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A new arginine-based dental adhesive system: formulation, mechanical and anti-caries properties

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ABSTRACT

Secondary caries at the margins of composite restorations has been attributed to adhesive failure and consequent accumulation of cariogenic biofilms.

Objectives: To develop and evaluate an etch-and-rinse adhesive system containing arginine for sustainable release and recharge without affecting its mechanical properties. Arginine metabolism by oral bacteria generates ammonia, which neutralizes glycolytic acids and creates a neutral environmental pH that is less favorable to the growth of caries pathogens, thus reducing the caries risk at the tooth-composite interface.

Methods: Experimental adhesives were formulated with methacrylate monomers and arginine at 5%, 7%, and 10% or no arginine (control). Adhesives were tested for: (i) mechanical properties of true stress (FS and UTS), modulus of elasticity (E), degree of conversion (DC), Knoop hardness number (KHN) and dentin microtensile bond strength (μ-TBS), (ii) arginine release and recharge, and (iii) antibacterial activities. Data was analyzed by *t*-test, one-way ANOVA and Tukey's tests.

Results: FS and UTS results showed no statistically significant differences between the 7% arginine-adhesive and control, while the results for *E,* DC, KHN and μ-TBS showed no difference among all groups. The 7% arginine-adhesive showed a high release rate of arginine (75.0 μmol/cm^2) at 2 h, and a more sustainable, controlled release rate (up to 0.2 μmol/cm^2) at 30 days.

Conclusions: Incorporation of 7% arginine did not affect the physical and mechanical properties of the adhesive. Arginine was released from the adhesive at a rate and concentration that exhibited antibacterial effects, regardless of shifts in biofilm conditions such as sugar availability and pH.

Clinical significance: Secondary caries is recognized as the main reason for failure of dental restorations. The development of an arginine-based adhesive system has the potential to dramatically reduce the incidence and severity of secondary caries in adhesive restorations in a very economical fashion.

1. Introduction

Secondary caries remains one of the main reasons for failure of dental composite restorations [1], and replacement of the failed restorations accounts for up to 75% of the operative work [2]. The inherent biodegradation of the interface between the tooth and the adhesive layer produces crevices that are readily colonized by caries pathogens such as *Streptococcus mutans*. Those crevices are also derived from polymerization shrinkage and improper resin-based composite layering [3,4]. In the microenvironment of a crevice, oral biofilms are protected from salivary flow and buffering capacity, which favors the continuous acid production by *S. mutans* and other bacteria leading to tooth demineralization. Although it is unlikely that biofilms can be eliminated from the crevices, the engineering of novel dentin adhesives that can shift the microbial ecology from a disease to a health state are greatly desirable. In this context, different "bio-active" polymer approaches with antibacterial properties have been proposed but none have been commercialized yet [5,6]. In this study, arginine was

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incorporated into an etch-and-rinse adhesive system for short-term moderation of acid challenges within the biofilm and long-term effects on the persistence of desirable health-related bacteria.

Arginine is an amino acid found in a variety of foods, and is also naturally produced by the human body and secreted in saliva in free form or as salivary peptides. Arginine entering the mouth can be metabolized by certain oral bacteria via the arginine deiminase pathway (ADS) to produce ammonia, which neutralizes glycolytic acids and contributes to the pH rise of oral biofilms [7]. Ammonia production via the ADS results in cytoplasmic and environmental pH increases and benefits oral bacteria by: (i) protecting them against acid killing [8,9], (ii) providing bioenergetic advantages that include increasing ΔpH and synthesizing ATP [8,10], and (iii) maintaining a relatively neutral environmental pH that is less favorable for the outgrowth of a cariogenic microflora [8,11]. A recent *in vitro* study suggested that arginine could also affect the adhesion properties of *S. mutans* [12]. A positive correlation between ADS activity in oral biofilms and absence of caries activity has been recognized clinically [13,14]. Thus, evidence accumulated from previous laboratory and clinical observations supports the premise that providing arginine regularly to oral biofilms can be an effective therapy to control caries at the tooth-composite interface. The aims of this study were to formulate an etch-and-rinse adhesive system containing different concentrations of arginine and to evaluate its mechanical properties and anti-caries activity. Our main hypothesis was that an adhesive formulation containing up to 10% arginine could release enough arginine into the environment to favor pH homeostasis of oral biofilms without compromising its mechanical properties and bond strength to dentin.

2. Materials and methods

2.1. Adhesive formulation

Experimental etch-and-rinse, two bottles, adhesive systems containing different concentrations of L-arginine (Sigma-Aldrich, Inc, St Louis, MO, USA) were fabricated. The primer solution consisted of 15% ethoxylated bisphenol-A dimethacrylate (BisEMA; Esstech Inc., Essington, PA, USA), 10% hydroxyethyl methacrylate (HEMA; Sigma-Aldrich), 10% urethane dimethacrylate (UDMA; Sigma-Aldrich), 10% triethylene glycol dimethacrylate (TEGDMA; Sigma-Aldrich), 15% distilled water and 40% ethanol, and at a ratio of 45% monomers and 55% solvents. The adhesive consisted of 40% UDMA, 30% TEGDMA, 17% BisEMA, 10% bisphenol A glycidyl methacrylate (BisGMA, Sigma-Aldrich), 1.5% diphenyliodonium hexafluorophosphate (DPIHP, Sigma-Aldrich) and 0.5% camphorquinone/1% amine (CQ/EDAB, Sigma-Aldrich) in the monomer mixture previously homogenized at a ratio of 97% monomers and 3% photo-initiator agents. L-arginine was added to the adhesive and homogenized at the weight ratio concentrations of 0% (Arg0; control), 5% (Arg5), 7% (Arg7), and 10% (Arg10). Of note, pilot tests were carried out in order to observe how arginine particles would dissolve with different dental adhesives monomers blends. Some properties were estimated such as arginine-dissolution, phase separation, wettability and viscosity. Once the adhesive blend presented clear-uniform appearance, proper viscosity for dental application and absence of phase-separation, arginine was added at concentrations ranging from 2 to 18%. The arginine concentrations of 5%, 7% and 10% were selected because these presented the best saturated mixtures. The adhesive systems were prepared in a dark room under controlled temperature and humidity, and then kept under refrigeration (4 °C). Prior to use, the adhesives were stirred for 15 min. All concentrations are provided here at a weight ratio.

2.2. Ultimate tensile strength (UTS)

Specimens (n = 10) were prepared using silicon molds with an hourglass shape of 10 × 4 mm and sectional area of 1.5 mm^2 (Odeme Dental Research, Luzerna, SC, Brazil). Each adhesive was placed into the molds, covered with a clear transparent Mylar-matrix and coverslip, and then light-cured at 1000 mW/cm^2 for 20 s (Valo, Ultradent, South Jordan, UT, USA). Specimens were fitted in a testing jig device and submitted to tensile strength test. Load was applied perpendicular to the plane of the cured adhesive in a semi-universal testing machine OM100 (Odeme Dental Research) at 0.75 mm/min. Ultimate tensile strength (UTS) was calculated in MPa using the formula: **UTS = F/A**, in which, F was the tensile strength (N) and A the transversal cross section area (mm^2).

2.3. Flexural strength (FS) and flexural modulus (E)

Bar-shaped specimens (n = 7) were prepared using a silicon mold of 10 × 2 × 2 mm (Odeme Dental Research). Each adhesive was placed into the molds, covered with a clear transparent Mylar matrix and coverslip, and then light-cured as described above. The cross sectional area was approximately 4.0 mm^2. Specimens were stored at 37°C for 24 h and subjected to three-point bending test in a universal testing machine (Instron, Norwood, MA, USA) with 8 mm span between supports and at crosshead speed of 0.5 mm/min. The maximum load for the specimens at fracture was recorded and the FS calculated using the following equation: $FS = 3FL/(2bh^2)$, where F was the maximum load (N), L the distance (mm) between supports, B the width (mm) and H the height (mm). B and H were measured immediately prior the testing. In the Instron machine, *E* data was based on the first load-displacement curve of the linear portion of the graphic obtained from the BlueHill 3 software, and used with the standard equation $E = L^3F/4wH^3d$, in which L is the support span length (mm), F the maximum load (N), *w* the specimen width (mm), H the specimen height and d is the deflection (mm) at load F.

2.4. Knoop hardness (KHN)

Disk shaped specimens (n = 5) were prepared by placing each adhesive into a rubber mold of 5 × 1 mm (Odeme Dental Research). A Mylar strip and a coverslip were placed over the adhesive/mold and light-cured as described above. Specimens were kept stored for 24 h in dry conditions at 37°C. Next, the top surfaces were polished under water with a 1200 grit SiC sandpaper to obtain a polished surface. KHN test was carried out in a microindenter HMV-2 (Shimadzu, Tokyo, Japan) with a load of 50 g and dwell-time of 15 s in order to obtain five measurements from each specimen. The mean KHN value was obtained by averaging the five indentations.

2.5. Degree of conversion (DC)

Disk shaped specimens (n = 5) were prepared in the same manner as for KHN and evaluated immediately after light-activation. DC was determined by a Fourier Transform Infrared spectrometer (Tensor 27, Bruker Optics GmbH, Ettlingen, Germany), coupled to an attenuated total reflectance (ATR). Absorbance spectra included 16 scans at a resolution of 1 cm^{-1}. Unpolymerized blends were scanned after been placed into a Teflon mold (Φ = 5 mm, 1 mm thick) and taken to the ATR. The adhesive blends were light-cured through a polyester strip using a light-curing unit (Valo, Ultradent, USA) for 20 s at 1,000m/Wcm^2. The polymerized samples were then scanned, and unconverted carbon double bonds were quantified by calculating the ratio derived from the aliphatic C=C (vinyl) absorption (1638 cm^{-1}) to the aromatic C=C absorption (1608 cm^{-1}) peaks for both polymerized and unpolymerized samples. The DC for each resin was calculated according to the follow equation: $DC(\%) = \{1-(X_a/Y_a)/(X_b/Y_b)\} \times 100$, where, X_a (polymerized) and X_b (unpolymerized) represent the bands of the polymerizable aliphatic double bonds, and Ya (polymerized) and Yb (unpolymerized) represent the bands of the aromatic double bonds.