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In-vitro Assessment of Bisphenol a Release from Orthodontic Retainers

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Abstract

Background: The adhesives used to bond lingual bonded retainers are low-loaded composite resins with a resinous matrix containing bisphenol A and other molecules. To investigate and quantify the release of BPA from these retainers.

Methods: Three types of orthodontic bond were selected randomly from the most popular orthodontic composites: Transbod XT, Master-Dent® Flow and Nanocomposite Proclinic Expert. Specimens were made using a metallic parallelepiped mold and polymerized for 20 seconds with an LED lamp. Each cured sample was immediately immersed in artificial saliva with different pH levels (7, 4, 2). Samples were analyzed at t0, t1 and t2 using a High-Performance Liquid Chromatography. Data were analyzed using a statistical package for social sciences. One-factor Anova and Anova for repeated measurements tests were used. A value of p less than 0.05 was considered statistically significant.

Results: The analyses showed BPA release from Proclinic, although both Transbond XT and Master-Dent[®] didn't show any release of BPA in saliva (p>0.05). Release from Proclinic was influenced by the pH of medium (p<0.05) and the immersion time (p<0.05), thereby the more the pH decreased the more the amount of BPA increased (p<0.05) and as the immersion time increased the amount of BPA increased (p<0.05). The maximum amount of BPA reached from Proclinic was 0.680µg/ml and 1.032µg/ml at t2-Ph4 and t2-Ph2 respectively.

Conclusions: The different amounts of BPA released from Proclinicremain relatively lower than the tolerable daily intake of BPA, but clinicians should be careful about the use of composites.

Keywords: Bisphenol A, Composites resin, Fixed retainers, In-vitro study, Orthodontic

Introduction

Currently, it is accepted that a retention phase is essential for stability of orthodontic treatment results and the application of a suitable retention method is important both for prevention of relapse and for increasing patient satisfaction[1]. Bonded lingual retainers have been used in orthodontics since the 1970s. These retainers are composed generally of metallic wires bonded to enamel with an adhesive [2,3]. Seen the complications related with lingual bonded retainers during the retention period, the retainers werekeptin mouth as long as possible[1,4]. The adhesives used to bond orthodontic retentions are low-loaded composite resins with a resinous matrix containing bisphenol A (BPA) and various other molecules. BPAis a synthetic component used in the manufacture of plastics since the 1950s[5]. This component is widely used in the dental practice. In fact, BPA and its derivatives are present in almost 80% of the composites used in dentistry [5]. Many prospective studies have focused on the biological effects generally observed in animals, especially at the endocrine level [6,7]. Carcinogenic effects [8,9], on the brain and behavior [10-13]. On obesity [14,15], on diabetes [16,17], on the cardiovascular system [18-21]. on the immune system and the enamel of teeth [22-24]. The tolerable daily intake (TDI) of BPA was reduced from 50 to 4mg/kg/day in 2015. BPA is banned for food contact in some European countries [25-26].

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In order to improve our practice and to protect the health of our patients during the retention period, we wanted to investigate and quantify the possible release of BPA from orthodontic bond used for fixed retainer.

Materials and Methods

Three types of orthodontic bond manufactured by different companies were selected randomly from the most popular orthodontic composites used in our clinic for bonding orthodontic wire retainers: Transbod XT (3M Unitek, Monrovia, Calif, USA), Master-Dent® Flow (Dentonics Inc, Monroe, USA) and Nanocomposite Proclinic Expert(CHL Medical solutions, Cavallotti, Milán – Italia). Composite samples were made using metallic parallelepiped molds (length of 18mm, thickness of 8mm)to better simulate the quantity of adhesive applied toan orthodontic retainer. Each sample was cured for 20seconds using a curing light with a LED lamp of 1000mw/cm2respecting a minimal distance of 1 mm between the light and the composite[27]. Each cured sample was immediately immersed in a solution of artificial saliva at different pH levels (pH7, pH4, and pH2) for 1 hour in a glass tube at 37°C. The saliva formula used for this study was Saliveze [28, 29], a therapeutic artificial saliva whose properties are close to those of real human saliva (Table 1). A total of 27 samples were analyzed att0, t1 and t2(Table 2) using a High-Performance Liquid Chromatography (HPLC). Thus, in each glass tube of 1ml of saliva, 1ml of acetonitril was added, then passed through a magnetic stirrer (vortex) for 15secs, the mixture was centrifuged at 1600rpm for 8min with a temperature of 6°C. The supernatant was filtered with a 0.2µm laying cellulose filter. Each 20µL of filtered solution was injected into the HPLC system for analysis. The characteristics of the HPLC used are as follows: a phenomenex synergi type column (150*2mm; 4 µm), a mobile phase H2O/Acetonitrile, a flow rate of 0.5ml/min, a column temperature at 35°C, the injection volume wasat 20µl and the detector was fluorescent with $\lambda ex=230$ nm and $\lambda em=316$ nm.

Statistical Analysis

The descriptive statistic of the items investigated obtained from the initial, t1and t2were presented. Quantitative values with a normal distribution are presented as means and standard deviation ($M\pm$ SD). Data were processed using the statistical package for social sciences, One-factor Anova test was carried out to compare the statistical means between the different independent groups. Anova test for repeated measurements was executed to compare the statistical means between the different matched groups. A value of P less than 0.05 was considered statistically significant.

Results

The quantitative and qualitative analyses showed no BPA release from both Transbond XT and Master-Dent® in artificial saliva at the 3 level of pH between t0 and t2 (p>0.05). A variable release of BPA was observed with Proclinic. The descriptive analysis and statistical comparisons of BPA release between the composites studied is presented intable 3a and b. Figures 1, 2 and 3showed BPA release curve from the different composites. Release from Proclinic was influenced by the pH of medium (p<0.05) and the immersion time (p<0.05), thereby the more the pH decreased the more the amount of BPA increased (p<0.05) and as the immersion time

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increased the amount of BPA increased (p<0.05). The maximum amounts of BPA reached in saliva from Proclinic was 0.680μ g/ml and 1.032μ g/ml at t1-Ph2 and t2-Ph2 respectively (table3).

Discussion

We followed the gold standards for sample preparation, material selection and biological testing. Liquid phase chromatography and mass spectrometry showed release of BPA in Proclinic, although both Transbond XT and Master-Dent® showed no BPA release in artificial saliva with the 3 level of pH between t0 and t2 (p>0.05). Release from Proclinic was influenced by the pH of medium (p<0.05) and the immersion time (p<0.05). The maximum amounts of BPA reached in saliva from Proclinic was 0.680µg/ml and 1.032µg/ml at t1-Ph2 and t2-Ph2 respectively. A comparison of published results was limited because there are diversity of protocols and inhomogeneity of methods analysis.

Our study did not show the release of BPA from Transbond XT. This result agrees withBationo et al [30], they didn't observe any release of BPA from Transbond XT, Transbond LV and MonoLok 2 in a Milli-Q water solution using a gas phase chromatography and mass spectrometry (GCMS) with a detection threshold of 0.02 ppm. They didn't study Master-Dent® and Proclinic but they highlighted the release of BPA from the Blugloo adhesive. Our result agrees also with Pelourde et al[31], they also didn't observe release from Transbond XT and Transbond LR above the detection threshold of 0.02 ppm using a GCMS method. But they highlighted the release of others several potentially toxic compounds. Our result agrees toowithEliades et al[32]and Malkiewicz et al[33]. Malkiewicz [33] didn't detect BPA in Transbond XT after 1 hour, 24 hours, 7 days and 31 days of material sample storage in water using the HPLC method, but the Resilence released 32.10 ppm of BPA at 1 hour. Eliades[31]didn't observe any release from Rely-a-bond and Reliance above a detection threshold of 0.1 ppm using a HPLC method at 1, 7, 21, 35 days of immersion in a pure alcohol solution medium.

However, regarding Transbond XT our result didn'tagree with Sunitha et al[34], Purushothaman et al[35], Eliades et al [36], Deviot et al[37], Kotyk and Wiltshire[38].

Sunitha[34] detected measurable amounts of BPA in all samples of Transbond XT at 1, 7, 21, and 35 days above a detection threshold of 0.1 ppm using a HPLC method; the maximum amount found reached 65.67 ppm at 21 days. The BPA release from Transbond XTwas greater when the composite was cured with a greater light-curing tip distance. Purushothaman[35] founded that, the light-emitting diode device (LED) demonstrated significantly less BPA release. The chemically cured group showed significantly less BPA release. Among the light-cured composites, those cured according to the manufacturers' recommendations released less BPA than did the self-etching primer and the moisture insensitive primer. Eliades[34]confirmed these results by identifying measurable amounts of BPA in all groups of Transbond XT studied in a double-distilled water for Immersion medium, using a GCMS method; with the highest found in

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the immersion media of the 1-month groups, whereas the control (tooth storage solution) had 0.16 mg/L.

Deviot[37]confirmed this result by detecting the BPA in all samples from Transbond XT stored in three extraction media: artificial saliva, acetonitrile and methanol, using a GCMS method. But, with liquid chromatography/mass spectrometry, BPA was detected only from samples immersed in methanol; Bis-GMA was detected, in varying amount according to the extraction media and the light-curing time. BPA was found after heating of the Bis-GMA solution. Kotyk and Wiltshire [38]observed BPA release from Transbond XT immerged in Artificial saliva of 0.34 ppm at 3 days using a GCMS method.

Our study showed the release of BPA in Proclinic, although LCMC showed no release of BPA from Master-Dent® in artificial saliva with the 3 level of pH between t0 and t2. A comparison of published results was impossible because there are no studies in literature about these composites. Kang [39] founded a significant high level of BPA from Filtek Flow (nanohybrid-filled flowable resin) and Z250 Universal Restorative (hybrid-filled restorative resin) in the saliva collected just after placement of the lingual bonded retainer light-cured by using a plasma xenon arc light curing unit (wavelength, 450-500 nm; intensity, 1900 mw/cm2) by radiating the occlusal and gingival surfaces of the resin for 3 seconds each. Flowable resin had lower BPA levels than those in the conventional hybrid resin. This result was performed using a HPLC method.

To minimize exposure for patient and staff to BPA, Eliades [40] suggested same measures like bonding with glass-ionomer cements; request details on BPA; optimize polymerization conditions (sufficient light exposure times, close proximity of tip to bracket and adequate light intensity); have the patient rinse with copious amount of water after bonding; use a sufficient adhesive hardness. For Debonding, Select a bracket base mesh-adhesive filler content combination that results in a resin cohesive/enamel-adhesive fracture, remove as much material as possible without using rotary instruments, during resin grinding have access to fresh air, use surgical suction and masks for all staff pregnant staff and operators must avoid continuous and long-term exposure to the aerosols produced during debonding, operatories with multiple dental units in the same area (open-bay plan) must allow sufficient space between chairs and avoid scheduling concurrent debonding appointments in multiple chairs [41,42]. Additional research is needed to determine the magnitude of change from pre-to post-dental treatment and the trajectory of uBPA concentrations posttreatment.[43] Purushothaman [35] suggested curing time should be according to the manufacturer's recommendations. These steps will ensure less BPA release and a greater degree of conversion. Sunitha [34] suggested the clinicians should ensure that the adhesive is completely cured by keeping the light-curing tip as close to the adhesive as clinically possible.

Conclusion

Under the conditions of this study, the analysis of the three orthodontic bonds used for fixed retainers allows us to highlighted the absence of BPA release from both Transbond XT and

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Master-Dent® in artificial saliva at the 3 level of pH between t0 and t2.A variable release of BPA was observed with Proclinic. Release from Proclinic was influenced by the pH of medium and the immersion time (p<0.05). The maximum amount of BPA reached from Proclinic was 1.032μ g/ml in saliva, Ph2 after 1 hour. The different amounts observed remain relatively lower than the reference dose for daily ingestion. The results of this study might have significant clinical implications by showing a variable BPA release from Proclinic influenced medium that can overcome some of the limitations of study. Clinicians should be careful about the type of composite used and must follow the guidelines of good practice.

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Disclosure of interest: The authors declare that they have no conflicts of interest concerning this article.

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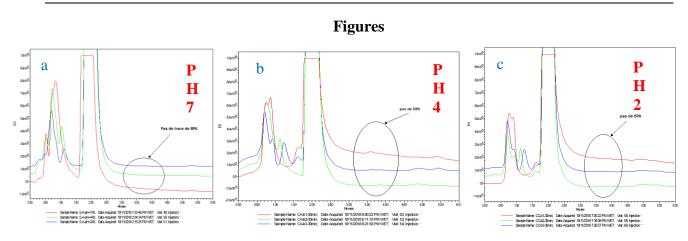


Fig. 1. Release of BPA from the **Transbond XT** in artificial saliva at pH7 (a), pH4 (b) and pH2 (c)

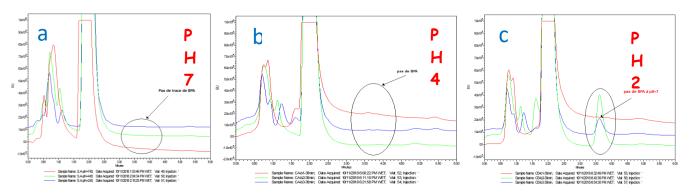


Fig. 2. Release of BPA from the Master-Dent® in artificial saliva at pH7 (a), pH4 (b) and pH2 (c)

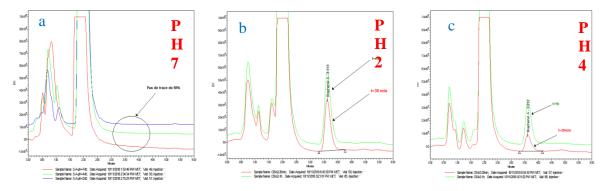


Fig. 3. Release of BPA from the **Proclinic** in artificial saliva at pH7 (a), pH4 (b) and pH2 (c)

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Component	Quantity en g/L/
Sodium carboxymethylcellulose	10
Potassium chloride	0.62
Sodium chloride	0.87
Magnesiumchloride	0.06
Calcium chloride	0.17
Di-potassium hydrogenorthophosphate	0.80
Potassium di-hydrogenorthophosphate	0.30
Sodium fluoride	0.0044
Sorbitol	29.95
Methyl p-hydroxybenzoate	1.00
Spirit of lemon	5 mL

Table 1: Saliveze formula, borrowed from Preetha and Banerjee.

Table 2: The different samples of composites under study

Code	Company (type)	PH	Time	Sample	Total of sample	
I	Transbond XT	PH7	T0(0mm)	9	27	
Π	Proclinic Nanohybrid "Expert"	PH4	T1(30mn)	9		
III	Master-Dent [®] Flow Composite	PH2	T2(1h)	9		

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Tables 3: Statistical comparisons of the amount of BPA (μ g/ml) released by the three composites studied depending of time and the pH of the immersion medium

	pH7			
	t0 (Initial)	t1 (30mn)	t2 (1h)	p value
3M Unitek, n=9	0.000001 ± 0.000001	0.000003±0.000004	0.000004±0.000001	0.6
Proclinic, n=9	0.000015 ± 0.000001	0.000020 ± 0.000004	0.000025±0.000003	0.5
Master-Dent, n=9	0.000011 ± 0.000001	0.000013±0.000004	0.000015±0.000002	0.4
	pH4			
	t0 (Initial)	t1 (30mn)	t2 (1h)	p value
3M Unitek (n=9)	0.00001 ± 0.000001	0.00001 ± 0.000004	0.000014 ± 0.000005	0.5
Proclinic (n=9)	0.00014 ± 0.00005	0.198±0.051	0.561±0.031	0.000*
Master-Dent	0.00001±0.000001	0.00001±0.000004	0.000012±0.000005	0.6
(n=9)				
	pH2			
	t0 (Initial)	t1 (30mn)	t2 (1h)	p value
3M Unitek (n=9)	0.00001 ± 0.000001	0.00001 ± 0.000002	0.000014±0.000003	0.6
Proclinic (n=9)	0.00015 ± 0.00005	0.680 ± 0.02	1.032±0.04	0.000*
Master-Dent	0.00001 ± 0.000001	0.00001 ± 0.000004	0.000014 ± 0.000005	0.5
(n=9)				

a: One-factor Anova test: *: statistically significant because p less than 0.05

	3M Unitek (n=9)		Proclinic (n=9)		Master-Dent (n=9)	
	Groups appariés	p value	Groups appariés	p value	Groups appariés	p value
pH7	t0-t1	0.5	t0-t1	0.6	t0-t1	0.5
	t1-t2	0.7	t1-t2	0.6	t1-t2	0.7
pH4	t0-t1	0.5	t0-t1	0.000*	t0-t1	0.5
	t1-t2	0.7	t1-t2	0.000*	t1-t2	0.7
pH2	t0-t1	0.5	t0-t1	0.000*	t0-t1	0.5
	t1-t2	0.7	t1-t2	0.000*	t1-t2	0.7

b: Anova test for repeated measurements, *: statistically significant because p less than 0.05