Journal of Oral Science, Vol. 65, No. 2, 121-126, 2023. doi.org/10.2334/josnusd.22-0374

Original Article

Effect of thickness on the degree of conversion, monomer elution, depth of cure and cytotoxicity of bulk-fill composites

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Received September 27, 2022; Accepted January 25, 2023

Abstract

Purpose: Today, bulk-fill composites are used as a single layer with a thickness of up to 4-5 mm. However, is proper polymerization achieved with this increased thickness?
Methods: This study was designed to investigate the effect of thickness on the degree of conversion (DC) (n = 6), the elution of monomers (n = 6), depth of cure (DoC) (n = 10) and cytotoxicity (n = 6) of the bulk-fill composites SDR Flow Plus (SDR), SonicFill2 SingleFill (SF) and ACTIVA Bioactive Restorative (ACT) in comparison to the conventional G-aenial Posterior (GC). Two-way analysis of variance (ANOVA) was used to assess the interaction between materials and surfaces, and one-way ANOVA and Tukey tests were used to compare the degree of conversion, monomer elution and cytotoxicity values ($P < 0.05$).
Results: The highest DC was found at the top surface of SDR, while the lowest DC was found at SF. The V2 mm/V0 mm DoC ratios of the composites except ACTs were appropriate according to the threshold. None of the composites were cytotoxic on day 1.
Conclusion: In bulk-fill composites, DC decreased and monomer elution increased with increasing depth. The V4 mm/V0 mm ratios of all bulk-fill groups were not appropriate. Additionally, only ACTs had a cell viability of <70% on day 7.

Keywords: bulk-fill composite, cell viability, FTIR, HPLC, Vickers

Introduction

Resin-based composites are used as the primary material for direct restorations of anterior and posterior teeth. They have certain advantages such as esthetic properties with a suitable color, adequate adhesion to dental tissues, good dental protection, and low cost [1]. However, there are some deficiencies that limit their use, including polymerization shrinkage, adhesion failure, and incomplete conversion of their monomers, leading to mechanical and biological issues [2].

Recently, bulk-fill resin composites have been developed to overcome these disadvantages [3]. It is recommended that these are applied in 2-mm layers to minimize the polymerization shrinkage and stress that occur with conventional composite restoration. This procedure requires operator knowledge and experience, as well as time. However, newly developed bulk-fill composites can be placed in a single increment of 4 mm. Bulk-fill composites, produced with a technology that facilitates the passage of light, allow dentists to achieve simpler and shorter treatment. However, the safety of bulk-fill composites used at the specified thickness should be evaluated in terms of the degree of conversion and monomer elution.

Bulk-fill composites show lower polymerization shrinkage and creep deformation than conventional composites [4]. Many previous studies have focused on the amount of eluted monomers [5,6], but few studies have investigated the relationship between the degree of conversion of bulk-fill composites and monomer elution [5]. The amount of eluted monomers can be influenced by the degree of conversion, the thickness and composition of the composite resins, and the solvent [6]. A lower degree of conversion increases the elution of monomers [5]. However, it can be said that the clinically acceptable range of conversion is >55% for bulk-fill composites [7].

The depth of cure (DoC) indicated as the thickness of a resin-based composite that has been adequately cured can be defined as “the maximum thickness that should be used for each successive RBC increment” [8]. DoC can be determined by calculating the ratio (hardness values) of the bottom and top surfaces of resin composites [9].

Residual monomers may be left in the structure of composite resins after polymerization, and these can increase the incidence and degree of toxic reactions. Due to their potential impact on the biocompatibility and structural stability of a restoration, many investigators have studied the elution of these unbound molecules into aqueous media. The quantity of leachable monomers has been correlated with the degree of cure of the polymer network [10].

The aim of the present study was to evaluate the degree of conversion, the amount of released urethane dimethacrylate (UDMA), triethylene glycol dimethacrylate (TEGDMA) and bisphenol-A glycidyl methacrylate (BisGMA) monomers, and the depth of cure and cytotoxicity of bulk-fill composites (SDR Flow Plus [SDR], SonicFill 2 SingleFill [SF] and ACTIVA Bioactive Restorative [ACT]) with layer thicknesses of 2 mm and 4 mm in comparison to a conventional composite (G-aenial Posterior [GC]) with a thickness of 2 mm.

Materials and Methods

The properties of the composite resins used in this study are given in Table 1.

Sample preparation

Sample preparation using titanium molds (2 molds superimposed, 6 mm in diameter and 2 mm in height) and transparent polyester strips is shown in Fig. 1. Disk I (6 mm Ø × 2 mm) with a top surface, cured directly, and disk II (6 mm Ø × 2 mm) with a top surface, cured by light coming from the bottom surface of disk I were prepared. Thus, the total distance traveled by the light at one time was 4 mm for the bulk-fill composite samples. The resins were polymerized using a LED curing light unit (Elipar FreeLight 2, approx. 1,000 mW/cm^2, 3M ESPE, Seefeld, Germany) in accordance with the manufacturers’ instructions (Table 1). Under the standard curing mode, an output wavelength range of 430-480 nm was applied. After polymerization, all samples were taken from the molds, and the transparent strips between the 2-mm layers were removed. To determine the top surface, a point was placed on the side edge of the bottom surfaces with an acetate pen. It was then stored in the dark to dry for 24 hours before analysis.

All tests were performed with 7 different groups, with bulk-fill composites as disk I and disk II, and with the conventional composite only as disk I. The experimental groups were: SDR Disk I, SDR Disk II, SF Disk I, SF Disk II, ACT Disk I, ACT Disk II, GC Disk I.

Degree of conversion

The top and bottom surfaces of the prepared disks were characterized by Fourier transform infrared spectroscopy using a Jasco 6800, equipped with an ATR accessory. The spectra were collected at 400-4,000 cm^{-1} wave-

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Table 1 Dental composites, types, matrix compositions, shades, polymerization times, composition of each material based on manufacturer's data, and manufacturers

Product name	Type	Matrix composition	Shade and cure time	Composition of materials based on manufacturer's data	Manufacturer
SDR Plus	flowable bulk-fill base	modified UDMA, TEGDMA, EBPDMA, filler; 68 wt%, 44 vol%	A2, 4 mm for 40 s	SDR patented urethane di-methacrylate, di-methacrylate resin, di-functional diluents, barium and strontium alumino-fluoro-silicate glasses (68% by weight, 45% by volume), photoinitiators, colorants	Dentsply Sirona, Charlotte, NC, USA
SonicFill 2 SingleFill	sonic-activated flowable and sculptable bulk-fill	Bis-GMA, bis-EMA, TEGDMA, filler; 83 vol%	A2, 5 mm for 20 s	barium glass, silicon dioxide (83.5% wt), Bis-GMA, Triethylene glycolidmethacrylate (TEGDMA), 3-trimethoxysilylpropyl methacrylate (TMSPMA), silicon dioxide, bisphenol-A-dimethacrylate, ethoxylated-bisphenol-A-bis- (2-hydroxy -3-methacryloxypropyl), Bis-EMAD	Kavo Kerr, Charlotte, NC, USA
ACTIVA BioActive-Restorative	flowable bulk-fill	blend of diurethane/ other methacrylates silica 6.7 wt%	A2, 4 mm for 20 s	mix of diurethane and methacrylates (without Bis-GMA), modified with polyacrylic acid (44.6%); reactive glass filler (21.8% by weight); inorganic filler (56% weight), patented rubber resin (Embrace), silica (ca. 3.0%), sodium fluoride (0.9%), water	Pulpdent, Watertown, MA, USA
G-aenial Posterior	micro-hybrid conventional composite	UDMA, dimethacrylate co-monomers (Bis-GMA free), filler; 81 wt%, 65 vol%	A2, 2 mm for 20 s	UDMA, DMA co-monomer, (Bis-GMA free), inorganic filler >100 nm, fluoroaluminosilicate, inorganic filler <100 nm, fumed silica, prepolymerized fillers (16-17 µm), silica, strontium and latanoid fluoride	GC, Tokyo, Japan

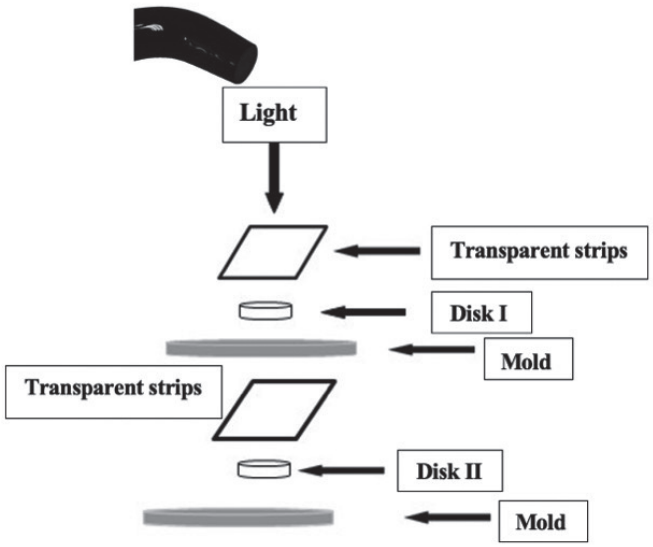


Fig. 1 Sample preparation using titanium molds and transparent polyester strips: Bulk-fill composite disks (6 mm Ø, 2 mm) I and II

length range with 16 scans at a resolution of 4 cm^{-1}. All samples (n = 6) were incubated at 37°C for 24 hours in the dark. The degree of conversion (DC) was calculated by assessing the variation in peak area of the aliphatic C = C bond peak of the methacrylate at 1,637 cm^{-1} by employing the carbonyl group C = O bond peak at 1,715 cm^{-1} as an internal standard during the polymerization, using the following equation [11]:

$$\%\,DC = \left(1 - \frac{(1{,}637\text{cm}^{-1}/1{,}715\text{cm}^{-1})_{\textit{peak area of polymer}}}{(1{,}637\text{cm}^{-1}/1{,}715\text{cm}^{-1})_{\textit{peak area of monomer}}}\right) \times 100$$

Monomer elution

After preparation of the disk samples, they were analyzed by high-performance liquid chromatography (Nexera 2, Shimadzu, Kyoto, Japan) to evaluate the elution of monomers from the polymerized samples. Six specimens (n = 6) were used for each material. Each sample was immersed in 5 mL of 75% ethanol/water solution and stored at 37°C for 24 hours in the dark [12]. After 24 h, the eluting compounds were analyzed on a HPLC-UV system with an Inertsil ODS-4 C18 column (250 mm × 4.6, 5 µm). The column was operated using 70% acetonitrile/30% water as the mobile phase with a flow rate of 1.0 mL/min at 25°C. The chromatograms were recorded at 210 nm wavelength. The injection volume was 20 µL. UDMA, TEGDMA, and BisGMA were used as standards for calibration of the system. The physical properties of these monomers, which are mostly used in composite resins, are given in Table 2. Calibration curves were drawn for each monomer using five different concentrations (5, 10, 20, 25, and 50 µg/mL). A representative chromatogram of the tested monomers is shown in Fig. 2.

Table 2 Physical properties of the resin monomers

Monomer	Molecular mass	Viscosity (Pa·s)	Tg (°C)	Limiting degree of double bond conversion (%)
Bis-GMA	512	1,369	−8	39
TEGDMA	286	0.05	−83	76
UDMA	470	28	−35	70
Bis-EMA	540	3	−46	52

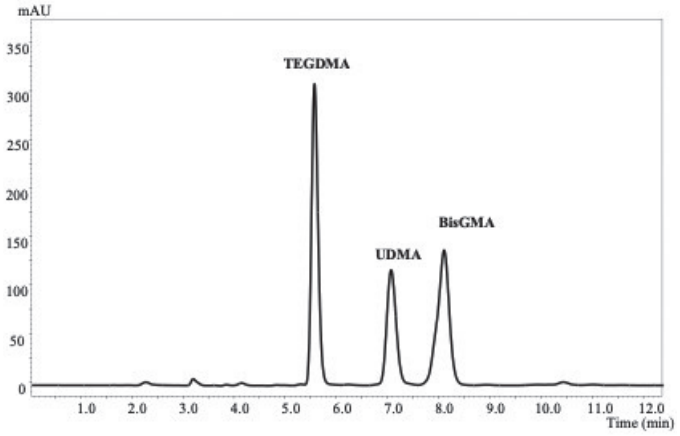


Fig. 2 The retention time of HPLC peaks of TEGDMA, UDMA, and BisGMA

Depth of cure

Microhardness measurements were made for different depths of composite. Ten specimens (n = 10) were used for each material and two measurements were obtained from each sample as top and bottom. No mechanical preparation or abrasion of the specimens was performed.

Surface Vickers hardness (VH) was determined using a Vickers hardness tester (Micro Hardness Tester, Wilson, Buehler, Lake Bluff, IL, USA) and a 100 g load (0.981 N) applied for 15 s, slope: 10 gf/s. Three indentations were recorded for each sample at different points of the irradiated top and non-irradiated bottom surfaces. For each surface, the mean value was then detrmined and converted into a Vickers hardness number (VHN = bottom VHN / top VHN × 100 (%), as N/mm^2 (MPa). The depth of cure (DoC) of the specimens calculated as the VHN ratio was determined as follows [13] :

VHN 2 mm ratio = VHN 2 mm / VHN 0 mm

VHN 4 mm ratio = VHN 4 mm / VHN 0 mm

Cytotoxicity

The L929 cell line (mouse fibroblast cells) was cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12) (PAN-Biotech GmbH, Aidenbach, Bavaria, Germany) with 10% FBS (PAN-Biotech) and 0.2% Primocin (Invivogen, San Diego, CA, USA) at 37°C in a 5% CO_2 atmosphere at 95% relative humidity. After reaching confluence in T25 and

T75 flasks, 5×10^3 cells were seeded per well into 96-well plates.

Prepared composite disks were sterilized under UV light in a laminar flow cabinet for 15 min for each surface. Each group had six composite disks to evaluate ($n = 6$) and according to ISO 10993-12, the composite disks were incubated in culture media with an extraction ratio of 3 cm^2/mL for 1-7 days in 48-well plates in a culture incubator. After 1-7 days, the eluates were aspirated for measurement of cytotoxicity. Thiazolyl Blue Tetrazolium Bromide (Gold Biotechnology Inc., St. Louis, MO, USA) dye was prepared at a final concentration of 5 mg/mL and filtered using a 0.22-µm syringe filter.

Twenty-four hours after the cells had been seeded into 96 well plates, the culture media were replaced with 200 µL composite eluates for 24 hours. MTT solution (20 µL) was added to each well and incubated for 3 hours. The culture media were then carefully removed, 100 µL DMSO was added, and each culture plate was incubated at 37°C for 15 min. The absorbance was measured at 570 nm using a BioTek Synergy H1 Hybrid plate reader (BioTek, Winooski, VT, USA). The absorbance values were used to calculate percentage cell viability. The assay was performed in biological duplicates.

Statistical analysis

The results were described as mean and standard deviation (mean ± SD). Data were primarily tested for normality with the Shapiro-Wilk test and for homoscedasticity with the Levene test. For comparing data with a normal distribution between groups, one-way ANOVA, two-way ANOVA and Tukey's multiple comparisons test were used. All statistical analyses were performed at MINITAB (Minitab Inc., State College, PA, USA) and GraphPad Prism 8.0 program (GraphPad Software, Inc., San Diego, CA, USA) with a significance level of 0.05 and a confidence level of 95%.

Results

Degree of conversion

The degree of conversion (DC) values are given in Table 3. A material depth from 0 mm to 4 mm affected DC for all materials (Table 3). The highest measured DC at 0 mm depth was for SDR (55.66 ± 6.35). DC significantly decreased at a depth of 4 mm for ACT, SDR, and SF ($P < 0.0001$). For top surfaces of disks I, the results showed that none of the materials reached significantly different DC values ($P = 0.068$). All materials except SF had significantly similar DC values at the bottom surfaces of the disks I ($P = 0.031$). At the top surfaces of disks II, the DC of SDR was significantly higher than that of ACT, but not SF ($P = 0.009$). The SF composite showed the lowest conversion value (19.46 ± 5.44) at a depth of 4 mm, but was not significantly different than that of ACT ($P = 0.023$). The degree ratios of conversion for all resin composites between 0 mm and 2 mm depth did not show any significant differences. The degree ratios of conversion of the resin composites at different depths are shown in Table 4. DC_4/DC_0 of SDR presented the highest point of 0.50 ± 0.12.

Monomer elution

The amounts of monomer eluted from the prepared composite disks are shown in Table 5. Comparison using Tukey's test demonstrated a significant difference between disk I (2 mm thick) of SF and the 2-mm-thick conventional composite GC in the case of BisGMA ($P = 0.034$). A 15-fold increase in the amount of BisGMA eluted was found at disk II for the SF composite. The TEGDMA elution from disk II of SF was significantly higher than for the other materials ($P < 0.0001$). No TEGDMA was detected in the GC samples. Among the bulk-fill composites, the ACT composite showed the lowest amount of TEGDMA eluted (1.78 ± 0.12) at disk I. At least a seven-fold higher amount of UDMA elution was observed in the case of ACT relative to the other materials, and there was a significant difference between disk I and disk II ($P < 0.0001$). The SDR and GC samples had significantly different UDMA values ($P < 0.0001$).

Depth of cure

The minimum value of the bottom-to-top surface hardness ratio is commonly calculated to establish the DoC of resin-based composites. For a material to be considered adequately cured, the value of the ratio needs to be in the range 0.8-0.85 [14].

The depth of cure results according to the Vickers values (data not shown) obtained from the bottom and upper surfaces of the prepared composite disks are presented in Table 6. The DoC ratios between the top surface and 2 mm for all bulk-fill composites except ACT were within the acceptable threshold value. The conventional composite (GC) used as the control group and the SF and SDR groups showed similar results at the level of V2 mm/V0 mm. When the bottom surfaces of the bulk-fill composites at 4 mm were evaluated (V4 mm/V0 mm), all of them were below this rate.

Table 3 Degree of conversion of the top and bottom surfaces of the resin composites at different depths

Depth (mm)	SDR	SF	ACT	GC
Disk I: 0	55.66 ± 6.35 A	44.37 ± 6.77 A	54.02 ± 12.15 A	47.74 ± 3.11 A
Disk I: 2	53.58 ± 4.91 AB	36.00 ± 13.82 A	44.26 ± 10.89 AB	45.37 ± 2.89 A
Disk II: 2	46.57 ± 3.75 B	42.11 ± 4.98 A	36.07 ± 6.05 B	-
Disk II: 4	27.41 ± 6.42 C	19.46 ± 5.44 B	19.65 ± 2.07 C	-

Letters correspond to results generated by one-way ANOVA using the Tukey method for multiple comparisons. Means that do not share a letter are significantly different from one another.

Table 4 Degree ratios of conversion of the resin composites at different depths

Depth (mm)	SDR	SF	ACT	GC
DC_2 / DC_0	0.97 ± 0.13	0.83 ± 0.32	0.85 ± 0.27	0.95 ± 0.10
DC_4 / DC_0	0.50 ± 0.12	0.45 ± 0.16	0.38 ± 0.11	-

Table 5 Amount of monomer eluted from the resin composites with different materials at the same depth (µg/mL)

Materials		UDMA	TEGDMA	BisGMA
SDR	Disk I	36.16 ± 3.11 C	9.56 ± 0.80 A	nd
SF		nd	8.94 ± 1.17 A	3.29 ± 1.18 A
ACT		257.33 ± 21.21 A	1.78 ± 0.12 B	nd
GC		144.54 ± 12.02 B	nd	2.09 ± 0.14 B
SDR	Disk II	71.85 ± 10.81 B	21.93 ± 3.95 B	1.13 ± 0.11 B
SF		3.44 ± 0.96 B	32.86 ± 1.50 A	47.57 ± 4.13 A
ACT		994.91 ± 87.97 A	5.94 ± 0.43 C	nd

Letters correspond to results generated by one-way ANOVA using the Tukey method for multiple comparisons. Means that do not share a letter are significantly different from one another.

Table 6 The depth of cure of composite discs

	SDR	SF	ACT	GC
V2 mm/V0 mm	0.83 ± 0.17	0.90 ± 0.08	0.68 ± 0.10	0.90 ± 0.14
V4 mm/V0 mm	0.55 ± 0.06	0.72 ± 0.27	0.57 ± 0.13	-

Cytotoxicity

In the present study, after exposure to the composite disk's eluate, cell viability was measured by MTT analysis for day 1 and day 7. No cytotoxic effect on any of the composite disks was noted for day 1 ($P > 0.05$). However, a high cytotoxic effect on cells treated with ACT Disk I and ACT Disk II was observed, and the cell viability was reduced to <70% on day 7 ($P < 0.001$). Cell viability results are shown in Fig. 3 (in brief, observed cell viability GC Disk I = SF Disk I = SF Disk II > SDR Disk I = SDR Disk II > ACT Disk I = ACT Disk II [$P < 0.0001$]).

Discussion

In this study, the degree of conversion using FTIR and the elution of unreacted monomers using HPLC were evaluated for different bulk-fill and conventional dental resin composites. The null hypothesis was rejected because there was a difference in the degree of conversion and the amount of monomers released from bulk-fill composite resins at different depths.

Bulk-fill resin composites are used as bases and liners during the restoration process. As the eluted monomers and other components can penetrate to the pulp, the regenerative ability of the pulp may be damaged [15]. Therefore, the amount of eluted components needs to be investigated to clarify the extent of the effect. The elution of dental resin composites can be increased by incomplete polymerization, in other words the degree of conversion. It is reported that polymerization of dimethacrylate resins is generally incomplete [16], which is why the degree of conversion remains

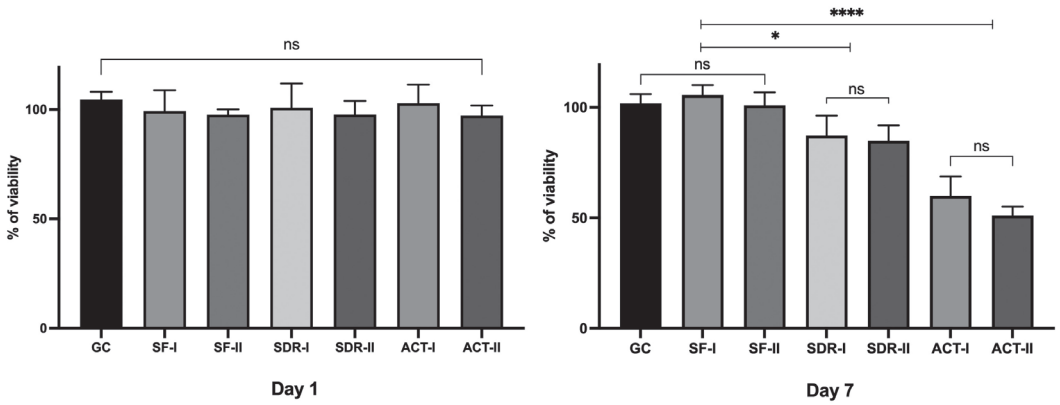


Fig. 3 Cell viability rates for the L929 cell line exposed to different composite disks for Day 1 and Day 7. Mean with SD values are given. ns = not significant **P* < 0.05 and *****P* < 0.0001. I = Disk I and II = Disk II

within the range 55-75% for bulk-fill resins. However, although for adequate clinical performance, a conversion rate has not yet been stated, a minimum of 55% is recommended, at least for occlusal restorative layers [7]. It has been reported that there is a negative correlation between *in vivo* abrasive wear depth and conversion within the range 55-65% [17]. Previous studies have shown that (co)monomers and other components released from composites cause cell death and membrane damage [18,19].

Resin polymerization may depend on the monomer type, filler characteristics, and curing conditions [20]. The studied composites varied in their filler and monomer composition (Table 1). Significant differences in DC for each resin composite were observed between different depths of each material except GC and different materials at the same depth, except at the 0 mm level. The materials with different compositions and different depths would have explained the differences in DC values while the other parameters remained constant. Notably, SDR showed the best performance at all depths.

In general, polymerization depth has recently been improved with an increase in translucency [21]. As light transmission used during the curing process is strongly dependent on the opacity of a resin composite, a higher DC was observed at 2 mm depth for the bulk fill composites except SF, in comparison to the conventional composite (GC). However, when the depth reached 4 mm, the DC of bulk fill composites was as low as 19.46%. Another factor that affects translucency is the filler content of resin composites. An increase in the filler ratio can significantly decrease monomer conversion [22]. In this study, a lower DC value was observed for SF compared with the other materials. This would have been consistent with the filler content, as SF has the highest filler content and this would cause an increase in light scattering.

Another factor, and perhaps the most significant one affecting the DC of monomers, is the monomer type and the viscosity (Table 2) [23]. The limiting degree of conversion of different monomer systems decreases in the following order: BisGMA < BisEMA < UDMA < TEGDMA [23]. BisGMA, which has strong hydrogen bonding between pendant hydroxyl groups and the rigid aromatic nuclei, is the most viscous monomer [5]. This interaction can decrease the mobility of the monomer during polymerization and the flexibility of the polymer after polymerization. BisGMA was not detected in ACT samples, and according to the manufacturer's information there is no BisGMA in SDR, ACT, or GC. UDMA also shows high viscosity due to the weaker hydrogen bonding between the imino and carbonyl groups compared to the hydroxyl groups [24]. The imino groups in UDMA can cause chain transfer reactions that increase the mobility of the radical sites in the network. This feature can increase the reactivity of UDMA for polymerization. This can explain the higher reactivity and the degree of conversion of SDR composite with UDMA relative to SF. TEGDMA with a low mW and a high concentration of double bonds has low viscosity and therefore can achieve a synergistic effect with other monomers in the composite mixtures in terms of polymerization rate, plasticization, and DC [25]. This might be another reason for the high DC of SDR. In a recent study, similarly to the present results, the highest DC was found for the flowable bulk-fill SDR, irrespective of the filling procedure and top or bottom measurement [26]. The DC of bulk-fill SonicFill was significantly lower at the bottom than at the top surface. The present results showed that the success of SDR was related to its relatively high translucency, allowing light to penetrate sufficiently deeply and thus making the polymerization-initiation system more efficient.

Elution analysis is crucial for confirming the stability of a polymerized composite resin and its biocompatibility. However, although this can yield some insight, it does not provide an absolute measure of the elution of monomers remaining in the composite after polymerization [27]. Some studies have reported that monomer elution is well correlated with the degree of double bond conversion measured by FTIR [27]. However, some double bonds detected by FTIR may remain in pendant groups bonded to the polymer chain, and these non-eluted bonds are not detected by HPLC.

Monomer elution may be affected by monomer conversion, solvent type, and the chemistry of the eluted monomers [6,28]. In this study, a 75% ethanol-water solution was chosen. The degradation of composite resins depends on the elution medium [28]. In the human mouth, saliva and other parameters such as acidic conditions and food/beverages may provide proteins that can bind to monomers, thus affecting their release from composites [29]. Ethanol is an aggressive organic solution, and its use may represent a worst-case scenario when mimicking oral conditions [29]. Furthermore, the United States Food and Drug Administration (US FDA) recommends a 75% ethanol-water solution as an oral/food simulating liquid [30]. This solution was found to be a more efficient elution medium than water or artificial saliva.

The present results showed that there was a strong correlation between DC and the amount of eluted monomers at any given depth for each composite. For instance, the increase in thickness of ACT resin increased the amount of eluted UDMA and TEGDMA four-fold while the DC decreased almost two-fold. As expected, the different degrees of conversion for different depths affected the amounts of monomers eluted from the composite disks. The disks II for all materials released significantly higher amounts of UDMA, TEGDMA, and BisGMA monomers than disks I. It was noteworthy that HPLC analysis of SDR composites showed almost double the amount of both UDMA and TEGDMA elutes from disk I to disk II. However, an increase in thickness may not always result in the same degree of increase in monomer elution [31]. The different elution values among the monomers could be explained by the chemical structure, molecular weight and reactivity of the monomers [31]. On the other hand, SF has the higher filler content (83.5%), and this could be the main reason why SF with the lowest DC value had the lowest total amount of eluted monomers. An increase in filler content leads to a decrease in the solvent absorption of composites, thus reducing the degree of monomer elution [32]. Khalid et al. showed that significantly high monomer elution and structural changes occurred at SDR [33]. It was observed that absence of the polymer network or cross-linking in resin composites decreased the stability. The free radicals that play an important role in the degree of conversion exist in large amounts between the loose cross-links of resin composite. These free monomers may leach out if they are soluble in aqueous media [34].

Recently, manufacturers claim to ensure a proper depth of cure even when higher composite increments are used at bulk fill resin composites. To allow larger incremental insertion, their content was changed by reduc-

tion or substitution of Bis-GMA, resulting in a lower-viscosity monomer, and/or by using monomers with a higher molecular weight, usually Bis-EMA, TEGDMA, EBPDMA and UDMA monomers [35].

In the present study, all resin composites except ACT showed similar results at the level of V2 mm/V0 mm. When V4 mm/V0 mm were evaluated, none achieved an acceptable DoC value. However, it can be said that the SF group had a better DoC value than the other bulk-fill composites and was similar to the control GC at V2 mm/V0 mm. In a previous study of the influence of sonic insertion of bulk-fill and conventional resin composites on cure depth [36], the influence of sonic insertion was found to be material-dependent, influenced only by the microhardness of the SonicFill resin, and did not interfere with the bond strength and cure depth of other bulk-fill and conventional resin composites. The similar results obtained in this study suggest that the good DoC values of SF are related to both the material molecular base and the application technique.

The Vickers hardness can be influenced by several factors such as resin type and filler content [37]. The composite resins with high filler content showed the high hardness mean values. As expected, the hardness mean values for the top surfaces were higher than the bottom ones. It is associated with the decrease in light reaching with depth, and thus polymerization. Some studies showed that as filler content increased, compressive strength, void content and micro-hardness increased significantly [22,37]. SF has the highest filler content (83.5%), and this could be another reason why SF with the lowest DC value has the good DoC values.

In studies evaluating cytotoxicity, cell viability is expressed as a percentage relative to the amount of viable cells in the negative control. According to ISO 10993-5 a material has cytotoxic potential if cell viability is lower than 70% (International Organization for Standardization, Biological evaluation of medical devices, 2009). In this study, all cells were viable at day 1 for bulk-fill and conventional composites. Cell viabilities were above 70% at day 7 except for the ACT group. The cytotoxic effect of ACT on day 7, regardless of its thickness, may be related to its glass ionomer content. Many studies have shown that composites with a high glass ionomer content tend to have high toxicity. In a study of the cytotoxicity of contemporary bulk-fill dental composites, Demirel et al. showed that pre-reacted glass-ionomer-containing bulk-fill composites were severely toxic (cell viability <70%) at 24, 48 and 72 hours [38]. Another study also evaluated the biocompatibility of contemporary bulk-fill resin-based composites including pre-reacted glass ionomer materials [39]. Among the bulk-fill composites, extracts of pre-reacted glass-ionomer materials resulted in the lowest cell viability. It was reported that the chemical composition, specimen thickness, and test concentrations of extracts had significant effects on cell viability and morphology. The conventional composite GC used as the control group and SF (regardless of thickness) from the bulk-fill composite group showed the best cell viability at all time points. This trend in the cell viability values was consistent with the data for monomer elution and hardness.

A similar study by Marigo et al. investigated whether the cytotoxic effects of different composite materials were correlated with the conversion degree, hardness, and depth of cure [40]. All tested materials showed slight cytotoxic effects, and there were no statistically significant differences in cytotoxicity in any of the experimental conditions, notwithstanding the differences in hardness and the degree of conversion. HPLC results showed that the amount of monomers leached from each specimen was influenced by thickness, although this did not affect cytotoxicity.

Within the limitations of this study, the following conclusions can be drawn: Among bulk-fill and conventional composites, SDR showed the highest degree of conversion at the top and bottom surfaces of the disk samples at different depths. Among the bulk-fill composites, SF with the lowest DC showed the lowest monomer elution while the highest amount of total monomers was eluted from ACT. Depth of cure values of bulk-fill composites except ACTs were evaluated as convenient according to the threshold only at V2 mm/V0 mm. None of the groups of composite resins were cytotoxic on day 1, while ACTs were cytotoxic on day 7 with a cell viability of <70%, regardless of layer thickness. In the light of these results, ACTIVA Bioactive Restorative should be improved in terms of biological side effects with a high residual monomer, low cure depth and cytotoxic effect.

It is biologically important to achieve sufficient polymerization of bulk-fill composites by curing at the recommended thickness and for the appropriate time. Clinical use of a material that does not meet this requirement is doubtful. For this reason, there appears to be a need to enhance the polymerization efficiency of bulk-fill composites with increasing depth.

Acknowledgments

This study was supported by Cukurova University Scientific Research Projects Coordination Unit (Project no: TSA-2020-13172). The authors thank to the research assistant Seda Süsgün and Bezmialem Vakif University, Beykoz Institute of Life Sciences and Biotechnology (BILSAB) for their contribution to the cytotoxicity analyses in this study.

Conflict of interest

The authors have no conflicts of interest to declare in relation to this work.

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