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The effect of zoledronate-containing primer on dentin bonding of a universal adhesive

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ABSTRACT

Objectives: To evaluate the bonding ability and nanoleakage of a universal adhesive applied to dentin pre-treated using a zoledronate-containing primer (zol-primer) before and after mechanical load cycling.

Materials and Methods: Flat dentin surfaces obtained from human molars were assigned to one of the following adhesion procedures (n = 6): 1-*Single Bond Universal* (SBU) applied in etch-and-rinse mode; 2- SBU applied as etch-and-rinse after the application of zol-primer; 3- SBU applied in self-etch strategy; 4- SBU applied as self-etch after the use of zol-primer. Half of the specimens were processed for microtensile bond strength test after 24 h, while the other half part was submitted to 200,000 mechanical cycles. Further specimens were silver-impregnated and assessed for interface nanoleakage by SEM. Data were analyzed with two-way ANOVA and Tukey's test ($p < 0.05$).

Results: At 24 h evaluation, the four groups presented similar bond strengths, whilst both groups bonded with etch-and-rinse technique showed significant bond strength reduction after mechanical load ($p < 0.05$), with the highest drop in bond strength for the specimens pre-treated with the zol-primer. No negative effects were found for self-etch strategy ($p > 0.05$) in microtensile test. Lower nanoleakage expression was observed for etch-and-rinse specimens treated with zol-primer. However, noteworthy reduction of adhesive layer thickness was observed when combining the zol-primer with the self-etch bonding approach.

Conclusion: It can be concluded that zol-primer should not be used along with a universal adhesive in etch-and-rinse mode, but its application before self-etch application may provide less degradation of the resin-dentin interface.

1. Introduction

The mechanism of bonding to enamel and dentin is essentially based on an exchange process in which minerals removed are replaced by resin monomers, that upon polymerization become micromechanically interlocked (Cardoso et al., 2011). Conversely, impregnation of synthetic resin monomers into demineralized dentin is challenging due to the humidity, permeability and physiologic hydrostatic pulpal pressure (Feitosa et al., 2012a). After adhesive polymerization, exposed collagen is easily detected and represents an area prone to degradation. Collagen degradation is accelerated by proteolytic enzymes, the so-called matrix metalloproteinases (MMPs) and cysteine cathepsins (CC) (Scaffa et al., 2012).

Several MMP inhibitors have been investigated (Breschi et al., 2008; Scaffa et al., 2012) such as galardin, batimastat and chlorhexidine, some have already been incorporated into experimental adhesives (Almahdy et al., 2012; Yiu et al., 2012). Synthetic MMP inhibitors should contain a functional group capable of chelating the zinc ions, which binds to the active site in the MMP molecule (Feitosa et al., 2012a; Tezvergil-Mutluay et al., 2014). A polyphosphonic acid such as zoledronate may provide additional inhibition of MMP due to the a potential bond which may create between zoledronate and proteins (Murphy et al., 2014). Moreover, it has been adovacated that the use of zolendric acid may reduce the activity of MMPs via dentin

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remineralization and reduce the degradation of resin-dentin interface especially when associated with ion-releasing adhesives (Tezvergil-Mutluay et al., 2014).

Recent multi-mode self-etch adhesives (so-called universal adhesives) may be applied in etched or non-etched enamel and dentin. The longevity and bond strength of these materials have been studied, but these remain questionable (Hanabusa et al., 2012; Perdigão and Loguercio, 2014). We hypothesize that zoledronate could improve the durability of dentin bonds created with universal adhesives, especially when dentin-etching is undertaken. Furthermore, zoledronate might also improve the resistance of partially demineralized collagen fibrils to mechanical stress. However, to our knowledge, this combination (zoledronate + universal adhesive) has never been investigated so far. Therefore, the objective of this study was to assess the effect of dentin pre-treatment using an experimental primer containing 7% zoledronate (Zol-primer), applied prior to the application bonding a universal adhesive in etch-and-rinse or self-etch mode, on the microtensile bond strength (µTBS) before and after mechanical cycling load challenge.

2. Materials and methods

2.1. Preparation of experimental zol-primer

The formulation of the zol-primer used has been described by Tezvergil-Mutluay et al. (2014), and was formulated with deionized water (50 vol%) saturated (7 mg/mL) with zoledronate [1-hydroxy-2-(1H-imidazol-1-yl) ethane-1,1-diyl]bis-phosphonic acid (MW 290; Santa Cruz Biotechnology, Santa Cruz, USA) and absolute ethanol (50 vol%) (pH adjusted to 6.8 with 0.1 M NaOH).

2.2. Sample preparation

Twenty-four third molars recently extracted for surgical reasons in the service of Dentistry and Maxillofacial Surgery Hospital General Universitario de Valencia under a protocol approved by the Biomedical Research Ethics Committee of the University and Polytechnic La Fe Hospital in Valencia, with registration No. 2014/00487/PI and. stored in deionized water (pH 7.4) at 4 °C no longer than 3 months,. Middle dentin specimens were obtained by removing the roots 2 mm below cemento–enamel junction (CEJ) and with a parallel cut at 2 mm above CEJ using a slow-speed water-cooled diamond saw (Isomet, Buehler, Lake Bluff, USA). The dentin surface was wet-polished with 600-grit SiC papers for 1 min to create a standard smear layer prior to bonding procedures (Feitosa et al., 2012b; Hamouda et al., 2011).

2.3. Experimental design and bonding procedures

Dentin specimens were divided randomly *by using Microsoft Excel (Windows) randomization* into two principal groups (n = 12) based on the bonding technique used: self-etch and etch-and-rinse. Subsequently, the specimens from each main group were divided into subgroups (n = 6), regarding the use or not of Zol-primer before adhesive application. Specimens of each principal group were divided into further sub-groups (n = 3), based on the challenge test: Control: water immersion for 24 h and MCL: mechanical-cycling load.

The universal adhesive employed in this study was the Single Bond Universal (3 M-ESPE, St. Paul, USA). The composition and application procedures are listed in Table 1. Resin composite build-ups were constructed in 3 horizontal layers (2-mm thick) up to 6 mm with Spectrum ® TPH® resin composite (Dentsply, Petropolis-RJ, Brazil) and light-cured for 30 s each layer.

Light-curing procedures were performed using the LED-curing unit DB85 (Dabi Atlante, Ribeirao Preto, Brazil). The output intensity was monitored with a Demetron Radiometer (Model 100, Demetron Research, Danbury, USA) to maintain a minimal light output intensity of 1000 mW/cm^2 throughout all experiments. All materials were used following the manufacturers' recommendations.

2.4. Mechanical-cycling challenge

The resin-bonded specimens were submitted to the mechanical cycling load executed using Chewing Simulator CS-4 (SD Mechatronik, Feldkirchen-Westerham, Germany) which has a stainless steel tip of 4 mm in diameter in contact with the central part of the restored specimens. All resin-bonded specimens were submitted to 200,000 mechanical cycles under a load of 30 N, at a rate of 2 Hz for one week (Ulker et al., 2010).

2.5. Microtensile bond strength (µTBS) and fracture type analysis

After 24 h immersion in distilled water (Control) or MCL, resin-bonded specimens were sectioned in resin-dentin sticks (0.9 mm × 0.9 mm) for microtensile bond strength testing. Sticks from the most peripheral area presenting remaining enamel were excluded. The sticks were attached to a jig with a cyanoacrylate cement (Super Bonder gel, Loctite, Henkel Corp., Rocky Hill, USA) and tested to tensile failure in a universal testing machine (DL2000, EMIC, Sao Jose do Rio Preto, Brazil) with a 500-N load cell and 0.5 mm/min cross-head speed. The exact cross-sectional area of each tested stick was measured with a digital caliper. The µTBS results were calculated and expressed in MPa. The µTBS values obtained from the sticks of the same resin-bonded tooth were averaged. Mean bond strength of each individual tooth was used as one unit for statistical analysis. Three resin-bonded teeth (n = 3) were evaluated for each sub-group. The µTBS data were statistically analyzed with two-way ANOVA (presence of zol-primer and aging regimen) and Tukey's post-hoc test at 5% significance level. Subsequent to the µTBS testing, the mode of failure of each fractured stick was determined using a stereomicroscope (Olympus SZ 40–50; Tokyo, Japan) at 100X magnification. The fractures were classified as adhesive, mixed, cohesive in composite or cohesive in dentin.

2.6. Nanoleakage analysis

Three resin-dentin sticks were selected from each bonded tooth and storage condition during the cutting procedure. These sticks were immersed in 50 wt% ammoniacal silver nitrate ($AgNO_3$ (aq)) solution in complete darkness for 24 h (Tay et al., 2002). Subsequently, the specimens were rinsed with distilled water to remove the excess of silver nitrate and immersed in photo-developing solution for 8 h under light to reduce silver ions into metallic silver grains. The silver-impregnated sticks were embedded in epoxy resin and polished using 600-, 1200-, 2000-grit SiC papers and diamond pastes (Buehler, Lake Bluff, IL, USA) with 1 and 0.25 µm particle sizes, and ultrasonically cleaned of 15 min after each abrasive/polishing step. Specimens were finally air-dried, dehydrated overnight in silica gel under vacuum, coated with carbon and analyzed using SEM (Inspect 50, FEI, Amsterdam, Netherlands) and observed in backscattered electron mode at 20 kV.

3. Results

3.1. Microtensile bond strength testing (µTBS)

The two-way ANOVA showed significant interaction ($p < 0.001$) between the specific treatments and the aging regimen. Tukey's test indicated no significant differences among all groups before load cycling ($p > 0.05$), while both groups treated in self-etch mode provided stable bond strength after load cycling ($p > 0.05$). Conversely, adhesive applied in etch-and-rinse mode depicted bond strength reduction after load cycling without zol-primer ($p = 0.006$), especially when zol-primer was used ($p < 0.001$); this latter group presented very low bond strength (8.0 ± 2.1 MPa). All numeric values in means and standard deviations are presented in Table 2. *Majority of fractures in failure mode*