Journal of the Mechanical Behavior of Biomedical Materials 77 (2018) 519–526

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

journal homepage: www.elsevier.com/locate/jmbbm

New adhesive system based in metals cross-linking methacrylate

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ARTICLE INFO

Keywords:
Antibacterial adhesive dental
Mechanical properties
Chemical properties
Antibacterial monomers
Dental materials

ABSTRACT

This study evaluated the anti-antibiofilm potential of silver methacrylate (Ag) or di-*n*-butyldimethacrylatetin (Sn) in experimental adhesive systems. Ag and Sn methacrylates were incorporated at 0.5 mol%, 1 mol% and 2 mol% in an adhesive resin. The anti-antibiofilm potential, degree of conversion (DC), microtensile bond strength (μTBS), water sorption/solubility ($W_{SR/SL}$), bonded interfaces pattern (SEM), cytotoxicity and leaching of Ag and Sn ions were evaluated. Data were statistically analyzed considering $\alpha = 0.05$. Only Ag at 2% affected DC and μTBS. Ag at 1% and 2% and Sn at 1% and 2% showed anti-biofilm potential against Mutans streptococci. Ag at 1% and 2% and Sn at 2% showed a statistically significant difference to the control in $W_{SR/SL}$ ($p < 0.05$). The additions of metal methacrylate did not affect cell viability, being the adhesive resins statistically similar to controls. Leached metals of Ag were more than 100x higher than for Sn. Between the concentration tested, Ag and Sn methacrylate at 1% presented an anti-biofilm effect without altering the mechanical properties evaluated.

1. Introduction

One of the most recurrent clinical problems in dentistry is caries adjacent to dental materials such as amalgam, composite resin, and glass-ionomer cements (Demarco et al., 2012; Kopperud et al., 2012; Mjor, 2005; Nedeljkovic et al., 2015). However, composites do not present anti-biofilm agents in their composition (Beyth et al., 2007; Karanika-Kouma et al., 2001), that differs from amalgam, which contains metal ions such as silver, mercury and copper (Beyth et al., 2007; Morrier et al., 1998), and glass-ionomer cements with fluorides (Cenci et al., 2009). Consequently, composite resins do not have the ability to prevent or reduce biofilm growth or to retard the progression of dental caries (Nedeljkovic et al., 2015).

To minimize this drawback, agents and/or monomers with anti-biofilm potential have been added to dental adhesive systems (Beyth et al., 2006; Cocco et al., 2015; Zhang et al., 2016). The advantage of an anti-biofilm adhesive is that it possibly disinfects the cavity before restoration placement and it inhibits bacterial leakage to the tooth-restoration interface (Nedeljkovic et al., 2015). However, the addition of anti-biofilm agents may have negative effects on the mechanical properties of adhesives and may have toxic effects on the dental tissue (Frassetto et al., 2016; Kurata et al., 2011; Ribeiro and Ericson, 1991). Unlike the addition of anti-biofilm monomers, such as small quantities (5%) of MDPB (12-methacryloyloxy-dodecylpyridinium bromide) (Zhang et al., 2013), mechanical properties could be maintained without causing biological effects (Hoshika et al., 2014; Imazato et al., 1998a; Imazato et al., 1998b; Imazato et al., 2003; Imazato et al., 1997; Imazato et al., 1998a, 1998b, 2003, 1997, 2006).

In addition, silver methacrylate and di-*n*-butyldimethacrylatetin may show anti-biofilm potential similar to other monomers described in the literature, such as MDPB and zinc methacrylate (Henn et al., 2012; Henn et al., 2012, 2011; Imazato et al., 1998b; Imazato et al., 1998b, 2003; Zhang et al., 2013). The silver ion has shown antibacterial, antifungal, antiviral activity against others microorganisms with a low toxicity.(Morones et al., 2005; Rai et al., 2009). Furthermore, organotin compounds that it has been showed anticancer (Ahmad et al., 2007; Cardarelli et al., 1984), antifungal (Sijpesteijn et al., 1962) and antibacterial affect (Ahmad et al., 2007; Salam et al., 2012). Also the tin has been used together with fluoride in dental materials and presents an inhibition of biofilm formation and reduction of acid production (Attramadal and Svatun, 1984; Svatun, 1978; Svatun and Attramadal, 1978). Thus, the incorporation of silver methacrylate and di-*n*-butyldimethacrylatetin into adhesive systems could improve the antibacterial properties of these materials.

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http://dx.doi.org/10.1016/j.jmbbm.2017.10.010
Received 4 August 2017; Received in revised form 5 October 2017; Accepted 8 October 2017
Available online 10 October 2017
1751-6161/

Table 1
Formulation of the experimental adhesive system.

Primer	Resin adhesive
30% HEMA	25% HEMA
30% GDMA-P	50% BisGMA
20% distilled water	25% TEGDMA
20% ethanol	0.4 mol% CQ*
	1 mol% EDAB**
	1 mol% DPI**

Hema – 2-hydroxyethylmethacrylate (Sigma-Aldrich, St. Louis, MO, USA); GDMA-P -glycerol dimethacrylate phosphate (is an equimolar mixture of glycerol dimethacrylate dihydrogen phosphate and glycerol tetramethacrylate hydrogen phosphate); ethanol (Labsynth Ltda., Diadema, SP, Brazil); BisGMA – bisphenol A diglycidyl methacrylate (Esstech Inc., Essington, PA, USA); TEGDMA – triethylene glycol dimethacrylate (Esstech); CQ – camphoroquinone (Esstech); EDAB – ethyl 4-dimethylaminobenzoate (Fluka, Milwalkee, WI, USA); DPI - diphenyliodonium hexafluorophosphate (Sigma-Aldrich). *The concentration of CQ was 0.398, 0.396 and 0.392 mol% (after the incorporation of respectively 0.5, 1 and 2 mol% of metal methacrylate). **The concentration of EDAB and DPI was 0.995, 0.990 and 0.980 mol % (after the incorporation of 0.5, 1 and 2 mol% of metal methacrylate, respectively).

Therefore, the purposes of this study were: (1) to develop an adhesive system with anti-biofilm potential through the addition of metal methacrylates for the first time; (2) to investigate the anti-biofilm potential, cell viability, and the chemical, physical and mechanical properties of these adhesive systems. The hypothesis tested was as follows: incorporation of these monomers will provide an anti-biofilm effect to adhesives without impairing the degree of C=C conversion, microtensile bond strength to dentin, the morphology of the bonded interfaces and water sorption and solubility.

2. Materials and methods

2.1. Formulation of the experimental adhesive system

Two-step self-etching adhesive systems were formulated as described in Table 1. Silver methacrylate (Ag) or di-*n*-butyldimethacrylatetin (Sn) (Aldrich Chemical Co., Milwaukee, WI, USA) (Fig. 1) were incorporated into the adhesive resin in three different molar concentrations: 0.5%, 1%, and 2%. An adhesive resin without incorporation of metal methacrylate was used as a negative control. A previous screening was performed to select the best concentrations in related to the mechanical properties tested in the study of these metal methacrylates (unpublished data). All experimental adhesive systems were subjected to tests mentioned below, and the data obtained were analyzed considering $p \leq 0.05$ as statistically significant using the software SigmaPlot 12.2 (Systat Software In., San Jose, USA).

2.2. Degree of C=C conversion (DC)

DC was evaluated using a real-time Fourier transform infrared spectroscopy (Prestige21; Shimadzu, Tokyo, Japan) with an attenuated total reflectance device incorporating a horizontal diamond crystal (PIKE Technologies, Madison, WI, USA). A micropipette was used to drop 10 µl of each material onto the total reflectance accessory and a preliminary reading for the uncured material (monomer) was taken using 24 scans coaddition, 4 cm^{-1} resolution, Happ-Genzel apodization and 2.8 mm s mirror speed (C=C). The adhesive resin was photo-activated for 20 s using a LED curing unit (Radii; SDI, Bayswater, Victoria, Australia) with 1400 mW cm^2 irradiance. Readings were carried out again after the polymerization of the specimens (C-C). The percentage of the degree of conversion was calculated as previously described (n = 3) (Ogliari et al., 2006). It was performed in triplicate for each material. Statistical analysis was performed using One-way ANOVA followed by Tukey's test.

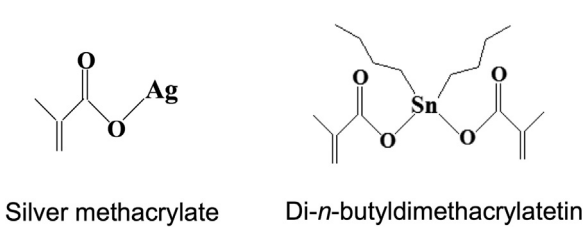

Fig. 1. Chemical structure of silver methacrylate and di-*n*-butyldimethacrylatetin.

2.3. Microtensile bond strength (µTBS) to dentin

The microtensile bond strength test was performance in according ISO 11405:2015 (ISO, 2015). The soft tissue of 70 freshly extracted bovine incisors was cleaned. Non-fractured teeth were stored in an aqueous solution of 0.5% chloramine-T for 7 days. Teeth were randomly assigned into 7 groups according to the adhesive system evaluated. At least 10 teeth were used per group. The buccal enamel was removed to expose the middle dentin layer. The exposed dentin surface was successively wet-ground with 400- and 600-grit SiC abrasive papers to create a standardized flat surface with consistent smear layer formation. After water-rinsing, the dentin substratum was dried with absorbent paper and then the experimental self-etching primer component was applied over the prepared surfaces for 30 s and was evaporated for 10 s, followed by the application of the adhesive resin, which was light activated for 20 s using the LED previously described. A restoration was performed over the top of the cured bonding agent using an incremental technique with a resin composite material (N'Durance; Septodont, Confi-Dental Division, Louisville, CO, USA); this was then light-cured for 60 s. Specimens were then stored in distilled water at 37 °C for 24 h. After this, they were sectioned perpendicularly to the bonding interfaces using a water-cooled, diamond saw at low speed (Isomet 1000; Buehler; Lake Bluff, IL, USA). This process was reproduced after turning the cut sections 90°, resulting in beams of bonded dentin to the composite with cross-sectional areas of 1 mm^2. At least two beams per tooth were produced for the evaluation of µTBS immediately (n = 20 per group). Beam dimensions were precisely measured using a digital caliper (Mitutoyo, Tokyo, Japan), after which they were attached to the tensile testing device using a special cyanoacrylate glue (Super Bonder Gel, Henkel Loctite, São Paulo, SP, Brazil). The dentin portion was attached to a fixed platform, and the composite side was attached to the upper, movable crosshead. The attached specimen was subjected to tensile vertical loading in a mechanical testing machine (DL500; EMIC, São José dos Pinhais, PR, Brazil) at a crosshead speed of 0.5 mm min, and the load at specimen failure was recorded. Bond strength values (MPa) were calculated by dividing the maximum load at failure by the cross-sectional area of the bond interface. The data regarding the microtensile bond strength were non-parametric, and statistical analysis was performed using Kruskal-Wallis followed by Tukey's test.

2.4. Scanning electron microscopy (SEM) analysis

The morphology of the bonded interfaces was analyzed using scanning electron microscopy (SEM) at 15 kV (JSM 6610, JEOL, Tokyo, Japan). Adhesive systems were applied as previously described. Dentin discs were bonded to each other using a thin layer of photo-activated, composite resin, generating a dentin-composite resin-dentin sandwich specimen. Specimens were embedded cross-sectionally in epoxy resin in order to make dentin-resin composite-interface visible. After 24 h, the specimens were wet polished with 600, 1200, 1500 and 2000-grit SiC papers and were polished with 3-, 1- and 0.5-µm diamond compounds (TED Pella, Inc., USA). The surfaces were etched with 50% phosphoric acid solution for 5 s and deproteinized by immersion in 2.5% NaOCl solution for 10 min. The specimens were ultrasonically cleaned with