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Assessing the Cytotoxicity of Materials Used as Bonding Agents in Orthodontic Fixed Retainers

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Objectives Fixed orthodontic retainers are pivotal in determining the success of treatment outcomes. However, the bonding materials utilized in their construction are not completely inert. These materials may release bisphenol A (BPA), a compound associated with various health risks. This study aimed to evaluate the cytotoxicity of potential bonding agents used in fixed orthodontic retainers.
Methods In this experimental study, human gingival fibroblasts were cultured and subjected to various dental materials. These materials included acrylic resin (representing removable retainers), Transbond LR, and Enlight LV (which are common adhesives for fixed retainers), as well as Gradia Direct, Direct Flo, and Herculite XRV (commonly used in restorative composites). A control group was also exposed to normal saline. Discs measuring 0.5×1×1 mm were prepared from these materials. The viability of the fibroblasts was assessed using the MTT assay, and a statistical analysis (one-way analysis of variance) was conducted to compare the groups at a significance level of 0.05.
Results The results of statistical analysis indicated no significant difference between the groups in terms of cytotoxicity for human gingival fibroblasts (p= 0.71).
Conclusion None of the tested materials negatively impacted the viability of human gingival fibroblasts. These results provide valuable clinical insights, reassuring orthodontic practitioners about the safety profile of these commonly used materials in the context of fixed orthodontic retention and restorative procedures.
Keywords Appliance; Orthodontic; Cytotoxic agent; Bonding; Dental.

Introduction

Retention plays a pivotal role in orthodontic treatment. It is essential to prevent unwanted tooth movement and preserve the results of treatment. [1] Retention in orthodontics can be achieved through two methods: removable retainers and bonded retainers. [2] Bonded and removable retainers are used under specific circumstances. Bonded retainers are often preferred by patients due to their convenience. They eliminate the need for regular wear and are seen as more aesthetically pleasing. Additionally, they do not require the patient to comply with a wear schedule. [3] While bonded retainers are often the preferred choice for patients, they come with their own set of challenges. These retainers can cause undesired tooth movements and interfere with oral hygiene practices, leading to gingival inflammation. There is also a risk of them becoming detached and loose. [4]

Bonded retainers, designed for permanent use, are continuously exposed to the oral environment and oral fluids. [5] It is important to note that these retainers are not completely inert. Resin-based materials, when used in the oral cavity, have been linked to a number of adverse effects. These effects can be both localized and systemic, resulting from the release of reactive substances into the oral cavity. These substances can include residual monomers, oxidation byproducts, and catalysts among others. [6] Specifically, the presence of bisphenol A (BPA) in the oral environment should be regarded as a potential health risk. [7]

BPA is frequently used in the production of polycarbonate plastics and epoxy resins. These materials are extensively used in a variety of modern applications, including the packaging of food and beverages. [8] In the field of dentistry, a significant number of orthodontic splints and plastic brackets are constructed from a polycarbonate matrix, which is produced using BPA. [9] Significantly, BPA has been associated with a range of health issues. These include hormonal imbalances, obesity, diabetes, asthma, behavioral changes, cancer, infertility, and genital anomalies. [10] The European Food Safety Authority (EFSA) has established the tolerable daily intake of BPA at 50 micrograms per kilogram of body weight. [11]

Given the significant role of fixed retainers in the success of orthodontic treatment and the potential toxicity of BPA on living cells, it becomes crucial to consider the quantity of BPA that might be released from these retainers. [9] Recent findings indicate that none of chemically cured and light-cured orthodontic adhesives used for bracket bonding show a detectable release of BPA, with levels falling below 0.1 ppm. Furthermore, these adhesives do not exhibit estrogenic activity. However, it is important to consider the extensive use of lingual fixed retainers, which require long-term exposure to the oral cavity. These retainers are in direct contact with the oral mucosa for extended periods, potentially allowing for the slow release of significant amounts of reactive substances. Therefore, this study aimed to evaluate the cytotoxicity of materials potentially used as bonding agents in orthodontic fixed retainers.

Methods and Materials

The study method was approved in the research committee of Tabriz University of Medical Sciences (code 398). Human gingival fibroblasts, obtained from the Pasteur Institute in Tehran, Iran, were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, NY, USA). The culture medium was supplemented with 10% fetal bovine serum (FBS; Gibco, NY, USA) and 1% penicillin-streptomycin (Gibco, NY, USA). [12] The cells were then incubated in a 96-well plate, with a density of 3000 cells per well for 24 hours at a temperature of 37ºC in a 5% CO2 atmosphere.

The materials evaluated in this study included acrylic resin, restorative composites, and bracket adhesives. Acrylic resin was tested for cytotoxicity as it is commonly used in removable Hawley retainers. Adhesives, such as Transbond LR (3M Unitek) and Enlight LV (Ormco), were tested since they are frequently used for bonding fixed retainers. Additionally, materials widely used in restorative dentistry, including Direct Flo, GC Gradia, Gradia Direct, GC Gradia, and Herculite XRV (Orange, Kerr/Sybron, CA, USA), were assessed for cytotoxicity.

In group 1, acrylic resin (Berkshire) was prepared as discs with dimensions of 0.5×1×1 mm (n=12). They were placed between two glass plates and cured for 20 seconds in a pressure pot. In group 2, light-cured resin-bonded composite (Transbond LR, 3M Unitek) was similarly prepared into 0.5×1×1 mm discs (n=12) and placed between two glass plates. The curing process for this group lasted 20 seconds, utilizing the LITEX 680A Curing Light (Dentamerica Inc., CA, USA). The power output during this process was approximately 650 mw per square centimeter, with a wavelength of 468 nm.

In a similar manner, for group 3, bonded retainers were fabricated using a specific light-cured resin-bonded composite (Enlight LV, Ormco). These were shaped into 0.5×1×1mm discs (n=12) and positioned between two glass plates. The same curing procedure as previously described was applied to this group. For group 4, a flowable restorative resin-bonded composite (Direct Flo, GC Gradia) was prepared into 0.5×1×1mm discs (n=12) and placed between two glass plates. The curing method used was identical to the one mentioned earlier.

For group 5, a restorative light-cured microfilled hybrid composite resin (Gradia Direct, GC Gradia) was prepared into 0.5×1×1mm discs (n=12). These were placed between two glass plates and cured using the previously described procedure. Similarly, in group 6, a restorative resin-bonded composite (Herculite XRV, Orange, Kerr/Sybron, CA, USA) was shaped into 0.5×1×1mm discs (n=12) using the same method. Finally, as a control group, fibroblasts were cultured in a complete medium without any treatment.

The composites were sterilized under the ultraviolet light of a laminar hood for 30 minutes. Each block was then stored in an equivalent amount of working culture medium for 24 hours at 4ºC. Following this, the cells were cultured with the conditioned media in 12 wells, repeating this process for each group. After a 24-hour incubation period, the conditioned medium was replaced with 200 µL of FBS-free DMEM medium. Then, 20 µL of a 4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (MTT; Sigma, USA) at a concentration of 5 mg/mL was added to each sample well and incubated for four hours. Subsequently, 100 mL of dimethyl sulfoxide was added to each sample to dissolve the formazan crystals. After 30 minutes in a dark room, the optical density was recorded using an ELISA reader at a wavelength of 540 nm. [13]

The data was analyzed using a one-way analysis of variance (ANOVA). This analysis was conducted using GraphPad Version 8. A P-value of less than 0.05 was considered to indicate statistical significance.

Results

Table 1 provides a detailed overview of the mean and standard deviation (error bars represent SEM) values for each group.

Table 1: Quantities of BPA Release Across the Groups

Material	Group code	SD	Mean
Acrylic resin	G1	0.072	0.794
Transbond LR	G2	0.07	0.805
enLight Ormco	G3	0.091	0.74
Gardia GC flow	G4	0.061	0.775
GCGradia composite	G5	0.074	0.768
LightBond composite	G6	0.071	0.767
Complete medium	CON	0.021	0.752

The ANOVA analysis revealed no statistically significant difference among the groups, as indicated by a P-value of

0.7106. This suggests that the cytotoxicity levels across the different materials tested are comparable. Figure 1 provides a visual representation of these findings, displaying a bar chart of the group means with error bars denoting standard deviations. This graphical illustration aids in understanding the data distribution and underscores the lack of significant differences among the groups studied (Figure 1).

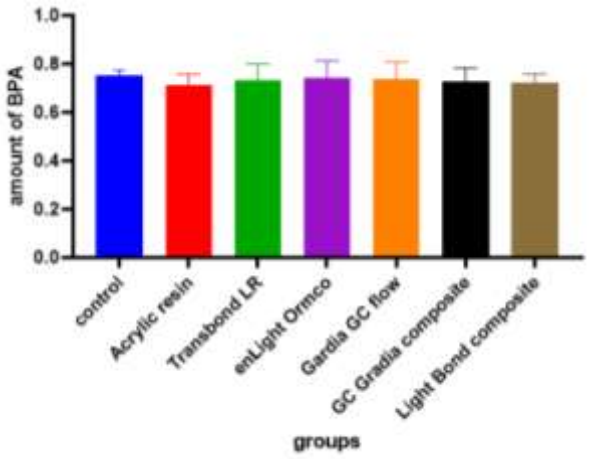

Figure 1: Illustration depicting BPA release among different groups. Blue: control. Red: Acrylic resin. Green: Transbond LR. Purple: enLight Ormco. Orange: Gradia GC flow. Black: GC Gradia composite. Brown: Light bond composite

Discussion

Fixed lingual retainers are crucial in maintaining the results of orthodontic treatment. Therefore, it is essential to assess the potential release of BPA from these retainers, considering their potential toxicity to living cells. [14] Fixed lingual retainers, compared to orthodontic brackets, contain a greater volume of composite adhesives and are typically kept in the mouth for extended periods. As a result, evaluating the release of BPA from these retainers is particularly important to ensure the safety and well-being of patients undergoing orthodontic treatment.

This study investigated six materials that are commonly utilized in dentistry and orthodontics. Notably, Transbond LR (3M Unitek) and Enlight LV (Ormco) are often used as adhesives for bonding lingual fixed retainers. Acrylic resin, a material frequently used in the fabrication of removable orthodontic retainers, was also examined. Other materials, such as Gradia Direct (GC Gradia), Direct Flo (GC Gradia), and Herculite XRV (Orange, Kerr/Sybron CA, US), which are used in tooth-colored restorations, were included due to their similar exposure conditions to lingual arch bonding.

The aim of this study was to compare the cytotoxicity of each material group with a control group, which was cultured in a complete medium. The findings revealed no significant difference between the cytotoxicity of study groups and the control group. However, Bationo et al,[15] Eliades et al, [16] and Moreira et al. [17] found that BPA was released in noticeable amounts. In the study by Bationo et al, [15] they used different light-cured composite resins for fixed retainer bonding from the ones used in the current research, which might explain the difference between the results. Also, in the study by Eliades et al,[16] the method of measuring BPA release was gas chromatography-mass spectroscopy analysis. Therefore, the observed differences between the studies could be attributed to the variations in the methods used for measurement.

Based on animal studies conducted by Jedeon et al. in 2013, it was found that exposure to BPA significantly affected amelogenesis, resulting in permanent enamel defects. [18-19] However, it is important to consider that the metabolism of BPA in rodents is significantly different from that in humans. Additionally, the concentration of BPA in rodents used in those studies is considerably higher than the levels typically observed in human subjects. [20] In that review study, the researchers determined that the release of BPA from dental composites used in restorative procedures has a negligible impact within clinical settings. Interestingly, the present study had similar findings, thereby corroborating the minimal influence of BPA in the application of dental materials. [20] The divergence in findings between the present study and the one conducted by Moreira et al. [17] may be due to the differences in the dimensions of the samples. In the present study, smaller samples were utilized, specifically discs measuring 0.5×1×1 mm. Conversely, Moreira et al. used larger samples for composite restoration materials, with a diameter of 5 mm and a thickness of 3 mm. This discrepancy in sample dimensions could potentially account for the variation in the results observed.

A key limitation of this study is the brief exposure duration, which may not accurately represent the long-term effects of the materials under investigation. This limitation should be kept in mind when interpreting the study results and drawing conclusions. Future research involving larger sample sizes and extended exposure periods would be advantageous for a more thorough understanding of the effects of these materials.

Conclusion

The present study found that the viability of human gingival fibroblasts was not adversely affected by the materials tested. However, it is important to exercise caution when considering the risks associated with BPA exposure. The study focused solely on cell viability, without examining the potential cellular toxicity or estrogenic effects of BPA. The results could be influenced by prolonged oral exposure to these materials.

Conflict of Interest

No Conflict of Interest Declared ■

References

1. Alassiry AM. Orthodontic retainers: A contemporary overview. J Contemp Dent Pract.. 2019; 20: 857-62.
2. Proffit WR, Fields HW, Larson B, Sarver DM. Contemporary orthodontics. Philadelphia: Elsevier. 2019; Chap18: 579-99.
3. Segner D, Heinrici B. Bonded retainers–clinical reliability. J Orofac Orthop. 2000; 61: 352-8.
4. Marinelli G, Inchingolo A, Inchingolo A, Malcangi G, Limongelli L, Montenegro V, et al. White spot lesions in orthodontics: prevention and treatment. A descriptive review. J Biol Regul Homeost Agents. 2021; 35: 227-40.
5. Chantarawaratit P-o, Yanisarapan T. Exposure to the oral environment enhances the corrosion of metal orthodontic appliances caused by fluoride-containing products: Cytotoxicity, metal ion release, and surface roughness. Am J Orthod Dentofacial Orthop. 2021; 160: 101-12.
6. Kloukos D, Sifakakis I, Voutsa D, Doulis I, Eliades G, Katsaros C, et al. BPA qualtitative and quantitative assessment associated with orthodontic bonding in vivo. Dent Mater. 2015; 31: 887-94.
7. Malkiewicz K, Turlo J, Marciniuk-Kluska A, Grzech-Lesniak K, Gasior M, Kluska M. Release of bisphenol A and its derivatives from orthodontic adhesive systems available on the European market as a potential health risk factor. Ann Agric Environ Med. 2015; 22.
8. Eliades T. Bisphenol A and orthodontics: An update of evidence-based measures to minimize exposure for the orthodontic team and patients. Am J Orthod Dentofacial Orthop. 2017; 152: 435-41.
9. Halimi A, Benyahia H, Bahije L, Adli H, Azeroual M-F, Zaoui F. A systematic study of the release of bisphenol A by orthodontic materials and its biological effects. Int Orthod. 2016; 14: 399-417.
10. Becher R, Wellendorf H, Sakhi AK, Samuelsen JT, Thomsen C, Bølling AK, et al. Presence and leaching of bisphenol a (BPA) from dental materials. Acta Biomater Odontol Scand. 2018; 4: 56-62.
11. Sabour A, El Helou M, Roger-Leroi V, Bauer C. Release and toxicity of bisphenol-A (BPA) contained in orthodontic adhesives: A systematic review. Int Orthod. 2021; 19: 1-14.
12. Jung YS, Ro ST, Kang SW, Lee H, Lee JS, Chae YK, et al. Bisphenol A release from commercially available 3-dimensionally printed resins and human cell apoptosis to bisphenol A: an in-vitro study. J Clin Pediatr Dent. 2023; 47: 89-95.
13. Sigaroodi F, Shafaei H, Karimipour M, Dolatkhah MA, Delazar A. Aloe vera/collagen mixture induces integrin α1β1 and PECAM-1 genes expression in human adipose-derived stem cells. Adv Pharm Bull. 2019; 9: 662.
14. Iliadi A, Koletsi D, Papageorgiou SN, Eliades T. Safety considerations for thermoplastic-type appliances used as orthodontic aligners or retainers. A systematic review and meta-analysis of clinical and in-vitro research. Materials. 2020; 13: 1843.
15. Bationo R, Rouamba A, Diarra A, Beugré-Kouassi MLA, Beugré JB, Jordana F. Cytotoxicity evaluation of dental and orthodontic light-cured composite resins. Clin Exp Dent Res. 2021; 7: 40-8.
16. Eliades T, Voutsa D, Sifakakis I, Makou M, Katsaros C. Release of bisphenol-A from a light-cured adhesive bonded to lingual fixed retainers. Am J Orthod Dentofacial Orthop. 2011; 139: 192-5.
17. Moreira MR, Matos LG, de Souza ID, Brigante TAV, Queiroz MEC, Romano FL, et al. Bisphenol A release from orthodontic adhesives measured in vitro and in vivo with gas chromatography. Am J Orthod Dentofacial Orthop. 2017; 151: 477-83.
18.Jedeon K, Loiodice S, Marciano C, Vinel A, Canivenc Lavier MC, Berdal A, et al. Estrogen and bisphenol A affect male rat enamel formation and promote ameloblast proliferation. Endocrinology. 2014; 155: 3365-75.
19.Jedeon K, De la Dure-Molla M, Brookes SJ, Loiodice S, Marciano C, Kirkham J, et al. Enamel defects reflect perinatal exposure to bisphenol A. Am J Pathol. 2013; 183: 108-18.
20.Schmalz GH, Widbiller M. Biocompatibility of Amalgam vs Composite-A Review. Oral Health Prev Dent. 2022; 20: 149-56.

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