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Original research article

HPLC analysis of monomers eluted from self-adhesive resin cements

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Abstract

Objective: The aim of this study was to determine the residual monomer leaching from two self-adhesive resin cements polymerized with Light Emitting Diode (LED) or halogen light curing unit.

Materials and Method: Clearfil SA (group A, n = 48) and BisCem (group B, n = 48) cements were inserted in plastic moulds. Each group was further divided into two subgroups. Specimens were light cured with LED light curing unit (LCU) in group A1 and group B1 and halogen LCU in group A2 and group B2 for 20 seconds. The following compounds released from the samples stored in distilled water were analyzed: triethylene glycol-dimethacrylate (TEGDMA) and bisphenol A glycidyl methacrylate (Bis-GMA). Analysis of substances was performed with the use of high performance liquid chromatography, after 1 hour and 24 hour incubation periods. Factorial experimental design and independent t-test was used for statistical analyses.

Results: Self-adhesive resin cements released more Bis-GMA and TEGDMA when they were polymerized with LED LCUs ($p<0.05$). Difference between different time periods was not statistically significant ($p>0.05$). Clearfil SA cement released more Bis-GMA than BisCem ($p<0.05$). BisCem released more TEGDMA than Clearfil SA ($p<0.05$).

Conclusion: The results of this study showed that the quantity of Bis-GMA and TEGDMA leached from self-adhesive resin cements was influenced by the type of LCU and by the type of self-adhesive resin cement.

Keywords: Dental curing lights; HPLC; resin cements

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Introduction

Self-adhesive resin cements were introduced as a new subgroup of resin cements and were designed with the intention of overcoming some of the shortcomings of both conventional and resin cements. Self-adhesive resin cements do not require any pretreatment of the tooth surface and application is accomplished in a single step. These luting cements are moisture tolerant, bond chemically to tooth tissues and restorative materials and offer aesthetics, optimal mechanical properties, dimensional stability and micromechanical adhesion. Because of these favourable features, self-adhesive resin cements are used in a wide range of applications (*e.g.* cementation of indirect restorations: ceramic, composite, metal, inlays, onlays, bridges, crowns, and posts including fibre posts).[1]

One of the most common drawbacks of resin-based materials is inadequate polymerization which results in high levels of residual monomers. Residual monomers resulting from incomplete conversion of monomers into polymer have the potential to cause irritation, inflammation, and an allergic response in oral mucosa.[2] In several *in vitro* studies, it was found that some of these monomers can show cytotoxic, genotoxic, mutagenic, or estrogenic effects and cause pulpal and gingival reactions.[3,4]

In order to identify a reliable method to achieve adequate polymerization in resin cements and overcome the formation of residual monomers, various types and modes of curing lights and devices have been studied extensively.[5-7] Halogen lights have been the most commonly used devices for accomplishing the polymerization of resin-based dental materials. These low-cost technology devices have drawbacks, such as a decline of irradiance over time due to bulb and filter ageing, which could lead to inadequate polymerization[8-10]. Light emitting diode (LED) is a highly efficient light source

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that produces light within a narrow spectral range and is designed to overcome some of the disadvantages of halogen lights[6]. Although many studies have reported on the mechanical properties of resin-based materials that have been cured with LED units, there is little information available about self-adhesive resin cements that have been cured with LED or halogen light curing units (LCUs).[11-13]

The aim of this study was to determine the residual monomer leaching from two self-adhesive resin cements polymerized with LED or halogen LCU. The hypothesis to be tested was that the type of self-adhesive resin cement, LCU and incubation time would affect the residual monomer elution from self-adhesive resin cements.

Materials And Method

Specimen preparation

Two different self-adhesive resin luting cements, Clearfil SA Cement (Kuraray, Osaka, Japan) and BisCem (BISCO Inc., Schaumburg, IL, USA) were used in this study. The chemical compositions and manufacturers of these materials are shown in Table 1.

In group A, Clearfil SA Cement (n = 48) and in group B, BisCem (n = 48) self-adhesive resin cements were inserted into plastic moulds (2 mm in height and 3 mm in diameter) and sandwiched between two layers of Mylar matrix strip and two glass slides. Each group was further divided into two subgroups according to the curing unit used. In groups A1 (n = 24) and B1 (n = 24), specimens were light-cured with LED-LCU (Elipar Freelight, 3M ESPE, St. Paul, MN, USA) and in groups A2 (n = 24) and B2 (n = 24), specimens were cured with a halogen LCU (Lunar Curing Light, Benlioğlu Dental Inc., Ankara, Turkey) above the glass slide from a distance of 0 mm for 20 seconds. The light intensity of the halogen and LED-LCUs were measured with a radiometer (Demetron, Kerr, Danbury, CT, USA) before exposure of the samples, and the levels were 600 mW/cm^2 and 400 mW/cm^2 respectively. Immediately after the curing process, the specimens were removed from the moulds and each was immersed in an Eppendorf tube containing 200 µL of distilled water at 37 ºC. Then each group (A1, A2, B1 and B2) was divided into two subgroups according to elution incubation periods: in the first subgroup from each pair, specimens were incubated for 1 hour (n = 12), in the second subgroup, the specimens were incubated for 24 hours (n = 12).

High performance liquid chromatography (HPLC) analysis

A stock solution containing 100.0 µg/mL Bis-GMA and TEGDMA (both from Aldrich Chemical Company Inc., Milwaukee, WI, USA) was diluted with methanol and calibration standards were prepared by appropriate quantitative dilution of the stock solution (Aldrich Chemical Company Inc., Milwaukee, WI, USA). Standard chromatographs of Bis-GMA and TEGDMA were obtained (Figure 1). The retention time for Bis-GMA and TEGDMA were 2.6 and 4.5 min respectively.

The analysis of eluted monomers released from the cements as well as the reference solutions of Bis-GMA and TEGDMA in water/acetonitrile (30:70) as a mobile phase was carried out by HPLC (Thermo Scientific-IG, Madison, WI, USA). The flow rate of the mobile phase was 1.0 mL/min; absorbance readings were performed at 210 nm. The stationary phase was C18, 150×4.6 mm^2 with a 5 µm particle size. In order to quantify the amount of residual monomers, data were confronted with the calibration standard curve peak area versus monomer concentration. The HPLC analysis was repeated three times. Data analysis was performed with SAS 9.2 statistical software (SAS Institute Inc., Cary, NC, USA) using the factorial experimental design to evaluate the effects of the three factors (the types of self-adhesive resin cement, LCUs and elution incubation periods) on the amount of residual Bis-GMA and TEGDMA monomers. Independent t-tests were performed to evaluate the differences between the groups.

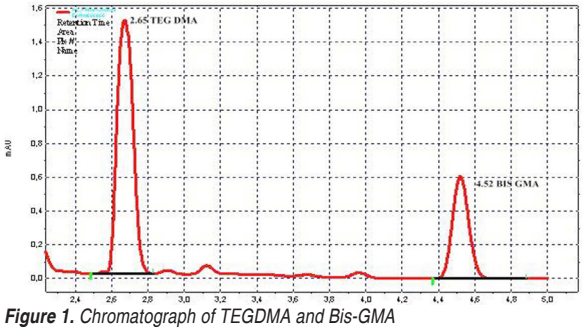

Figure 1. *Chromatograph of TEGDMA and Bis-GMA*

Table 1. *Composition of the materials*

Materials	Manufacturer	Lot no	Composition
BisGMA	Aldrich Chemical Company Inc, Milwaukee, WI, USA,	494356	Bisphenol A glycerolate dimethacrylate
TEGDMA	Aldrich Chemical Company Inc, Milwaukee, WI, USA,	409510	Triethylene glycol dimethacrylate
Clearfil SA	Kuraray Medical Inc. Tokyo, Japan	15AAA	MDP, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, colloidal silica, barium glass fillers
BisCem	BISCO Inc, Schaumburg, IL, USA	0900011325	Bis (hydroxyethyl methacrylate) phosphate, tetra ethylene glycol dimethacrylate, dental glass

Table 2. *Factorial experimental design results of Bis-GMA residual monomer*

	1 hour					24 hours				
Source	**DF**	**SS**	**MS**	**F value**	**p value**	**DF**	**SS**	**MS**	**F value**	**p value**
Material	1	590.73	590.73	699.41	0.0001	1	452.99	452.99	308.79	0.0001
LCUs	1	7.21	7.21	8.54	0.0005	1	15.792	15.792	10.77	0.002
Material-LCU	1	5.46	5.46	6.47	0.014	1	15.431	15.431	10.52	0.002
Error	44	37.16	0.84	-	-	43	63.081	1.467	-	-
Total	47	640.57	-	-	-	46	539.767	-	-	-

DF: degree of freedom, SS: sum of squares, MS: mean square, LCU: light curing unit

Table 3. *Mean and standard deviation values of the Bis-GMA monomer (ppm) eluted from self-adhesive resin cements*

	1 hour	24 hours
Group A1 (Clearfil SA + LED LCU)	7.8 (1.3) aA	7.7 (1.8) aA
Group A2 (Clearfil SA + halogen LCU)	6.3 (1.3) bA	5.4 (1.6) bA
Group B1 (BisCem + LED LCU)	0,1 (0,3) cA	0.4 (0.05) cB
Group B2 (BisCem + halogen LCU)	0.0 (0.0) cA	0.4 (0.05) cB

Different capital letters (for rows) and different lowercase letters (for columns) indicate a statistically significant difference (p<0.05; t-test).

Results

Bis-GMA

The statistical analysis showed that there were statistically significant differences between the amount of Bis-GMA residual monomer values according to the type of self-adhesive resin cement and the type of curing light (p<0.05; Table 2). Clearfil SA cement released statistically more Bis-GMA monomer than BisCem (p<0.05; Table 3). Polymerization with LED-LCUs caused more residual Bis-GMA monomer release from both types of self-adhesive resin cements. The mean amounts of eluted residual Bis-GMA monomer are shown in Table 3. When groups were compared; Group A1 released significantly more Bis-GMA for both 1 hour and 24 hour incubation periods (p<0.05). Also there were significant differences between Group A2 and the other groups for both incubation periods. There was not any significant difference between Group B1 and Group B2 in neither of the incubation periods (p>0.05). When the incubation periods were compared, there were no significant differences between two incubation periods for Group A1 and Group A2 (p>0.05). The differences between the two incubation periods were significant for Group B1 and B2 (p<0.05). Amounts of released residual Bis-GMA monomer were significantly higher in 24 hour incubation period than 1 hour in Group B1 and also in Group B2 (p<0.05).

TEGDMA

Depending on the statistical analysis, it was determined that the amount of TEGDMA values varied according to the type of self-adhesive resin cement and the type of LCU (p<0.05; Table 4). BisCem released significantly more residual TEGDMA than Clearfil SA (p<0.05; Table 5). Self-adhesive resin cements released significantly more TEGDMA residual monomers when they were polymerized with LED-LCUs (p<0.05). Incubation periods did not affect the TEGDMA residual monomer release in any of the groups (p>0.05). The mean amounts of eluted TEGDMA are shown in Table 5. When the groups were compared, there was no statistically significant difference between the TEGDMA amounts of Group A1 and A2 after 1 hour (p>0.05); however, significant difference was determined between these two groups after 24 hours (p<0.05). There was a significant difference between Group B1 and B2 after 1 hour (p<0.05) but no significant difference was found between them after 24 hours (p>0.05). There was not a significant difference between the two incubation periods for TEGDMA release from all groups (p>0.05).

Discussion

The current study investigated the amounts of residual Bis-GMA and TEGDMA eluted from two different self-adhesive resin cements (Clearfil SA and BisCem) following polymerization with LED and halogen LCUs. The results from statistical analysis partially supported the original hypothesis that the type of self-adhesive resin cement and LCU affect the amounts of residual Bis-GMA and TEGDMA monomers. However, the results did not support the other part of the hypothesis, which predicted that the amount of residual monomer elution changes depending on the duration of the incubation period.

Among different factors studied, it was found that the type of self-adhesive resin cement affected the elution of Bis-GMA and TEGDMA monomers. Clearfil SA cement released more residual Bis-GMA monomer while BisCem cement released more residual TEGDMA monomer. These differences between the amounts of residual monomers may depend on the different chemical composition and monomer content of self-adhesive resin cements. Interestingly, detectable levels of TEGDMA eluted from all of the BisCem samples, although based on the information received from the

Table 4. *Factorial experimental design of TEGDMA residual monomer*

	1 hour					24 hours			
Source	**DF**	**SS**	**MS**	**F value**	**p value**	**DF**	**SS**	**MS**	**F value**	**p value**
Material	1	5211.6	5211.6	74.28	0.0001	1	5983.3	5983.3	71.81	0.0001
LCUs	1	708.71	708.7	10.10	0.003	1	243.0	243.05	2.92	0.094
Material-LCU	1	102.20	102.2	1.46	0.234	1	143.9	143.98	1.73	0.196
Error	44	3087.3	70.16	-	-	43	3582.7	83.32	-	-
Total	47	9109.9	-	-	-	46	10044.0	-	-	-

DF: degree of freedom, SS: sum of squares, MS: mean square, LCU: light curing unit

Table 5. *Mean and standard deviation values of the TEGDMA monomer (ppm) eluted from self-adhesive resin cements*

	1 hour	24 hours
Group A1 (Clearfil SA + LED-LCU)	27.8 (3.7) aA	32.08 (4.9) aA
Group A2 (Clearfil SA + halogen LCU)	23.04 (3.5) aA	24.02 (3.3) bA
Group B1 (BisCem + LED-LCU)	51.6 (13.4) bA	51.16 (9.9) cA
Group B2 (BisCem + halogen LCU)	40.9 (8.6) cA	50.1 (13.9) cA

Different capital letters (for rows) and different lowercase letters (for columns) indicate a statistically significant difference (p<0.05; t-test).

manufacturer, there was no TEGDMA in the chemical composition of this resin cement.

The elution process is consequently dependent on the size and the chemical composition of the leachable molecules.[14] Tanaka *et al.*[15] determined that smaller molecules like TEGDMA move more easily than larger, bulkier ones such as Bis-GMA. The results of a recent study showed higher concentrations of eluted TEGDMA than of the other monomers (Bis-GMA, Bis-EMA, HEMA) from different bulk fill composites.[16] Similar to these findings, in our study TEGDMA monomer leached more than Bis-GMA monomer from both of the self-adhesive resin cements.

In the current study LED (400 mW\cm^2) and halogen (600 mW\cm^2) LCUs were used for polymerizing the self-adhesive resin cements. The specimens which were polymerized with the LED-LCU released more residual monomers. In contrast with our result, Tabatabaee *et al.*[6] reported that halogen light induced greater monomer elution than LED light. Ak *et al.*[7] compared the release of residual monomers from composite resins and fissure sealants polymerized with LED and halogen LCUs and determined that curing these materials with a LED-LCU eluted less residual monomers in comparison to a halogen LCU. However, in both of these HPLC studies, the halogen LCUs used had a lower intensity (around 400-450 mW\cm^2) than the one used in our study. Halvorson *et al.*[17] determined that higher light intensities promoted a higher wavelength peak at 470 nm and more camphorquinone molecules were excited. Consequently, higher light intensities cause the generation of more free radicals and induce faster monomer conversion, resulting in lower residual monomer elution.

Ceballos *et al.*[13] reported that the curing efficiency of composite resins did not only depend on the LCU type, but was also influenced by the composite resin brand. In our study, after 24 hours, BisCem released similar amounts of Bis-GMA and TEGDMA when polymerized with LED and halogen LCUs. Unexpectedly, Clearfil SA cement released more Bis-GMA and TEGDMA after 24 hours when it was polymerized with LED. This difference could be attributed to the different contents of the BisCem and Clearfil SA cements.

An important point about the leaching of residual monomers from resin-based dental materials is the time needed for the complete elution. In the literature there are contradictory claims about the length of time needed for the complete elution of unreacted monomers. Although some studies have suggested that elution is completed in 1 to 7 days, others argued that it takes a longer amount of time.[5,6] Altıntaş *et al.*[18] measured the elution of residual monomers at time intervals of 1 h and 1, 3, 7, 14, and 21 days and reported significant differences in the amount of HEMA eluted from total-etch adhesive resin (Single Bond) at the different time intervals. Similar to this study Ak *et al.*[7] determined changes in the elution rate of Bis-GMA and TEGDMA monomers from resin-based composites over time. Contrary to these results Siderou and Achilias[19] reported no significant difference between the amounts of residual Bis-GMA and TEGDMA monomers released from resin-based composites after 3 and 30 days. Similarly, in the current study, two observation periods were used to determine the effect of shorter and longer incubation time on residual monomer elution and the results showed that duration of time did not affect the release of residual Bis-GMA and TEGDMA monomers from self-adhesive resin cements.

Another parameter that affects the amount of unreacted monomers is the solvent used for the elution. Numerous solvents such as distilled water, saliva, ethanol, methanol or acetonitrile have been used in previous HPLC studies.[6,15,19,20] Organic solvents such as ethanol or methanol or mixtures of these solvents with water are preferred to simulate oral conditions.[21] In the current study, distilled water was used as a solvent to mimic oral conditions as in some of the previous dental resin and composite extraction studies.[6,15]

Previous cytotoxicity studies showed that TEGDMA and especially Bis-GMA have a high number of cytotox-

ic features.[4,22,23] Heil *et al.*[24]also showed that TEGDMA and Bis-GMA might have mutagenic effects at subtoxic concentrations. Additionally, Hansel *et al.*[25] determined that TEGDMA might promote the proliferation of the important cariogenic microorganisms *Lactobacillus acidophilus* and *Streptococcus sobrinus*. In the current study, Bis-GMA and TEGDMA monomers that were not converted to polymers during polymerization and were subsequently released as residual monomers from self-adhesive resin cements were analyzed. The amounts of the residual monomers eluted from self-adhesive resin cements in the current study were lower than the toxic values stated in previous cytotoxicity studies.[4,22-24] However, leaching of monomers from self-adhesive resin cements not only decreases the biocompatibility of the material, but also can affect their mechanical properties negatively. Therefore, even small amounts of residual monomer release should be critical for clinical success.

Conclusion

All samples released Bis-GMA and TEGDMA. The quantity of monomers leached from self-adhesive resin cements was influenced by the type of LCU and self-adhesive resin cement used. Incubation periods (1 hour and 24 hours) did not affect the amounts of residual Bis-GMA and TEGDMA monomers released from samples. Both types of self-adhesive resin cements released more residual Bis-GMA and TEGDMA monomers when they were polymerized with LED LCUs. Clearfil SA cement released more residual Bis-GMA monomers while BisCem cement released more residual TEGDMA monomers.

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Conflict of interest disclosure: The authors declare no conflict of interest related to this study.

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Kendinden bağlanabilen rezin simanlardan salınan monomerlerin HPLC ile incelenmesi

Özet

Amaç: Bu çalışmanın amacı, Light Emitting Diode (LED) veya halojen ışık kaynaklarıyla sertleştirilmiş iki kendinden bağlanabilen rezin simanın artık monomer salımını belirlemektir.

Gereç Ve Yöntem: Clearfil SA (grup A, n = 48) ve BisCem (grup B, n = 48) simanlar plastik kalıplara yerleştirildi. Her grup kendi içinde ikiye ayrıldı. Grup A1 ve B1'deki örnekler LED, grup A2 ve B2'deki örnekler halojen ışık kaynaklarıyla polimerize edildi. Distile suda tutulan örneklerden salınan trietilen glikol-dimetakrilat (TEGDMA) ve bisfenol A glisidilmetakrilat (Bis-GMA) bileşikleri, 1 saat ve 24 saat enkübasyon sonrasında yüksek performanslı likit kromotografisi (HPLC) ile incelendi. İstatistiksel değerlendirmeler için faktöriyel deneysel dizayn ve t testleri kullanıldı.

Bulgular: Kendinden bağlanabilen rezin simanlar LED ışık kaynaklarıyla sertleştirildiğinde daha fazla Bis-GMA ve TEGDMA monomeri saldılar ($p<0.05$). Zaman periyodları arasında istatistiksel olarak anlamlı bir fark bulunamadı ($p>0.05$). Clearfil SA siman BisCem'den daha fazla Bis-GMA saldı ($p<0.05$); BisCem ise Clearfil SA'dan daha fazla TEGDMA saldı ($p<0.05$).

Sonuç: Bu çalışmanın bulguları, kendinden bağlanabilen rezin simanlardan salınan Bis-GMA ve TEGDMA miktarlarının ışık kaynağı ve rezin simanın tipinden etkilendiğini göstermiştir.

Anahtar Kelimeler: Dental polimerize edici ışık kaynakları; HPLC; rezin simanları