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Evaluation of bisphenol- A release from orthodontic adhesives measured with high performance liquid chromatography- An in vitro study

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Abstract

Introduction: The objectives of this study were to quantify in vitro the Bisphenol A (BPA) release from 3 orthodontic Composites.

Methods: For the in-vitro portion of this study, 3 orthodontic composites were evaluated: GROUP I: Light cure 3M XT Orthodontic Adhesives, GROUP II: Self-cure 3M XT Orthodontic Adhesives and GROUP III: Flowable 3M XT composite resins. 8 specimens were tested from each group across 4 different time intervals of 30 minutes, 24 hours, 1 week and 1 month.

The specimens were immersed in a water/ethanol solution and the liquid chromatography system was used. The peak was detected in JASCO-BORWIN Chromatography software. Data was subjected to statistical analysis using Statistical package for social sciences (SPSS v 21.0, IBM). Inter group comparison (>2 groups) was done using Kruskall Wallis ANOVA followed by pair wise comparison using Mann Whitney U test. For all the statistical tests, $p<0.05$ was considered to be statistically significant

Results: Results showed BPA release from all the specimens of Group I and Group III except Group II at 30 minutes, 24 hours and 1 week time intervals. However, BPA release was not detected from any

specimen belonging to all 3 groups at 1 month time interval.

Conclusions: BPA is released from light cure as well as flowable composite resins during different time intervals up to the first week after which it diminishes and is undetectable at 1-month time interval. Release of BPA was not detected from self-cure composite resins at any time interval, suggesting it to be a safer and better choice of adhesive for orthodontic bonding.

Introduction

Orthodontic treatment with fixed appliances requires the application of adhesive materials for securing brackets to the tooth enamel. Both light-cured and chemically cured composites have been shown to be clinically effective and acceptable for orthodontic bonding. [1]

The properties of composite materials result from two major components used in their production, namely, inorganic fillers, such as silica, barium-aluminum glass, or powdered ceramics and an organic matrix made of oligomers and monomers, of which the most frequently used include:glycidyl methacrylate (BIS-GMA), DEGMA 2-(2-hydroxyethoxy) ethyl methacrylate, triethyleneglycoldimethacrylate (TEGDMA), or urethanedimethacrylate (UDMA).[2]

The compounds constituting organic matrix of composite materials are not biologically inert substances.[3] Orthodontic bonding resins are exposed to oral fluids and are in contact with tissues for a prolonged duration of treatment time ranging from 2 to 3 years. Incomplete polymerization and biodegradation can result in the release of resin monomers into the oral cavity.[4] Among the potentially harmful bio-chemicals that are released from polymers, Bisphenol A deserves special attention. It is a decomposition product from Bis-GMA which is widely-used in the manufacture of composite resin fillings. It is also an essential component of Bis-GMA resin, constituting up to 70% weight of materials used as enamel and dental fillings .[5]

BPA has been classified as a category 3 toxic substance and a significant risk factor for human fertility and reproductive system.[6-8] Numerous studies conducted in the laboratory involving tests on animals have shown that BPA compounds are potent allergens and have cytotoxic and mutagenic potential.[9] Some of them also indicate parahormonal action by activating receptors for the estrogen group of hormones.[10]

It has been suggested that clinicians should be cautious in preventing extended contact of any of these materials with patient's skin, mucosa, and gingiva.[6] Preliminary studies have revealed mixed results while evaluating BPA release from various orthodontic products and the jury is still out on the position of BPA exposure. With this background, the present study was undertaken to evaluate Bisphenol A release from routinely used orthodontic adhesives.

The aim of the study was to evaluate and compare Bisphenol-A release from light cure, self-cure and flowable composite resins at 4 different time intervals using High Performance Liquid Chromatography (HPLC).

Materials and Methods

Orthodontic adhesives were divided into three groups as shown in **Figure 1**. GROUP I: Light cure 3M XT Orthodontic Adhesives, GROUP II: Self cure 3M XT Orthodontic Adhesives and GROUP III: Flowable 3M XT composite resins. Sample size was calculated based on a study done by Yoon-Goo Kang et al [11]. Keeping α error at 5%, β error at 20% and power of study 80%, the sample size obtained was 6.09. Hence, 8 specimens were tested from each group across 4 different time intervals of 30 minutes, 24 hours, 1 week and 1 month.

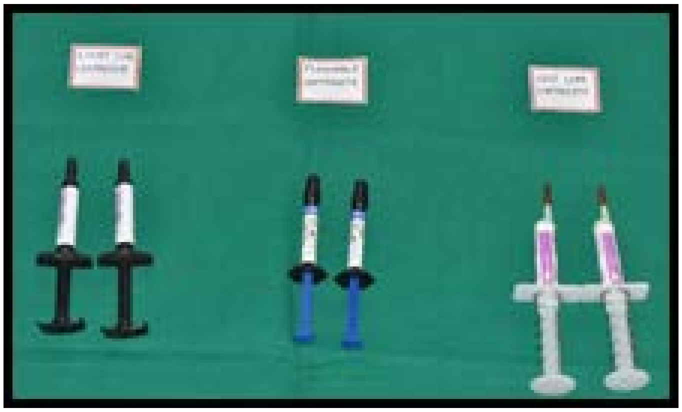

Figure 1: 3 Types of Orthodontic Adhesives Used In the Study: Light Cure, Self-Cure and Flowable Adhesive Resins

A total 24 disc shaped specimens with 5mm diameter and 2 mm thickness were prepared 8 from each resin matrix group. The light cure and Flowable discs were cured for 60 sec. at 0 mm tip distance.

Each prepared disc was individually weighed on an electronic weighing machine to ensure equal weight of all discs. The discs were then immersed separately in 5 ml glass vials containing 4ml of ethanol /water solution (70:30) as shown in **Figure 2**. All the glass vials containing discs were vigorously agitated for 60 seconds and then allowed to settle. Aliquots from each glass vials were tested for BPA release using High Performance Liquid Chromatography (HPLC) unit at different time intervals of 30 min, 24 hours, 1 week and 1 month.

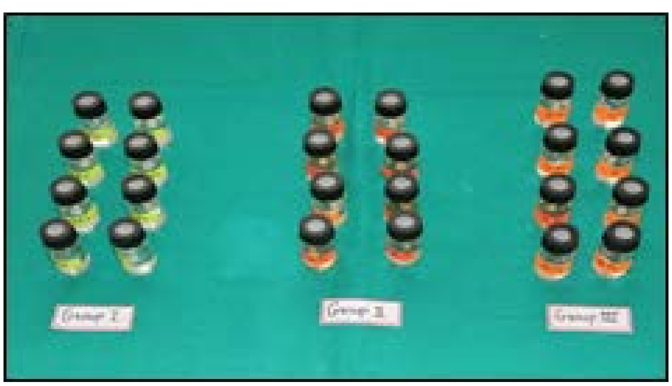

Figure 2: Discs Immersed Separately In 5 Ml Glass Vials Containing 4ml of Ethanol /Water Solution (70:30)

In the present study, JASCO Model:LC-Net II/ADC High Performance Liquid Chromatography system was used to determine BPA release **Figure 3**. The samples were processed with a column EC 250/46 NUCLEOSIL. The mobile phase was carried out with 60:40 mixture of acetonitrile/water solution. The flow rate was 1ml per millimetre. The peak was detected in JASCO-BORWIN Chromatography software which was a UV detector and the wavelength for scanning was kept at 227 nm. **Figure 4** shows HPLC chromatographs of a few samples from each group. The working temperature was kept at 24^{0}C.The limit of detection in Liquid chromatography was 50 microliter.

Figure 3: HPLC Unit (JASCO , MX-2080-31)

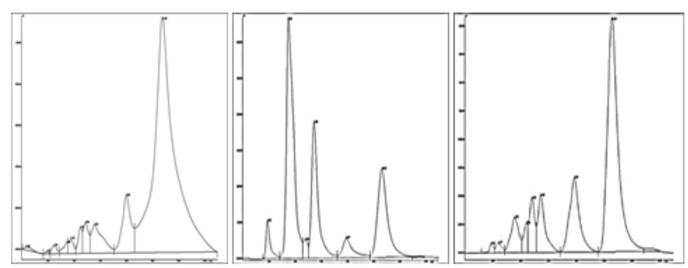

Figure 4: Shows HPLC of Few Samples from Each Group

The amount of BPA released from each specimen was measured and recorded as shown in **Table 1.** Data obtained was compiled on a MS Office Excel Sheet. Data was subjected to statistical analysis using Statistical package for social sciences (SPSS v 21.0, IBM). Inter group comparison (>2 groups) was done using Kruskall Wallis ANOVA followed by pair wise comparison using Mann Whitney U test. Intra group comparison was done using Friedman's test followed by pair wise comparison using Wilcoxon Signed rank test.

For all the statistical tests, $p<0.05$ was considered to be statistically significant.

Table 1: Inter Group Comparision of Mean Values of BPA Release and Standard Deviation Time Intervals

Groups & Time Interval	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum	Chi square value	P value of KW ANOVA
30 I MIN	2	4.542000	.0353553	.0250000	4.5170	4.5670		
II	2	.000000	.0000000	.0000000	.0000	.0000		
III	2	4.579000	.0056569	.0040000	4.5750	4.5830	4.706	.0.095#
Total	6	3.040333	2.3551446	.9614838	.0000	4.5830		
24 I HRS	2	4.471000	.0296985	.0210000	4.4500	4.4920		
II	2	.000000	.0000000	.0000000	.0000	.0000	3.824	.148#
III	2	4.458000	.0353553	.0250000	4.4330	4.4830		
Total	6	2.976333	2.3055577	.9412400	.0000	4.4920		
1 WEEK I	2	4.533500	.0120208	.0085000	4.5250	4.5420		
II	2	.000000	.0000000	.0000000	.0000	.0000	4.706	.095#
III	2	4.462500	.0417193	.0295000	4.4330	4.4920		
Total	6	2.998667	2.3230554	.9483834	.0000	4.5420		
1 MONTH I	2	.00	.000	.000	0	0		
II	2	.00	.000	.000	0	0	0.000	1.000#
III	2	.00	.000	.000	0	0		
Total	6	.00	.000	.000	0	0		

Result

Results showed BPA release from all the specimens of Group I and Group III except Group II at 30 minutes, 24 hours and 1 week time intervals as shown in **Figure 5**. BPA assessment based on Area [µV.Sec] measurement showed more release in Group III compared to Group I, but the differences were not statistically significant ($p > 0.05$). However, BPA release was not detected from any specimen belonging to all 3 groups at 1 month time interval.

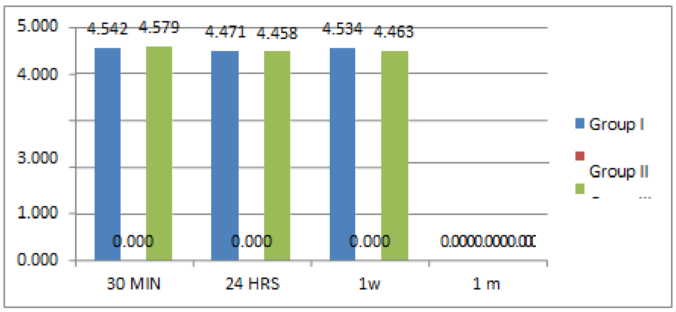

Figure 5: Inter Group Comparison of Mean Value of BPA Release and Standard Deviation at Different Time Intervals

Discussion

Use of BPA, the chemical substance utilized for manufacturing products of epoxy resin and polycarbonate plastics, has dramatically increased in recent years. In dentistry, composite resins for restorative and orthodontic bonding, including pit and fissure sealants are largely based on BPA.[12] Due to this increased use of BPA based materials in our daily life, exposure to this chemical, which is the most common environmental disruptor, has increased conspicuously. The daily human consumption of BPA should be less than 1 µg per kg of body weight, and greater doses may lead to destructive adverse effects on the endocrine system.[13-15] Even though medical research has identified the potential problems associated with increased exposure to BPA, orthodontic research in this regard is scarce.[16]

In the available literature there are very few reports describing research on the chemical stability of orthodontic adhesive resins. In contrast to composite restorative materials, which undoubtedly release potentially harmful chemical compounds into the external environment, it is assumed that orthodontic adhesives are biologically safe.

The phenomenon of basic monomer release from polymerized orthodontic adhesive to the external environment was first described by Eliades et al [17]

To date, evidence from orthodontic literature indicates that degradation of orthodontic adhesives can result in leaching out of BPA and the process is accelerated by mechanical and chemical aging.[3,4] Leached components from monomers or amines used for intiation of polymerization have been associated with a variety of cytotoxic reactions in tissues[9]. In-vitro studies have shown that residual TEGDMA can lead to DNA chain sequence deletion, causing chromosomal anomalies.[18]

The present study revealed that BPA was released from light cure orthodontic adhesives at time intervals of 30 mins, 24 hours and 1 week using HPLC after which it diminished and was not detectable at 1 month time interval. This is in accordance with a few similar studies [19-22] which concluded that the highest concentration of bisphenol A was obtained initially after1 hour and later decreased. Bisphenol A (BPA) release from light cure adhesive was found to be greater with longer distance of curing tip [23]. This could be a result of incomplete polymerization occurring with longer tip distances. However, the results obtained in our study are contradictory to study which concluded that no trace of BPA was identified for any orthodontic adhesive across different time intervals.[24] Very few reports have been published in orthodontic literature regarding release of BPA from self cure adhesives. In the present study no

BPA release was detected from self-cure adhesive at any tested time interval. This is in accordance withthe results of a similar study done by Eliades et al[25] while contradictory to another study which concluded that the amount of BPA release detected from self-cure adhesive was much higher than light cure adhesive.[26]

Information regarding BPA release from Flowable composite resins in past literature is scarce and requires greater research to confirm the associated risk. The present study revealed that BPA was released from Flowable composite resins at short time intervals with no release detected at 1 month. This partly agrees with result of a similar studydone by Kang et al[27] Who found high level of BPA release in saliva only shortly after retainer placement, after which it diminished. In general, thickness of the adhesive resins might predispose for less curing and concomitantly greater leaching. Adhesive resins containing a higher ratio of filler to matrix ratio have greater strength and at the same the time the lower organic content reduces the potential for BPA release. In general, the degree of conversion of adhesives modulates the physical and mechanical properties of the material, particularly solubility and degradation. Decreased conversion results in monomer leaching and the release of substances such as plasticizers and polymerization initiators and inhibitors.[2]

Light cure and self-cure orthodontic-adhesive exposure to the oral environment involves only the peripheral margins of the bracket with an average thickness of 150 to 250 µm, and thus the effect of aging might not be potent. This is unlike flowable composite resins that are commonly used for bonding fixed lingual retainers resulting in exposure of a substantial portion of material to the oral environment, there by maximizing the effect of mechanical and chemical ageing. The oral environment, which is characterized by wide temperature and pH changes, by the presence of digestive enzymes and periodic supply of a variety of chemical compounds, can promote poly-BPA molecule degradation to bisphenol A. Furthermore, it has been confirmed that constituents of orthodontic adhesives exhibit estrogenic actions in cells after simulated debonding procedures.[28] Despite of the relatively large number of studies dealing with the quantitative determination of monomer leaching, the lack of standardized protocols is an obstacle in interpreting the actual amount released, even under simulated clinical conditions.

Conclusion

The results of this study allow formulation of the following conclusion

- In conditions of the current experiment it was demonstrated that BPA is released from light cure as well as flowable composite resins during different time intervals up to the first week after which it diminishes and is undetectable at 1-month time interval.
- BPA release was found to be more with flowable composite resins compared to light cure adhesive.
- Release of BPA was not detected from self-cure composite resins at any time interval, suggesting it to be a safer and better choice of adhesive for orthodontic bonding.

In the context of the results of present study and reports published in the available literature, it seems necessary to undertake further research on the biosafety of use of light cure orthodontic adhesive and flowable composite resins for orthodontic bonding. Alternative materials should be explored to overcome potential concerns arising from the use of bonding materials, till clarity emerges in this regard. According to the precautionary principle, it would be beneficial to reduce exposure to monomer as far as possible.

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