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Impact of pH and application time of meta-phosphoric acid on resin-enamel and resin-dentin bonding

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ABSTRACT

Purpose: To evaluate the immediate microshear resin-enamel bond strength (μSBS) and the immediate and 6-month microtensile bond strength (μTBS) and nanoleakage (NL) of the adhesive interface performed by different pHs of 40% meta-phosphoric acid (MPA) were compared with conventional 37% ortho-phosphoric acid (OPA) under different application times. Additionally, the enamel etching patterns were evaluated and the chemical/morphological changes induced by these differents groups were evaluated.

Materials and methods: One hundred and ninety-eight extracted human molars were randomly assigned into experimental groups according to the combination of independent variables: Acid [37% ortho-phosphoric acid (OPA), 40% meta-phosphoric acid (MPA) at pHs of: 0.5, 1 and 2] and Application Time [7, 15 and 30 s]. Enamel-bond specimens were prepared and tested under μSBS. Resin-dentin beams were tested under μTBS tested immediately or after 6-months of water storage. Nanoleakage was evaluated using bonded-beams of each tooth/time-period. Enamel etching pattern and chemical and ultra-morphology analyses were also performed. The μSBS (MPa) data were subjected to a two-way repeated measures ANOVA (Acid vs. Application time). For μTBS, Acid vs application time vs storage time data were subjected to three-way ANOVA and Tukey's test ($\alpha = 0.05$).

Results: MPA pH 0.5 showed μTBS similar to OPA, independently of the application time on enamel ($p > 0.05$) or dentin ($p > 0.05$). OPA provided higher nanoleakage values than MPA ($p = 0.003$). Significant decreases in TBS and increases in NL were only observed for OPA after 6 months ($p = 0.001$). An increase in the application time resulted in a more pronounced etching pattern for MPA. Chemical analysis showed that dentin demineralized by MPA depicted peaks of brushite and octacalcium phosphate. MPA exposed less collagen than OPA. However, optimal results for MPA were dependent on pH/application time.

Conclusion: The use of 40% meta-phosphoric acid with a pH of 0.5 is an alternative acid-etching agent for dentin and enamel bonding. Furthermore, the use of MPA preserves the resin-dentin interface over a 6-months period, due to presence of brushite and octacalcium phosphate and a reduced demineralization pattern.

1. Introduction

There is a consensus that the mechanism of bonding resin-based materials to dentin with most dental adhesives relies on micro-mechanical retention through forming a resin-impregnated "hybrid layer", which, upon polymerization of resin, offers a mechanical coupling zone (Nakabayashi et al., 1982; Pashley et al., 1993; Van Meerbeek et al., 1993).

Usually, an etch-and-rinse (ER) adhesive is applied by a clinician using a strong ortho-phosphoric acid etchant (32–40%), followed by a primer and a bonding resin (3-step ER) or a combined primer and bonding resin (2-step ER) to promote adhesion. This ortho-phosphoric acid (OPA) completely demineralizes 5–8 μm into the intertubular dentin matrix to create nanometer-sized porosities around collagen fibrils (Pashley et al., 2011). Furthermore, OPA also partially demineralizes to a greater depth (Feitosa et al., 2013).

Unfortunately, full infiltration of resin monomers within OPA-etched dentin is a complex task. Excessive acid conditioning causes deep demineralization that jeopardizes complete infiltration of resin monomers, thereby resulting in the formation of a weaker and

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unprotected demineralized dentin zone at the base of these hybrid layers (Hashimoto et al., 2000, 2002b). Furthermore, the resin–dentin bonds created with contemporary hydrophilic dentin bonding systems deteriorate over time (Breschi et al., 2008; Liu et al., 2011). For ER hydrophilic adhesives, there is a decreasing gradient of resin monomer diffusion within the hybrid layers (Breschi et al., 2004; Wang and Spencer, 2002). This results in incomplete resin infiltration at the bottom the hybrid layer, leaving exposed collagen fibrils (Armstrong et al., 2004; Breschi et al., 2004; Mazzoni et al., 2006) that are susceptible to enzymatic degradation by host-derived collagen-bound matrix metalloproteinases (MMPs) (Mazzoni et al., 2006; Nishitani et al., 2006; Osorio et al., 2011) and by cysteine cathepsins (Scaffa et al., 2012).

Several approaches have been tested to inhibit the degradation of ER adhesion to dentin, but regardless the strategy used, the conditioning employed in all procedures has been OPA (Reis et al., 2013). This is because enamel etching with OPA is considered the gold standard in terms of retention and marginal seal of bonded anterior and posterior restorations (Heintze and Rousson, 2012; Mahn et al., 2015). Recently, the meta-phosphoric acid (MPA) was proposed as an alternative dentin conditioning agent for ER adhesives and a suitable strategy to create more durable resin-dentin bonds (Feitosa et al., 2013; Millan Cardenas et al., 2017).

Meta-phosphoric acid achieved successful results due its milder acidity (pH around 2.6) and particular acid-ionization constant (Ka) which contributed to the creation of a thinner partially demineralized collagen layer characterized by the presence of mineral precipitation (Feitosa et al., 2013; Millan Cardenas et al., 2017). However, in a recent study, MPA was applied for 60 s (Feitosa et al., 2013). This long application time is in contrast to the clinician's need for simplification and rapid procedures (Reis et al., 2013). It was recently showed that lowering the pH of MPA reduced the application time whilst maintaining its etching pattern and immediate bonding to dentin (Millan Cardenas et al., 2017).

However, to the extent of our knowledge, the immediate resin-enamel bond, as well as the longevity of resin-dentin bond produced by MPA with different pHs and application times has not been evaluated. Thus, the aim of this study was to evaluate the immediate microshear resin-enamel bond strength (μSBS) and the immediate and 6-month microtensile resin-dentin bond strength (μTBS) and nanoleakage (NL) of an adhesive interface when using MPA and OPA of varying pHs and etching times. Additionally, the chemical/morphological changes induced by the use of MPA and OPA on dentin surface will be analyzed. The following null hypotheses were tested: different pHs and application times of the 40% meta-phosphoric acid have no difference when compared to OPA on (1) μSBS to enamel, (2) enamel etching pattern, (3) μTBS, (4) NL tested either immediately or after 6-months of water storage, and (5) chemical/morphological differences of etched dentin.

2. Methods and materials

2.1. Tooth selection and preparation

One hundred and ninety-eight extracted, caries-free human molars were used. The teeth were collected after obtaining the patients' informed consent. The Ethics Committee Review Board of the local university approved this research project under protocol number 1,302,629. The teeth were disinfected in 0.5% chloramine, stored in distilled water, and used within six months after extraction.

2.2. Experimental design

The teeth were randomly assigned into 12 experimental conditions (n = 60 enamel specimens for μSBS; 84 dentin specimens for μTBS and NL; 21 for ATR-FTIR vibrational analysis; 21 for CLSM analysis and 12 for enamel-etching pattern evaluation) according to the combination of the independent variables: **Acid** [37% of ortho-phosphoric acid (OPA; pH = 0.5, Alpha-Acid, Nova DFL, Rio de Janeiro, Brazil); 40% of meta-phosphoric acid (MPA, Sigma Aldrich, St. Louis, USA) adjusted to the following pHs – 0.5, 1 and 2] and **Application Time** [7, 15 and 30 s]. Since specimens for μTBS and NL were tested at different **Storage Times** [immediate and 6 moths], it was also considered as an independent variable.

2.3. Microshear bond strength test (μSBS)

The roots of 60 teeth were removed by sectioning at the enamel-cementum junction. Each dental crown was then sectioned along the diagonals across the long axis of the tooth to produce four enamel specimens (buccal, lingual, and two proximals) (Loguercio et al., 2015). Two hundred and forty enamel specimens were embedded in a polyvinyl chloride tube (10 mm high × 13 mm diameter) using a chemically cured acrylic resin (Jet Clássico, São Paulo, SP, Brazil) so that the enamel surface was left exposed at the top of the cylinder. The bonding area was isolated according to the protocol suggested by Shimaoka and others. (Shimaoka et al., 2011) Four to six perforations, with an internal diameter of 0.8 mm, were made in an acid-resistant, double-faced adhesive tape (Adelbras Ind e Com Adesivos Ltda, São Paulo, SP, Brazil) that was adapted to the enamel surface. This procedure was performed using the Hygienic Ainsworth–style rubber-dam punch (Coltene, Alstatten, Switzerland). The variation in the number of perforations for each enamel surface was dependent on the dimensions of the enamel specimens.

The randomization of the specimens for the μSBS testing was done using block randomization. A person not involved in the research protocol performed this procedure using computer-generated numbers. The gel etchants were applied on enamel and left in place for the appropriate application times. The surfaces were thoroughly rinsed for 60 s and air dried with an air spray for 5 s keeping the enamel moist before adhesive application. The two-steps etch-and-rinse adhesive (Ambar, FGM, Joinville, SC, Brazil) was applied strictly in accordance with the manufacturer's instructions, as described in Table 1.

After application of the adhesive system, polyethylene transparent Tygon tubes (Tygon Medical Tubing Formulations 54-HL, Saint Gobain Performance Plastics, Akron, OH, USA), with the same internal diameter as the perforations and a height of 0.5 mm, were positioned on the perforations over the double-faced tape, ensuring that their lumen coincided with the circular areas exposed by the perforations. Resin composite (Opallis, FGM, Joinville, SC, Brazil) was carefully packed inside each tube and a clear Mylar matrix strip was placed over the filled Tygon tube and pressed gently into place. The resin composite was light cured for 20 s using an LED light-curing unit set at 1200 mW/cm^2 (Radii- cal, SDI Limited, Bayswater, Victoria, Australia). A radiometer (Demetron L.E.D. Radiometer, Kerr Sybron Dental Specialties, Middleton, WI, USA) was used to check the light intensity every five specimens. These procedures were carried out under 10x magnifying loupes.

After storage of the specimens in distilled water for 24 h at 37 °C, the Tygon tubes and the double-faced adhesive tape were carefully removed using a blade, to expose the composite cylinders. Each specimen was examined under a stereomicroscope at 10× magnification. The bonded cylinder was discarded if there was evidence of porosities or gaps at the interface (Muñoz et al., 2014).

The specimens were attached to a shear-testing fixture (Odeme Biotechnology, Joaçaba, SC, Brazil) and tested in a universal testing machine (Kratos IKCL 3-USB, Kratos Equipamentos Industriais Ltda, Cotia, São Paulo, Brazil). Each specimen was positioned in the universal testing machine and a thin wire (0.2-mm diameter) was looped around the base of each composite cylinder. The wire contacted the composite resin cylinder along half of its circumference. The setup was maintained in alignment (resin–enamel interface, the wire loop, and the center of the load cell) to ensure the correct orientation of the shear forces