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Surface characteristics and bacterial adhesion on single-shade composite resins: A comparative *in vitro* study of one-step versus multi-step polishing techniques

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This study evaluated the effects of two polishing systems, OptraGloss (G; one-step) and OptiDisc (D; multi-step), on surface roughness (SR), contact angle (CA), surface free energy (SFE), and bacterial adhesion on three single-shade composite resins: Omnichroma (O), ZenChroma (Z), and Charisma Diamond One (C). Data for SR, CA, SFE, and adhesion of *Streptococcus mutans* (*S. mutans*) and *Streptococcus mitis* (*S. mitis*) were analyzed using one-way ANOVA, Tukey *post-hoc* tests, and Pearson correlation (α=0.05). Multi-step polishing groups (OD, ZD, and CD) exhibited significantly lower SR (0.18, 0.18, and 0.29 μm, respectively) compared to OG (0.46 μm), ZG (0.30 μm), and CG (0.44 μm) (p<0.05). The highest CA was observed in ZG (91.6°). *S. mitis* adhesion was greater than *S. mutans* in all groups except OG. A significant correlation was observed between SR and the adhesion of *S. mutans* (r=0.693, p<0.001). Polishing systems applied to single-shade composite resins did not impact the SFE but affected SR, and bacterial adhesion.

Keywords: Bacterial adhesion, Single-shade, Polishing, Roughness, *Streptococcus mutans*

INTRODUCTION

The terms single-shade or monochromatic have been introduced to describe composite resins equipped with smart chromatic technology, which are designed to esthetically simulate the entire spectrum of tones using just a single nominal color shade[1-3]. It is postulated that these materials, formulated according to the broad color-matching concept, seamlessly integrate with the surrounding dental structure irrespective of the tooth's intrinsic color[1]. Comprising nano-fillers (nanomers) and nanoclusters, these composite resins are noted for their 'chameleon effect', which purportedly enhances their color harmony with dental tissues[4,5]. While these composite resins exhibit a novel structure that assists in overcoming esthetic challenges in restorations, the primary reason for the frequent replacement of restorations continues to be the development of secondary caries due to cariogenic biofilm formation[6-11].

Dental plaque hosts a variety of species and strains; some are identified as early colonizers, while others are known to be cariogenic microorganisms[12]. On freshly cleaned tooth surfaces, the predominantly initial colonizing microorganisms such as *Streptococcus mitis* (*S. mitis*), *Streptococcus oralis*, *Streptococcus sanguinis* and constitute a significant portion of the microflora[11,13-15]. Additionally, it has been reported that *S. mitis* is the most highly represented streptococcal species in coronal tooth plaque[16,17]. Numerous cariogenic microorganisms present in plaque are linked to dental caries, with Streptococcus *mutans* (*S. mutans*) being identified as the main causative agent in caries development[6,7,11,18,19].

The influence of composite resins' surface properties on their interactions with oral microorganisms is notable[7]. These surface characteristics, together with bacteria's adhesion capacity, promote bacterial attachment to the material. Crucial factors in this process include surface roughness (SR), surface free energy (SFE), the hydrophobicity of the restoration, and its material composition[11,18]. Specifically, SR and the chemical composition of the surface are key in facilitating bacterial adhesion and the formation of biofilms[6]. Surfaces with a high SFE (more hydrophilic) tend to be more prone to biofilm development than those with a lower SFE (more hydrophobic)[20].

Moreover, by carrying out finishing and polishing procedures, the surface quality of composite resin restorations can be improved. This enhancement in surface properties may contribute to prolonging the functional life of the restoration[21,22]. The purpose of the polishing procedure is not only to confer an esthetically pleasing appearance and surface gloss to the restoration but also to eradicate retention sites for bacteria and diminish plaque adherence[21,23]. Multi-step polishing systems, consisting of discs impregnated with aluminum oxide such as OptiDisc, have been proven effective in dentistry and are commonly used in routine practice. Concurrently, one-step polishing systems, which provide time-saving benefits for both the patient and the dentist, are becoming increasingly prevalent in today's dental landscape. Among these, the OptraGloss polishing system, which boasts a high diamond content and obviates the need for an additional polishing paste step[21].

Due to the limited research available on the surface characteristics and bacterial adhesion of single-shade composite resins incorporating nano-fillers, the objective of this research is to evaluate the SR, SFE, and bacterial adhesion of various single-shade composite resins subjected to different polishing techniques. The hypotheses being tested are as follows: i) The SR of single-shade composite resins will be unaffected by different

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polishing systems, ii) The contact angle (CA) and SFE of single-shade composite resins will not be influenced by different polishing systems, and iii) The adhesion of *S. mutans* and *S. mitis* to single-shade composite resins will not be impacted by different polishing systems.

MATERIALS AND METHODS

The Cukurova University Faculty of Medicine Clinical Research Ethics Committee granted approval for this study (approval no: 2022/126.30). Drawing from a prior study[8], G*Power 3.1 (Heinrich Heine University, Düsseldorf, Germany) was utilized to ascertain the necessary minimum specimen size for each group. In the case of SR analysis, considering a 95% power, a 0.05 alpha level, and a 1.32 effect size, a minimum of 4 specimens per group was determined. For bacterial adhesion, with the same power and alpha level but an effect size of 0.74, at least 7 specimens per group were required. This *in vitro* study employed 10 specimens per group.

Specimen preparation

In the current study, the restorative materials utilized and their characteristics are provided in Table 1. For this study, a total of 120 specimens were created, with 40 specimens for each of the three restorative material using Teflon molds with dimensions of 10 mm in diameter and 2 mm in thickness. The composite resin materials were placed into these molds in a single layer and covered on the top and bottom surfaces with a mylar strip. The specimens were then exposed to polymerization using a light-curing device (VALO Ultradent, South Jordan, UT, USA) in standard power mode, applying 1,000 mW/cm^2 for 20 s. To maintain consistent light output, it was checked every 5 specimens using a radiometer (SDI radii plus, SDI, Bayswater, Australia). Following their preparation, the specimens were submerged in distilled water and maintained at a temperature of 37°C for a period of 24 h. This procedure was conducted to allow composite resins to continue polymerizing, eliminate as much unpolymerized monomer as possible, and to achieve optimal mechanical properties of the materials after light curing.

GraphPad QuickCalcs software (GraphPad, San Diego, CA, USA) was employed for all randomization procedures. These included the order of creating composite resin specimens, assigning to experimental groups as well as randomly selecting specimens for images 3D profilometer and scanning electron microscope (SEM), and for energy distribution X-ray spectroscopy (EDS) analysis.

Polishing procedure

Each specimen surface abraded in a single direction and with gentle pressure for 1 min under running water using silicon carbide papers of 400-, 600-, and 1000-grit (Mayer Scientific, Dexter, MI, USA), respectively, to obtain a standard initial surface. Subsequently, the specimens were ultrasonically cleaned in distilled water for a duration of 10 min and then air-dried. After the finishing procedure, the specimens were categorized into two subgroups based on the polishing system used (n=20). The polishing systems used in the study are shown in Table 2. In the groups subjected to one-step polishing (G), OptraGloss was applied using a low-

Table 1 Single-shade composite resins used in the study and their properties

	Manufacturer/Lot number	Composition		Filler w/v%
		Monomer	Filler type and size	
Omnichroma (O)	Tokuyoma Dental, Tokyo, Japan/056E51	UDMA, TEGDMA	Uniform sized supra-nano filler (spherical SiO_2-ZrO_2) and composite filler (260 nm)	79/68
ZenChroma (Z)	President Dental, Germany/2022003395	UDMA, Bis-GMA, TEMDMA	Ultrafine hybrid filler (SiO_2) and glass powder (5 nm–3 μm).	75/53
Charisma Diamond One (C)	Kulzer Dental, Wehrheim, Germany/K010021	UDMA, TCD-DI-HEA, TEGDMA	Nanohybrid filler, B_2O_3-F-Al_2O_3-SiO_2, silica, TiO_2, fluorescent pigments, metallic oxide pigments, organic pigments (5 nm–20 μm)	81/64
Soda-lime-silica glass (ST)	Paul Marienfeld, Lauda-Koenigshofen, Germany	—	72.6% SiO_2, 13.0% Na_2O, 8.8% CaO, 4.3% MgO, 0.6% Al_2O_3, 0.3% K_2O, 0.2% SO_3, 0.1% Fe_2O_3	—

Bis-GMA: Bisphenol A-glycidyl methacrylate; TEGDMA: triethylene glycol dimethacrylate; UDMA: urethane dimethacrylate; TCD-DI-HEA: 2-propenoicacid (octahydro-4,7-methano-1H-indene-5- diyl) bis (methyleneiminocarbonyloxy-2,1-ethanediyl) ester; SiO_2: silicon oxide (silica); ZrO_2: zirconium oxide; TiO_2: Titanium dioxide, B_2O_3-F-Al_2O_3-SiO_2: Boro-fluoro-aluminosilicate; Na_2O: Sodium oxide; CaO: Calcium oxide; MgO: Magnesium oxide; Al_2O_3: Aluminum oxide; K_2O: Potassium oxide; SO_3: Sulfur trioxide; Fe_2O_3: Iron(III) oxide TEMDMA: Tetra-ethylen Di methacrylate; ST: Standard.
*The data were provided by the manufacturers.

Table 2 Polishing systems used in the study and their properties

Polishing system	Type	Manufacturer/Lot number	Compositions
OptraGloss (G)	One-step polishing system	Ivoclar Vivadent, Schaan, Liechtenstein/ZL008G	Polyurethane, diamond granules
OptiDisc (D)	Multi-step polyester disc	Kerr, Orange, CA, USA/7120288	Aluminum-oxide particles (Coarse-Medium-Fine-Extrafine)

speed handpiece. The application involved maintaining medium pressure with constant sweeping motion at a speed of 10,000 rpm for 30 s, accompanied by a water spray. In the groups subjected to multi-step polishing (D), OptiDiscs —coarse, medium, fine, and extra-fine— were sequentially applied using a low-speed handpiece. Each application maintained medium pressure and employed a constant circular motion at 10,000 rpm for 30 s, accompanied by a water spray. For each specimen, a fresh rubber or disc polisher was employed during the polishing processes. The polishing process for each group —OmniChroma+Optra Gloss (OG), ZenChroma+Optra Gloss (ZG), Charisma Diamond One+Optra Gloss (CG), OmniChroma+Optidisc (OD), ZenChroma+Optidisc (ZD), and Charisma Diamond One+Optidisc (CD)— was conducted on separate days. In the polishing process, the one-step groups underwent a 10-min interval after processing every four specimens while the multi-step groups took a 10-min break following every two specimens. To minimize potential variations on the specimen surfaces due to operator differences, a single operator performed all preparation, finishing, and polishing procedures. After completing the polishing process, surface residues on the specimens were rinsed off with distilled water for 20 s. Subsequently, the specimens were air-dried using a spray for 5 s. Soda-lime-silica glass, employed as a reference in bacterial adhesion studies, was used as the standard material (ST) in the current research, with the relevant details presented in Table 1.

SR measurement

The measurement of SR was conducted by a second operator who was unaware of the specific polishing system applied to each specimen. To maintain measurement precision, the profilometer underwent calibration using a reference block prior to initiating the measurements and was recalibrated at the start of each new set of measurements. SR was determined employing a stylus profilometer (NanoMap–LS, AEP Technologies, Santa Clara, CA, USA), which utilized a contact force of 0.34 mN for measurement. Two-dimensional surface profiles of the specimens were acquired using a diamond-tipped instrument with a 2.5 μm diameter, operating at a scanning velocity of 60 μm/s. Measurements were relatively made at the center of specimens to avoid areas potentially not reached by the polishing system. The SR of each specimen was measured in three distinct regions (one central, one right, and one left); the average of these three readings was recorded as roughness values (Ra) in micrometers (μm).

CA and SFE determination

The wettability and CA of the specimen surfaces were ascertained using an automatic CA and surface tension measurement device (OCA-50, Dataphysics, Filderstadt, Germany). This was conducted using the sessile drop method for measuring CA. Specifically, the CA for each surface was assessed by measuring the angle formed by a drop of distilled water on the surface. The average of the left and right CA of the water droplets was calculated and recorded as the value of the CA. To calculate the SFE of the specimens, CA measurements were performed using four different liquids, each with varying polarities (distilled water, ethanol, ethylene glycol, and dimethyl sulfoxide).

Average CA values were used to compute the components of the SFE, and calculations were based on the approach presented by Owens and Wendt[24]. The SFE (γ_S^T) value was ascertained by summing the dispersive (γ_S^D) and polar (γ_S^P) components.

SEM and EDS analysis

An additional specimen from each group, post-polishing, was secured onto aluminum stubs. These were subsequently gold-coated using the Q150R Plus Sputter Coater Combined System (Quorum Technologies, Lewes, UK). For detailed examination, a SEM (FEI, Quanta 650 FEG, Eindhoven, The Netherlands) was employed, operating at 15 kV and magnifying at 12,000×.

For elemental analysis, SEM coupled with EDS was utilized. A specific region, measuring 20×20 μm, of the specimens was scrutinized at a magnification of 500×. This produced a spectrum, revealing the fundamental elemental composition of the material as distinct peaks.

Subsequent to the bacterial adhesion study, one specimen was randomly picked from each batch. To visualize the biofilm formation on the specimen surfaces *via* SEM, they were initially fixed in a 2.5% glutaraldehyde solution for 1 h. A series of ethanol washes were then applied, progressing in concentration from 10% to 100%. Following this, the specimens were allowed to dry in a bacteriological incubator set at 37°C overnight. The aforementioned SEM protocol was reiterated, maintaining consistent magnification. Representative micrographs highlighting the biofilm's presence on the specimen surfaces were captured, and a subsequent analysis of these images was carried out.

Microbiological procedures

In the course of this study, a microbiological method was conducted in accordance with a prior investigation[18]. To examine bacterial adhesion, twenty specimens were prepared from each group. These specimens were then divided into two subgroups, each consisting of ten specimens, to specifically evaluate the adhesion of *S. mutans* and *S. mitis*. Before initiating the bacterial adhesion procedure, the prepared specimens were sterilized in an autoclave (NC 90M, Nuve, Zurich, Switzerland) at 122ºC for 15 min and placed in sterile petri dishes.

To foster bacterial adhesion by forming a pellicle layer on the specimen surfaces, a designated artificial saliva recipe was employed and included 8.4 mg of NaF, 2,560 mg of NaCl, 332.97 mg of $CaCl_2$, 250 mg of $MgCl_2$ ($6H_2O$), 189.48 mg of KCl, 3,015 mg of CH_3COOK, 772.00 mg K_3PO_4 ($3H_2O$) and 0.1 mL of H_3PO_4 (Merck, Darmstadt, Germany)[7]. The pH of the solution was then adjusted using a pH meter to a range of 6.5–7 by adding 30 mL of 0.1 M NaOH[9]. Post-sterilization, 140 mg of Type II mucin (Sigma-Aldrich Chemie, Deisenhofen, Germany) was integrated for every 100 mL of the artificial saliva.

S. mutans (ATCC 25175) and *S. mitis* (ATCC 49456) strains was obtained from the bacteriology laboratory of the Medical Microbiology, Department at Cukurova University Medical Faculty. Previously cultured and frozen (−80°C) strains were thawed. Subsequently, the strains were inoculated onto 5% sheep blood agar (HiMedia Laboratories, Mumbai, India). Cultured bacteria were transferred into tubes filled with 5 mL of brain-heart infusion broth (BHI; Oxoid, Basingstoke, UK), and these tubes were then incubated at a temperature of 35°C in an environment containing 5% CO_2 for a period of 24 h. After the incubation period, the tubes with bacteria were subjected to agitation in a centrifuge (EBA 21, Hettich Zentrifugen) for a duration of 5 min. This was followed by the addition of 5 mL of phosphate-buffered saline (PBS; Sigma–Aldrich, St. Louis, MO, USA) to each tube, which were then centrifuged for a brief period of 15 s. From the sediment formed at the base of these tubes, a bacterial suspension was produced with a density of 10^9 cfu/mL. The concentration of the suspension was verified using a Den-1 McFarland Densitometer (Biosan, Riga, Latvia).

1. Bacterial adhesion

For the study of bacterial adhesion, 20 specimens in each group were divided into two subgroups: 10 for *S. mutans*, 10 for *S. mitis* from each group including control (glass; n=20). The specimens, placed in sterile petri dishes, were coated with 5 mL of mucin-enriched artificial saliva, aiding in the development of a pellicle layer. They were then subjected to incubation at 37°C in a 5% CO_2 atmosphere for 1 h. Post incubation, the specimens were cleansed using 5 mL of sterile saline and shifted to fresh sterile petri dishes. Each specimen was then covered with a 200 μL portion of the prepared bacterial suspension. Following a 15-min period, 5% glucose-enriched BHI was added to completely cover the specimens in each petri dish. Subsequently, these dishes were placed in an incubator (Thermo/Forma Thermo Fisher Scientific CO_2 Water Jacketed Incubator, Waltham, MA, USA) and maintained for 24 h at 37°C in a 5% CO_2 atmosphere to enable the evaluation of bacterial adhesion.

Following a 24-h period, each specimen was transferred to a tube containing 2 mL of PBS. The specimens were then agitated for 60 s using a vortex mixer (NM 110, NÜVE, Ankara, Turkey) to remove loosely attached bacteria. After this, 200 μL of BHI broth was allocated into each well of a 96-well microplate, and 20 μL of the wash solution from the vortex-treated specimens was introduced to the wells. The BHI broth in the microplates was incubated at 35°C in a 5% CO_2 atmosphere for another 24 h. Post-incubation, the optical densities at 630 nm of the solutions in the microplates were measured using an automatic microplate reader (Thermo Scientific Multiskan FC, Thermo Fisher Scientific, Vantaa, Finland). The bacterial load was quantified and reported as $\times 10^9$ CFU/mL.

Statistical analysis

Statistical analysis of the collected data was carried out using IBM SPSS Statistics software for Mac, version 26 (IBM SPSS, Chicago, IL, USA) To evaluate the normality of the data distribution, the skewness and kurtosis coefficients, along with the Shapiro-Wilk test, were employed. The data related to SR, CA, SFE, and bacterial adhesion underwent statistical examination using one-way analysis of variance (ANOVA) and Tukey's *post-hoc* tests. Differences between the adhesion of different bacterial species were analyzed using Student's *t*-test. Additionally, the Pearson correlation coefficient was calculated to investigate the relationships between the parameters of SR, SFE and CA, and bacterial adhesion (α=0.05).

RESULTS

SR

The mean, standard deviation, and statistical differences of the experimental groups were presented in Fig. 1. The relationship between the different polishing systems and the composite resins studied is illustrated in the three-dimensional representations of the surface structure (Fig. 2). There were differences between the OG and OD groups, ZG and ZD groups, and CG and CD groups, with the multi-step polishing groups showing lower roughness values (p<0.0001, p=0.022, and p=0.002, respectively). The OD group exhibited the lowest roughness value among the experimental groups, showing no significant difference compared to the ST groups (p=0.058). Conversely, the OG group displayed the highest roughness value, significantly differing from the other groups (p<0.0001). When comparing groups subjected to one-step polishing system, no significant difference was found between the OG and CG groups (p=0.913). However, the surface roughness in the ZG group was significantly lower than in these two groups (p<0.0001).

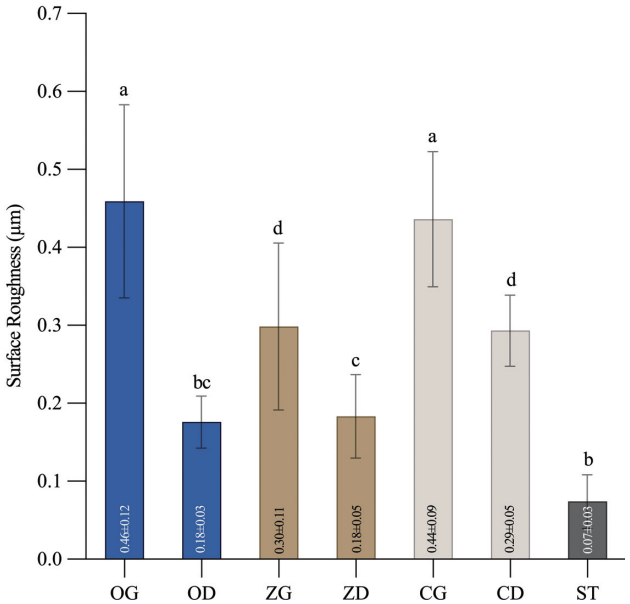


Fig. 1 Mean and standard deviations of surface roughness values. (different letters indicate statistical differences among the groups.) (p<0.05).
The abbreviations represent the composite resins and polishing groups: "O" (Omnichroma), "Z" (ZenChroma), "C" (Charisma Diamond); "G" (OptraGloss), "D" (OptiDisc); "ST" (Standard, soda-lime-silica Glass).

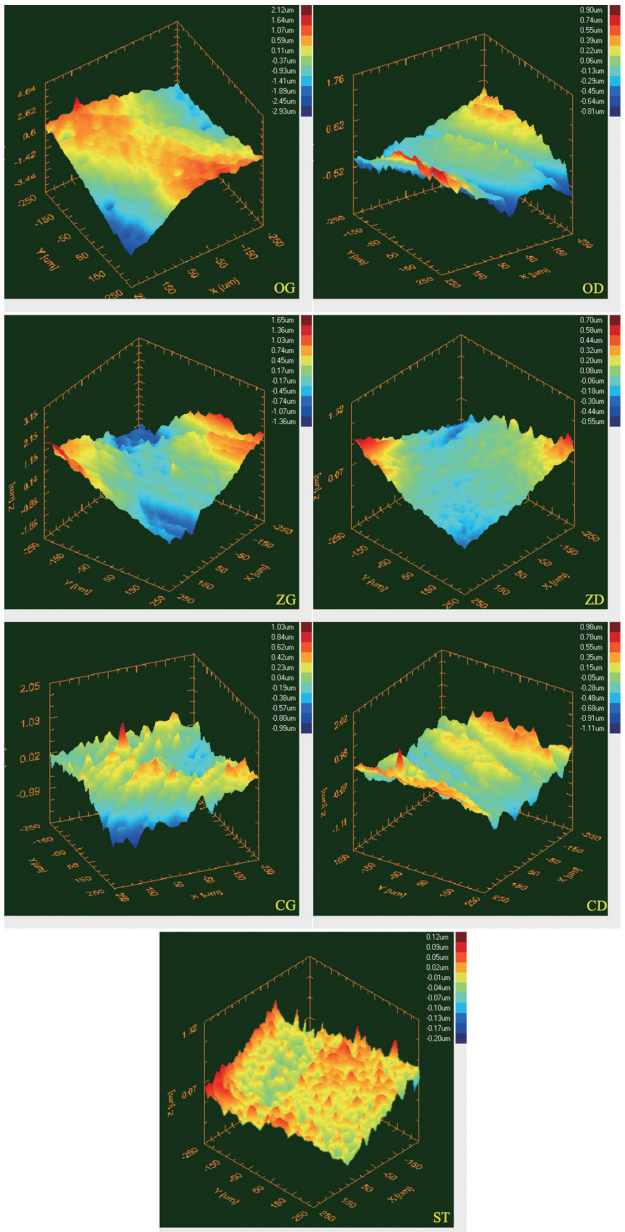


Fig. 2 Representative 3D profilometer images of the investigated materials.
The abbreviations represent the composite resins and polishing groups: "O" (Omnichroma), "Z" (ZenChroma), "C" (Charisma Diamond); "G" (OptraGloss), "D" (OptiDisc); "ST" (standard, Glass).

Among the groups treated with the multi-step polishing, OD and ZD groups were similar (p=0.999), and both showed significantly lower roughness values compared to the CD group (p=0.012 and p=0.022, respectively).

CA and SFE

1. CA

The mean CA values, standard deviations, and statistical differences of all materials are presented in Fig. 3. The lowest CA value was found in the ST group (51.55°) which was statistically significant than all experimental composite resin groups (p<0.0001). Different polishing systems created differences in the CA of the same composite group, with the OD (82.76°) and ZD (81.89°) groups showing lower CA compared to the OG (88.85°) and ZG (91.55°), respectively (p=0.033 and p<0.0001). However, the differences between CG (85.23°) and CD (83.31°) was not significant (p=0.949). One-step polishing groups OG and ZG showed similar CA (p=0.788), while the CG group had a significantly lower CA than the ZG group (p=0.024). Within the multi-step polished groups, there was no significant statistical difference among the OD, ZD, and CD groups (p>0.05).

2. SFE

The SFE findings were shown in Fig. 4. The dispersive component (γ_S^D) contributed more than the polar component (γ_S^P)to the SFE (γ_S^T) of the experimental groups. However, in the ST group, γ_S^P has a significant effect on γ_S^T. The ST group has the highest SFE compared to all other groups (p<0.0001). Regarding $\gamma_S^T$of the experimental groups, there is no significant difference between the OG–OD groups, ZG–ZD groups, and the CG and CD groups (p=0.999, p=0.224, and p=0.096 respectively). There is also no significant difference among the groups polished with one-step system (OG, ZG and CG) (p>0.05). In groups polished the

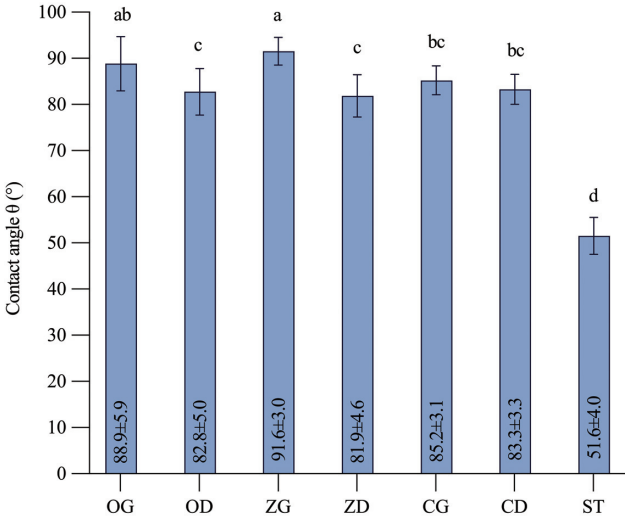


Fig. 3 Mean and standard deviations of CA values (different letters indicate statistical differences between groups) (p<0.05).
The abbreviations represent the composite resins and polishing groups: "O" (Omnichroma), "Z" (ZenChroma), "C" (Charisma Diamond); "G" (OptraGloss), "D" (OptiDisc); "ST" (Standard, soda-lime-silica Glass).

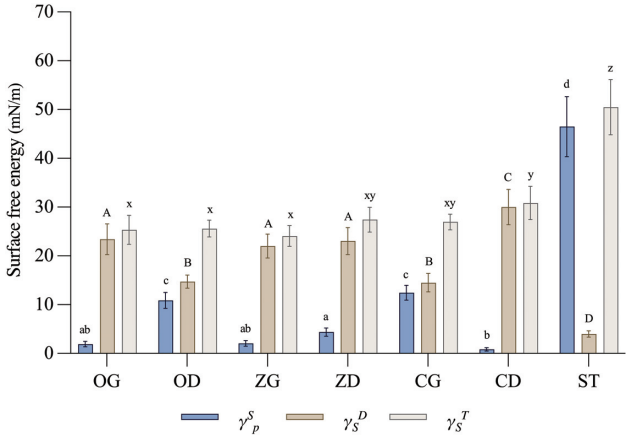


Fig. 4 Mean and standard deviation of the surface free energy (SFE) values.
γ_S^T: Total SFE, γ_S^D: Dispersive component, γ_S^P: polar component) Lowercase letters signify differences in γ_S^T. Uppercase letters signal differences in γ_S^D. "x, y, z" mark differences in γ_S^P (p=0.05). The abbreviations represent the composite resins and polishing groups: "O" (Omnichroma), "Z" (ZenChroma), "C" (Charisma Diamond); "G" (OptraGloss), "D" (OptiDisc); "ST" (Standard, soda-lime-silica Glass).

multi-step system, OD showed the lowest SFE, similar to ZD (p=0.851) while CD was significantly different from OD (p=0.007).

While the γ_S^D component significantly decreased from OG to OD (p<0.0001), it remained stable between ZG and ZD (p=0.965). For CG and CD, γ_S^D component was significantly higher in CD than in CG (p<0.0001). Compared groups applied the one-step polishing system, no significant differences were found between the OG and ZG groups (p=0.858) whereas the CG group displayed a significantly lower γ_S^D compared to both OG and ZG (p<0.0001). With the multi-step system, significant differences were observed among the OD, ZD, and CD groups (p<0.0001).

In the context of γ_S^Pcomponent, ST demonstrated the highest value, which was statistically different from the other groups (p<0.0001). In contrast to the γ_S^D, the γ_S^P component significantly increased from OG to OD (p<0.0001), while it significantly decreased from CG to CD (p<0.0001). No significant differences were noted between ZG and ZD (p=0.395). In the groups applied one-step polishing system, OG and ZG displayed similar γ_S^P values (p=0.999), whereas CG significantly exceeded both (p<0.0001). For the multi-step polished groups, significant differences were noted among the OD, ZD, and CD groups (p<0.0001).

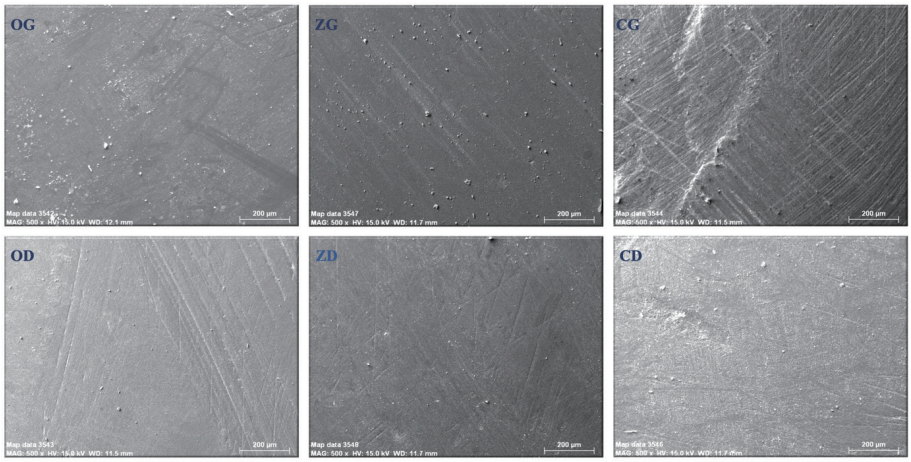
EDS

Figure 5 illustrates the differences in elemental composition *via* EDX analysis for the materials tested. Oxygen (O), carbon (C), silicon (Si), and aluminum (Al) elements were detected across all groups. Carbon content, representing the organic phase, was considered. The ST group's elemental atomic weight percentages were identified as O (56.77%), Si (29.48%), sodium (Na; 6.52%), calcium (Ca; 5.6%), and magnesium (Mg; 1.63%), with no carbon detected. EDS analysis indicated variations in elemental composition of the composite resin groups polished with different systems. The OG group exhibited higher percentages of O, Si, and Al but a reduced C percentage compared to the OD group. The ZG group showed higher percentages of Si, Al, Ni, and F, but lower O and C than the ZD group. The CG group had lower percentages of Ba, Si, C, and Al and higher O and F compared to the CG group.

Bacterial adhesion

Figure 6 presents the mean and standard deviation values along with the statistical analysis for the *S. mutans* and *S. mitis* bacterial adhesion of the groups. According to the pairwise comparisons between the bacterial species, *S. mitis* exhibited higher bacterial adhesion than *S. mutans* in all groups, except the OG (p<0.0001). The lowest bacterial adhesion of *S. mutans* was observed in the ST group, significantly differing from other groups, except ZD (p<0.0001).

According to the pairwise comparison of the polishing systems, OD, ZD, and CD groups showed significantly less *S. mutans* adhesion than OG, ZG, and CG, respectively (p<0.0001). Compared the composite resins applied the same polishing system regarding *S. mutans* adhesion, OG was the highest, significantly differing from ZG and CG (p<0.0001). Among the groups applied the multi-step system, ZD was similar with and CD



	O	Si	C	Al	Ni	F	Ba	Na	Ca	Mg
OG	69.07	12.56	18.30	0.07						
OD	68.68	11.77	19.52	0.02						
ZG	47.50	15.99	12.38	2.80	4.67	16.65				
ZD	53.75	12.54	14.77	2.15	2.50	14.29				
CG	40.68	19.93	12.26	3.51		7.09	16.53			
CD	39.45	20.19	13.41	3.69		5.86	17.40			
ST	56.77	29.48						6.52	5.60	1.63

Fig. 5 SEM images of the focused region of the investigated materials along with the percentage of weight of the groups from the EDS analysis (scale bar: 200 μm).
The abbreviations represent the composite resins and polishing groups: "O" (Omnichroma), "Z" (ZenChroma), "C" (Charisma Diamond); "G" (OptraGloss), "D" (OptiDisc); "ST" (Standard, soda-lime-silica Glass).

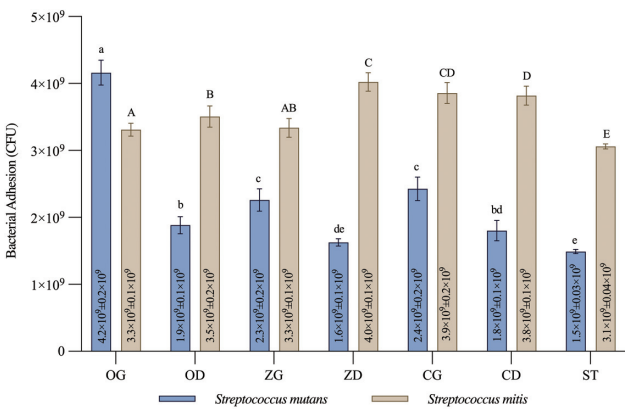


Fig. 6 The mean and standard deviations of *S. mutans* and *S. mitis* bacterial counts.
Different uppercase letters indicate statistical differences between the material groups to which *S. mitis* adhered. Different lowercase letters signify statistical differences between the material groups to which *S. mutans* adhered (p<0.05). The abbreviations represent the composite resins and polishing groups: "O" (Omnichroma), "Z" (ZenChroma), "C" (Charisma Diamond); "G" (OptraGloss), "D" (OptiDisc); "ST" (Standard, soda-lime-silica Glass).

(p=0.085) but significantly lower than OD (p<0.0001).

Similarly, the lowest *S. mitis* adhesion was observed in the ST group, significantly different from all other groups (p<0.0001). The highest *S. mitis* adhesion was in the ZD group, which was significantly different from all groups, except CG (p<0.0001). Compared the polishing systems, OD and ZD groups had significantly higher *S. mitis* adhesion compared to OG and ZG groups (p=0.021 and p<0.0001, respectively), while CG and CD groups were similar (p=0.993). Compared the composite resins polished with the same system, OG and ZG groups were similar (p=0.999), but the CG group was significantly higher *S. mitis* adhesion than the other two (p<0.0001). Among the groups polished with multi-step system, *S. mitis* adhesion ranked from the highest to lowest as ZD, CD, OD, with significant differences among the groups (p<0.05).

SEM

Representative SEM images are presented for each group in Fig. 7. The first column displays the experimental groups post-polishing, including the ST group. Subsequent columns illustrate the adhesion of *S. mutans* and *S. mitis* bacteria to the surfaces of these groups. The SEM images post-polishing indicated differences in surface appearance and bacterial adhesion. The post-polishing images show similar surfaces for OG and OD groups. In the representative image for the ZD group, an increase in relatively opaque areas compared to ZG was observed. Interestingly, while the CD group displayed a surface similar to the other groups, the CG group showed

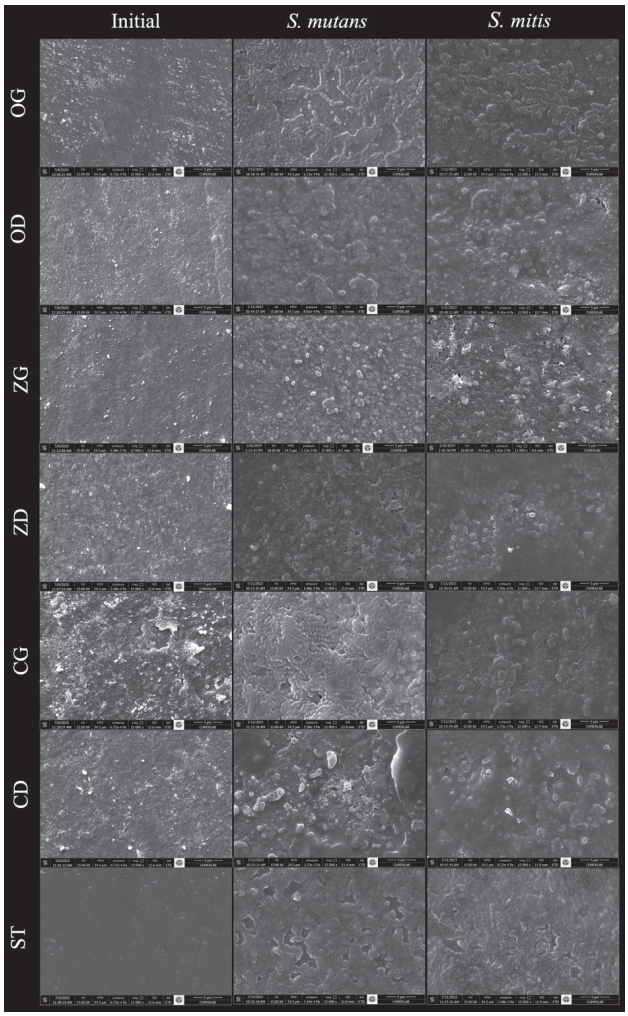


Fig. 7 Representative SEM images of the investigated materials at the magnification of 12,000×.
The abbreviations represent the composite resins and polishing groups: "O" (Omnichroma), "Z" (ZenChroma), "C" (Charisma Diamond); "G" (OptraGloss), "D" (OptiDisc); "ST" (Standard, soda-lime-silica Glass).

opaque areas and a more layered surface appearance. All materials used in this study exhibited adhesion of *S. mutans* and *S. mitis*, indicative of advanced bacterial accumulation or aggregation.

DISCUSSION

Smart shade matching allows for color adjustment based on the absorption and emission of light by composite resin and is closely linked to the surface properties of the restoration. The color matching is achieved by adjusting the morphology of filler types in composite resins. There is insufficient information regarding the impact of surface polishing procedures on bacterial adhesion to both currently used composite resins and those still under development with this new particle technology. Therefore, this study focused on evaluating single-shade composite resins following two different polishing systems in terms of the parameters related to surface properties. Three different single-shade composite resins were investigated in this study: Omnichroma, known for its advanced smart shade matching; Charisma Diamond One, noted for its improved physical properties; and the recently developed ZenChroma, which features an ultrafine grain structure. Significant differences were observed in the SR, CA, and bacterial adhesion of investigated composite resins following the application of various polishing systems. Consequently, the null hypotheses concerning these parameters were rejected. However, the null hypothesis regarding SFE was not rejected, as no differences were detected between the polishing systems in terms of SFE.

Various studies in the literature indicated that aluminum oxide discs are most effective in achieving the lowest roughness values on composite resin surfaces[7,21,25]. Furthermore, Cazzaniga *et al.*[6] attributed the reduced SR achieved with aluminum discs, compared to one-step rubber point, to the multi-step protocol. The current study indicated that single-shade composite resins polished with the multi-step system exhibited less SR compared to those polished with the one-step system. In addition to our findings consistent with the current general consensus, this study provided a detailed examination of whether innovative single-shade composite resins with self-adjusting color matching properties, featuring varying particle sizes and compositions, respond similarly to different polishing systems. Thus, this study aimed to fill a gap in the literature by addressing the specific aspects.

In composite resin studies, it is generally anticipated that plaque accumulation on the surfaces of these materials intensifies when the SR surpasses a critical value of 0.2 μm[26]. In this study, the SR of the groups of Omnichroma and ZenChroma polished with the multi-step system remained under this threshold. The higher roughness of the CD group compared to the OD and ZD groups, and the fact that it remains above the critical roughness threshold, can be attributed to its grain structure, which can reach up to 20 μm, as well as its additive content, which enhances the physical properties of the composite resin. The one-step polishing system demonstrated lower effectiveness compared to the multi-step system in the SR analysis of single-shade composite resins.

One of the significant aspects of SR studies is to develop a prediction related to bacterial adhesion and retention. Salehi *et al.*[27] focused on investigating the initial adhesion of *S. mutans* on the surface of polymer-based restorative materials and their findings revealed that the level of bacterial adherence is substantially affected by the SR of the investigated materials. Studies evaluating the impact of different finishing and polishing procedures on composite resins showed that in groups with increased live *S. mutans* counts, SR values were higher[7,18]. The prevailing consensus is that smoother surfaces are less likely to lead to biofilm formation

on their surfaces, regardless of the type of restorative material employed. Similarly, a significant positive correlation was observed between SR and the formation of *S. mutans* biofilm in this study (r= 0.693, p<0.0001).

In contrast to the positive correlation between SR and *S. mutans* adhesion, there was no significant correlation between SR and *S. mitis* adhesion (r=0.082, p=0.679). Additionally, the multi-step polished groups, which exhibited smoother surfaces, demonstrated higher or comparable adhesion of *S. mitis* in this study. The results of *S. mitis* adhesion in this study suggest that factors beyond SR may influence that bacterial adhesion. Previous studies demonstrated that, in addition to SR, factors such as electrical properties, hydrophobicity, SFE, and fluoride release might also play a significant role in bacterial adhesion[8,22]. Therefore, the hydrophobicity and SFE of polished composite resins were also evaluated in the current study.

CA, a parameter influencing biofilm formation is utilized to define the "hydrophilicity" or "hydrophobicity" of restorative materials[28]. One-step groups with higher CA, indicating greater hydrophobicity exhibited the higher *S. mutans* adhesion, while multi-step groups with lower CA showed higher or comparable *S. mitis* adhesion. This suggests that CA values may indeed have an impact on bacterial adhesion. The findings also indicated certain correlations between CA values and bacterial adhesion. Specifically, a significant positive moderate correlation was observed between CA and *S. mutans*, while a significant negative moderate correlation was identified between CA and *S. mitis* (r=0.414, p=0.028 and r=0.442, p=0.019; respectively). The observed correlation between parameters such as SR or CA and bacterial adhesion in this study indicates that these relationships could be dependent on the surface properties of the bacterial species.

A CA greater than 62° indicates the presence of hydrophobic characteristics in the material[29].

Hydrophobicity, a potentially critical factor in bacterial adhesion is a property associated with the material surface and can also be characterized for the bacterial surface[30]. Olivera *et al.*[31] found that hydrophobic bacteria exhibit increased adhesion on hydrophobic surfaces. Specifically, *S. mitis* strains show a preference for hydrophilic surfaces, whereas *S. mutans* is more inclined to adhere to hydrophobic surfaces[32]. One of the primary species-specific factors influencing bacterial adhesion might be the hydrophilic or hydrophobic nature of the bacterial surface. In this study, the results indicated that all polished composite resins exhibited CA greater than 62°, indicating hydrophobic surfaces. Conversely, the glass surface (the standard group) was found to be hydrophilic. This difference might explain the relatively lower adhesion of *S. mutans* to the glass surface compared to the higher adhesion of *S. mitis*.

Yuan *et al.*[33] observed that bacterial adhesion is not always solely related to SR but also to surface energy. Bilgili *et al.*[18] identified a positive correlation between the SFE of the investigated composite groups and *S. mitis* adhesion whereas *S. mutans* adhesion was unaffected by the SFE. However, unlike the CA results, the polishing systems did not have a significant effect on the SFE values of the composite resin groups in this study. Concerning relation SFE and bacterial adhesion, no significant correlation was found between *S. mutans* adhesion and SFE (r=−0.326, p=0.09), though a moderate significant positive correlation was observed with *S. mitis* adhesion in this study (r=0.506, p=0.006). The findings presented above underscore the existence of a nuanced interaction between the surface properties of composite resins and bacterial adhesion, emphasizing once again that this interaction may vary depending on the specific bacterial species involved. Indeed, there is no consensus in the literature regarding the relationship between surface energy and bacterial adhesion[9,10,18]. The complexity of these relationships highlights the need for further research to elucidate the contributing factors in bacterial adhesion to composite resins.

EDS analysis was performed to explore the connection between the elemental composition of the examined surfaces and the formation of biofilms. The analysis revealed that all composite resins shared common elements, including O, C, Si, and Al. Additionally, Ni was found in the ZenChroma group, and Ba was found in the Charisma group. Furthermore, the ZenChroma and Charisma groups contained the element F (fluorine). However, the existence of a direct correlation between the elemental content of the surfaces assessed through EDS and the formation of biofilms continues to be an ambiguous aspect in existing literature[6,19,34]. Consistent with existing literature data, this study did not find a direct relationship between the surface elemental composition of composite resins and biofilm formation[6,19]. In studies by Cazzaniga *et al.*, the presence of fluoride-containing Filtek Supreme XTE resin was expected to reduce biofilm formation on the surface, but bacterial adhesion data did not support these findings[6]. They suggested that this could be due to the fluoride content or release in the material composition being too low to significantly reduce biofilm formation. In a recent study using three different bulk-fill composite resins with EDS analysis, fluoride was detected in their compositions. However, despite its proven antimicrobial and bacteriostatic effects even at low concentrations, fluoride did not have any effect on microbial adhesion or biofilm formation. One possible explanation for this phenomenon is that fluoride may be tightly incorporated into the material and resin matrix after polymerization without significant release[20]. In the current study, it was also observed that the presence of fluoride detected by EDS analysis in ZenChroma and Charisma did not have an impact on bacterial adhesion.

The formation of biofilms is impacted by multiple factors associated with the physical and chemical properties of a material's surface. These include the size, shape, and distribution of fillers, as well as the composition of the matrix. The relationship between filler size and biofilm formation in resin composite resins has been a subject of debate in the literature.

Cazzaniga *et al*.[6] observed that composite resins containing nano-fillers had considerably less biofilm formation than those that are bulk-fill. Similarly, Pereira *et al*.[22] showed that nano-filled composite resins had reduced biofilm formation when compared to both nanohybrid and microhybrid composite resins. However, Steinberg and Eyal[35] found differences in bacterial adhesion when testing three microhybrid composite resins. In the present study, despite using nano-filled and nanohybrid single-shade composite resins, different bacterial adhesion patterns were observed. Similarly, this study demonstrated that composite resins with smart chromatic technology, despite having relatively similar structures and undergoing the same polishing system, exhibited different bacterial adhesion behaviors.

The outcomes of this study should be considered in light of the constraints inherent in an *in vitro* setup. In the employed *in vitro* model, the adhesion of *S. mutans* and *S. mitis* was examined under specific conditions, including neutral pH and an artificial saliva environment, without the presence of fundamental oral protective elements. The determination of both live and dead bacteria on the composite surface, which serves as a parameter indicating the surface's capacity to host bacterial adhesion initially, could provide valuable insights for planning future studies on bacterial adhesion. Additional *in vitro* investigations are necessary to elucidate the effects of the tested parameters on the formation of a multispecies biofilm in a controlled environment. Consequently, subsequent to these initial evaluations, in situ and *in vivo* studies are essential to corroborate the findings observed in the *in vitro* experiments and provide more comprehensive insights into the development of restorative materials with optimized surface characteristics to minimize bacterial colonization and biofilm formation.

CONCLUSIONS

This study highlighted the complex interplay between the surface properties of single-shade composite resins and bacterial adhesion, with a particular focus on the effects of different polishing systems. Smoother surfaces, achieved through multi-step polishing systems, tend to have lower SR and are less conducive to *S. mutans* adhesion, while the higher roughness observed with one-step systems correlated with increased *S. mutans* biofilm formation. Conversely, *S. mitis* adhesion was found to be less dependent on SR and more influenced by hydrophobicity. However, different polishing system had no effect single-shade composite resins of SFE. Moreover, the current study underscores the importance of considering the specific bacterial species when evaluating the impact of composite resin surface properties on bacterial adhesion.

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CONFLICT OF INTEREST

The authors do not have any financial interest in the companies whose materials are included in this article.

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