Institute of Clinical Dentistry, University of Oslo, Oslo, Norway, [2]Institute of Oral Biology, University of Oslo, Oslo, Norway, [3]Department of Pharmacy, University of Oslo, Oslo, Nauru

Objectives: Most products on the market for the treatment of dry mouth have very limited and short-term effect. We aim to develop an ideal product for long-term relief of dry mouth, by combining mucoadhesive properties of biopolymers and moisturizing properties of liposomes. The first step is to assess the liposome-coating ability of three different biopolymers, and to evaluate stability over time, considering shelf life.

Methods: Cationic (SoyPC/DOTAP) and anionic (SoyPC/EggPG) liposomes were produced by the thin-film method in two concentrations (3 and 6mM), and hydrated with phosphate buffer (PB) or glycerol-phosphate buffer (G-PB). The liposomes were added to three specific biopolymer solutions of opposite charge, inducing spontaneous formation of chitosan-coated liposomes (CcLs), alginate-coated liposomes (AcLs) or gellan gum-coated liposomes (GcLs). Stability and precipitation were evaluated by size- and zeta potential measurements over time (t_{CcL}=8weeks, $t_{AcL\ and\ GcL}$=16weeks).

esults: Sizes of uncoated liposomes were between 120 and 135nm, whereas biopolymer-coated liposomes increased in size varying from 200 to 590nm (AcLs<GcLs<CcLs).

Both AcLs and CcLs maintained sufficient stability in three of the four samples, while the GcLs only were stable in 3mM PB, as shown in Table 1. The zeta potential remained stable for all samples.

Conclusions: All three biopolymers showed good liposome-coating abilities. Alginate and chitosan are the most promising liposome-coating biopolymers demonstrating good stability, and will be focused upon in further studies regarding oral mucoadhesive properties and hydration effects.

0309

Salivary Protective Effects of Enalapril in Diabetics: Does Gender Matter?

Ljiljana Djukic

School of Dental Medicine, University of Belgrade, Belgrade, Serbia

Objectives: It has been shown that ACE inhibitors (ACEIs) moderately reduce diabetes-induced oral complications (xerostomia, hyposalivation) risk development due to pharmacodynamic and antioxidant properties. It is unknown if there are gender-associated differences in ACEIs oral tissues protective effects, especially at saliva level which is critical in maintaining oral health. Present study aimed to investigate gender differences in ACEIs salivary protective effects by evaluating salivary angiotensin system components and antioxidant marker levels in hypertensive men (Hm) and hypertensive women (Hw) with/without T2D compared to healthy.

Methods: Study enrolled 36 50-75 years old men and age-matched postmenopausal women: 12 Hm+Hw treated with enalapril (ACEIs); 12 Hm+Hw with T2D on enalapril and metformin (antidiabetic); 12 healthy men+women. Levels of salivary ACE, ACE2, angiotensin II (Ang-II), angiotensin-(1-7) (Ang-(1-7)) were measured by ELISA; total antioxidant capacity (TAC) was measured spectrophotometrically.

Results: Gender analysis revealed similar levels of all investigated parameters between men and women, healthy and enalapril-treated regardless of T2D. In men as well as women, enalapril, while not affecting significantly ACE levels, increases ACE2 and decreases Ang-II (vasoconstrictor, prooxidant) and Ang-(1-7) (vasodilator, antioxidant), significantly in hypertensives than in hypertensives with T2D compared to healthy. In enalapril-treated Hm as well as Hw with/without T2D, TAC (antioxidant protection marker) was similar in all groups.

Conclusions: Present study revealed that there are no significant gender-associated differences in salivary angiotensin system components and antioxidant marker in enrolled healthy individuals and that enalapril beneficially affects investigated parameters in both, hypertensive men and women regardless of T2D presence. In men and women, enalapril induces salivary protective effects to the similar extent and in the similar manner.

0309.1

Administration of Systemic Antibiotics for Dental Treatment in Kosovo Major Dental Clinics - National Survey

Lirim Mustafa[1, 2], Ivana Sutej[1], Hilmi Islami[3], Hajrullah Fejza[4], Agon Hoti[4]

[1]Department of Pharmacology, Univeristy of Zagreb- Dental Faculty, Zagreb, Croatia, [2]Health Management, Iliria College, Prishtina, Albania, [3]Department of Pharmacology, University of Prishtina-Medical Faculty, Prishtina, Albania, [4]UBT College, Prishtina, Albania

Objectives: The appropriate prescription of antibiotics has become a challenge in most health care systems, because of misuse and a high level of antibiotics resistance. Kosovo in last decade is facing challenges regarding antimicrobial resistance, because majority resistance of bacteria and corresponding antibiotics groups compared with the means in EU countries is two to five times higher. The purpose of the present study is to investigate patterns of antibiotics prescription of dentists in Kosovo major dental clinics.

Methods: Data collection based on Point-Prevalence Survey (PPS) study, was made in 10 regional Dental Clinics and in Tertiary Health Center, during years 2015 -2019. Quantitative data analysis was made using descriptive statistics and were processed using MS Excel.

Results: Most prescribed antibiotic during observed time period from 2015 to 2019 in Kosovo, was Amoxicillin, although a drastic increase of amoxicillin with clavulanic acid, as broad-spectrum antibiotic, is observed. The trend of antibiotics' use in tertiary

health institutions is in overall decrease in Kosovo with an exception in year 2017. Despite this overall decrease, inconsistency in prescribing is observed when pattern is analyzed for each region separately. The highest number of patients in health care dental clinics received antibiotics for maxilla-related health conditions and the lowest number of them for oncologic ones.
Conclusions: The patterns of antibiotics' prescriptions by dental practitioners in Kosovo during years 2015-2019 oscillate, based on obtained results. Even that there are improving trends, compared to the global health care standards, the irrational use of antibiotics in dental health care clinics in Kosovo still is present and this issue should be further addressed by health care professionals.

0310

Recol: Introducing the French National Practice-Based Research Network

Marie Tohmé[1, 2], Jean-Pierre Attal[7, 8], franck Decup[3, 4], Sophie Doméjean[5, 6], Valentin Garyga[1, 2], Julien Laupie[9, 4], Jean-Noël Vergnes[10, 11], Brigitte Grosgogeat[12, 13]

[1]Hospices civils de Lyon, Pôle d'0dontologie, Université Claude Bernard Lyon 1, Faculté d'Odontologie, Lyon, France, [2]Private Pratice, Lyon, France, [3]Service de Médecine Bucco-Dentaire, Hôpital Charles Foix, Assistance Publique des Hôpitaux de Paris, Université de Paris, Faculté d'Odontologie, Montrouge, France ; Université de Paris, Laboratoire de Pathologies, Imagerie et Biothérapie Orofaciales (UR 2496), Montrouge, France, Ivry/Seine, France, [4]Private Pratice, Paris, France, [5]Centre de Recherche en Odontologie Clinique EA 4847, Université Clermont Auvergne, UFR d'Odontologie,, Clermont-Ferrand, France, [6]Service d'Odontologie, CHU Estaing, Clermont-Ferrand, France, [7]Service de Médecine Bucco-Dentaire, Hôpital Charles Foix, Assistance Publique des Hôpitaux de Paris, Université de Paris, Faculté d'Odontologie, Montrouge, France Université de Paris, Unité de Recherche Biomatériaux (UR4262), Montrouge, France, Ivry/Seine, France, [8]Private Pratice, Paris, France, [9]Association Dentaire Française, Paris, France, [10]CHU Toulouse, Service d'Odontologie, Université Paul Sabatier, UFR d'Odontologie, Toulouse, France, [11]Division of Oral Health and Society, McGill University, Montreal, Quebec, Canada, [12]Laboratoire des Multimatériaux et des Interfaces (UMR CNRS 5615), Université Claude Bernard Lyon 1, Faculté d'Odontologie, Villeurbanne, France, [13]Pôle d'0dontologie, Hospices civils de Lyon, Lyon, France

Objectives: Evidence-based dentistry (EBD) integrates the practitioner's clinical expertise, the patient's needs and preferences, and clinically relevant evidence. While research conducted in university hospitals often has good internal validity, this kind of research may suffer from cumbersome protocols and various biases in the representativeness of patients and practitioners. Thus, it is necessary to recast the way clinical research is conducted in order to facilitate knowledge transfer. To this end, research networks involving private dental practitioners (PDPs) have been organized worldwide (USA, UK, Japan, Australia, NZ) and more recently in France.
Methods: The first Dental Practice-Based Research Network was born in the USA. Inspired by this example, a DPBRN was launched in France in 2018. ReCOL (*Recherche Clinique en Odontologie Libérale* – Clinical Research in Private Dental Practice) is a nonprofit organization bringing together PDPs and academicians to collaborate and investigate research questions
Results: Strong of 500+ members, ReCOL facilitated so far four questionnaire surveys and two clinical research projects based in private practices. The convivial atmosphere facilitates expertise sharing and ReCOL's anchorage in routine practice ensures knowledge translation is achievable. The management is collaborative and inclusive, to reconcile academic dentistry and private practice. Questions addressed range from patient-centered outcomes (e.g. post-operative pain in implant-surgery) to epidemiological surveys (e.g. clinical performance of existing dental restorations in the French population and the need for reintervention).
Conclusions: The steps taken to launch the ReCOL project will be exposed, as well as participants' rating of their experience so far. The ReCOL project will be used as a case-study, to inspire other dental communities worldwide to adopt similar translational organizations. Guidance on how to proceed towards a DPBRN will be offered.

0311

Evaluation of Restored Teeth in Adults: a Practice-Based Study.

Marie-Agnès Gasqui[1, 2], Emmanuelle Dantony[6, 5], Charlène Chevalier[2], Alexandra David[2], Valentin Garyga[1], Marie Tohme[1], François GUEYFFIER[4, 5], Patrice NONY[3, 5], Delphine MAUCORT-BOULCH[6, 7], Franck DECUP[8, 9], Brigitte Grosgogeat[1, 2]

[1]Service d'Odontologie, Hospices Civils de Lyon, LYON, France, [2]Laboratoire des Multimatériaux et Interfaces, Université de Lyon, Université Claude Bernard Lyon 1, LYON, France, [3]Hôpital Cardiologique, Hospices Civils de Lyon, LYON, France, [4]Hôpital Cardiologique, Hospices Civils de Lyon, LYON, France, [5]Laboratoire de Biométrie et Biologie Évolutive, UMR CNRS 5558, VILLEURBANNE, France, [6]Service de Biostatistique-Bioinformatique, Pôle Santé Publique, Hospices Civils de Lyon, LYON, France, [7]Équipe Biostatistique-Santé, Laboratoire de Biométrie et Biologie Évolutive, CNRS UMR 5558, VILLEURBANNE, France, [8]Service de Médecine Bucco-Dentaire, Hôpital Charles Foix, Assistance Publique des Hôpitaux de Paris, PARIS, France, [9]Pathologies, Imagerie et Biothérapie Orofaciales, Université de Paris, UR 2496, PARIS, France

Objectives: To evaluate the clinical performance of dental coronal restorations in adults seen by French general dental practitioners, members of ReCOL, new network of practice based.
Methods: Forty practitioners responded to the call for participation and 400 patients were included in this multicenter observational cross-sectional study. Coronal restorations were assessed using a simplified rating based on 8 criteria from the FDI World Dental Federation. Whenever a restoration received a score equivalent to a 4 or 5 according to the FDI classification, whatever the criterion(s) selected among the 7 studied, it was considered a failure. Other variables were also considered: oral health status, risk factors, and patient's oral health quality of life (OHIP-14 questionnaire). Restoration characteristics (extent, material, technique) are presented according to failure versus success, age group, and risk profile.

Results: 74% (95% CI: 70; 79) of patients had at least one failing restoration. Need for reintervention increased with age, thigh risk profile (83% v. 62% for low risk), and extent of the filling (32%, 39%, 44%, and 44% respectively for 1, 2, 3 surfaces and crowns). There were more posterior than anterior teeth restored (median per patient: 6 v. 1) or needing re-intervention (median per patient: 1 v. 0). Inadequate color match, proximal contact or marginal adaptation were the top 3 reasons for a restoration to be failing. Also, the OHIP-14 score were higher for patients needing reintervention (median: 5 v. 2).
Conclusions: The number of dental filling failures, requiring a reintervention in adults in France is very high are high in a context of ever-changing materials and techniques. To meet these needs, large, detailed, standardized databases, powered by practice-based research networks, are welcome to follow up and analyze successes and failures and to assist practitioners in their clinical decision makings and to respond to patient requests.

0312

Accuracy of Intraoral Scanners vs. Conventional Impressions: Pilot Study

Viriato Dias[2], Leonardo Martins[1], João Rua[1], Félix S. Manuel Antunes[1]
[1]Instituto Universitário Egas Moniz, Almada, Portugal, [2]Master of Dental Medicine, Setúbal, Portugal

Objectives: The purpose of this in-vitro study was to evaluate one of the most important aspects: trueness and precision of digital models obtained by intraoral scanners when compared with the reference model, and to compare them with conventional impressions models.
Methods: A digital model was produced and printed in three dimensions (3D). It was scanned with a laboratory scanner to obtain the reference data. Then, it was scanned by three intraoral scanners: TRIOS® 3 (3SHAPE™), i500 (MEDIT™) and 3M™ TRUE DEFINITION (3M™ ESPE). Conventional impressions were followed by subsequent type IV plaster casting. Finally, laboratory scanner readings were performed. In the three-dimensional analysis program, GOM Inspect 2019, the images were submitted to the measurements proposed for the evaluation of the degree of trueness and precision of the different techniques.
Results: Concerning all the measurements, Trios 3 shows the highest trueness (46.3±52.1µm), followed by conventional impressions (49.7±54.6µm), i500 Medit (76.7±59.3µm) and 3M True Definition (91.7±70.1µm). In turn, conventional impressions are the one with the best precision (21.8±12.9µm) followed, by Trios 3 (27.0±12.2µm), 3M True Definition (36.6±23.8µm) and lastly i500 Medit (38.2±15.8µm).
Conclusions: In conclusion, digital vs. conventional systems are very close in terms of precision and trueness, but digital systems have greater advantages in clinical and laboratory use, where Trios 3 intraoral scanner showed the highest degree of accuracy (trueness and precision) in full arch readings, as well as results superior to those obtained by conventional impressions.

0313

Comparative Evaluation of Wettability Between Alginate and Silicone Impression Materials

Naofumi Niizeki, Atsushi Tachino, Futoshi Fusejima
GC Corporation, Itabashi-ku, Tokyo, Japan

Objectives: In Japan, Aroma Injection (ARI), paste-type alginate impression material filled in a cartridge, is available for combined impressions that can take precise impressions. Silicone impression materials are generally used for obtaining a precise impression to make a prosthesis. An impression taken by silicone impression materials has high accuracy, but because they are hydrophobic, there is a risk of re-impression if moisture protection is neglected. On the other hand, ARI has the same accuracy as silicone impression material and is hydrophilic. The aim of this study was to evaluate the wettability of ARI and silicone impression materials.
Methods: ARI (GC, Japan), Paradigm Light Body (PDL: 3M, USA), Aquasil Ultra LV, XLV (AU LV, AU XLV: Dentsply Sirona, USA) were used. Dynamic wettability and contact angle were measured by the following methods. For the dynamic wettability, the surface tension before setting was measured 1 minute after the start of mixing of the pastes. Regarding the contact angle, each material was flattened and 2.0 µL of distilled water was dropped onto the surface of the material 1 minute after the start of mixing of the pastes, and the contact angle was measured 10, 30 and 60 seconds after the dropping.
Results: From the results of dynamic wettability evaluation, it was clarified that ARI had the highest hydrophilicity before setting of the material. In addition, from the results of the contact angle evaluation, ARI showed the smallest contact angle, and it was clarified that the hydrophilicity of ARI was significantly higher than that of silicone impression materials evaluated in this study.
Conclusions: ARI is more hydrophilic than the silicone impression materials and can take a precise impression even if exudate remains, suggesting that the risk of clinical failure is reduced by using ARI.

0314

Accuracy of fit of Digital vs. Conventional Impression: Systematic Review

Katia Sarafidou, Maria Chatziparaskeva, Dimitris Chatzikamagiannis, Vasileios Mpotskaris, Athina Bakopoulou, Maria Kokoti
Prosthodontics, AUTH, Thessaloniki, Greece

Objectives: Nowadays, intraoral scanners (IOS) are gaining popularity for the 3-dimensional capturing of abutments of tooth-supported prostetic restorations in place of conventional impression making. However, experimental data regarding adequate fit of digital impression-derived restorations remain limited. Aim of the present systematic review was to compare marginal and internal fit of dental prostheses manufactured either after digital vs. conventional impression making.

Methods: The review followed the guidelines of the preferred reporting items for systematic reviews and meta-analyses (PRISMA). Pubmed and Scopus were searched by two independent reviewers, while a hand search was also conducted. Inclusion criteria were: in-vitro or clinical articles in English, published between 2010 and 2020, reporting on fixed prosthetic restorations.
Results: A total of 457 articles were collected, while 35 where finally included in the review. The publications presented heterogeneity regarding sample size, span length, restorative material, manufacturing step in which digitization was applied, or type of IOS. 22 articles were in vitro- and 13 clinical studies. Restorative materials included zirconia, lithium-disilicate, and alloys (all types). The majority of studies referred to restorations of four untis or less. In the alloy group, 4 studies supported superior fit of digitally fabricated restorations, two studies showed no significant difference, and one a larger marginal gap for digital techniques. Regarding lithium-disilicate restorations, 3 studies supported superior fit of digital manufacturing, 5 studies reported no significant difference, while one supported superiority of conventional impression. For zirconia restorations, the majority of studies (8) supported better fit of fully digital manufacturing, 7 articles showed no significant difference, whereas in one study conventional techniques resulted in superior fit.
Conclusions: Digital manufacturing of dental prostheses appears to have reached clinically acceptable limits. Especially for zirconia restorations, the application of a fully digital workflow seems to be already incorporated in every-day clinical practice.

0315

Effectiveness of Retraction Techniques for Dental Impressions - Clinical Trial Results

Heike Rudolph[1], David Zügel[2], Victor Korbay[1], Sigmar F. Schnutenhaus[1], Ralph G. Luthardt[1], Katharina Kuhn[1]
[1]Department of Prosthetic Dentistry, Ulm University, Ulm, Germany, [2]Private Practice, Lenggries, Germany

Objectives: Dental impressions need an adequate soft-tissue management with retraction materials and techniques in order to achieve the required accuracy e.g. for making fixed partial dentures. We asked probands to participate in a clinical trial analyzing the depth and width of the sulcus representation after using either the double cord technique or a kaolin-based paste. We also examined a possibly persisting gingival recession.
Methods: Forty probands gave informed consent. After making the baseline impression, we asked them to refrain from tooth brushing for up to 14 days to induce experimental gingivitis. We than made the intervention impressions after using either a kaolin-based aluminum chloride containing paste (3M Astringent Retraction Paste, 3M Oral Care) or cords impregnated with the same astringent (RoekoRetracto, Coltène/Whaledent). The clinical investigator put the retraction materials into the palatal sulci of the upper premolars. The laboratory team made gypsum master models, which were digitized (digiSCAN, AmannGirrbach). The analysis team measured the achieved depth and width of the sulci using reverse engineering software (Surfacer10.6 and geomagic studio/qualify 9). Thereafter, we made control impressions every three months to assess any gingival recession. In addition to descriptive statistics, we used the one-sample t-test and univariate analysis of variance (IBM SPSSS 24.0).
Results: The mean sulcus width was 0.6mm (SD 0.1; maximum 0.8mm) for both retraction techniques. The mean sulcus depth using the cord technique was 0.8mm (SD 0.2; max. 1.1mm) and using the paste 0.7mm (SD 0.15; max. 1.0mm). Only the maximum sulcus depth showed a significant difference between cord and paste application (p=0.011).
Conclusions: With both methods, we achieved a clinically suitable sulcus representation in probands with mild artificial gingivitis. Both materials initially cause gingival recession but neither technique resulted in persisting gingival recession after one year under observation. This requires a non-damaging application of the retraction techniques.

0316

Protocol for a New Index Validation in Prosthodontics Clinical Research

Anoushka Davarpanah[1], Anh Nguyet Diep[2], Anne-Françoise Donneau[2], Amélie Mainjot[1, 3]
[1]Dental Biomaterials Research Unit (d-BRU), University of Liège (ULiege), Liege, Belgium, [2]Biostatistics Unit, Public Health Department, University of Liège (ULiege), Liege, Belgium, [3]Department of Fixed Prosthodontics, University of Liège Hospital (CHU), Liege, Belgium

Objectives: Protocols to validate indices in prosthodontics research have been scarcely reported. Meanwhile, there is no appropriate index gathering all different aspects of implant restorations. This work introduces a protocol to validate a new index to Score Implant Restorations (SIR index).
Methods: The index items are developed by experts in the field of implant prosthodontics clinical research based on literature and practical needs. The first step consists in the validation of the index items to establish content validity. Experienced clinical researchers (prosthodontists and surgeons) are asked to rate the relevance of each item on a 4-point Likert scale. The number of experts ranges from 3 to 10 depending on criteria of expertise and knowledge diversity. If the content of items is not validated, modifications are conducted and a new evaluation round is carried on until the content validity upholds. The second step concerns the validation of the index across measures of discriminant validity, internal consistency, inter-rater agreement and intra-rater reliability on a sample of clinical researchers. An online platform containing pictures of clinical cases is designed. The examiners are calibrated following a 4-step procedure (blind rating, educational session, second round rating, debriefing). Subsequently, examiners (n=X) evaluate a sample of restorations and repeat the evaluations at a time interval ranging from 2 to 4 weeks (n=Y for the first round and n=Y' for the second). X and Y are calculated on the basis of a 95% confidence interval (CI) of the pre-defined intra-class correlation coefficients (ICCs). Evaluation time is also considered when determining Y and Y'.
Conclusions: This original protocol could be used by researchers to validate any index in prosthodontics clinical research. It will first be used to validate a new index to score implant restorations (SIR index), which is currently needed to improve clinical research in implant prosthodontics.

0316.1

Learning Curve Exists in CAD, but Does it Matter?

Yanning Chen[1], Edmond H. Pow[2], James Kit-Hon Tsoi[1]

[1]Applied Oral Sciences and Community Dental Care, Faculty of Dentistry, The University of Hong Kong, Hong Kong, China, [2]Restorative Dental Sciences, Faculty of Dentistry, The University of Hong Kong, Hong Kong, China

Objectives: This study aimed to compare single crown restorations designed by experienced dental technician and by dental students using Computer-Aided Design (CAD) software.

Methods: Digital datasets of models were obtained and 3D-printed (n=12). Teeth #45 on these models were prepared and scanned again. For each model, crown designs were done by an experienced technician (group TD), and by two dental students after 3-hour standard training of the same technician (group AD), respectively, with CAD software (Zfx Manager 2.0). The original tooth morphology and crown designs were superimposed (Geomagic Control 14.0), and occlusal morphological parameters, including average positive and negative profile discrepancy, standard deviations (SD), estimated root mean square ($RMS_{estimate}$), volume discrepancy, volume/area profile discrepancy, and cusp angle, were analyzed. Fracture resistance was determined using compressive load-to-fracture test (Instron E3000, crosshead speed 0.5mm/min) on monolithic lithium disilicate crowns (IPS e.max CAD) that were milled, sintered, and adhesively luted to the 3D-printed dies. The failure mode was recorded and examined under microscopy, while representative samples were examined using SEM. Paired t-test, repeated measurements of ANOVA, Fisher's exact test, and Pearson's correlation were used in statistical analysis (α=0.05).

Results: For profile and volume discrepancy parameters, no significant differences were found between crowns designed by the dental technician and students except average positive profile discrepancy ($p<0.05$). Both group TD and AD had significantly higher cusp angles than the original teeth ($p<0.05$). No significant difference was found in load capacity values, while group TD has a significantly higher percentage of bulk fracture ($p<0.05$).

Conclusions: With the aid of CAD software, no significant discrepancies in occlusal morphology exist between technician- and student-designed crowns except average positive profile discrepancy and cusp angle. Furthermore, both groups can achieve clinically acceptable fracture resistance.

0316.2

Optical Properties of CAD-CAM Milling Materials Influenced by Cigarette Smoking

Elena-Bianca Varvara[1], Corina M. Prodan[1], Adrian-Mihai Varvara[1], Cristina Gasparik[1], Amelia A. Boitor[1], Jean-Baptiste Neel[2], Diana Dudea[1]

[1]Prosthetic and Dental Materials, University of Medicine and Pharmacy "Iuliu Hatieganu" Cluj Napoca, Romania, Cluj-Napoca, Cluj, Romania, [2]Student, Faculty of Dental Medicine, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania

Objectives: CAD-CAM milling materials are becoming nowadays one of the main options for the esthetic rehabilitation of prosthetic patients. Choosing the right restoration material for a smoker patient could be a challenging task. The objective of the study was to evaluate the influence of cigarette smoking on the optical properties of a group of CAD-CAM milling materials.

Methods: An in vitro study was conducted using samples of 1mm thickness (n=10) of two different CAD-CAM milling materials: feldspar ceramic (Vita Mark II- VM) and composite resin (Brilliant Coltene - BC) (A2 Shade). The samples were placed in a sealed cabin to mimic the act of smoking and exposed to the smoke of 1, 10, and respectfully 100 cigarettes. The lightness (L*), and color coordinates (a* and b*) of each sample were measured before and after each exposure, using a spectrophotometer (Vita Easyshade Advanced 4.0). The color difference ΔE_{00} was calculated, and the data were statistically analyzed and compared with the thresholds of perceptibility and acceptability PT_{00}=0.8, AT_{00}=1.8.

Results: The color differences after the exposure to the smoke of 1, 10, and 100 cigarettes were ΔE_{00} =0.4, 2.6, and respectively 15.5 units for BC and ΔE_{00} =0.2, 2.6 and respectively 12.4 units for VM. Even after 10 cigarettes, PT and AT have been exceeded.

Conclusions: The optical properties of the two CAD-CAM milling materials varied due to the exposure to smoke, with the color becoming darker, reddish, yellowish (decreasing of L*, increasing of a* and b*).

0317

The Brittle Balance After a Regenerative Endodontic Procedure

Valerie Peeters[1], Gertrude V. Van Gorp[1, 2], Olaf Strijbos[1], Mariano Simón Pedano De Piero[1], Paul Lambrechts[1]

[1]Endodontology, University Hospitals of Leuven, Leuven, Belgium, [2]Paediatric Dentistry and Special Dental Care, University Hospitals of Leuven, Leuven, Belgium

Objectives: This case report describes and evaluates the ten-year follow-up and long-term outcome of a regenerative endodontic procedure (REP).

Methods: An eight-year-old girl presented with a history of a subluxated right central upper incisor combined with an uncomplicated enamel-dentin fracture. After two months, clinical signs of pulp necrosis were evident on tooth 11. Cone-Beam CT (CBCT) revealed an apical radiolucency and open apex. A REP with Pro-Root MTA (Dentsply Sirona, Baillaigues, Switzerland) was performed according to the European Society of Endodontology position statement: Revitalization procedures. Two years later, the tooth remained asymptomatic and CBCT evaluation confirmed satisfactory periodontal healing, no ankylosis, apical closure, intracanal deposition of mineralized tissue, lengthening and thickening of the root canal walls. Five years post-trauma, a second subluxation occurred on the same tooth. During follow-up, a new radiolucency could be noticed. An apexification with Pro-Root MTA (Dentsply Sirona, Baillaigues, Switzerland), with disruption of the formed dentin bridge, was performed. Four years later, nine years after the first trauma, the patient presented with a pulsating pain in her tooth. Endodontic microsurgery was conducted showing remaining biofilm as well as reparative dentin. After this intervention, the tooth remained asymptomatic.

Results: Tooth discoloration after REP could have been avoided by using a calciumsilicate cement without bismuthoxide. A REP is a biological-based treatment of a necrotic immature permanent teeth resulting in continued root development, increased thickness in the dentinal walls and apical closure. Any intervention after a REP or a second trauma, could disturb an existing brittle balance between healing and some controlled residual infection. Tooth extraction and replacement with a prosthetic device or an implant should be postponed as long as possible.
Conclusions: This case report illustrates the brittle balance after a REP of a traumatized tooth and emphasizes the need for long-term follow-up after dental trauma.

0318

Amniotic Epithelium - a Possible Stem Cell Source for Pulp Regeneration?

Ella Ohlsson[1], Kerstin M. Galler[1], Cristina Bucchi[2], Melanie Wölflick[1], Andreas Rosendahl[1], Wolfgang Buchalla[1], Matthias Widbiller[1]
[1]Department of Conservative Dentistry and Periodontology, University Hospital Regensburg, Regensburg, Germany, [2]Faculty of Dentistry, Research Centre for Dental Sciences, Universidad de La Frontera, Temuco, Chile

Objectives: The aim of regenerative endodontics is to replace damaged pulp tissue and restore its biological function. Transplantation of multipotent dental pulp stem cells (DPSC) may facilitate pulp-like tissue formation, however, their availability is limited. Therefore, we aimed to investigate the use of pluripotent human amniotic epithelial cells (HAEC), which can be obtained in large numbers from the placenta, as an alternative stem cell source.
Methods: HAEC were isolated from placentas by trypsinization of the amniotic membrane and characterized by flow cytometry (CD44/CD49f/CD105/CD326), whereas DPSC were obtained from primary cultures of human pulp after magnetic-activated cell sorting (STRO-1). Dentine matrix proteins (eDMP) were extracted and concentrated as previously described. Both cell types were cultured with eDMP to induce odontogenic differentiation, with osteogenic differentiation medium (StemPro™) to promote osteogenic lineage commitment and with 10% fetal bovine serum as a control. Morphological changes were documented after 7 days by fluorescence imaging after DAPI and phalloidin staining to visualize cell nuclei and the actin cytoskeleton. Expression of genes related to dentinogenesis and proliferation (COL1A1/IBSP/BGLAP/BMP4/TGFB1/NES/GPX3/IGFBP2/S100A4) was investigated by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) after 1, 7 and 14 days (n=4). The mineralization capacity was assessed by alizarin red staining after 21 days. Data were analysed nonparametric tests (Mann-Whitney *U*) at an $\alpha = 0.05$ level of significance.
Results: Isolated HAEC expressed all surface antigens (CD49f>CD105>CD44>CD326), but neither morphological changes nor an increased expression of differentiation-related genes were observed during the culture period. In contrast to DPSC, which differentiated and mineralized under osteogenic and odontogenic culture conditions, HAEC only showed calcification after induced osteogenic differentiation.
Conclusions: Overall, HAEC appear to not differentiate into an odontogenic, mineralizing phenotype, which makes them an unsuitable cell source for regenerative endodontic approaches.

0319

Gene Expression Profiling of Dental Pulp- and Apical Papilla-Derived Cells

Martyna Smeda[1], Kerstin M. Galler[1], Melanie Wölflick[1], Andreas Rosendahl[1], Cristina Bucchi[2], Wolfgang Buchalla[1], Matthias Widbiller[1]
[1]Department of Conservative Dentistry and Periodontology, University Hospital Regensburg, Regensburg, Germany, [2]Faculty of Dentistry, Research Centre for Dental Sciences (CICO), Universidad de La Frontera, Temuco, Chile

Objectives: The application of both dental pulp stem cells (DPSC) and stem cells from the apical papilla (SCAP) in regenerative endodontic protocols is conceivable. The aim of this study was to identify similarities and differences between DPSC and SCAP regarding their gene expression profiles during induced osteogenic differentiation.
Methods: Primary cells from dental pulp and apical papilla were isolated from an extracted third molar of an 18-year-old patient and analysed for mesenchymal stem cell markers (CD73, CD90, CD105) by flow cytometry. Multi-lineage differentiation was assessed by adipogenic, chondrogenic and osteogenic culture protocols. Transcriptome-wide gene expression of DPSC and SCAP during osteogenic differentiation was profiled by Clariom™ S Human Arrays over 14 days (n = 2). A control was set up by minimum essential medium α supplemented with 10 % fetal bovine serum. Transcriptome Analysis Console 4.0 Software was used for microarray data analysis and genes were ranked by the empirical Bayes method. Gene regulation (fold-change > 10; P-value < 0.01) was evaluated in the context of culture parameters (timepoint, type of medium, cell type) and group selections were made to identify similarities and differences.
Results: A high proportion of DPSC and SCAP expressed mesenchymal stem cell markers and both cell types were able to enter all the lineages investigated. Transcriptome analysis revealed the greatest gene regulation over the differentiation period, whereas medium and cell type were only secondary variables. During osteogenic differentiation, fewer genes were regulated for both cell types compared to the undifferentiated control (10 % FBS). However, 13 common key genes were identified during induced osteogenic differentiation for DPSC and SCAP: GPX3, PIP, IGFBP2, SAA2/SAA2-SAA4/SAA4, SAA1, GPM6B, FAM107A, LEPR/LEPROT, PAPPA, ABCA6, VWA5A, PDE1A, ID3.
Conclusions: DPSC and SCAP appear to share similarities not only in terms of origin, but also in terms of regulation of gene expression during the process of cell differentiation.

0320

Biological Responses of Calcium-Silicate-Based Cements on a 3D Pulp Analogue

Anna Koutrouli[1], Konstantinos Arapostathis[1], Maria Kokoti[2], Athina Bakopoulou[2]

[1]Paediatric Dentistry, Aristotle University of Thessaloniki, Thessaloniki, Greece, [2]Prosthodontics, Aristotle University of Thessaloniki, Thessaloniki, Greece

Objectives: Aim of this study was to evaluate biological responses of two bioactive calcium silicate-based cements (MTA and Biodentine™) on a three-dimensional, tissue-engineered organotypic pulp analogue.

Methods: For the establishment of the pulp analogue, Human Umbilical Vein Endothelial Cells (HUVEC) and Stem Cells from Human Exfoliated Deciduous teeth (SHED) were first co-cultured in 2D conventional culture flasks at a ratio of HUVEC:SHED=3:1. After 24h, the co-cultures were trypsinized and the pellet incorporated into a collagen I/fibrin hydrogel (total protein content 3.5mg/ml), at a concentration of (2.5-6.5)×10^5cells/hydrogel, depending on the experimental procedure. Biodentine™ and MTA cylindrical specimens (6.5×2mm) were prepared and placed in direct contact with the cell-seeded hydrogels 48h later, whereas hydrogels without specimens served as control. Live/dead staining, MTT assay and Scanning Electron Microscopy (SEM) were used to evaluate cell viability/proliferation and morphology. Real-time PCR to evaluate expression of angiogenic markers (PECAM-1, VEGFa, VEGFR1, VEGFR2, ANGPT-1, Tie-2).

Results: Live/dead staining at day 3 after treatment showed that viable- dominated over non-viable cells (not exceeding 10% for both materials). Cells were evenly distributed inside the hydrogels exposed to the bioactive cements without any differences compared to the control (untreated) cultures. MTT analysis at day 1 and 3 showed that viability/proliferation inside the cell-seeded hydrogels was not influenced by the presence of the bioactive cements. Real-time PCR at day 3 showed upregulation of VEGF and ANGPT-1 in both treated and untreated hydrogels, whereas the respective receptors VEGFR2, VEGFR1 and Tie-2 were downregulated. These alterations were more pronounced in the silica cement-treated groups.

Conclusions: Both calcium silicate-based materials are biocompatible and exert a positive angiogenic effect on the organotypic pulp analogue, validating further application in vital pulp therapy of deciduous teeth.

0321

Could Dental Clinicians Improve the Detection of Sjogren's Syndrome?

Indre Stankeviciene[1], Alina Puriene[1], Lina Stangvaltaite-Mouhat[2, 5], Diana Mieliauskaite[3], Jolanta Aleksejuniene[4]

[1]Vilnius University, Vilnius, Vilniaus, Lithuania, [2]UiT The Arctic University of Norway, Tromso, Norway, [3]State Research Institute Centre for Innovative Medicine, Vilnius, Vilniaus, Lithuania, [4]The University of British Columbia, Vancouver, British Columbia, Canada, [5]Oral Health Centre of Expertise in Eastern Norway, Oslo, Norway

Objectives: To assess the prevalence and predictors of xerostomia among adults and identify how many of the ones experiencing xerostomia had sicca and Sjogren's syndromes.

Methods: The cross-sectional study included 1405 35-74-year-old Lithuanians (51.7% response rate). Xerostomia was determined by a self-reported experience of dry mouth as "often" or "always". A dentist diagnosed sicca syndrome by unstimulated whole sialometry and Schirmer's test, and all cases were referred to a rheumatologist to confirm the Sjogren's syndrome. Self-reported questionnaire collected data about predictors.

Results: The prevalence of xerostomia was 8.0% (n=112), sicca syndrome was diagnosed for eight (0.6%) participants, Sjogren's syndrome for two (0.14%) participants, and this was their first-time diagnosis. Self-reported xerostomia positively associated with older age (OR 1.7, 95%CI 1.1-2.6), urban residence (OR 3.3, 95%CI 1.6-5.0), presence of systemic diseases (OR 2.5, 95%CI 1.4-3.3), and negatively with the use of alcohol (OR 0.6, 95%CI 0.4-0.9). Eight participants had sicca syndrome, which was more prevalent among females, participants in older age group, having systematic diseases, and using medications.

Conclusions: The prevalence of xerostomia was 8.0% and the predictors of xerostomia were older age, urban residence, the presence of systemic diseases, and not using alcohol. Sjogren's syndrome was diagnosed in two participants (0.14%). Dental clinicians if trained can suspect Sjogren's syndrome cases, therefore, continuous education courses for dental clinicians about dry mouth conditions are needed.

0322

Saliva Viscosity of Frozen Saliva Samples of Healthy Respondents in Different Ages (Pilot Study)

Fabian Wippermann[1], Tudor Sandulescu[1], Wing-Kee Lee[2], Anja Kodet[3], Xandra Puntigam[3], Andree Piwowarczyk[4], Wolfgang H. Arnold[1], Ella A. Naumova[1]

[1]Biological and Material Sciences in Dentistry, Witten/Herdecke University, Witten, Germany, [2]Institute for Physiology, Pathophysiology and Toxicology, Witten/Herdecke University, Witten, Germany, [3]Anton Paar Germany, Ostfildern-Scharnhausen, Germany, [4]Prosthodontics and Dental Technology, Witten/Herdecke University, Witten, Germany

Objectives: Human saliva has many different characteristics, which influences oral health considerably. The aim of the study was to measure the viscosity of frozen saliva samples of healthy subjects at different ages. Further, sialometric characteristics (flow rate, weight of saliva sediment and total salivary protein) have been determined.

ethods: The ethics committee of Witten/ Herdecke University approved the protocoll (98/2018). Twelve healthy subjects, who had no oral diseases and were not under medication that influences the water household, have been divided into two groups: younger (ages 18-25, n=6) and older (ages 70-85, n=6) subjects. Every subject collected saliva at three days in spring 2019 during period of ten minutes at four different times each day (T1: 7:15 a.m.; T2: 11:15 a.m.; T3: 3:15 p.m., T4: 7:15 p.m.). Saliva was then disposed, portioned, centrifuged and frozen. Flow rate, weight of sediment and total salivary protein was determined and viscosity was measured with a rheometer MCR 302 (Anton Paar, Graz, Austria).

Results: Viscosity of saliva decreases with increasing shear forces. At the different loadings, viscosity of the older group showed significantly lower viscosity in daily profile than viscosity of the younger group. Weight of sediment in the daily profile of the older group, except T1, was considerably lower than the weight of sediment of the younger group. Total-protein weight and flow rate in the daily profile showed no significant differences between age groups.
Conclusions: Saliva of the older group in the daily profile showed less sediment (epithelial tissue and bacteria). It showed lower viscosity under pressure and was less able to glide than saliva of the younger group. It can be concluded that saliva of the older group loses the ability of "washout". The method of viscosity measuring of the frozen saliva samples demonstrated usable and convincing results, so more extended studies could be carried out.

0323

Changes in Salivary Biomarkers After Physical Activity in Football Players

Ioannis Kouvaras[1], Sergio Bizzarro[1], Toon Ligtenberg[4], Henny Solleveld[2], Arnold Goedhart[2], Marja L. Laine[1], Luc Vanden Bossche[3]
[1]Periodontology, Academic Centre for Dentistry Amsterdam, University of Amsterdam and Vrij Universiteit Amsterdam, Amsterdam, Netherlands, [2]Sports Injury Lab, Dronten, Netherlands, [3]Department of Physical Rehabilitation and Sport Medicine, Ghent University Hospital, Ghent, Belgium, [4]Oral Biochemistry, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrij Universiteit Amsterdam, Amsterdam, Netherlands

Objectives: Professional football players are expected to perform optimally and therefore their health and fitness are closely monitored. However, high stress levels and poor oral health in athletes are common findings, and both can affect performance and injury incidence. Saliva, as an easy-to-collect liquid biopsy, could be used to monitor athletes' response to stress from physical activity.The main objective was to study changes in salivary cortisol levels before and after a regular training session. Furthermore, changes in other salivary biomarkers and the response to similar training sessions at two different stages of the soccer season, winter and summer, were studied. Finally, the oral health condition of the footballers was assessed.
Methods: Professional football players (age >18 years) from two U21 teams in Belgium were recruited. Oral rinses before and after two afternoon training sessions were collected. Oral examination was carried out before the winter training. ELISA, capillary electrophoresis and enzymatic fluorescence measurements were used to determine salivary cortisol, protease, amylase, lactate, ammonium, potassium, sodium, magnesium and calcium as markers for stress and physical performance.
Results: In winter (n=36), a significant increase was found after training for saliva levels of cortisol (p=0.001), protease (p=0.001) and sodium (p=0.002). In summer (n=15), only magnesium (p=0.014) and calcium (p=0.012) showed significant increases. Significant seasonal differences in response to exercise were found for sodium and calcium ($p<0.05$). Considerable inter-individual variability among the players in the response to physical activity was noted. On average, the players showed suboptimal oral health, with high prevalence of caries, erosion, gingivitis and periodontitis.
Conclusions: Physical exercise affects salivary biomarkers. Athletes need to improve their oral health. Inter-individual variability in the response to physical activity should be further investigated in relation to performance and risk of injuries.

0324

Cytotoxicity and Apoptosis Effect of Newly Designed Florescent Dyes on SCAP

Marina Miteva[1], Nikolay Ishkitiev[1], Aleksey Vasilev[2], Tanya Stoyanova[3], Mariya Dragneva[1]
[1]Medical Chemistry and Biochemistry, Medical University Sofia, Sofia, Stolichna, Bulgaria, [2]Chemistry and Pharmacy, Sofia University St. Kliment Ohridski, Sofia, Sofia city, Bulgaria, [3]Biology, Sofia University St. Kliment Ohridski, Sofia, Sofia city, Bulgaria

Objectives: The aim of this research is to test the cytotoxicity of new developed florescent dyes. Two types of dyes were checked, one that is specifically connecting to proteins and another that connects to the RNA. The nucleoid acids binding dyes are checked for specificities for RNA and/or DNA. The difference of the structure, charge and the hydrophilicity is expected to influence cellular permeabilisation and cellular toxicity.
Methods: We used mesenchymal stem cells that are isolated from the apical papilla. When cells reached 80–90 % confluence, they were trypsinized (0.05 % trypsin-EDTA, Gibco) (5 min, 37 °C). Further on the cells are transferred into two different vessels. Half of the cells are seeded in 48 well plate and the other half in 25 cm2 plastic flask. The dyes are administered in the 48 well plate and the cell were immediately placed for observation in InCell Analyzer 6000 (GE HealthCare). In the flasks the florescent dyes were added 24 hours prior the flow-cytometry. Cellular penetration was observed and recorded on Incell Analyzer 6000 (GE HealthCare) for a period on every 30 minutes for 6 hours. The differentiation between the apoptotic and alive cells was made by Anexine V kit for flow-cytometry on Navios (BioRad). For High through-put florescent cellular analysis, the apoptotic cells were visualized with FITC- conjugated anti-anexine V antibody. The nuclei were counterstained with DAPI.
Results: The results show that most of the tested dyes have a significant cytotoxic effect on the cells. Some of the dyes were hydrophilic or negatively charged and could not penetrate the membrane. They were packed in nano-particles and their specifity and cytotoxicity was evaluated. For some of the tested dyes the was dose dependent.
Conclusions: New specific dyes are urgently needed for understanding cellular processes. Only one RNA specific binding dye is available on the market. Development of new nontoxic dyes discriminating between RNA and DNA or binding specific regions in the proteins will give us valuable tools for understanding the transmembrane transport, RNA translation and protein folding and interactions.

0325

Expression of Senescence Markers in Human Pdl Stem Cells After Long-Term Cultivation *in Vitro*

Zornitsa Mihaylova, Marina Miteva, Nikolay Ishkitiev
Medical University - Sofia, Sofia, Bulgaria

Objectives: Cell senescence is a continuous irreversible process, ending with cell cycle arrest. It has been stated that long-term *in vitro* cultivation leads to decreased proliferation ability, disruption in cellular morphology and function in somatic cells. The aim of the present study is to investigate the markers of cell cycle arrest and senescence, i.e. beta-galactosidase and telomerase activity, after long term cultivation (up to 16 passages) of periodontal ligament cells.

ethods: Periodontal ligament stem cells were isolated from routinely extracted third molars via enzymatic digestion (3 mg/mL collagenase type I and 4 mg/mL dispase) and cultured continuously up to passage 16. Cell count and population doubling were evaluated at each passage. The enzymatic activity of telomerase and beta-galactosidase were assessed, as these are well-known markers for cell senescence and aging. The following kits were used for these purposes, following the manufacturer`s instructions: Human TERT / Telomerase Reverse Transcriptase ELISA Kit (ELISAGenie, Dublin, Ireland) and ELISA Kit for Galactosidase Beta (GLb) (Cloud Clone Corp, Katy, TX, USA). Total protein amount was previously identified in all samples using Nanodrop 1000 (Thermo Scientific).

Results: The results demonstrate 2 peaks with significantly increased cellular proliferation rate at passage 6 and passage 12. Slight differences in the proliferative ability were identified between cells from 1st and 16th passages without any statistical significance. Significant decrease in telomerase activity was observed starting after the first passage. Beta-galactosidase activity was found to be uninterrupted following long-term *in vitro* cultivation.

Conclusions: Our study indicates that PDL stem cells do not enter cell proliferation arrest phase after long-term *in vitro* cultivation (16 passages – about 40 doublings), as the PDL stem cells did not show significant decrease in the proliferation ability. The telomerase activity is known to be quite typical for neoplastic cells. Therefore, our data suggest lack of tumorigenic potential in human PDL stem cell culture as we revealed suppression of telomerase activity following continuous cultivation.

0326

Epithelial Cell Culture from Reduced Enamel Epithelium

Nikolay Ishkitiev[1], Marina Miteva[1], Zornitsa Mihaylova[2], Silvia Kalenderova[1]
[1]Medical Chemistry and Biochemistry, Medical University - Sofia, Sofia, Bulgaria, [2]Medical University - Sofia, Sofia, Bulgaria

Objectives: Our goal is to isolate and characterize epithelial cellular culture from reduced enamel epithelium in dental follicle of retained third molars.

Methods: Dental follicle of retained third molars were collected after routine extractions in Dental faculty (Medical university – Sofia) after informed consent was obtained from the patients. Follicle tissue was dissected with scalpel and minced, after which it was digested with 3 mg/ml Collagenase I / 4 mg/ml Dispase solution in PBS for 30 min. Heterogenous cell culture was then trypsinised and mesenchymal cells were separated from epithelial cells. Further epithelial cells were grown in KBM-Gold medium (Lonza, Basel, Switzerland) supplemented according to the manufacturer's instructions. Epithelial cell cultures were characterised for expression of variety of dental specific and epithelial markers with immunofluorescence.

Results: After enzymatic digestion the follicle tissues were allowed to attach to the bottom of 6 cm. dishes and after 3-7 days we observed cells migrating and proliferating. We recognised two types of cells: spindle like mesenchymal cells and oval, tightly lined next to each other epithelial cells. Trypsin disattached quickly the mesenchymal cells and the epithelial colonies remained attached longer. Morphological and immunofluorescent observations confirmed we managed to isolate epithelial cell culture, expressing CK10, CK14, P63, Amelogenin, Ameloblastin, Tuftelin.

Conclusions: Dental follicles of retained third molars have viable epithelial cells, relative to the enamel organ epithelium and rests of Malassaez, which can be isolated and studied further.

0327

Isolation of Mesenchymal Stem Cells from Adult and Deciduous Teeth

Tanya Stoyanova[1], Nikolay Ishkitiev[2], Marina Miteva[2], Aleksey Vasilev[3], Meglena Kandinska-Vasileva[3]
[1]Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria, [2]Medical Chemistry and Biochemistry, Medical University Sofia, Sofia, Bulgaria, [3]Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria

Objectives: The aim of this research is to extract different types of mesenchymal stem cells from teeth. Types of stem cells isolated from the tooth include Stem cells from the apical papilla (SCAP), Desiduous pulp stem cells (SHED), Dental pulp stem cells (DPSC), Periodontal ligament stem cells (PDL), Bone marrow-derived mesenchymal stem cells (BMSC), oral mesenchymal stem cell and gingiva mesenchymal stem cells (GMSC).

Methods: The mesenchymal stem cells are isolated form third molars, which are extracted in routine procedure, from patients between the age of 18-30 years old. First tissue that is isolated from the tooth is SCAP. It is isolated when tooth is not fully developed. The apical papilla that is separated from the apex with a scalpel. The DPSC are isolated from the pulp tissue. With dental bud the crown is separated from the root. Further the pulp tissue is gently separated from the crown and root for DPSC. When the root is separated from the crown and the pulp is removed, the tissue on the surface of the root with the whole root is seeded for the isolation of the periodontal ligament (PDL). SHED is isolated from deciduous teeth. BMSC type of cells are gathered with a routine extraction. GMSC were isolated from the gingiva. The tissue is digested in a solution of 3mg/mL collagenase Type I and 4mg/ml dispase dissolved in DMEM for 1 hour at CO_2 incubator. The suspensions of cells are cultured into 25 cm^2 plastic flasks with DMEM supplemented with 20% fetal bovine serum (FBS, Gibco BRL), 100U/mL antibiotic/antimicotic (ThermoScinetific), for

primary culture and then incubated at 37°C .and 5% CO2. When cells reached 80–90 % confluence, they were trypsinized (0.05 % trypsin-EDTA, Gibco) (5 min, 37 °C).
Results: We successfully isolated the tissue, incubated and cultivated the cells. After that we checked with specific stem cells markers.
Conclusions: With these experiments we have proven that there is a possibility to extract multipotent mesenchymal stem cell types from adult teeth. If successfully differentiated, they can be used to develop new techniques for stem cell therapy and drug development.

0328

Mesenchymal Stem Cells Secretome Alleviate Oxidative Stress Related Impaired Osteogenesis.

Ragda Saleem[1, 3], Ellen Berggreen[2, 4], Kamal B. Mustafa[1], Niyaz Al-Sharabi[1]
[1]Department of Clinical Dentistry, Faculty of Medicine, University of Bergen, Bergen, Norway, [2]Oral Health Centre of Expertise in Western Norway, Bergen, Norway, [3]Centre for International Health, University of Bergen, Bergen, Norway, [4]Department of Biomedicine, Faculty of Medicine, University of Bergen, Bergen, Norway

Objectives: Oxidative stress has been connected to various diseases including bone diseases. Mesenchymal stem cells conditioned medium (MSC-CM) contains a set of antioxidants and anti-apoptotic factors among other factors. However, the effect of MSC-CM in reversing impaired osteogenesis related to oxidative stress still needs to be investigated. Therefore, this in vitro study aimed to investigate the efficacy of MSC-CM in alleviating the impaired osteogenic capacity of bone marrow MSC (BMSC) under oxidative stress conditions.
Methods: Oxidative stress was induced on BMSC by exposure to hydrogen peroxide, before treating them with MSC-CM. Cell viability and cellular apoptosis were assessed using Alamar blue assay and FITC Annexin V kit using flow cytometry, respectively. Activity of the antioxidant enzyme superoxide dismutase (SOD) was measured using SOD assay kit. Osteogenic capacity of the cells was investigated by alkaline phosphatase staining, osteogenic genes analysis, and Alizarin red staining.
Results: Exposure to hydrogen peroxide significantly decreased BMSC viability and increased cellular apoptosis. Osteogenic capacity was significantly impaired with minimal mineralization detected after 21 days. Treating BMSC with MSC-CM for 7 days significantly increased cells viability compared to non-treated cells (control). A decrease in cellular apoptosis was also observed in cells treated with MSC-CM compared to the control. Significantly higher SOD activity was detected in cells treated with MSC-CM. After 14 days, cells treated with MSC-CM showed more alkaline phosphatase activity and significantly higher expression of the osteogenic genes collagen type I and osteopontin compared to the control. Furthermore, significantly greater mineralization was detected in cells treated with MSC-CM compared to the control after 21 days.
Conclusions: These results indicate that beside the antioxidant and anti-apoptotic properties, MSC-CM enhances the osteogenic capacity of BMSC under oxidative stress conditions. Therefore, MSC-CM has a great potential in treatment of oxidative stress related bone diseases.


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