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ResearchArticle

Evaluation Of BIS-GMA Release In Different Types Of Composites

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Abstract

BIS-GMA is frequently is involved in the structure of composite materials and may remain unpolymerized after polymerization. Residual Bis-GMA has harmful effects, it is crucial to determine the amounts released monomer from dental materials. The aim of this study is to compare residual BIS-GMA amounts in three types of composite. A total of 45 (n=15) composite (Filtek Ultimate Universal Restorative,3M ESPE,Germany), flowable composite (Nextcomp Flow,Meta Biomed,Kore), bulk fill composite(Tetric N Ceram, Ivoclar,Lichtenstein) cylindrical samples were polymerized in Teflon molds in accordance with the manufacturer's instructions. The samples were placed in 1.5ml ethanol (Carlo Erba, Pharm. Grade, 99.9%) and kept at 37ºC. Monomer release of the samples was evaluated on the first, third and seventh days using the High Performance Liquid Chromatography technique. BIS-GMA release was shown with the highest amounts within all groups on the first day. The elution in composite fillings was significantly lower than the other materials on the first day($p<0.05$). The highest total emission rates were observed in the flowable composite. The material selected in clinical practice should be considered because the monomer release may vary depending on the composite type.

Keywords: BIS-GMA, composite dental resin, flowable hybrid composite, bulk fill composite

Farklı Kompozit Dolguların BIS-GMA Salınımlarının İncelenmesi

Öz

Kompozit malzemelerin sıklıkla yapısında yer alan BIS-GMA, polimerizasyondan sonar reaksiyona girmeden kalabilmektedir. Artık monomerlerin bilinen zararlı etkileri nedeniyle, dental malzemelerden salınan monomer miktarlarının belirlenmesi çok önemlidir. Mevcut çalışmanın amacı, kompozitrezin, akışkan kompozitve bulk fill kompozit materyallerden salınan BIS-GMA miktarlarının değerlendirilmesidir. Toplamda 45 adet kompozit (Filtek Ultimate Universal Restorative,3M ESPE,Germany), akışkan kompozit (NextcompFlow,MetaBiomed,Kore), bulk fill kompozit (Tetric N Ceram, Ivoclar,Lichtenstein) silindirik örnek (n=15)Teflon kalıplarda üretici firma talimatları doğrultusunda polymerize edildi. Örnekler 1,5ml etanol (Carlo Erba, Pharm. Grade, 99.9%) içine yerleştirilerek 37ºC'de muhafaza edildi. Örneklerin monomer salınımıYüksekPerformanslıSıvıKromatografitekniğikullanılarakbirinci, üçüncü ve yedinci günde değerlendirildi. BIS-GMA salınımının ilk gün tüm gruplarda en yüksek miktarlarda görülmüştür. Kompozit grubunda elüsyon birinci gün diğer malzemelere göre anlamlı olarak düşük bulunmuştur($p<0.05$). En yüksek toplam salınım oranları akışkan kompozitte görülmüştür. Klinikte malzeme seçiminde, monomer salınımının kompozit tipine göre değişebileceği dikkate alınmalıdır.

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AnahtarKelimeler: BIS-GMA, kompozit dental rezin, akışkanhibritkompozit, bulk fill kompozit

1. Introduction

Resin methacrylates are filling materials which are most frequently used in dentistry. Resin based dental materials include mono, di and / or multiple methacrylate monomers and are widely used in the formation of Bisphenol A glycidyldimethacrylate (BIS-GMA), urethane dimethacrylate (UDMA), triethylene glycol dimethacrylate (TEGDMA) and hydroxyethyl methacrylate (HEMA). (Schwengberg et al. (2005), Van Landuyt et al. (2011)) The monomer content is expected to transform into a polymeric form during the polymerization of an ideal resin-based restorative material. The remaining monomers that do not react are called "residual monomers". After the resin-based restorative materials are placed in the tooth, un-polymerized monomer residues are released into the oral environment. (Van Landuyt et al. (2011)) The cytotoxic, genotoxic and allergic effects of residual monomers and their effects on the reproductive system have been reported in the literature previously. (Goldberg (2008), Schweikl et al. (2007))

BIS-GMA is a monomer universally found in composite materials. Bisphenol A (BPA) is one of the initial components of BIS-GMA production. Mainly BPA derivatives rather than pure BPA, form the structure of dental resins. These products are liquid monomers which polymerize into a solid following either chemical or light curing. (Al-Hiyasat and Darmani (2006)) BPA may be found as a pollutant in dental resins but is not used in their formulation for the reason that moisture from saliva inhibits its polymerization by causing hydrolysis of the 2 end hydroxyl groups. Hence, BIS-GMA, which is a derivative that has methyl methacrylate groups added to the hydroxyl groups of BPA via a glycidyl spacer, is used most frequently as the base of the resin. (Söderholm and Mariotti (1999)) Mechanical, bacterial, thermal or saliva enzymatic biodegradation end with BPA release in the oral cavity as a result of incomplete polymerization in BIS-GMA-based restorative materials. (Marzouk et al. (2019)) In previous literature, it was stated that BPA causes neurological and behavioral disorders in children, affects the prostate and mammary glands, and causes premature puberty in girls. (Akyüz et al. (2011))

In this study, it was aimed to measure the amount of BIS-GMA that did not react after polymerization. In the hypothesis of the study, it is stated that "There is no difference between different types of composite materials in terms of BIS-GMA release".

2.Material-Methods:

Three different types of resin-based composite fillings were evaluated in terms of monomer elution. The materials used and their contents are shown in **Table 1**.

Table 1: Composite filling materials and their monomer intent

Material	Commercial name	Monomer intent	LOT	Manufacturer
Flowable composite	Nextcomp Flow	Bis-GMA UDMA	NXF1509241	Meta Biomed, Kore
Composite	Filtek Ultimate Universal Restorative	Bis-GMA UDMA TEGDMA	N923376	3M ESPE,Germany
Bulk fill composite	Tetric N Ceram	Bis-GMA UDMA TEGDMA	N930551	Ivoclar, Vivadent, Schaan, Liechtenstein

Polytetrafluoroethylene molds of 10 mm diameter and 4 mm depth were used to prepare 45 samples (n = 15 for each composite) to be measured for monomer release. After the non-polymerized materials were placed in the mold, cellulose acetate strip tape and glass coverslip were placed on it and polymerized with the light device (Elipar, 3M, ESPE, USA) in accordance with the manufacturer's instructions. The light device was cured to the filling materials over glass coverslip. After polymerization, the samples were removed from the mold and the excess edges were smoothed. Each sample was stored at 37^0C in 1.5 mL storage medium until the time of measurement. After 24 hours, the samples were transferred to the freshly prepared storage solution and previous solution was filtered through the syringe filter and vialed for subsequent measurement. The release of the BIS-GMA in resin-based restorative materials was monitored by using the high performance liquid chromatography technique (High Performance Liquid Chromatography-HPLC). Samples polymerized in layers of 4 mm thickness and were kept in 0.5 mL of 75% (v/v) ethanol solution (Ethanol, Carlo Erba, Pharm. Grade, 99.9%) for 1 day, 3 days or 1 week under 175 rpm agitation and at 36.5 °C in a shaking incubator.

Monomer release measurements were performed with ThermoDionex Ultimate 3000 series HPLC system equipped with PDA detector set at 206 nm. The analytical column was Zorbax ODS (250mm * 4,6mm * 5µm). The mobile phases were consisted of aqueous solution of trifluoroacetic acid (0.01% v/v) (A) and acetonitrile (B) and the constant flow rate was set at 1 mL / min. where the column temperature was fixed at 30°C. The chromatographic separation was achieved under gradient flow conditions given as follows; 0 min, 25 % B; 0-1 min, 15–40% B; 1–1.5 min,40-45 % B; 1.5–2.0 min, 45–55% B; 2.0–2.5 min, 55-65 % B; 4.0 min,70 % B; 8.5 min, 70 % B 8.5–9.0 min, 70-25 % B 10.0 min, 25% B. The injection volume was 10 µL. The analytical merits of HPLC method was given in **Table 2**.

Table 2: Analytical method validation parameters for BIS-GMA

	BIS-GMA
Linear Range ($mg.L^{-1}$)	0.5-500
Linear Equation	$0.4454C_{Bis\text{-}GMA}$
R^2	0.9993
Limit of Detection ($mg.L^{-1}$)	0.17
Limit of Quantification ($mg.L^{-1}$)	0.5
Accuracy (25 mgL^{-1})	97.3
Precision (25 mgL^{-1} N=7)	2.1

The concentration of BIS-GMA in supernatant solutions was calculated from the 8-point calibration curve in the concentration range of 0.5-500 µg/mL. Quality control samples containing 25 µg/mL BIS-GMA were analyzed before each batch in order to control the accuracy of the measurements and the samples re-analyzed in case of QC samples recoveries lower than 85%.

The distribution of normality of numerical data was verified by Shapiro-Wilk test. Differences in BIS-GMA release between groups were verified using one-way ANOVA test. Tukey's HSD analysis was performed for post-hoc analysis. Statistical analyzes were tested in SPSS (V 25, IBM, NewYork) program at a significance level of 0.05.

3.Research Findings:

The BIS-GMA release of the composite, flowable composite and bulk-fill composite filling materials on the 1st, 3th and 7th days is shown in **Table 3**. When the differences between the groups in terms of monomer release are examined, it was seen that there was a difference between the first day BIS-GMA release of the composite, bulk fill and flowable composite ($p < 0.001$). In the post-hoc analysis, a statistically significant difference was found between composite and bulk fill and flowable composite at the end of the first day ($p < 0.001$). It was found that BIS-GMA release in composite fillings was significantly lower than flowable composite and bulk fill composite.

There was a statistically significant difference between the tested groups in terms of BIS-GMA release in third day ($p < 0.001$). Post-hoc investigations found a statistically significant difference between flowable composite and bulk fill and composite materials. The BIS-GMA release of the flowable composite was significantly higher than the bulk fill and composite materials.

In addition, the difference of BIS-GMA release between groups was statistically significant after the first week ($p < 0.05$). When the difference among the groups was examined, it was observed that the BIS-

GMA release of the flowable composite is significantly higher than that of the composite and bulk fill composite.

Table 3: Mean values of the residual BIS-GMA eluted from each resin-based filling materials

Time (day)	Bulk Fill	Flowable Composite	Composite	
	BIS-GMA (nmol)	BIS-GMA (nmol)	BIS-GMA (nmol)	P value
1	208.73[a]	204,86[a]	109.78[b]	<0.001*
3	47.75[a]	206,48[b]	48.57[a]	<0.001*
7	36,65[a]	174.70[b]	48.57[a]	<0.01*

4.Results:

Although the estrogenic effects of BPA have been identified since the 1930s, the number of studies on this subject has increased after the 2000s. The reported harmful effects of BPA are remarkable.

There are numerous studies about the BIS-GMA release of different materials. (Putzeys et al. (2019), Alshali et al. (2015)) In the current study, the BIS-GMA release of 3 different composite materials were examined on the 1st, 3th and the 7th days. According to the data obtained as a result of the research, the tested null hypothesis was rejected. BIS-GMA release varied among the composite materials.

Monomer elution depends on the molecular properties and material composition of the monomers. Elution should be performed at different time intervals to investigate the kinetics of monomer release. Generally, the highest BIS-GMA release was seen in the first 24 hours, and the amount of residual monomer decreased in the following days. (Moilanen et al. (2013), Polydorou et al. (2009), Ferracane (1994)) In our study, the highest Bis-GMA release was observed in 3 different composite materials within the first 24 hours. Composite contains 1-10% Bis-GMA, flowable composite contains 1-10% Bis-GMA, bulk fill composite contains 2.5-10% Bis-GMA. Although they have similar monomer compositions, a difference has been observed between the emission of different types of composite materials.

The lowest total BIS-GMA release was observed in the composite filling material and the highest release was observed in the flowable composite material. Filler particle type and content, resin porosity and homogeneity of polymer matrix composition affect the residual monomer amounts of materials. (Reichl et al. (2008)) The release differences obtained in our study were thought to be a result of content differences within the materials used.

HPLC or gas chromatography methods were widely used to measure monomer release analysis. In this study, HPLC analysis, which were used frequently during in-vitro studies, were preferred to determine the monomer release of

composite materials. As the monomers are soluble in the mobile phase, it provides a great level of control over the separation process. (Moharamzadeh et al. (2007)) In our study, we preferred to make monomer release by HPLC analysis.

Two different solvents were used in the previous studies which examined monomer release: 1: Aqueous media (such as artificial saliva, real saliva), 2: Organic extraction solutions (such as ethanol, methanol, acetone). The Food and Drug Administration (FDA) has reported that a 75% ethanol/water solution, which is similar to the oral conditions, can be used in monomer release studies. (Alshali et al. (2015), Moharamzadeh et al. (2007)) We used the 75% ethanol/water solution according the FDA recommendations

There are some limitations to this study. It was conducted under laboratory conditions where all conditions were optimized. The oral environment differs between individuals under clinical conditions and is difficult to compare in this respect. Nevertheless, in vitro studies guide clinical studies. In this study, the monomer release amount of merely composite filling materials after being polymerized with light was investigated. Since there may be differences between the monomer release that occurs when the filling materials are used with adhesive systems in the oral environment and the singular release, it is inappropriate to compare. Therefore, more clinical studies are needed on this subject.

Along with the limitations of the study, it was observed that BIS-GMA was released as a monomer from all of the evaluated filling materials, and the highest release occurred within the first 24 hours. The highest total emission rates were observed in the flowable composite. The material selected in clinical practice should be considered because the monomer release may vary depending on the composite type.

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