Original Article

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Is a Single Rinse Effective on Evacuating the Residual Monomers After Orthodontic Bonding? An *In Vivo* Study

Braketleme Sonrası Tek Çalkalama Artık Monomerlerin Uzaklaştırılmasında Etkili Midir? Bir *İn Vivo* Çalışma

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ABSTRACT

Objective: Orthodontic adhesives are known to release potentially harmful bio-chemicals such as Bisphenol A, a derivative from Bisphenol-A-Glycidyl-Methacrylate (Bis-GMA). The aim of this study was to evaluate the amount of Bis-GMA released after the use of orthodontic adhesives polymerizing chemically or with light. We also aimed to check whether a single rinse is effective on evacuating all the residual monomers.

Methods: Light curing (Transbond XT, Unitek, CA, USA and Opal Seal Ultradent, Utah, USA) and chemically curing adhesives (Rely-a bond, Reliance Orthodontic Products, Inc., USA and Unite, 3M/Unitek, CA, USA) were used to bond upper and lower braces of 48 patients. Patients gargled 25 mL drinking water for 1 minute; before bonding (T0), immediately after bonding (T1) and immediately after the first rinse (T2). The samples were placed in amber colored glass bottles, preserved in -20 °C and filtered through a 0.45-micron filter and analyzed with Liquid Chromatography Tandem Mass Spectrometry.

Results: Bis-GMA release was recorded with all the adhesives. Higher amount of residual monomer was recorded for the light curing composite adhesives; Transbond XT and Opal Seal, followed by chemically curing; Unite and Rely-a bond. There was no statistically significant difference in Bis-GMA concentration at T1 and T2 ($p>0.05$).

ÖZ

Amaç: Ortodontik adezivlerin kullanımı sonrası Bisfenol-A-Glisidil-Metrakrikattan (Bis-GMA) türeyen Bisfenol A gibi potansiyel zararlı biyokimyasal ajanların ortaya çıktığı bilinmektedir. Bu çalışmanın amacı; Kimyasal ya da ışıkla sertleşen adezivlerin kullanımıyla ortaya çıkan Bis-GMA miktarının değerlendirilmesinin yanı sıra braketleme sonrası tek çalkalamanın tüm artık monomerlerin tahliyesinde etkili olup olmadığının değerlendirilmesidir.

Yöntemler: Işıkla (Transbond XT, Unitek, CA, USA and Opal Seal Ultradent, Utah, USA) ve kimyasal (Rely-a bond, Reliance Orthodontic Products, Inc., USA and Unite, 3M/Unitek, CA, USA) polimerizasyon gösteren adezivlerle 48 hastanın alt ve üst braketleri yapıştırılmıştır. Hastalar braketleme öncesi (T0), braketlemeden hemen sonra (T1) ve ilk çalkalamayı takiben (T2) 25 mL'lik içme suyunu 1 dakika boyunca çalkalamıştır. Örnekler kehribar renkli şişelerde -20 °C'de korunmuş ve 0,45-mikron inceliğinde filtrelerden geçirilmiş ve likit kromatografi kütle spektrometresi ile ölçümler gerçekleştirilmiştir.

Bulgular: Tüm gruplarda braketleme sonrası Bis-GMA ortamda tespit edilmiştir. Işıkla sertleşen adezivlerde daha çok miktarda olmak üzere Transbond XT, Opal Seal, Unite ve Rely-a bond gruplarında sırasıyla azalacak miktarda Bis-GMA tespit edilmiştir. T1 ve T2 ölçümleri arasında istatistiksel olarak fark yoktur ($p>0,05$).

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Conclusion: Significant release of Bis-GMA was observed following orthodontic bonding with either light or chemically curing adhesives. No significant difference was recorded between the first and the second rinses following bonding, meaning that a single rinse may not be effective in evacuating all the residual monomers.

Keywords: Bis-GMA, orthodontic adhesives, liquid chromatography

Sonuç: Kimyasal ve ışıkla sertleşen adeziv kullanımı sonrası ortamdaki Bis-GMA miktarı anlamlı derecede artmıştır. Braketleme sonrası ilk ve ikinci çalkalama örneklerindeki Bis-GMA miktarında anlamlı farklılık bulunmaması, tek çalkalamanın tüm artık monomerleri uzaklaştırmada etkili olamayacağı yönünde yorumlanabilir.

Anahtar Sözcükler: Bis-GMA, ortodontik adeziv, likit kromatografi

Introduction

Advancements in material science offered practitioners a variety of adhesive materials with different properties in clinical practice. Composite adhesives not only shorten treatment time but also provide easier handling.

The adhesive composites in orthodontics have similar chemical composition with dental restorative composite materials. They consist of inorganic fillers, a solvent base and an organic matrix. The organic matrix of composite materials changes into polymers with polymerization. However, some monomers not joining the polymer structure may remain (1).

The organic matrix part of composites most frequently includes glycidyl methacrylate (Bis-GMA), which decomposes into a potentially harmful bio-chemical called Bisphenol A. Bisphenol A is known to have undesirable effects on the reproduction capacity of rats and estrogenic activities (2,3). Its cytotoxicity and mutagenic properties have been confirmed in tissue cultures (4,5).

There are different studies in the literature to measure the residual monomer quantities (6-10). However, the concentration of the Bis-GMA is depending on factors such as the temperature, the pH, the solvent solution since it can dissolve to other components. The evacuation of the Bis-GMA from the oral medium in a short time prior to its conversion to BPA is important, however, its low concentration makes it difficult to detect in chromatography devices (11). However, there are no studies in the literature evaluating the short time release of Bis-GMA *in vivo* from different adhesives.

In the clinical practice, it is advised to ask patients to rinse their mouth with water following the bonding procedure to reduce the BPA presence to before bonding levels (12). However, there is no study in the literature evaluating the Bis-GMA concentration following orthodontic bonding cured by neither light nor chemically *in vivo*.

This study aims to investigate whether rinsing following bonding is effective on evacuating the residual Bis-GMA and to evaluate Bis-GMA concentration following bonding with light and chemically curing orthodontic adhesives *in vivo* with a sensitive method.

Methods

The study was approved by the Ethics Committee of Bezmialem Vakıf University (approval number: 71306642-050.01.04). The volunteers signed a consent form prior to participating in the study.

A power analysis was performed based on a former study (12). Accordingly, 12 patients in each of the groups would guarantee the power of 80% at the 5% confidence level allowing detection of differences.

Bonding Process

Forty-eight patients with no composite dental restoration and bonding the upper and lower jaws was possible at the same appointment were included (1st molar to 1st molar of the contralateral side). Patients with missing or extra tooth, with crowding requiring extraction, appliances containing acrylic parts such as a Nance appliance were excluded. The patients were randomly assigned into two groups (n=24) using a randomization web site (https://www.randomizer.org/) based on the adhesive type; light or chemically curing. As four brands were going to be tested, the groups were subdivided using the same software. Two light curing [Transbond XT, Unitek, CA, USA (n=12) and Opal Seal Ultradent, Utah, USA (n=12)] and two chemically curing adhesives [Rely-a bond, Reliance Orthodontic Products, Inc., USA (n=12) and Unite, 3M/Unitek, CA, USA (n=12)] were used to bond upper and lower braces. The chemical compositions of the adhesives are shown in Table 1.

The buccal surfaces of the teeth were cleaned with prophylaxis pumice and rinsed with a water-air spray. The teeth were etched with 37% orthophosphoric acid for 15 s, rinsed and dried for 4-5 s. Every patient received the same type of braces and molar tube set (18-inch slot, Mini Master Series; Roth, American Orthodontics, Sheboygan, USA).

The composite adhesives were applied in accordance with the manufacturer's instructions and finally light-cured by using a LED curing unit (VALO LED, Ultradent, South Jordan, USA) in power mode. The primer was cured for 3 s, the paste was cured for 3 s from the mesial and 3 s from the distal sides. For the chemically curing group, the primer was applied to both tooth and bracket surfaces, and the medium was isolated for 4 minutes following the last bracket placement.

The patients were given 25 mL of drinking water to rinse in a glass for 1 minute. The samples were collected at three instances: before bracket bonding (T0), immediately after bracket bonding (first rinse; T1) and immediately after the first rinse (second rinse; T2). 144 samples were collected and placed in amber colored glass flasks and stored at -20 °C until analysis.

The samples were filtered through a 0.45-micron filter. 1 μL from each sample was collected and analyzed in a liquid chromatographer (Agilent 1200, San Jose, CA, USA) and a mass spectrometer (Agilent 6460, San Jose, CA, USA). Analytical distinction was achieved by a Poroshell 120 EC-C18 3x50 mm with a particle size of 2.7 microns set at a flow speed of 0.2 mL per minute and column temperature of 35 °C. The gradient values are given in Table 2.

Electrospray ionization was carried out using N_2 in negative and positive ion mode sets at 300 °C and a flow rate of 11 mL per minute, while the nebulizer was set at a pressure of 45 psi. Capillary voltage was set at 3,500 V. The retention time of the peak value for Bis-GMA was 4.81 minutes.

Statistical Analysis

The Statistical Package for the Social Sciences for Windows 22.0 (Armonk, NY: IBM Corp, 2013) was used for the

Table 1. Chemical composition of the adhesives

	Chemical name	(C.A.S. No.)	wt% range)
Transbond XT adhesive paste	Silane treated quartz	100402-78-6	70 - 80
	Bisphenol a diglycidyl ether dimethacrylate	1565-94-2	10 - 20
	Bisphenol a Bis (2-hydroxyethyl ether) dimethacrylate	24448-20-2	5 - 10
	Silane treated silica	68611-44-9	< 2
	Diphennyliodonium hexafluorophosphate	58109-40-3	< 0.02
Transbond XT adhesive primer	bisphenol a diglycidyl ether dimethacrylate	1565-94-2	45 - 55
	triethylene glycol dimethacrylate	109-16-0	45 - 55
	Triphenylantimony	603-36-1	< 1
	4-(dimethylamino)-benzeneethanol	50438-75-0	< 0.5
	dl-camphorquinone	10373-78-1	< 0.3
	Hydroquinone	123-31-9	< 0.03
Opal bond adhesive	Bisphenol a diglycidyl ether dimethacrylate	1565-94-2	< 20
	Ethoxylatebisphenoldimethacrylate	41637-38-1	< 10
	Triethylene glycol dimethacrylate	109-16-0	< 5
	Aluminum oxide	1344-28-1	< 10
Opal seal adhesive	Bisphenol a diglycidyl ether dimethacrylate	1565-94-2	< 15
	Hydroxypropyl methacrylate	27813-02-1	45 - 55
	Ethyl alcohol	64-17-5	< 10
	Methacrylic acid	79-41-4	< 10
Rely bond adhesive paste	Bisphenol a diglycidyl ether dimethacrylate	1565-94-2	10-30
	Amorphous silica	60675-86-0	50-75
	Triethylene glycol dimethacrylate	109-16-0	5 - 10
	Aluminum oxide	1344-28-1	1 - 5
Rely bond adhesive primer	bisphenol a diglycidyl ether dimethacrylate	1565-94-2	10-30
	tetrahydrofurfuryl methacrylate	2455-24-5	30 - 50
	2-Hydroxyethyl methacrylate	868-77-9	10 - 30
	Triethylene glycol dimethacrylate	109-16-0	10 - 30
Unite adhesive paste	Silane treated quartz	100402-78-6	50 - 60
	Bisphenol a diglycidyl ether dimethacrylate	1565-94-2	10 - 20
	Benzoyl peroxide	94-36-0	< 2
	Silane treated silica	68611-44-9	5 - 10
	Dimethyl siloxane	67762-90-7	< 2
Unite adhesive primer	bisphenol a diglycidyl ether dimethacrylate	1565-94-2	5 - 15
	triethylene glycol dimethacrylate	109-16-0	70 - 80
	2,2'-(P-Tolylimino) diethanol	3077-12-1	5 - 15
	3- Methacryloxypropyltrimethoxysilane	2530-85-0	< 5
	Poly (Methyl methacrylate) (PMMA)	9011-14-7	< 5

statistical analysis. The statistical analysis was performed with Mann-Whitney U test for comparing the data between two independent groups and Kruskal-Wallis test for data in two or more independent groups. Data within a group were compared using Wilcoxon signed-rank test. All tests were performed at a significance level of α=0.05.

Results

The mean amounts of Bis-GMA detected at T0, T1 and T2, and comparison of the light and chemically curing adhesive groups are presented in Table 3. A statistically significant difference was observed between the light and chemically curing adhesive groups' samples at T1 and T2 (p=0.000). The light-cured group showed higher values than the chemically cured group.

Table 4 reports the results of the Mann-Whitney U test within the groups in terms of sample collection time. In all groups, the differences between T0 and T1 and between T0 and T2 were found to be statistically significant (p=0.000). The change between T1 and T2 was insignificant.

The mean Bis-GMA amounts recorded for the subgroups at T0, T1 and T2 and evaluation of the values for the same time period between the subgroups are given in Table 5. The differences between the means in the T0 period showed no significance (p=0.982), whereas significant differences were found for the T1 and T2 periods (p=0.000). Mann-Whitney U test was used to determine which group caused the difference (Table 6). For both the T1 and T2 periods, higher Bis-GMA concentrations were recorded in the Transbond group followed by Opal and Unite. The Bis-GMA levels for the Rely-a Bond group were significantly lower than the other groups.

Table 2. Gradient values related to analytical division

Time	Function	Parameters
1. min	Solvent composition change	Solvent change 95% A, 5% B
3. min	Solvent composition change	Solvent change 2% A, 98% B
7. min	Solvent composition change	Solvent change 2% A, 98% B
7.1 min	Solvent composition change	Solvent change 95% A, 5% B

A: 1 mM Ammonium formate, 0.1% formic acid in water
B: 0.1% formic acid In acetonitrile

Table 3. Bis-GMA amount comparison between light and chemically curing adhesive groups

	Groups	Mean (ppb)	SD	p
T0	Light cure	0.603	0.226	0.797
	Chemical cure	0.609	0.247	
T1	Light cure	29.683	5.324	**0.000****
	Chemical cure	13.693	4.888	
T2	Light cure	29.715	5.175	**0.000****
	Chemical cure	13.688	4.838	

Mann-Whitney U test, *p<0.05, **p<0.01, SD: Standard deviation

Table 4. Evaluation of the Bis-GMA amount within the groups according to sample collection time

Groups	p		
	T0-T1	T1-T2	T0-T2
Light cure	0.000**	0.732	**0.000****
Chemical cure	0.000**	0.943	**0.000****

Mann-Whitney U test, *p<0.05, **p<0.01

Table 5. Evaluation of the Bis-GMA amount within the subgroups according to sample collection time

	Groups	N	Mean (ppb)	SD	p
T0	Transbond	12	0.590	0.249	0.982
	Opal	12	0.615	0.212	
	Unite	12	0.601	0.252	
	Rely a bond	12	0.616	0.253	
T1	Transbond	12	34.892	0.214	**0.000****
	Opal	12	24.473	0.124	
	Unite	12	18.475	0.204	
	Rely a bond	12	8.910	0.133	
T2	Trans bond	12	34.772	0.229	**0.000****
	Opal	12	24.657	0.351	
	Unite	12	18.421	0.180	
	Rely a bond	12	8.955	0.141	

Kruskall-Wallis H test. *p<0.05. **p<0.01, SD: Standard deviation

Table 6. Evaluation of the group differences at T0. T1 and T2

	T0			T1			T2		
Groups	**Opal**	**Unite**	**Rely a bond**	**Opal**	**Unite**	**Rely a bond**	**Opal**	**Unite**	**Rely a bond**
Transbond	0.982	0.982	0.982	**0.00****	**0.00****	**0.00****	**0.00****	**0.00****	**0.00****
Opal		0.982	0.982		**0.00****	**0.00****		**0.00****	**0.00****
Unite			0.982			**0.00****			**0.00****

Mann-Whitney U test. *p<0.05. **p<0.01

Table 7. Within group comparison of the samples collected at T0. T1. and T2

Groups	p		
	T0-T1	T1-T2	T0-T2
Transbond	**0.002****	0.347	**0.002****
Opal	**0.002****	0.209	**0.002****
Unite	**0.002****	0.182	**0.002****
Rely-a Bond	**0.002****	0.170	**0.002****

Mann-Whitney U test. *p<0.05. **p<0.01.

The results for the intragroup comparison of the samples collected at T0, T1 and T2 are given in Table 7. The mean amount of Bis-GMA recorded at T1 and T2 for each group was found significantly higher in comparison to the T0 measurements, whereas the difference between the mean values at T1 and T2 was insignificant for all groups.

Discussion

There is a general belief that orthodontic adhesives are biologically safe, and this opinion is supported by the argument that adhesive resins are used in small quantities. However, the excess adhesive is not always completely removed prior to polymerization. Moreover, some unreacted material may remain since the composite located under the metallic components access light only by transillumination. Małkiewic et al. investigated the levels of BPA, BPA polymers and Bis-GMA resin in eluates of six commonly used orthodontic adhesives *in vitro* and demonstrated that most of the orthodontic adhesives released Bisphenol A or its derivatives. In the literature, it was reported that even the pellicle layer formation immediately after brushing may decrease monomer release proving that laboratory studies may not reflect precise clinical conditions (13), this is the reason why the present *in vivo* study was designed.

Studies analyzing residual monomers used various solvents such as distilled water, ethanol, saline, cell culture medium or artificial saliva (14). ISO recommends using distilled water to determine the chemicals that are released from resin-based materials, whereas US FDA recommends using a 75% water-ethanol solution. Accordingly, Moreira et al. (15) reported higher amounts of monomers with water-ethanol solution in comparison to using water only. In this study, drinking water presented in a glass was used as sample collection liquid since it is a routine to rinse with water following bonding.

Storage conditions are essential for specimens containing biological fluids, e.g. saliva. The saliva specimens were stored at -80 °C or -70 °C in the literature (16,17). Kloukos et al. (12) kept from rinsing specimens at 4 °C. In this study, the specimens were placed at -20 °C as described by Olea et al. (18).

Liquid chromatography combined with a tandem mass spectrometer was selected in our study for investigating monomers with high molecular weight since combining the mass spectrometer with chromatographic methods is proven to provide more accurate and sensitive results compared to other chromatographic methods (19). While only liquid or gas chromatography devices can measure up to 1 ppm (one millionth), the chromatography combined with mass spectrometry can measure up to 1 ppd (one billionth) of monomers (20), which means that the last one provides 1000 times more sensitive results.

It was shown that Bis-GMA released from composite resins hydrolyzes into Bis-HPPP after 25 hours (21). In another study, chemical and light cured composites were kept in 0.9% saline at 37 °C for 2 months. Following this period, Bis-GMA was not detected in the samples because of the fact that Bis-GMA hydrolyzes and transforms into Bis-HPP with time (22). Moreira et al. (15) found that 75% of the monomer release occurred within a few hours and 95% of the residual monomer was released within 48 hours, whereas Polydorou et al. (23) reported higher monomer release within the first 24 hours. Bis-GMA monomer levels may be affected from the water, esterase enzyme and the temperature of the oral cavity over time. Therefore, the amount of released Bis-GMA was measured in this study immediately after polymerization. Although the samples were collected at different times, similar results were obtained for both groups. This may be proving that Bis-GMA did not deteriorate under storage conditions in our study. On the other hand, in the study of Kloukos et al. (12), the amount of BPA, which is the product of Bis-GMA deterioration, was evaluated. In contradiction with our results, the authors reported that the increase in BPA concentration was reduced after the 2nd post-bonding rinse. Since the concentration of the Bis-GMA is known to be varying with the media temperature, these differences might be related to the fact that their samples were conserved at 4 °C till the measurements were performed while our samples were stored at -20 °C.

In some studies evaluating residual monomer release from chemical or light curing adhesives, a higher degree of monomer release was reported in the chemically-cured group (8,24). In the present study, higher Bis-GMA release was found in the light-cured groups, contradicting these studies. It was reported that increasing curing time leads to decreased residual monomer release (24). Depending on that, it may be hypothesized that using QTH light sources with longer exposure time would cause less residual monomer formation in comparison to high-power LED sources. This study demonstrates that reduced

exposure time with high intensity LEDs can result in composite restorations with inferior curing depth and increased leaching of monomers.

In our study, higher Bis-GMA concentrations were recorded with light-cured composites, especially in the Transbond XT followed by the Opal Seal, Unite and Rely a Bond groups. Considering the chemical contents of these composites, monomer concentrations appeared to be similar. The composites were used in the clinic with bonding agents from their own brands. Similarly, the concentration of Bis-GMA in the bonding agent content was in harmony with the adhesives. Therefore, the presence of unreacted monomers could be expected to be higher. Furthermore, the higher Bis-GMA concentration in the Transbond XT group may be explained by the high amount of Bis-GMA (wt. 45-55%) within the primer and the exposure of the primer to a relatively large surface.

In a study by Ratanasathien et al. (25), it was reported that Bis-GMA has a cytotoxic effect on fibroblasts when its concentration is above 4.78 ppm. On the other hand, increased estrogenic activity of HeLa cells was reported when the Bis-GMA concentration was above 5 ppm (26). In our study, the highest release amount of Bis-GMA was 34.8 ppd, which was considerably lower than those limits. According to these results, it may be assumed that the amount of Bis-GMA release does not reach toxic levels following orthodontic bonding.

Study Limitations

In our study, we found that light-curing composite adhesives, which are commonly used in orthodontic clinics, may be more disadvantageous in terms of biocompatibility compared to chemically curing counterparts. For this reason, manufacturers can plan studies to improve the chemical composition in order to reduce the amount of residual monomers of the light-curing composites. In addition, the use of chemically cured composite adhesives is more difficult clinically. Although fewer Bis-GMA release was found, the repositioning of incorrectly positioned brackets can cause the patients to be more exposed to additional residual monomers. Therefore, it can be suggested to use chemically curing composites with indirect bonding.

It is known that the residual monomer released in the first minutes following polymerization of adhesive resins is related to the oxygen inhibition layer, and the later release is related to residual monomers remaining in the adhesive resin matrix. One of the aims of our study was to check whether one-single rinsing was effective on evacuating the Bis-GMA immediately after orthodontic bonding. Thus, we only analyzed the pre-bonding, post-bonding and the second rinse samples. We proved that a single rinse was not effective in reducing the Bis-GMA concentration, but the number of effective rinsing was not presented. This might be considered as a limitation. Different rinsing solutions and the effective rinsing number may be determined with repeated rinsing procedures. Additionally, the amount of Bis-GMA can be monitored by long-term follow-up studies.

Conclusion

Bis-GMA release was observed immediately after orthodontic bonding in all groups. The highest amount of Bis-GMA was found in the Transbond group, whereas the lowest measurements were recorded in the Rely-A Bond group. The light-cured composite adhesives released higher amounts of residual monomer. The difference between the Bis-GMA levels in first and second rinses was statistically insignificant, meaning that a single rinse may not be effective in evacuating all residual monomers. However, mouth rinsing with water following orthodontic bonding may still be considered to moisten the oral mucosa and remove the possible residual acid.

Ethics

Ethics Committee Approval: Bezmialem Vakıf University (SBW_MF405-1210318105400.pdf).

Informed Consent: Obtained.

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Authorship Contributions

Surgical and Medical Practices: B.Ş.Y., Concept: E.G., B.Ş.Y., S.İ.R., Design: E.G., B.Ş.Y., S.İ.R., Data Collection or Processing: E.G., B.Ş.Y.,Analysis or Interpretation: E.G., B.Ş.Y., S.İ.R., Literature Search: E.G., B.Ş.Y., S.İ.R., Writing: E.G., B.Ş.Y., S.İ.R.

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