



Original Article

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# Biodegradation of orthodontic composites by *Streptococcus mutans*: An *in vitro* qualitative and quantitative assessment

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# ABSTRACT

**Objectives:** The purpose of this study was to evaluate the degradation products of orthodontic composites (Grengloo, Blugloo, Transbond XT, and Transbond LR) by *Streptococcus mutans* and then to quantify the levels of released bisphenol A (BPA) using gas-phase chromatography and mass spectrometry (GC–MS).

**Materials and Methods:** Orthodontic light-cured composite discs were incubated at  $37^{\circ}$ C in brain heart infusion (BHI) (control group) and in a culture of *S. mutans* with BHI (test group). Incubation solutions were collected every 48 h in each group and replaced with fresh solutions. These incubation solutions were accumulated and grouped. The assessment of degradation products from composites was done at 1 and 30 days. Detected BPA was then quantified. The limit of quantification was 0.01 µg/mL.

**Results:** Degradation products were present at day 30. For the test group, BPA was detected in Blugloo at day 1 (0.38  $\mu$ g/mL) and triethylene glycol dimethacrylate (TEGDMA) was detected in Grengloo and Transbond LR at day 1.

**Conclusion:** *S. mutans* can hydrolyze long-term orthodontic composites. Monomers such as BPA and TEGDMA may be present in degradation products. It is possible to separate and identify leaching compounds by GC–MS technique.

**Keywords:** Orthodontic composites, Bisphenol A, Triethylene glycol dimethacrylate, *Streptococcus mutans*, Gas-phase chromatography and mass spectrometry

# INTRODUCTION

Brackets bonding has become a daily act in orthodontics since Newman has proposed in 1965 to paste directly orthodontic brackets using an epoxy resin in replacement of sealing systems.<sup>[1]</sup>

The composite resins used in dentistry or orthodontics are complex polymers containing a variety of monomers, initiators, activators, stabilizers, plasticizers, and other additives. Two monomers are mainly used in orthodontic adhesive resins: Bisphenol A (BPA) diglycidyl dimethacrylate (Bis-GMA) and triethylene glycol dimethacrylate (TEGDMA). BPA is used as a raw material for the formulation of Bis-GMA.<sup>[2]</sup>

Several monomers can enter the manufacturing of composite resins. In general, the main used monomer is Bis-GMA. However, its high viscosity prevents optimum handling. It should be

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diluted with other monomers such as TEGDMA or urethane dimethacrylate (UDMA).<sup>[3]</sup>

The proportions of the monomers vary considerably according to the products and their clinical use. This is a proprietary trade secret maintained by the manufacturers.

In the intraoral environment, composite resins are exposed to extreme thermal changes, pH variances, mechanical erosion, and degradation occurrence from bacterial and salivary enzymes, which can cause BPA release.<sup>[4,5]</sup>

Several monomers contained in composite resins (such as Bis-GMA, ethoxylated bisphenol A dimethacrylate [Bis-EMA], UDMA, and TEGDMA declared by manufacturer) are known to diffuse from partially polymerized materials and to be cytotoxic *in vitro*.<sup>[6,7]</sup>

The toxicity of resin-based materials is due to residual monomers as well as to the degradation products linked to the activity of salivary esterases.<sup>[8,9]</sup>

BPA is an endocrine disruptor with potential toxicity *in vitro*<sup>[10]</sup> and *in vivo*.<sup>[11]</sup>

Other compounds including TEGDMA and Bis-GMA, released by restorative and bonding composites, also present potential toxicity.<sup>[12-19]</sup> Infants, young children, and pregnant or lactating women are the most sensitive.<sup>[20]</sup>

*Streptococcus mutans*, one of the primary inhabitants present in saliva and at the restoration material-tooth interface, is regarded as the chief etiological agent responsible for dental caries.<sup>[21,22]</sup>

During the demineralization of the enamel organ, it is possible to observe white opaque lesions of the enamel to the junction between the brackets and the enamel. These lesions are minimal in general and not extended.<sup>[23]</sup>

The demineralization process is fast and can appear in the 4<sup>th</sup> week of orthodontic treatment. These lesions are mainly related to inadequate plate control and ill-fitting orthodontic attachments.<sup>[24]</sup>

In orthodontics, because of the many sites of bacterial retention, the amount of *S. mutans* is increased during fixed treatments.<sup>[25-31]</sup>

*Streptococcus* species have been shown to contain esterases.<sup>[32]</sup> A study by Lara-Carrillo *et al.*<sup>[27]</sup> highlights the change in the saliva flow and the saliva buffer with the bonding of the orthodontic attachments.

It has been shown that the enzyme activity of saliva<sup>[33,34]</sup> and bacteria<sup>[34-36]</sup> has an impact on composite resins. This degradation of composite resins is done by an activity that affects the ester links of the organic matrix. These ester links are present in Bis-GMA and TEGDMA. In addition, the release of some products through the degradation of

composite resins can have an effect on the growth of some bacteria species of *Streptococcus* and *Lactobacillus*.<sup>[37]</sup>

The purpose of this study was to evaluate the degradation products of orthodontic composites by *S. mutans* and then to quantify the levels of released BPA using gas-phase chromatography and mass spectrometry (GC–MS).

# MATERIALS AND METHODS

### **Biodegradation experiment**

Orthodontic light-cured composites were used for this study [Table 1]. Resin discs (10 mm in diameter and 1 mm in thickness) were prepared following the ISO 10993–12:2012 standard for medical device testing in biologic systems (n = 4 per resin).<sup>[38]</sup> They were then cured for 20 s using BA Optima 10 LED Curing Light (light intensity 1000 ~ 1200 mW/cm<sup>2</sup> and wavelength 420 ~ 480 nm).

*S. mutans* strain (ATCC 25175 from Kwik-Stik microorganisms) was cultured on LB agar medium supplemented with brain heart infusion (BHI) broth.

The resin discs (n = 2 per resin and group) were incubated in 12-well plates at 37°C [Figure 1]. Each well was filled with 400 µL, either of BHI (control group) or *S. mutans* in BHI (test group). Incubation solutions were collected every 48 h in each group and replaced with fresh solutions. These incubation solutions were accumulated and grouped. The assessment of degradation products from the composites was done at day 1 and day 30.<sup>[35]</sup> Bacitracin solution was added to the BHI to achieve a pure culture of *S. mutans*. The final concentration in bacitracin of culture medium was 0.2 U/mL.

# Analytical method

The eluates of incubation solutions were extracted using solid-phase extraction ( $NH_2$  cartridge) and then analyzed by gas-phase chromatography and mass spectrometry (Agilent

Table 1: Composite resins used in thy study.						
Product (lot)	Resin matrix	Manufacturer				
Grengloo (6623923)	TEGDMA, UDMA, HEMA, Bis-EMA6, GMA, EO-TMPTA, 3-trimethoxysilylpropyl methacrylate	Ormco				
Blugloo (6556174)	UDMA, Bis-EMA6, GMA, EO-TMPTA, 3-trimethoxysilylpropyl methacrylate					
Transbond XT (N921496)	Bis-GMA, Bis-MEPP	3M				
Transbond LR (N919866)	Bis-GMA, TEGDMA					



Figure 1: Resin samples immersed in brain heart infusion.

6890 Series – Agilent 7673). A capillary column 30 m in length, internal diameter of 320  $\mu$ m, and film thickness of 0.25  $\mu$ m were used with helium carrier gas at a flow rate of 1.2 mL/min. The column temperature program was set as follows: Initially, 80°C for 1 min, increasing to 150°C at a rate of 20°C/min, and then increasing to 280°C for 2 min at a rate of 10°C per min. The injector temperature was 280°C and the transfer line was 280°C. Mass spectra were obtained using electron impact ionization (69.9 eV, 34.6  $\mu$ A, 230°C).

Data were acquired by scan mode and selected ion monitoring (SIM) mode and were processed with MSD ChemStation software.

BPA and TEGDMA were identified by searching for their fragments in SIM mode.

The calibration curve and response factor were computed with reference BPA and caffeine as internal standard. Linear correlation with efficiency of 0.996 was obtained between BPA amount and corresponding peak area. BPA was quantified after his identification. The limit of quantification was 0.01  $\mu$ g/mL.

#### RESULTS

Many chemical molecules were identified in tested resin materials [Tables 2 and 3].

For the test group, BPA was detected in Blugloo at day 1 (0.38  $\mu$ g/mL) [Figure 2]. TEGDMA was present in Grengloo and Transbond LR at day 1 [Figure 3].

#### DISCUSSION

Resin composites and adhesives are subject to a significant amount of biological breakdown in the oral cavity due to the presence of condensation type bonds within the resin.<sup>[39]</sup> These bonds, which include esters, urethanes, and amides, are predominantly found in the di-vinyl monomers, and they

time (BHI: Control group, SM: Test group, d1: Day 1, d30: Day 30).							
Compound	BHI d1	SM d1	BHI d30	SM d30			
$C_7 H_{10} N_2 O_2$	Х	Х	Х	Х			
$C_{11}H_{18}N_2O_2$	Х	Х	Х	Х			
$C_{14}H_{16}N_2O_2 \\$	Х	Х	Х	Х			
$C_{15}H_{20}N_2O_2$	Х	Х	Х	Х			
$C_{15}H_{20}N_2S$	Х	Х	Х	Х			
Uric acid C <sub>5</sub> H <sub>4</sub> N <sub>4</sub> O <sub>3</sub>	Х	Х		Х			
Oleic acid C18H34O2			Х	Х			
$C_{12}H_{14}N_2O_2 \\$	Х						
$C_7 H_{12} N_2 O_3$	Х						
TEGDMA C14H22O6		Х					
BPA $C_{15}H_{16}O_2$		Х					
$C_{21}H_{44}$			Х				
$C_{17}H_{36}$			Х				
$C_{20}H_{36}O_2$				Х			
$C_{20}H_{38}O_2$				Х			
Octadecanoic acid				Х			
$C_{18}H_{36}O_2$							
$C_{20}H_{25}NO_{3}S$				Х			
$C_{20}H_{40}O_2$				Х			
$C_{18}H_{32}O_2$				Х			
$C_{23}H_{32}O_2$				Х			
C <sub>5</sub> H <sub>9</sub> NO				Х			

Table 2: Compounds found according to group and incubation

**Table 3:** Compounds found according to materials.

Compound	Grengloo	Blugloo	Transbond XT	Transbond LR
$C_7 H_{10} N_2 O_2$	Х	Х	Х	
$C_{11}H_{18}N_2O_2$	Х	Х	Х	
$C_{14}H_{16}N_2O_2$	Х	Х	Х	Х
$C_{15}H_{20}N_{2}O_{2} \\$	Х	Х	Х	Х
$C_{15}H_{20}N_2S$	Х	Х	Х	Х
Uric acid	Х	Х	Х	Х
$C_5H_4N_4O_3$				
Oleic acid	Х	Х	Х	
$C_{18}H_{34}O_2$				
$C_{12}H_{14}N_2O_2$	Х			
$C_7 H_{12} N_2 O_3$	Х			
TEGDMA	Х			Х
$C_{14}H_{22}O_{6}$				
BPA C <sub>15</sub> H <sub>16</sub> O <sub>2</sub>		Х		
$C_{21}H_{44}$	Х			
$C_{17}H_{36}$	Х			
$C_{20}H_{36}O_2$		Х	Х	Х
$C_{20}H_{38}O_2$			Х	
Octadecanoic	Х	Х	Х	Х
acid C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>				
$C_{20}H_{25}NO_3S$		Х		
$C_{20}H_{40}O_2$			Х	
$C_{18}H_{32}O_2$			Х	
$C_{23}H_{32}O_2$		Х		
C5H0NO	Х			

are all prone to chemical hydrolysis, catalyzed by acids, bases, or enzymes.<sup>[40]</sup>



Figure 2: Spectrum and concentration of bisphenol A detected in Blugloo.



Figure 3: Mass spectrum showing triethylene glycol dimethacrylate detected in the test group with Grengloo.

*S. mutans* has esterase activities at levels capable of degrading dental resin composites and adhesive system.<sup>[35]</sup> Consequently, the formation of bacteria dense biofilm can result in the ongoing destruction of the resin composite.

Many groups have studied the degradation of resin composites in the oral cavity. In the early 1990s, the focus of the studies shifted toward the chemical breakdown of these restorative materials because it was suggested that enzymes in the oral cavity may contribute to the degradation of resin composites.<sup>[41,42]</sup> Since then, a number of studies have investigated the degradation of resin composites in the presence of salivary-like enzymes<sup>[43-46]</sup> and the subsequent biological effects of the by-products on the surrounding bacteria and mammalian cells.<sup>[47-53]</sup> These biological processes that render commercial resins vulnerable to premature failure are currently beyond the control of the clinicians.

While there have been studies investigating the impact of composite degradation products on bacterial growth and virulence gene expression,<sup>[54,55]</sup> the potential effect of bacterial degradative activity on resin composites and adhesives has yet to be explored. Therefore, we hypothesized that, in addition to acid production, cariogenic bacteria contain esterase activities that degrade dental resin composites and adhesives.

The results of analyses of leachable substances (monomers, additives, and degradation products) from dental polymerbased materials may be influenced by the type of extraction media, the time and temperature of the extraction procedure, as well as the degree of curing and composition of the material.<sup>[39,56,57]</sup> It is known that methacrylates may degrade hydrolytically in aqueous environments.<sup>[39,58]</sup>

The results of this study support the hypothesis that cariogenic bacteria (*S. mutans*) contain esterase activities at levels capable of hydrolytic-mediated degradation of cured resin composites and adhesives.<sup>[35]</sup>

Orthodontic treatments increase the risk of the occurrence of carious lesions,<sup>[59]</sup> constituting a prejudice for patients and greatly compromising the success of these treatments. This risk is inherent both in the apparatus which causes an increase in plaque retention sites but also in a modification of the bacterial flora and in the age of patients.<sup>[60]</sup> The installation of orthodontic devices is followed by a modification in the oral ecosystem with an increase in the number of *S. mutans* and *Lactobacilli*.<sup>[23]</sup>

Human saliva has also been shown to hydrolyze resin composites and adhesives.<sup>[61]</sup>

Many bacterial species express esterases; however, the overall function of *S. mutans* esterases and, more specifically, their importance in contributing to the biodegradation process of dental resin composite restorations is not well-understood. In other bacteria, esterases have been linked to virulence and pathogenesis.<sup>[32,62,63]</sup>

# CONCLUSION

- *S. mutans* can hydrolyze long-term orthodontic composites
- Monomers such as BPA and TEGDMA may be present in degradation products
- Leaching compounds can be separate and identify by GC–MS technique.

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#### Declaration of patient consent

Patient's consent not required as patients identity is not disclosed or compromised.

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Nil.

#### **Conflicts of interest**

There are no conflicts of interest.

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