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Biosilicate as a dentin pretreatment for total-etch and self-etch adhesives: In vitro study

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ABSTRACT

Purpose: To evaluate the effect of dentin pre-treatment using biosilicate microparticle suspension prior to the application of total-etch and self-etch adhesives on dentin microtensile bond strength.
Material and methods: The labial enamel of forty bovine central incisors was removed using 600-grit SiC disks to expose the flat dentin surface. Six bonding protocols were evaluated ($n=10$): Group 1 (etching with 35% phosphoric acid+adhesive Adper Single Bond™ 2—Control group); Group 2 (10% biosilicate suspension prior to etching with 35% phosphoric acid+adhesive Adper Single Bond™ 2); Group 3 (10% biosilicate suspension after etching with 35% phosphoric acid+adhesive Adper Single Bond™ 2); Group 4 (self-etch adhesive Adper Easy One™—Control group); Group 5 (10% biosilicate suspension prior to the self-etch adhesive Adper Easy One™); Group 6 (10% biosilicate suspension after the self-etch adhesive Adper Easy One™). Composite build-ups were performed with Filtek™ Z350. The specimens were stored in distilled water for 24 h at 37 °C and sectioned into sticks with a 1.0 mm^2 cross-sectional area. Each stick was tested in a Universal testing machine (0.5 mm/min), and the mean microtensile bond strength data (MPa) were analyzed by two-way ANOVA and Bonferroni's multiple comparisons test ($\alpha=0.05$). Representative SEM micrographs were taken to compare the microstructure of different treatments of dentin. The relative amounts of calcium (Ca) and phosphorus (P) were determined by EDS.
Results: Group 3 (10% biosilicate suspension after acid etching with 35% phosphoric acid+adhesive Adper Single Bond™ 2) showed the highest bonding values ($p<0.05$). Regarding the self-etch adhesive, there was no difference ($p>0.05$) between Group 5 and Group 6. SEM images demonstrated biosilicate microparticles in groups 2 and 4. EDS data analysis showed higher amounts of Ca and P ions in groups treated with biosilicate than control groups.
Conclusion: The application of biosilicate microparticle suspension as dentin pre-treatment positively influenced the bond strength of the total-etch adhesive when used after acid etching; meanwhile, it does not interfere with the bonding ability of self-etch adhesive to dentin.
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1. Introduction

Currently, the concept of minimally invasive restorative treatment is to perform restorations that can stabilize the carious process and remineralize the dental hard tissues [1–3]. Furthermore, some remineralizing substances have been applied over dentin as a pretreatment before adhesive procedures [4].

Bioglasses, or bioactive glasses, are a type of dentin remineralizing agent, and have shown good results in clinical and laboratory studies [5–7]. In general, bioglasses react with tissue fluid, promoting hydroxyapatite formation and tissue remineralization [8]. Bioactive glasses are able to bind chemically to dental tissues by forming a layer of carbonated hydroxyapatite which has a similar chemical composition to the mineral phase of these tissues [9].

Biosilicate is the designation of a particular composition of a group of fully crystallized glass-ceramics. Micron-sized biosilicate particles were developed for treating dentin hypersensitivity [7,10]. When in contact with dentin, biosilicate particles rapidly react with the surrounding tissue inside the dentin tubules [7]. In addition, biosilicate exhibits a wide spectrum of antimicrobial properties, including against anaerobic bacteria; its lowest minimal inhibitory concentration values were obtained for oral microorganisms [11].

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However, the application of a remineralizing agent can promote changes to the dentin surface and influence the process of adhesion [5,12–14]. Accordingly, it seems that the incorporation of biosilicate microparticles in the hybrid layer formed by total-etch and self-etch adhesives may play an important role during restorative procedures, but this hypothesis has never been studied.

Therefore, the present study evaluated the influence of dentin pretreatment with biosilicate microparticle suspension on the microtensile bond strength (μTBS) and compared the micromorphology and mineral composition of dentin treated with total-etch and self-etch adhesives. The research hypothesis to be tested was that biosilicate application on dentin surface prior to total-etch and self-etch adhesive systems would not influence μTBS to dentin and there would be no differences in the micromorphology of the hybrid layer.

2. Materials and methods

2.1. Experimental design

The experimental design of this study followed a randomized complete block design in a 3×2 factorial scheme. The following factors were under study:

- Biosilicate pretreatment at three levels: 1 – biosilicate prior etching; 2 – biosilicate after etching; 3 – no biosilicate application (control);
- Adhesive systems at two levels: 1 – etch-and-rinse adhesive; 2 – self-etch adhesive.

The characteristics of the materials used in this study are described in Table 1.

2.2. Specimen preparation

Sixty non-carious bovine lower central incisors were used to obtain dentin substrates for bonding [15]. The incisors were used within one month of extraction and stored in a 0.5% chloramine T aqueous solution at 4 °C until use. The roots of the teeth were removed 2 mm below the enamel–cementum junction using a diamond-embedded disk mounted in a precision electric saw machine (Isomet 1000, Isomet, Buehler, Lake Bluff, IL, USA). Tooth crowns were individually included in chemically cured acrylic resin before the flat superficial dentin surfaces were exposed by grinding the labial enamel surfaces with 180-grit silicon carbide paper (Struers, Ballerup, Denmark) under running water in a metallographic grinder (Polipan-U, Panambra Zwick, São Bernardo do Campo, São Paulo, Brazil). The exposed dentin surface of each tooth was finished with 600-grit silicon carbide paper (Struers) under running water in a metallographic grinder (Polipan-U) in order to standardize the resulting smear layers.

2.3. Bonding protocols

The teeth were randomly divided into six groups, according to the biosilicate pretreatment and the adhesive system used ($n=10$).

The biosilicate suspension was prepared with distilled and deionized water at a concentration of 10%. A microtube of 1.5 mL was used to hold 0.1 mg of biosilicate microparticles (Vitrovita, São Carlos, SP, Brazil), which was mixed with 1 mL of distilled and deionized water immediately before the application [6].

- *Group* 1- *Total-etch adhesive (control group)*: dentin surfaces were etched with 35% phosphoric acid gel (3M ESPE Scotchbond™ Etchant) for 15 s, rinsed and excessive water was removed with absorbent paper. A total-etch adhesive (Adper™ Single Bond 2, 3M ESPE Dental Products, St. Paul, MN, USA) was applied and polymerized for 20 s following the manufacturer's instructions with an LED dental curing light unit (Flash lite 1401, Discus Dental, Culver City, CA, USA).
- *Group* 2- *Biosilicate prior acid etching+adhesive*: the 10% biosilicate suspension was applied using a microbrush on the dentin surface and left for 5 min [6], then it was gently removed with absorbent paper; subsequently, a 35% phosphoric acid gel (3M ESPE Scotchbond™ Etchant, 3M ESPE Dental Products, St. Paul, MN, USA) was applied to the surface for 15 s, rinsed and excessive water was removed with absorbent paper. The total-etch adhesive (Adper™ Single Bond 2) was applied and polymerized as described for Group 1.
- *Group* 3- *Biosilicate after acid etching + adhesive*: dentin surfaces were etched with 35% phosphoric acid gel (3M ESPE Scotchbond™ Etchant) for 15 s, rinsed and excessive water was removed with absorbent paper; subsequently, the 10% biosilicate suspension was applied using a microbrush on the dentin surface and left for 5 min [6], then it was gently removed with absorbent paper. The total-etch adhesive (Adper™ Single Bond 2) was applied and polymerized as described for Group 1.
- *Group* 4- *Self-etch adhesive (Control group)*: dentin surfaces were etched with a self-etch adhesive (Adper™ Easy One), then the adhesive was polymerized for 20 s.
- *Group* 5- *Biosilicate prior self-etch adhesive*: the 10% biosilicate suspension was applied on the dentin surface as described for Group 1; subsequently, a self-etch adhesive (Adper™ Easy One, 3M ESPE Dental Products, St. Paul, MN, USA) was applied and

Table 1
Materials used in the study.

Material	Composition	Manufacturer
Biosilicate microparticles	Fully crystallized glass-ceramic of the $Na_2OCaO–SiO_2–P_2O_5$ system, with the addition of Li_2O and K_2O; particle size: approximately 4 μm.	Vitrovita, São Carlos, SP, Brazil
Scotchbond™ Etchant – phosphoric acid gel	Water, phosphoric acid (35% by weight), fumed synthetic amorphous silica, polyethylene glycol, aluminum oxide; pH approximately 0.6.	3M ESPE Dental Products, St. Paul, CA, USA
Adper™ Single Bond 2 – total-etch adhesive	BisGMA[a], HEMA[b], dimethacrylates, ethanol, water, a novel photoinitiator system and a methacrylate functional copolymer of polyacrylic and polyitaconic acids; 10% by weight of 5 nm-diameter spherical silane treated-silica particles.	3M ESPE Dental Products, St. Paul, CA, USA
Adper™ Easy One – self-etch adhesive	HEMA, BisGMA, methacrylated phosphoric esters, 1.6 hexanediol dimethacrylate, methacrylate functionalized polyalkenoic acid, finely dispersed bonded silica filler with 7 nm primary particle size, ethanol, water, initiators based on camphorquinone, stabilizers; pH approximately 2.3.	3M ESPE Dental Products, St. Paul, CA, USA

[a] BisGMA: Bisphenol A-glycidyl dimethacrylate.
[b] HEMA: 2-hydroxyethylmethacrylate.

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