

## Research Article

# Evaluation of Monomer Releasing from Dentin Replacement Materials

Nurgül Çetin Tuncer,<sup>1</sup> Sevede Gül Batmaz,<sup>2</sup> Sergen Özdemir,<sup>1</sup> Çağatay Barutçugil ,<sup>1</sup> Ayşe Dünder,<sup>1</sup> and Kardelen Yıldırım<sup>3</sup>

<sup>1</sup>Department of Restorative Dentistry, Faculty of Dentistry, Akdeniz University, Antalya, Türkiye

<sup>2</sup>Department of Restorative Dentistry, Faculty of Dentistry, Çukurova University, Adana, Türkiye

<sup>3</sup>Private Clinic, Snowdrop Dental Clinic, Antalya, Türkiye

Correspondence should be addressed to Çağatay Barutçugil; [cagatay@akdeniz.edu.tr](mailto:cagatay@akdeniz.edu.tr)

Received 29 October 2023; Revised 5 February 2024; Accepted 13 February 2024; Published 12 March 2024

Academic Editor: Zhonghua Peng

Copyright © 2024 Nurgül Çetin Tuncer et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The aim of this study is to determine and quantify the monomer elution from four different resin-based composite dentin replacement materials for 3 months using HPLC. Four different composite dentin replacement materials were used in the present study: EverX (EVX), X-tra base (XTB), SDR (SDR), and GrandioSO Heavy Flow (GHF). Fifteen samples from each material were prepared (5 × 5 × 4 mm). After preparation, each specimen was immersed in a 10 ml 75% ethanol/distilled water solution for three different periods: 1 h, 24 h, and 3 months ( $n = 5$ ). After the immersion period, 0.5 ml of solutions were taken from each bottle and analyzed using HPLC. At the end of the 3-month immersion period, the elution of monomers was determined mostly from SDR, GHF, EVX, and XTB, respectively. TEGDMA, the most released monomer of all groups, was released from all samples after 1 h, 24 h, and 3 months. The amount of monomer released in all composite groups at the end of the 3-month immersion period was significantly higher than the monomer amounts released after the 1-hour immersion period. The monomers were eluted from the composite dentin replacement materials during all immersion periods, and the amount of eluted monomers was increased with time.

## 1. Introduction

In recent decades, resin-composite materials have been frequently used due to the aesthetic demands of patients and their ease of application. However, the use of resin composites is not recommended for large restorations exposed to high occlusal stress and masticatory load in the posterior region [1, 2]. A long-term clinical study revealed that a composite fracture in the molar teeth is one of the most common causes of restoration failure [3]. Flowable composites and cements as dentin replacement materials have been used in the past to overcome these problems [4]. However, the use of flowable composites as dentin replacement materials leads to enamel fractures in large cavities without adequate dentin support [5]. Today, there are composite dentin replacement materials that are used instead of flowable com-

posites or cements that are produced specifically to address these problems. When the resin composite shrinks, the underlying dentin replacement material absorbs the stress and distributes the contraction force by transmitting it to the adjacent structures. The use of such intermediate material modifies the configuration factor of the cavity [6]. Also, it has been reported that the use of composite dentin replacement materials strengthens the conventional composite and thus provides stronger restorations [3]. For these reasons, it may be beneficial to use dentin replacement materials under conventional composites in deep cavities in the posterior region.

Aside from the beneficial properties of composite dentin replacement materials, there is also the possibility of the release of unreacted monomers. Since monomers form the main part of the resin matrix (20–40 wt%), they represent

the greatest risk in terms of biotoxic effects and mechanical properties [7]. These monomers may be released from the restoration into the oral cavity or from the dentinal tubules into the pulp chamber over time [8, 9]. These released monomers have cytotoxic, genotoxic [10, 11], and estrogenic [12] effects and may cause reactions in the pulp [13] and soft tissues [14, 15]. Unreacted monomers may cause pulp damage if there is insufficient protection at the bottom of the cavity [13]. When dentin thickness decreases, there is a high risk of unreacted monomers passing through the dentinal tubules and irritating the pulp [9]. In deep cavities, pulp damage has been occurring even after 3 years [16].

Although there have been studies on the different properties of composite dentin replacement materials [3, 6, 17, 18], there is a serious lack of knowledge about the elution of monomers. Therefore, the aim of this study was to determine and quantify monomer elution from four different composite dentin replacement materials for 3 months using high-performance liquid chromatography (HPLC) after immersion in ethanol. The null hypothesis was that there would be no monomer release from the composite dentin replacement materials even after 3 months.

## 2. Materials and Methods

**2.1. Sample Preparation.** Four different composite dentin replacement materials were used in the present study: EverX Posterior (GC, Tokyo, Japan), X-tra base (Voco, Cuxhaven, Germany), SDR (Dentsply, Konstanz, Germany), and GrandioSO Heavy Flow (Voco, Cuxhaven, Germany). Detailed information about the composition and manufacturers of composites are given in Table 1.

Fifteen samples from each composite material were prepared in Teflon molds ( $5 \times 5 \times 4$  mm). The molds were filled with the composite materials and sealed with a Mylar strip. The samples were built up in one increment (4 mm) except for the GHF composite resin samples. GHF composite resin samples were built up in two increments (2 mm + 2 mm), and all specimens were polymerized according to the manufacturer's instructions with a LED curing unit (Elipar S10, 3M ESPE, St. Paul, MN, USA) with a light output power of  $1200 \text{ mW/cm}^2$ . The radiometer system on the device was used to verify the output intensity for each use of the light curing unit.

After preparation, each specimen was immediately immersed in a 10 ml 75% ethanol/distilled water solution in the amber-colored glass bottles at room temperature for three different immersed periods: 1 h, 24 h, and 3 months. The specimens were divided randomly into three subgroups according to the immersion period ( $n = 5$ ). At the end of the immersion period, 0.5 ml of the solution from each vial containing the specimen was taken up in sterile glass vials with the help of a micropipette and stored at room temperature for analysis with HPLC.

**2.2. HPLC Analyses.** The monomer releasing analyses were performed using chromatographic measurements including a thermodiode array detector (DAD) and autosampler (Accela HPLC, Thermo Fisher Scientific, San Jose, CA,

USA) with a C18 analytical column ( $250 \text{ mm} \times 4.6 \text{ mm}$ ,  $5 \mu\text{m}$ – $100 \text{ \AA}$  particle size, Luna C18, Phenomenex, Torrance, CA, USA) and software to control instruments and data handling (Thermo Xcalibur v.2.2, Thermo Fisher Scientific, San Jose, CA, USA). As a solvent, 80% HPLC-grade acetonitrile (Merck KGaA, Darmstadt, Germany)/20% ultrapure water (obtained using the Millipore refinement system in ultrasound at  $18.2 \text{ MWcm}$  at  $25^\circ\text{C}$ ), and the diluted samples were passed through a  $0.45 \mu\text{m}$  membrane filter prior to injection) with a flow rate of  $1 \text{ ml/min}$  was used. For preparing the calibration curves and reference monomers' retention times, pure monomers bisphenol-A glycidyl methacrylate (BisGMA), triethylene glycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA), hydroxyethyl methacrylate (HEMA), and ethoxylated bisphenol-A dimethacrylate (BisEMA) were used (Figure 1), and data about the substances are given in Table 2.

Each of the standard solutions at a concentration of 5, 10, 25, 50, and  $100 \mu\text{g/ml}$  was left in 75% ethanol solution for 24 h. The solutions obtained were stored at  $4^\circ\text{C}$  until the monomer analysis. The released monomer amounts from samples were calculated by using standard curves, and identification and quantitative analysis were made by comparing the elution time and by the integration of the elution peak area with those of the authentic sample which used the linearity of the calibration curve, based on the quantitative determination of standard monomers in the six solutions. The peak areas, correlation coefficients, and retention times were obtained for each monomer, and plotted versus the concentration using linear regression analysis for BisGMA, BisEMA, UDMA, TEGDMA, and HEMA and are represented in Figure 2. The correlation coefficients of the regression equations were higher than 0.95; accuracy within the interval was 73–111% of the target level, and repeatability with a relative standard deviation was lower than 10%, demonstrating that the method was accurate and appropriate for quantitative analysis (Figure 2).

The limit of detection (LOD) and the limit of quantification (LOQ) were determined from the calibration curve according to the following formula;

$$\text{LOD} = \frac{3.3s}{S} \quad \text{LOQ} = \frac{10s}{S}, \quad (1)$$

where  $S$  is the slope and  $s$  is the standard deviation of intercept. The absolute amount of each monomer in the extract was calculated in  $\mu\text{g/ml}$ .

**2.3. Statistical Analyses.** Data normality was analyzed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Multiple comparisons were performed using the one-way ANOVA, independent sample  $t$ -test, and Tukey HSD test. In addition, the paired sample  $t$ -test was used to assess the time-dependent changes in the same materials. The level of significance for the statistical analysis was 0.05. A software program (G\*Power 3.1; Universitat Düsseldorf) was used to perform the power analysis.

TABLE 1: The compositions and manufacturers of resin-based composite tested in present study.

Material	Manufacturer	Code	Organic matrix	Filler loading	Inorganic filler ratio % (wt/vol)
EverX Posterior	GC Corporation, Tokyo, Japan	EVX	BisGMA, TEGDMA, PMMA	E-glass fibres, barium borosilicate glass filler	74.2/53.6
X-tra base	Voco GmbH, Cuxhaven, Germany	XTB	UDMA, BisEMA, EBPADA, DEGDMA	Barium glass ceramic, fumed silica	75/61
SDR	Dentsply, Konstanz, Germany	SDR	BisEMA, TEGDMA, modified UDMA resin	Ba-Al-F-borosilicate glass, Sr-Al-F-silicate glass, SiO <sub>2</sub> , titanium dioxide	68/44
GrandioSO Heavy Flow	Voco GmbH, Cuxhaven, Germany	GHF	BisGMA, BisEMA, TEGDMA	Glass ceramic, SiO <sub>2</sub>	83/68

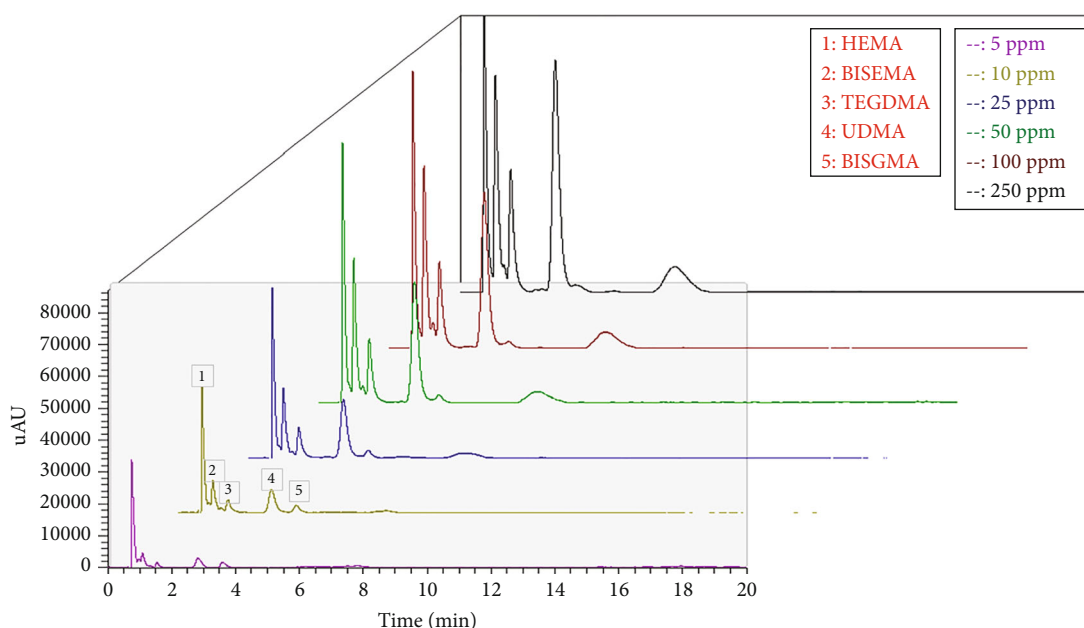


FIGURE 1: HPLC chromatogram of a mixture of reference monomers (BisGMA, BisEMA, UDMA, TEGDMA, and HEMA).

TABLE 2: Monomers used as reference standard.

Monomer	Name	Chemical type	Mol. W.	CAS no.
Hydroxyethyl methacrylate	HEMA	C <sub>6</sub> H <sub>10</sub> O <sub>3</sub>	130.14 g/mol	868-77-9
Triethylene glycol dimethacrylate	TEGDMA	C <sub>14</sub> H <sub>22</sub> O <sub>6</sub>	286.32 g/mol	109-16-0
Bisphenol-A ethoxylated dimethacrylate	BisEMA	C <sub>25</sub> H <sub>32</sub> O <sub>6</sub>	Av. M <sub>n</sub> ~1,700	41637-38-1
Urethane dimethacrylate	UDMA	C <sub>23</sub> H <sub>38</sub> N <sub>2</sub> O <sub>8</sub>	470.56 g/mol	72869-86-4
Bisphenol-A glycidyl dimethacrylate	BisGMA	C <sub>29</sub> H <sub>36</sub> O <sub>8</sub>	512.59 g/mol	1565-94-2

All monomers in this study were from Aldrich (Sigma-Aldrich, St. Louis, MO, USA) [19].

### 3. Results

The amount of monomer released from the composite dentin replacement materials and the statistical differences for each immersion period are shown in Table 3.

At the end of the 3-month immersion period, the elution of monomers was determined mostly from SDR, GHF, EVX, and XTB, respectively. TEGDMA, the most released monomer of all groups, was released from all samples after 1 h, 24 h, and 3 months. No quantifiable TEGDMA release was

observed in the SDR samples that were immersed for only 1 hour. At the end of the 3-month immersion period, EVX and GDH showed the highest, and XTB showed the lowest TEGDMA release ( $p < .05$ ). Except for the samples of the SDR group immersed for 3 months, no quantifiable HEMA release was detected in any of the samples. BisEMA was released from all groups except EVX. At the end of 3 months, there was no significant difference between the amounts of BisEMA released in the GHF, XTB, and SDR groups ( $p > .05$ ). UDMA was released from the XTB and

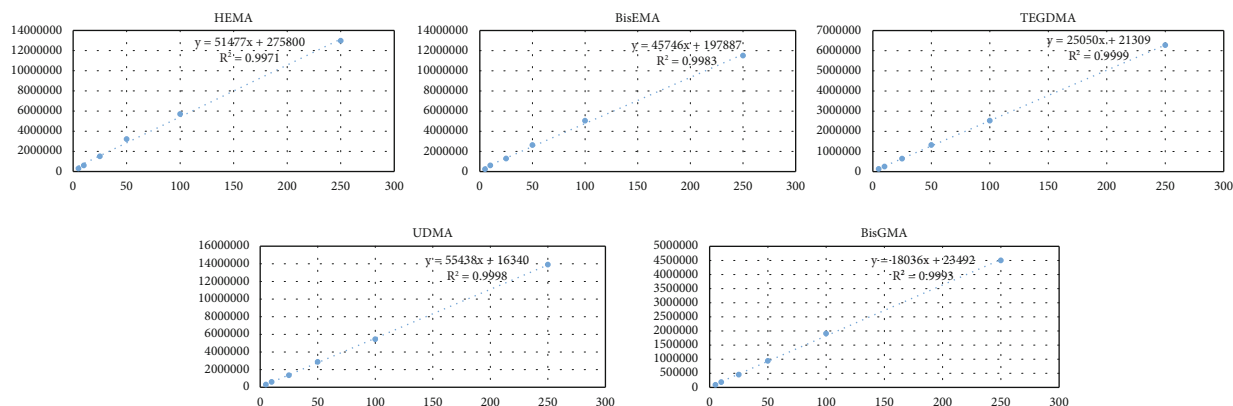


FIGURE 2: HPLC calibration curves for reference monomers.

SDR groups in all time periods, and the amount of UDMA released from SDR was found to be significantly higher in all immersion periods ( $p < .05$ ). No quantifiable level of BisGMA release was detected from XTB and SDR. The amount of BisGMA released from the GDH group at the end of 3 months was significantly higher than that from the EVX group ( $p < 0.05$ ). The amount of monomer released in all composite groups at the end of the 3 month immersion period was significantly higher than the monomer amounts released after 1-hour immersion period ( $p < .05$ ; Figure 3).

#### 4. Discussion

Bulkfill composite resins have been produced with the claim of eliminating the disadvantages of the layering technique in clinical applications. Although it is stated by the manufacturers that ideal polymerization can be achieved in bulk use up to 4 mm or even 5 mm in these materials, there have been many studies on the degree of polymerization, monomer release, etc. Recently, Süsgün Yildirim et al. [20] evaluated the relationship between the layer thickness of bulkfill composites and monomer release and conversion rates and reported that as the layer thickness increases, the conversion rate decreases and monomer release increases. It should also be noted that monomers and other contents released from resin composites can penetrate the pulp, damage the pulp, and damage its regenerative properties [21]. For this reason, it is important to determine the amount of residual monomer released from dentin replacement materials, especially used in the area closest to the pulp. In the present study, BisEMA, HEMA, TEGDMA, UDMA, and BisGMA elution from four composite dentin replacement materials was evaluated using HPLC over 3 months. According to the results, monomer elution from composites was determined even after 24 hours. Thus, the null hypothesis that there would be no monomer release was rejected.

The amount of monomers released from resin-based dental materials is generally determined by the HPLC method. High molecular weight monomers, such as BisGMA and UDMA, may be degraded by the gas chromatography technique, which is another method for determining the amount of monomers, and only degradation products can be detected [22]. Since monomers can dissolve

in the mobile phase in the HPLC method, the elution process occurs in a more controlled manner [23]. Therefore, in this study, the HPLC method was used to determine the amount of monomer released from composite dentin replacement materials. Another important point for determining monomer elution is the immersion time. Ferracane [24] reported that after the polymerization of composite materials, 50% of the monomers were eluted in the first 3 hours, and 85–100% in the first 24 hours. More recent studies using the HPLC method have reported that monomer release from composite materials continues after 24 hours, and even up to 1 year [25, 26]. In another recent study, monomer release was detected even from resin-containing CAD/CAM blocks during the long immersion period [19]. In the present study, the amount of monomer released during 1 hour, 24 hours, and 3 months was quantified, and it was found that the release of all monomers continued until the 3-month immersion period, even though some monomers were released at a quantifiable level after 3 months. Also, Alshali et al. [27] stated that monomer release from resin-based composite materials is expected even after 3 months according to the results of their study. There are several factors that affect monomer release from resin-based dental materials [24]. For example, there is an inverse correlation between the amount of monomer released and the degree of conversion (DOC), which reaches its highest level 24 hours after polymerization. Second, the type of immersion solution can affect the amount of monomer released. Additionally, the filler content and rate of composites may affect monomer release. In the present study, 75% ethanol-water solution was used for the immersion process. This solution is recommended by the United States Federal Drug Administration as it mimics the oral environment well and has a solubility similar to the resin-composite matrix.

Among the tested base composites, the highest monomer release was detected in SDR and showed the highest vulnerability. This may be related to the low initial conversion degree [28], as well as the density and heterogeneity of the weak cross-links in the polymer structure of SDR [27]. This causes the polymer structure to swell, thus opening pores and pathways, and eluding residual monomers. In addition, considering the filler content of the tested composites, it was observed that SDR has the lowest filler ratio. In previous

TABLE 3: The mean eluted monomer amounts ( $\mu\text{g/ml}$ ) in different intervals for resin-based composites.

	BisEMA			HEMA			TEGDMA			UDMA			BisGMA		
LOD	1.97			2.43			0.47			0.63			1.18		
LOQ	5.97			7.36			1.43			1.90			3.51		
EVX	1 h	*		1 h	24 h	3 m	1 h	24 h	3 m	1 h	24 h	3 m	1 h	24 h	3 m
		*		*	*	*	4.410 $\pm$ 0.026 <sup>a</sup>	26.483 $\pm$ 0.722 <sup>c</sup>	30.673 $\pm$ 0.141 <sup>c</sup>	*	*	*	*	*	6.339 $\pm$ 0.629 <sup>a</sup>
							6.376 $\pm$ 0.754 <sup>b</sup>	8.167 $\pm$ 0.839 <sup>a</sup>	9.033 $\pm$ 0.346 <sup>a</sup>	1.974 $\pm$ 0.048 <sup>a</sup>	4.280 $\pm$ 0.051 <sup>a</sup>	7.078 $\pm$ 0.426 <sup>a</sup>	*	*	*
XTB	10.029 $\pm$ 0.255	10.925 $\pm$ 0.474	14.338 $\pm$ 1.093 <sup>a</sup>	*	*	*	8.713 $\pm$ 0.085	9.485 $\pm$ 0.781 <sup>a</sup>	12.511 $\pm$ 0.627 <sup>b</sup>	17.232 $\pm$ 1.267 <sup>b</sup>	22.991 $\pm$ 0.256 <sup>b</sup>	60.919 $\pm$ 0.781 <sup>b</sup>	*	*	*
SDR	*	*	14.976 $\pm$ 0.325 <sup>a</sup>	*	*	*	8.485 $\pm$ 0.343 <sup>c</sup>	12.841 $\pm$ 0.500 <sup>b</sup>	28.297 $\pm$ 4.677 <sup>c</sup>	*	*	*	*	*	*
GHF	*	*	14.099 $\pm$ 0.317 <sup>a</sup>	*	*	*							12.886 $\pm$ 0.702	14.982 $\pm$ 0.909	19.360 $\pm$ 5.570 <sup>b</sup>

Different superscript lowercase letters indicate statistically different groups in the same column according to the one-way ANOVA and Tukey HSD and independent sample *t*-test ( $p < 0.05$ ). \* Nondetectable and nonquantified according to LOQ. LOD: limit of detection; LOQ: limit of quantification (LOD and LOQ values were represented in a previous study [19] from authors).



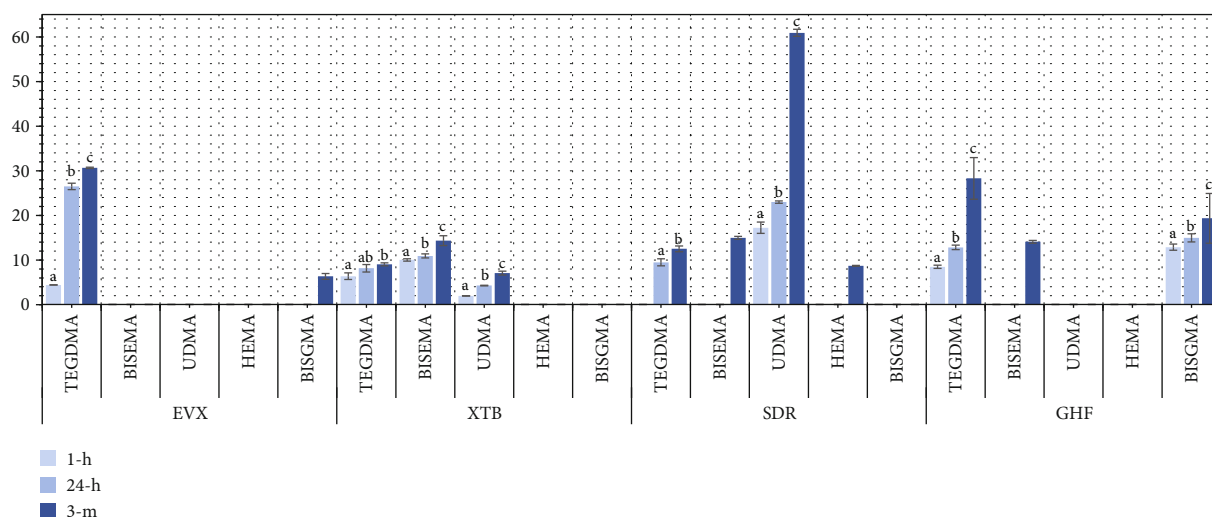


FIGURE 3: The eluted monomer from resin-based composites separately for each immersion period. Letters indicate statistically different group for each monomer released from resin-based composites according to the paired sample *t*-test ( $p < 0.05$ ).

studies, it was reported that SDR showed the highest monomer release, which is in accordance with the results of the present study [8, 27, 29]. In a previous study [30] that examined TEGDMA elution from SDR, it was reported that there was no TEGDMA elution after 1 week, while at the end of the 1-month immersion,  $9.4 \mu\text{g/g}$  TEGDMA elution was detected in 75% ethanol solution. The results of the present study showed that  $12.511 \mu\text{g/ml}$  TEGDMA elution from SDR was detected at the end of 3 months. In accordance with the results of the present study, BisEMA, TEGDMA, and UDMA release from SDR were also detected in previous studies [8, 27, 31].

In previous studies, XTB was investigated in terms of monomer release in an ethanol/water solution for 3 months. The authors stated that DEGDMA, BisEMA, and UDMA were eluted from the XTB [27, 29]. In accordance with the results of that study, BisEMA, TEGDMA, and UDMA were eluted from XTB. Since the retention times of TEGDMA and DEGDMA monomers are very similar to each other, these monomers are likely to interfere in HPLC. In contrast to the results of the present study, Lagočka et al. [8] could not detect UDMA elution from XTB composite. XTB samples showed the lowest monomer elution in the present study, and these results are consistent with those of previous studies [27, 29]. In another study, Al-Hiyasat et al. [32] reported that the change in the filler and monomer ratio of the composite has a significant effect on the monomer release from the material. Some studies have found a lower absorption rate in composite materials with a high filler ratio than those with a lower proportion of filler [33, 34]. This can be explained by the fact that XTB samples absorb less solution, showing lower monomer elution. In only one study investigating the monomer elution from EVX, it was found that BisGMA and TEGDMA were detected after a week of immersion in an ethanol (100%) solution [35]. Therefore, in the present study, monomer elution from EVX was comprehensively analyzed, and it was found that  $6.339 \mu\text{g/ml}$  BisGMA and  $30.673 \mu\text{g/ml}$  TEGDMA eluted even after 3

months of immersion. Additionally,  $14.099 \mu\text{g/ml}$  BisEMA,  $28.297 \mu\text{g/ml}$  TEGDMA, and  $19.360 \mu\text{g/ml}$  BisGMA elution from GHF were found in the present results, but there has been no study evaluating monomer elution from GHF up to now. According to the manufacturers' data, none of the tested materials contained HEMA, and the XTB composite did not contain TEGDMA. However, in this study, TEGDMA elution from the XTB samples and HEMA elution from the SDR samples were detected. Manufacturers are responsible for providing information about the compounds found in the material safety data sheet (SDS) for any concentrations above 1% [7]. In addition, it has been concluded that HEMA might be a degradation product of UDMA-like monomers, which are included in SDR [31, 36]. Cebe et al. [31] could not detect HEMA elution at the end of 1 month from the SDR composite; however, Lagočka et al. [8] detected HEMA elution from SDR. HEMA elution was detected at the end of 3 months in the present study.

The structure, size, and weight of the monomers contained in the resin matrix are another important factors affecting the amount and rate of monomer release. Smaller and lower molecular weight monomers, such as TEGDMA, are eluted faster and with greater amounts than high molecular weight monomers, such as BisGMA [27, 37–39]. In addition to the rigid structure of BisGMA, TEGDMA has a flexible and linear structure that makes it easier to pass through intermolecular spaces [38]. Furthermore, TEGDMA is hydrophilic and prone to elute from aqueous solutions. The results of the present study showed that TEGDMA was eluted in a shorter time and in higher amounts than other monomers.

The evaluation of residual monomers eluted from composite materials is also important in determining the biocompatibility of these materials. There are previous studies on the cytotoxicity of monomers eluted from composite resins. For instance, Urcan et al. [40] analyzed the EC50 value of HEMA, TEGDMA, UDMA, and BisGMA monomers on human gingival fibroblasts. The EC50 values of

the HEMA, TEGDMA, UDMA, and BisGMA monomers were 11.20 (0.60) mmol/l, 3.60 (0.20) mmol/l, 0.10 (0.04) mmol/l, and 0.09 (0.01) mmol/l, respectively. The comparative toxicity of these monomers was listed as BisGMA (most toxic) > UDMA > TEGDMA > HEMA (least toxic). When these mmol values were converted to  $\mu\text{g}$ , it was found that only UDMA monomer elution from SDR after 3-month immersion was above the EC50 dose in the present study.

It should be considered that monomer release in the oral environment is affected by the amount of wear of the composite restoration, saliva flow, and enzymatic degradation due to saliva enzymes, contrary to the laboratory conditions in which this study was conducted. It is appropriate to evaluate cytotoxicity and monomer release together, considering the remaining dentin thickness and dentin permeability, in order to better simulate the clinical conditions in future studies. In addition, according to the information obtained from the manufacturers, the monomers forming the organic matrix of the resin-based composite materials used in the study were taken into consideration, and the monomers whose release was examined in this study were preferred. Therefore, in vitro studies using different analysis methods and different monomer types and, if possible, in vivo clinical studies or studies that can further simulate the clinical environment can be conducted in future studies.

## 5. Conclusion

Within the limitations of the present study, the following conclusions were reached:

- (1) The monomers were eluted from the composite dentin replacement materials during all immersion periods
- (2) Monomer release at the end of 3 months was significantly higher than the 1-hour amount for all composite dentin replacement materials
- (3) The characteristics of the resin matrix and the filler ratio of the composite dentin replacement materials used affected the monomer elution
- (4) SDR was the only composite that UDMA released above the value considered cytotoxic

## Data Availability

All data from the research project used to support the findings of this study are available from the corresponding author upon request.

## Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors./All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964

Helsinki Declaration and its later amendments or comparable ethical standards.”

## Conflicts of Interest

Nurgül Çetin Tuncer declares that she has no conflict of interest. Çağatay Barutçugil declares that he has no conflict of interest. Ayşe Dünder declares that she has no conflict of interest. Sevede Gül Batmaz declares that she has no conflict of interest. Kardelen Yıldırım declares that she has no conflict of interest.

## Authors' Contributions

Nurgül Çetin Tuncer was responsible for writing and editing. Çağatay Barutçugil was responsible for conceptualization, formal analysis, writing, reviewing, editing, and supervision. Sevede Gül Batmaz was responsible for the investigation. Ayşe Dünder was responsible for methodology, resources, and supervision. Kardelen Yıldırım was responsible for the investigation. Seren Özdemir was responsible for reviewing and editing.

## Acknowledgments

This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) (grant number: 216S723).

## References

- [1] P. A. da Rosa Rodolpho, M. S. Cenci, T. A. Donassollo, A. D. Loguercio, and F. F. Demarco, “A clinical evaluation of posterior composite restorations: 17-year findings,” *Journal of Dentistry*, vol. 34, no. 7, pp. 427–435, 2006.
- [2] D. C. Sarrett, “Clinical challenges and the relevance of materials testing for posterior composite restorations,” *Dental Materials*, vol. 21, no. 1, pp. 9–20, 2005.
- [3] S. Garoushi, L. V. Lassila, A. Tezvergil, and P. K. Vallittu, “Load bearing capacity of fibre-reinforced and particulate filler composite resin combination,” *Journal of Dentistry*, vol. 34, no. 3, pp. 179–184, 2006.
- [4] J. H. T. Payne, “The marginal seal of class II restorations: flowable composite resin compared to injectable glass ionomer,” *Journal of Clinical Pediatric Dentistry*, vol. 23, no. 2, pp. 123–130, 1999.
- [5] R. Haak, M. J. Wicht, and M. J. Noack, “Marginal and internal adaptation of extended class I restorations lined with flowable composites,” *Journal of Dentistry*, vol. 31, no. 4, pp. 231–239, 2003.
- [6] J. C. Castaneda-Espinosa, R. A. Pereira, A. P. Cavalcanti, and R. F. Mondelli, “Transmission of composite polymerization contraction force through a flowable composite and a resin-modified glass ionomer cement,” *Journal of Applied Sciences*, vol. 15, no. 6, pp. 495–500, 2007.
- [7] K. L. Van Landuyt, T. Nawrot, B. Geebelen et al., “How much do resin-based dental materials release? A meta-analytical approach,” *Dental Materials*, vol. 27, no. 8, pp. 723–747, 2011.
- [8] R. Lagocka, M. Mazurek-Mochol, K. Jakubowska, M. Bendyk-Szeffer, D. Chlubek, and J. Buczkowska-Radlińska, “Analysis of base monomer elution from 3 flowable bulk-fill composite

- resins using high performance liquid chromatography (HPLC)," *Medical Science Monitor*, vol. 24, pp. 4679–4690, 2018.
- [9] E. Putzeys, R. C. Duca, L. Coppens et al., "In-vitro transdental diffusion of monomers from adhesives," *Journal of Dentistry*, vol. 75, pp. 91–97, 2018.
  - [10] N. H. Kleinsasser, K. Schmid, A. W. Sassen et al., "Cytotoxic and genotoxic effects of resin monomers in human salivary gland tissue and lymphocytes as assessed by the single cell microgel electrophoresis (comet) assay," *Biomaterials*, vol. 27, no. 9, pp. 1762–1770, 2006.
  - [11] H. Schweikl, G. Spagnuolo, and G. Schmalz, "Genetic and cellular toxicology of dental resin monomers," *Journal of Dental Research*, vol. 85, no. 10, pp. 870–877, 2006.
  - [12] H. Darmani and A. S. Al-Hiyasat, "The effects of BIS-GMA and TEG-DMA on female mouse fertility," *Dental Materials*, vol. 22, no. 4, pp. 353–358, 2006.
  - [13] L. Accorinte Mde, A. D. Loguercio, A. Reis, A. Muench, and V. C. de Araújo, "Adverse effects of human pulps after direct pulp capping with the different components from a total-etch, three-step adhesive system," *Dental Materials*, vol. 21, no. 7, pp. 599–607, 2005.
  - [14] J. Blomgren, T. Axell, O. Sandahl, and M. Jontell, "Adverse reactions in the oral mucosa associated with anterior composite restorations," *Journal of Oral Pathology & Medicine*, vol. 25, no. 6, pp. 311–313, 1996.
  - [15] Y. L. Lai, Y. T. Chen, S. Y. Lee, T. M. Shieh, and S. L. Hung, "Cytotoxic effects of dental resin liquids on primary gingival fibroblasts and periodontal ligament cells in vitro," *Journal of Oral Rehabilitation*, vol. 31, no. 12, pp. 1165–1172, 2004.
  - [16] J. M. Whitworth, P. M. Myers, J. Smith, A. W. G. Walls, and J. F. McCabe, "Endodontic complications after plastic restorations in general practice," *International Endodontic Journal*, vol. 38, no. 6, pp. 409–416, 2005.
  - [17] A. Moorthy, C. H. Hogg, A. H. Dowling, B. F. Grufferty, A. R. Benetti, and G. J. P. Fleming, "Cuspal deflection and microleakage in premolar teeth restored with bulk-fill flowable resin-based composite base materials," *Journal of Dentistry*, vol. 40, no. 6, pp. 500–505, 2012.
  - [18] T. A. Omran, S. Garoushi, A. A. Abdulmajeed, L. V. Lassila, and P. K. Vallittu, "Influence of increment thickness on dentin bond strength and light transmission of composite base materials," *Clinical Oral Investigations*, vol. 21, no. 5, pp. 1717–1724, 2017.
  - [19] K. Barutçigil, A. Dundar, S. G. Batmaz, K. Yıldırım, and Ç. Barutçigil, "Do resin-based composite CAD/CAM blocks release monomers?," *Clinical Oral Investigations*, vol. 25, pp. 329–336, 2021.
  - [20] Z. Susgun Yildirim, E. Eyiler, and Z. G. Bek Kurklu, "Effect of thickness on the degree of conversion, monomer elution, depth of cure and cytotoxicity of bulk-fill composites," *Journal of Oral Science*, vol. 65, no. 2, pp. 121–126, 2023.
  - [21] S. Krifka, C. Seidenader, K. A. Hiller, G. Schmalz, and H. Schweikl, "Oxidative stress and cytotoxicity generated by dental composites in human pulp cells," *Clinical Oral Investigations*, vol. 16, no. 1, pp. 215–224, 2012.
  - [22] W. Geurtsen, W. Spahl, and G. Leyhausen, "Variability of cytotoxicity and leaching of substances from four light-curing pit and fissure sealants," *Journal of Biomedical Materials Research*, vol. 44, no. 1, pp. 73–77, 1999.
  - [23] K. Moharamzadeh, R. Van Noort, I. M. Brook, and A. M. Scutt, "HPLC analysis of components released from dental composites with different resin compositions using different extraction media," *Journal of Materials Science: Materials in Medicine*, vol. 18, no. 1, pp. 133–137, 2007.
  - [24] J. L. Ferracane, "Elution of leachable components from composites," *Journal of Oral Rehabilitation*, vol. 21, no. 4, pp. 441–452, 1994.
  - [25] E. C. Munksgaard, A. Peutzfeldt, and E. Asmussen, "Elution of TEGDMA and BisGMA from a resin and a resin composite cured with halogen or plasma light," *European Journal of Oral Science*, vol. 108, no. 4, pp. 341–345, 2000.
  - [26] O. Polydorou, R. Trittler, E. Hellwig, and K. Kummerer, "Elution of monomers from two conventional dental composite materials," *Dental Materials*, vol. 23, no. 12, pp. 1535–1541, 2007.
  - [27] R. Z. Alshali, N. A. Salim, R. Sung, J. D. Satterthwaite, and N. Silikas, "Analysis of long-term monomer elution from bulk-fill and conventional resin-composites using high performance liquid chromatography," *Dental Materials*, vol. 31, no. 12, pp. 1587–1598, 2015.
  - [28] R. Z. Alshali, N. Silikas, and J. D. Satterthwaite, "Degree of conversion of bulk-fill compared to conventional resin-composites at two time intervals," *Dental Materials*, vol. 29, no. 9, pp. e213–e217, 2013.
  - [29] E. Lempel, Z. Czibulya, B. Kovacs et al., "Degree of conversion and BisGMA, TEGDMA, UDMA elution from flowable bulk fill composites," *International Journal of Molecular Sciences*, vol. 17, no. 5, p. 732, 2016.
  - [30] R. Lagocka, K. Jakubowska, D. Chlubek, and J. Buczkowska-Radlinska, "Elution study of unreacted TEGDMA from bulk-fill composite (SDR Dentsply) using HPLC," *Advances in Medical Sciences*, vol. 60, no. 2, pp. 191–198, 2015.
  - [31] M. A. Cebe, F. Cebe, M. F. Cengiz, A. R. Cetin, O. F. Arpag, and B. Ozturk, "Elution of monomer from different bulk fill dental composite resins," *Dental Materials*, vol. 31, no. 7, pp. e141–e149, 2015.
  - [32] A. S. Al-Hiyasat, H. Darmani, and M. M. Milhem, "Cytotoxicity evaluation of dental resin composites and their flowable derivatives," *Clinical Oral Investigations*, vol. 9, no. 1, pp. 21–25, 2005.
  - [33] M. Braden and K. W. Davy, "Water absorption characteristics of some unfilled resins," *Biomaterials*, vol. 7, no. 6, pp. 474–475, 1986.
  - [34] U. Ortengren, H. Wellendorf, S. Karlsson, and I. E. Ruyter, "Water sorption and solubility of dental composites and identification of monomers released in an aqueous environment," *Journal of Oral Rehabilitation*, vol. 28, no. 12, pp. 1106–1115, 2001.
  - [35] A. R. Sajjani and M. N. Hegde, "Leaching of monomers from bulk-fill composites: an in vitro study," *Journal of Conservative Dentistry*, vol. 19, no. 5, pp. 482–486, 2016.
  - [36] V. B. Michelsen, G. Moe, R. Skalevik, E. Jensen, and H. Lygre, "Quantification of organic eluates from polymerized resin-based dental restorative materials by use of GC/MS," *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, vol. 850, no. 1–2, pp. 83–91, 2007.
  - [37] A. Nalcaci, N. Ulusoy, and O. Atakol, "Time-based elution of TEGDMA and BisGMA from resin composite cured with LED, QTH and high-intensity QTH lights," *Operative Dentistry*, vol. 31, no. 2, pp. 197–203, 2006.



- [38] I. D. Sideridou and D. S. Achilias, "Elution study of unreacted Bis-GMA, TEGDMA, UDMA, and Bis-EMA from light-cured dental resins and resin composites using HPLC," *Journal of Biomedical Materials Research Part B B Appl Biomater*, vol. 74-B, no. 1, pp. 617–626, 2005.
- [39] K. Tanaka, M. Taira, H. Shintani, K. Wakasa, and M. Yamaki, "Residual monomers (TEGDMA and Bis-GMA) of a set visible-light-cured dental composite resin when immersed in water," *Journal of Oral Rehabilitation*, vol. 18, no. 4, pp. 353–362, 1991.
- [40] E. Urcan, H. Scherthan, M. Styllou, U. Haertel, R. Hickel, and F. X. Reichl, "Induction of DNA double-strand breaks in primary gingival fibroblasts by exposure to dental resin composites," *Biomaterials*, vol. 31, no. 8, pp. 2010–2014, 2010.