Leaching of monomers from bulk-fill composites: An in vitro study

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Abstract

Aim and Objectives: To evaluate the elution of bisphenol A-glycidyl methacrylate (BisGMA) and triethylene glycol dimethacrylate (TEGDMA) from two bulk-fill composites at different polymerization times, for different storage periods when cured with quartz-tungsten-halogen (QTH) curing unit.

Materials and Methods: Tetric N-Ceram bulk fill and EverX Posterior were analyzed using high-performance liquid chromatography unit. Totally, 68 samples were prepared, two groups (n = 17) from both the composites, one for each tested polymerization time. Each sample was cured with a QTH curing unit, using soft-start curing technique and stored in 2 ml of ethanol for 24 h. Storage medium was renewed and then stored again for 1 week. Data acquired were statistically analyzed.

Results: The elution of BisGMA was significantly higher from Tetric N-Ceram bulk fill and BisGMA and TEGDMA from EverX Posterior composite at the end of 24 h, irrespective of the curing time. In EverX Posterior, a higher amount of TEGDMA was eluted at the end of 24 h, while at the end of 1 week, significantly higher amount of BisGMA was released.

Conclusion: A significant amount of the release of BisGMA as well as TEGDMA was seen from both the composites when stored for different time intervals.

Keywords: Bisphenol A-glycidyl methacrylate; bulk-fill composites; high-performance liquid chromatography; triethylene glycol dimethacrylate

INTRODUCTION

Composite resins are made up of a complex mixture of the organic resin matrix, inorganic fillers, and a silane coupling agent connecting the two. Base monomers such as bisphenol A-glycidyl methacrylate (BisGMA) and urethane dimethacrylate (UDMA) along with low-viscosity monomer like triethylene glycol dimethacrylate (TEGDMA) constitute the polymerizable resin matrix.[1] The common drawbacks of dental composites include polymerization shrinkage and high residual monomer content.[2]

Dentists use incremental placement techniques to manage the polymerization shrinkage. This technique is time-consuming, and there is a risk of contamination while placing, adapting, and curing each increment. Recently, a new class of resin-based composite, called “bulk fill” composites, has been introduced with the purpose of saving clinical chairside time.[3] Its unique advantage is that it can be placed and cured in an increment of 4-mm thickness, without adverse effect on polymerization shrinkage.

One of the alternatives to overcome polymerization shrinkage is the use of soft-start curing which involves, low-intensity light during the initial phase of polymerization and later switched to high-intensity light for the remaining curing time reducing the rate of polymerization.[4]

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The elution of residual monomers, oligomers, and other degradation products into the oral environment has raised concerns regarding composites. It occurs by diffusion of the resin matrix or by its degradation or erosion over a period.\textsuperscript{[5,6]}

According to Ferracane,\textsuperscript{[7]} the factors affecting elution are: (i) amount of polymerization reaction, (ii) chemistry of the solvent, and (iii) chemical nature and size of the released components. The unpolymerized monomers can get eluted directly into the pulp by means of dentinal microchannels.\textsuperscript{[8]} BisGMA has a strong hemolytic potency which can cause time- and concentration-dependent cytotoxicity in human gingival and pulp fibroblasts.\textsuperscript{[9]} TEGDMA can easily penetrate cell membranes and react with intracellular molecules, thereby causing cytotoxicity.\textsuperscript{[10]}

Thus, the aim of this study was to evaluate the amount of the elution of BisGMA and TEGDMA from these composite resins cured by a quartz-tungsten-halogen (QTH) light using “Soft Start” curing technique, when stored at a storage period of 24 h and 1 week using two polymerization times: 30 and 40 s.

MATERIALS AND METHODS

Sample preparation

Two nanohybrid dimethacrylate bulk-fill composite resins were used [Table 1]. From each of them, 34 samples were prepared. A total of 68 samples. These samples were divided into two groups of 17 samples each based on different polymerization times: 30 and 40 s.

- Group 1: 30 s polymerization (30 s)
- Group 2: 40 s polymerization (40 s).

A standard teflon mold of 2 mm × 2 mm × 2 mm dimension was used to prepare the specimens. It was placed on a glass plate, and underneath the mold, a transparent plastic matrix strip was positioned. The material was inserted into the mold followed by the placement of one more transparent plastic matrix strip over them, to prevent oxidation of the superficial layer. Each specimen was cured using “Soft Start curing technique” with QTH curing units for 30 and 40 s, i.e., initial curing with the low intensity of 450 mW/cm\textsuperscript{2} for 10 s using QHL-75 curing light (Dentsply) and the remaining 20 and 30 s, respectively, were cured with a higher intensity of 750 mW/cm\textsuperscript{2} using Astralis 7 curing light (Ivoclar Vivadent). A calibrated radiometer system was used to verify the irradiance at each use of the light cure unit. A 1-cm glass plate was used to standardize the distance between the light source and the specimen.

Immediately after curing, the specimens were immersed in 2 ml of 99.99% ethanol\textsuperscript{[10-12]} (Hayman Limited, Eastways Park, Witham, Essex, CM88YE, England). The storage of these samples was done for a time period of 24 h at room temperature. After 24 h, the storage medium was changed, and the specimens were again stored for 7 days.

Sample evaluation

After 24 h and 7 days, the samples were measured using the high-performance liquid chromatography (HPLC) unit (Shimadzu, Model SPD 20A, Shimadzu Corporation, Kyoto, Japan). The separation of monomers took place with a CC 125/4 Nucleodur 100-5 C18 ec HPLC -Column. The mobile phase was acetonitrile/water (75/25% v/v) at a flow rate of 1 mL/min, and detection was performed at a wavelength of 254 nm for 30 min. For the analysis of extracted residual monomers, reference standards of BisGMA (CAS No. 261548, Sigma-Aldrich Chemical Co., USA) and TEGDMA (CAS No. 494356, Sigma-Aldrich Chemical Co., USA) were purchased and stock solutions were prepared by appropriate quantitative dilution. Twenty microliters from the solution was injected into HPLC system and standard chromatograms were obtained for both the monomers individually.

| Material used               | Composition                                    | Manufacturer                   |
|-----------------------------|------------------------------------------------|--------------------------------|
| EverX Posterior             | Dimethacrylates monomers                        | GC, UK                         |
|                            | BisGMA, TEGDMA                                  |                                |
|                            | Fillers                                         |                                |
|                            | Silicon dioxide: 1-5 (weight %)                  |                                |
|                            | Barium glass: 60-70 (weight %)                  |                                |
|                            | Glass fibers: 5-15 (weight %)                   |                                |
| Tetric N-Ceram Bulk Fill    | Dimethacrylates monomers                        | Schaan, Liechtenstein, Germany |
|                            | BisGMA, BisEMA, UDMA                            |                                |
|                            | Fillers                                         |                                |
|                            | Barium glass filler (0.4 μm-0.7 μm),            |                                |
|                            | ytterbium                                       |                                |
|                            | Trifluoride (200 nm), mixed                     |                                |
|                            | Oxide (160 nm): 61 (weight %)                   |                                |
|                            | Polymer filler: 17 (weight %)                   |                                |

UDMA: Urethane dimethacrylate, BisGMA: Bisphenol A-glycidyl methacrylate, BisEMA: Bisphenol A polyethylene glycol diether dimethacrylate, TEGDMA: Triethylene glycol dimethacrylate
**Statistical analysis**

The acquired data were statistically analyzed using Student’s $t$-test and paired $t$-test. Statistical Package for Social Science software version 15 (IBM SPSS Statistics, NY, USA) was used.

The level of significance was set at $p = 0.05$.

**RESULTS**

Significantly higher amount of BisGMA was eluted from Tetric N-Ceram bulk-fill composite at the end of 24 h as compared to 1 week when cured for 30 s, i.e., $P < 0.001$ and 40 s, i.e., $P = 0.004 < 0.05$. Furthermore, significantly higher amount of BisGMA was eluted from EverX Posterior composite at the end of 24 h as compared to 1 week when cured for 30 s, i.e., $P < 0.001$ and 40 s, i.e., $P = 0.022 < 0.05$. On comparing the elution of TEGDMA from EverX Posterior, significantly higher amount was eluted at the end of 24 h as compared to 1 week when cured for 30 s, i.e., $P < 0.001$ and 40 s, i.e., $P < 0.001$ [Table 2].

Student’s $t$-test showed that from EverX Posterior, a higher amount of TEGDMA was released at the end of 24 h as compared to BisGMA, when cured for 30 s, i.e., $P = 0.166$ and when cured for 40 s, i.e., $P = 0.456$, but it was not statistically significant. At the end of 1 week, significantly higher amount of BisGMA was released as compared to TEGDMA, when cured for 30 s, i.e., $P < 0.001$ and when cured for 40 s, i.e., $P < 0.001$ [Table 3].

**DISCUSSION**

The degree of conversion for dental composites is approximately between 35% and 77%. Asmussen and Peutzfeldt reported that a linear polymer structure with a fewer cross-links is significantly more prone to softening in ethanol. Thus, incomplete polymerization may increase residual monomer.

Studies show the cytotoxic potency of basic monomers to be in the following order: BisGMA > UDMA > TEGDMA >> HEMA.[1] BisGMA is cytotoxic and has shown synthetic estrogenic effects.[15,16] According to the National Institute for Occupational Safety and Health, since TEGDMA is lipophilic nature, it can penetrate the cytosol and membrane lipid compartments of mammalian cells.[17] 99.99% ethanol was used as a solvent, owing to its maximum ability to extract residual monomers and solubility parameter similar to BisGMA.[13]

The maximum elution, i.e., 85–100% of monomers was reported to occur within 24 h by Ferracane and Condon and within 7 days by Ortengren et al.[18,19] Hence, the elution of monomers after polymerization was tested at the end of 24 h and 7 days in the present study. The molecules of BisGMA and UDMA, which are high molecular weight base monomers, get decompose in the gas chromatograph and, hence, only their decomposition products can be evaluated.[19] HPLC, being an accurate and common separation method, was preferred to qualify and quantify the elutable monomers.[20]

In the present study, the overall elution of BisGMA was higher than TEGDMA, for both composite materials [Table 2], which correlates with the results of Komurcuoglu et al., Polydorou et al., and Hegde et al.[1,13] Predominantly, it could be because BisGMA had low double-bond conversion as compared to TEGDMA or due to the degree of conversion for dental composites.

### Table 2: Paired $t$-test was applied to compare the mean amount of release of bisphenol A-glycidyl methacrylate from Tetric N-Ceram bulk fill and EverX Posterior and triethylene glycol dimethacrylate from EverX Posterior based on different storage times.

| Paired samples statistics | Leaching agent | Curing time | Mean | $n$ | SD | Paired differences | Mean | $SD$ | $t$ | $P$ |
|---------------------------|---------------|-------------|------|----|----|-------------------|------|------|-----|-----|
| Tetric N-Ceram bulk fill  | BisGMA        | 30 s        | 38.995 | 17 | 7.797 | 5.508             | 3.912 | 5.804 | <0.001 |
|                           |               | After 24 h  | 33.487 | 17 | 7.669 |                   |       |       |       |
|                           |               | After 1 week| 38.875 | 17 | 11.517 | 5.353             | 6.590 | 3.349 | 0.004 |
|                           |               | 40 s        | 33.521 | 17 | 6.911 |                   |       |       |       |
| EverX posterior            | BisGMA        | 30 s        | 37.369 | 17 | 6.102 | 6.201             | 4.476 | 5.712 | <0.001 |
|                           |               | After 24 h  | 31.168 | 17 | 5.314 |                   |       |       |       |
|                           |               | After 1 week| 37.148 | 17 | 13.217 | 6.098             | 9.890 | 2.542 | 0.022 |
|                           |               | 40 s        | 31.050 | 17 | 7.923 |                   |       |       |       |
|                           | TEGDMA        | 30 s        | 40.344 | 17 | 6.120 | 18.934             | 6.012 | 12.983 | <0.001 |
|                           |               | After 24 h  | 21.409 | 17 | 7.113 |                   |       |       |       |
|                           |               | After 1 week| 40.091 | 17 | 9.180 | 18.892             | 9.705 | 8.026 | <0.001 |
|                           |               | 40 s        | 21.198 | 17 | 5.919 |                   |       |       |       |

BisGMA: Bisphenol A-glycidyl methacrylate, TEGDMA: Triethylene glycol dimethacrylate, SD: Standard deviation
variation in the reactive potentials and chemical properties of the two.[21] In addition to the above, 99.99% ethanol used for the storage of the samples could also be one of the probable reasons for higher elution of BisGMA.[1] When the elution of BisGMA from both the bulk-fill nano hybrid dimethacrylate resins was compared, paired t-test revealed that 24 h samples showed significantly higher release at \( P < 0.05 \), irrespective of the different polymerization time. The release of TEGDMA was also significantly higher from the 24 h samples, irrespective of the curing time [Table 2], and is in accordance with Ferracane and Condon.[13] Varying polymerization time groups of 30 and 40 s showed no significant difference in the elution of BisGMA and TEGDMA from the two bulk-fill composite resins [Graph 1].

At the end of 24 h, a higher amount of TEGDMA was eluted from EverX Posterior as compared to BisGMA but not significantly, i.e, \( P = 0.166 \) \( (P > 0.05) \) when cured for 30 s and \( P = 0.456 \) \( (P > 0.05) \) when cured for 40 s [Table 3]. It is in agreement with Hegde et al.[1] and Nathanson et al., who have shown that within the first 4 min, the maximum amount of TEGDMA was released which reduced thereafter.[22] In addition, Durner et al. reported that initially, the elution of TEGDMA was higher than BisGMA.[23] This could be because of its hydrophilicity and small molecular weight, exhibiting higher mobility.[24] While at the end of 7 days, the elution of BisGMA from the same material was significantly higher than TEGDMA, irrespective of the curing times, i.e, \( P < 0.05 \) [Table 3], which is similar to the results of the study by Polydorou et al.[10]

Of the two bulk-fill composites evaluated, Tetric N-Ceram Bulk fill showed higher elution of BisGMA than EverX Posterior, irrespective of the varying polymerization and storage time. This might be due to the absence of TEGDMA. Furthermore, it contains prepolymer fillers, due to which any remaining unreacted or pendant \( \mathrm{C} = \mathrm{C} \) double bond increases the amount of elutable monomers [Graph 2].[25]

**CONCLUSION**

Within the limitations of the study, the following conclusions were drawn:

- For BisGMA and TEGDMA elution, a significantly higher amount of monomer was eluted after 24 h than after 1 week
- Higher quantity of BisGMA was eluted from Tetric N-Ceram bulk fill in comparison to EverX Posterior, irrespective of the polymerization time and storage periods
- Although samples were cured using soft-start curing technique, the elution of monomers from bulk-fill composites was still observed.

Manufacturers recommend 20 s curing time with QTH unit to polymerize bulk-fill composites.

### Table 3: Student’s t-test was applied to the mean amount of release of bisphenol A-glycidyl methacrylate and triethylene glycol dimethacrylate from EverX Posterior bulk-fill composite based on different storage times

| Leaching agent | n | Mean | SD | t   | P   |
|----------------|---|------|----|-----|-----|
| EverX Posterior |   |      |    |     |     |
| 30 s           |   |      |    |     |     |
| After 24 h BisGMA | 17 | 37.36977 | 6.102676 | -1.419 | 0.166 |
| TEGDMA         | 17 | 40.34424 | 6.120872 | <0.001 | 0.456 |
| After 24 h BisGMA | 17 | 31.16824 | 5.314855 | -0.456 | <0.001 |
| TEGDMA         | 17 | 21.19859 | 7.919885 |
| 40 s           |   |      |    |     |     |
| After 24 h BisGMA | 17 | 37.14853 | 13.21705 | -0.754 | 0.456 |
| TEGDMA         | 17 | 40.09129 | 9.180097 |
| After 24 h BisGMA | 17 | 31.05041 | 7.923286 | 4.107 | <0.001 |
| TEGDMA         | 17 | 21.19859 | 5.919885 |

BisGMA: Bisphenol A-glycidyl methacrylate, TEGDMA: Triethylene glycol dimethacrylate, SD: Standard deviation

**Graph 1:** Bar graph depicting the mean amount of the release of bisphenol A-glycidyl methacrylate from Tetric N-Ceram Bulk fill and EverX Posterior and triethylene glycol dimethacrylate from EverX Posterior based on different polymerization times

**Graph 2:** Bar graph depicting the mean amount of the release of bisphenol A-glycidyl methacrylate between Tetric N-Ceram Bulk fill and EverX Posterior composite resins
However, from the present study, even though 30 and 40 s of curing time, composite resins showed no notable effect in the elution of monomers. Further considerations and research on suitable modifications in the composite materials, curing lights, or curing techniques to reduce monomer elution from composite resins are required.

**Clinical relevance**
Residual monomers were eluted from bulk-fill composite resins at all time periods, even when cured using an alternative curing technique like “Soft Start” curing technique.

**Limitation**
Since absolute alcohol was used to immerse the cured composite samples, it did not simulate the oral cavity environment, but quantifies the amount of residual monomer which can be correlated.

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**Conflicts of interest**
There are no conflicts of interest.

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