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Evaluation of dentin desensitization protocols on the dentinal surface and their effects on the dentin bond interface

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ABSTRACT

Objectives: To evaluate the effect of desensitizing agent containing calcium phosphate nanoparticles on the bond strength of *etch-and-rinse* adhesive system (Scotchbond Multi-Purpose), presence of precipitate, dentinal tubule obliteration and hybrid layer formation in dentin in comparison with potassium nitrate plus sodium fluoride or strontium chloride compounds.

Methods: 150 bovine incisors were treated with (n = 10): G1, Desensibilize Nano P ($Ca_3(PO_4)_2$ + 5% KNO_3 + 0.9%NaF); G2, Desensibilize (10%$SrCl_2$ + 5%KNO_3); G3, Desensibilize KF2% (5%KNO_3 + 0.2%NaF); G4, Ultra EZ (3%KNO_3 + 0.25%NaF) and G5, no treated (control). Scanning electron microscopy was used to assess the incidence of precipitates (500×) and obliterated dentinal tubule counts (1.000×). The adhesive system was used after all desensitization treatments. The bond strength (n = 40) and the fracture pattern were evaluated. Confocal laser microscopy was used to quantify the hybrid layer formation in dentin.

Results: G1 and G2 presented higher adhesive system bond strength (MPa) than G4 and G5, however no significant differences were observed in comparison with G3. Cohesive fracture was frequently found: G1 (58.5%), G2 (51.3%) and G3 (43.8%). G1 showed the highest incidence of precipitates and the highest number of blocked dentinal tubules. G1 and G2 presented similar hybrid layer formation and the highest hybrid layer formation values.

Conclusions: Desensibilize Nano P (G1) favored the bond strength of the adhesive system to dentin, increased the precipitation of residues, obliteration of dentinal tubules, and hybrid layer formation in comparison with other agents.

Clinical relevance: Desensitizers promote dentin obliteration, however, may affect dentin bonding.

1. Introduction

Dentin hypersensitivity (DH) is pain resulting from dentin exposure in response to chemical, thermal, tactile or osmotic stimuli and cannot be considered a pathology or any other defect. This pain does not occur spontaneously and disappears after the stimulus is removed [1–3].

DH affects a large part of the population in all ethnic groups. A study on Dentin Hypersensitivity conducted by the Canadian Advisory Board reported a prevalence ranging from 8 to 57% in adults between 20 and 40 years old [1]. However, the treatments are not effective and definitive.

Cervical root dentin exposure and presence of open dentinal tubules are two fundamental conditions for DH development [4–6]. Thus, therapeutic procedures for DH are based on substances that depress neural transmission, and also, they may be associated with obliteration of exposed dentinal tubules [7,8].

Several DH protocols mainly containing potassium nitrate associated with sodium fluoride and / or strontium chloride have been proposed, however showing disappointing clinical results [9,10]. On the other hand, calcium phosphate nanoparticles associated with potassium nitrate and sodium fluoride (Desensibilize Nano P) provide calcium and fluoride ions deposition on dentin surface, favoring dentinal tubules obliteration [11]. However, many cases require an adhesive restoration after desensitization protocol, and the effects of DH treatments on the bond strength of *etch-and-rinse* adhesive systems to dentin, and on the hybrid layer formation are still unclear.

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Therefore, the aim of this study was to evaluate the effect of a desensitizing agent containing calcium phosphate nanoparticle (Desensibilize Nanop) on the bond strength of *etch-and-rinse* adhesive system (Scotchbond Multi-Purpose), the presence of precipitate, dentinal tubule obliteration and hybrid layer formation in dentin in comparison with potassium nitrate with sodium fluoride (Ultra EZ and Desensibilize KF 2%) and / or strontium chloride (Desensibilize) compounds. The null hypothesis was that desensitizing agents would have no effect on the parameters analyzed.

2. Materials and methods

This present study was approved by the Ethics Committee of Araraquara Dental School, São Paulo State University (Proc. CEUA no 2/2015). A hundred and fifty bovine incisors with similar crowns and root anatomies were stored in 0.1% thymol solution at 4 °C until the research began.

Fragments (10 mm long × 10 mm wide × 5 mm thick) were obtained from the middle third of the tooth by using a hard tissue cutting machine (Isomet 100, Buehler, Lake Bluff, IL) cooled under running water. The buccal surfaces of fragments were flattened with a polishing machine (DP-10; Panambra, Struers, Ballerup, DI) using #600 sandpaper for 20 s (ISO 6344-1), to expose the dentin, and planing to standardize the smear layer. The dentin surface was previously treated by using 17% EDTA (Biodinâmica, Ibiporã, PR, Brazil) for 3 min to simulate an exposed and sensitive dentin model [9,10]. Afterwards, the specimens were rinsed with distilled water and stored in artificial saliva until they were used.

2.1. Application of dentin desensitization protocols

Four protocols using dentin desensitizer agents were used in this study. The specimens were randomly divided into four groups (G1 to G4) and a control (G5) as described in Table 1 (n = 10). All desensitization protocols were performed in accordance with the respective manufacturer's instructions. In G1, the desensitizer agent was rubbed on the dentin surface with a rubber cup for 10 s and left undisturbed for 5 min during every session. All groups were submitted to 4 treatment sessions at 1week intervals [12]. Between each session, the teeth were stored in artificial saliva (0.375 g/l $CaCl_2.2H_2O$, 0.125 g/l $MgCl_2.6H_2O$, 1.2 g/l KCl, 0.85 g/l NaCl, 2.5 g/l $NaHPO_4.12H_2O$, 1 g/l sorbic acid, 5 g/l hydroxyethyl cellulose-sodium, and 43 g/l sorbitol solution) (Arte & Ciência, Araraquara, São Paulo, Brazil), at 37 °C. The artificial saliva was replaced in every session. G5 (negative control) received no treatment and was kept in artificial saliva.

2.2. Evaluation of residue precipitation and dentinal tubule occlusion

Fifty specimens were randomly divided into experimental groups and control (n = 10). After the desensitization protocols, the specimens were kept in artificial saliva for 24 h. The specimens were dried inside a closed chamber containing colloidal silica for 7 days. Then the specimens were mounted onto metal stubs, gold-sputter coated (single cycle-120 s) under vacuum, in a metalizing chamber (MED 010, Balzers Union, Balzers, Liechtenstein) and examined by means of scanning electron microscopy with JEOL 6060 (JEOL 6060; JEOL Ltda, Tokyo, Japan) operating at 20 kV. Four different fields were initially assessed and the most representative image was obtained at 500× magnification.

Another image at 1000× magnification was obtained from this same site to count the unblocked and open dentinal tubules. All images were obtained by only one operator. Another two calibrated examiners classified the presence of residues according to Kuga et al. [13]. Two different examiners counted the open dentinal tubules in each image of the specimens. The results obtained by the two examiners were used to calculate the mean value that was determined for the specimen.

Table 1
Composition of dentin desensitizing agents.

Group	Composition	Manufacturer
G1 – Desensibilize NanoP	$Ca_3(PO_4)_2$ (nm) + KNO_3 (5%) + NaF (0.9%)	FGM, Brazil
G2 – Desensibilize	$SrCl_2$ (10%) + KNO_3 (5%)	FGM, Brazil
G3 – Desensibilize KF 2%	KNO_3 (5%) + NaF (2%)	FGM, Brazil
G4 – Ultra EZ	KNO_3 (3%) + NaF (0.25%)	Ultradent, USA
G5 – Negative Control	–	–

$Ca_3(PO_4)_2$ (nm), calcium phosphate nanoparticles; KNO_3, potassium nitrate; NaF, sodium fluoride; $SrCl_2$, Strontium chloride.

2.3. Energy-dispersive X-ray analysis (EDX)

Four specimens of each group were analyzed using scanning electron microscopy (SEM) and submitted to carbon coverage (BalTec SCD 004 Sputter Coater; Balzers,Vaduz, LI), at 15 kV for 180 s. Afterwards, dentin surface was submitted to SEM and EDX (JEOL 6060; JEOL Ltda, Tokyo, Japan) to assess the precipitate formation.

2.4. Bonding procedure

After the treatment protocols, one hundred specimens were randomly divided: fifty were subjected to testing to evaluate the bond strength of the adhesive system to cervical dentin; and fifty to access the hybrid layer formation. Acid etching was performed with 37% phosphoric acid (Condac 37; FGM, Joinville, Brazil), for 15 s and rinsed with distilled water for 30 s. The surface was slightly dried using air spray and the *etch-and-rinse* adhesive system (Scotchbond Multi-Purpose; 3 M ESPE, St. Paul, MN, USA) was immediately applied according to manufacturer's instructions. The adhesive system was light polymerized by using a LED system (LED Bluephase; Ivoclar Vivadent, Schan, Liechtenstein, AL), with an intensity of 1200 mW / cm^2 for 10 s.

2.5. Bond strength evaluation

After all treatment protocols, four cylinders made of composite resin specimens were prepared on the buccal surface, two in the mesial and two in the distal region (n = 40). A transparent Tygon tube matrix (Tygon tube, R-3603; Saint-Gobain Performance Plastics, Miami Lakes, FL, USA) with an internal diameter of 0.7 mm, and 1.0 mm high was used for the composite resin filling (Filtek Z-250; 3 M, St. Paul, MN, USA) and light polymerized for 40 s.

After preparation, the specimens were stored in a 100% relative humidity environment at 37 °C and the shear bond test was performed after 24 h. All fragments were fixed in a metal matrix so that the composite cylinder specimens were placed perpendicularly to a load cell of 0.5 N. An orthodontic wire (0.2 mm in diameter) held the composite cylinder base. All specimens were subjected to compressive loading at a crosshead speed of 0.5 mm/min in an EMIC DL2000 electromechanical testing machine (EMIC, São José dos Pinhais, PR, Brazil) until the displacement of the composite specimens. The bond strength was obtained from the maximum force (N) divided by the bond area (mm^2), in MPa. An arithmetic average was calculated for the four composite specimens from each fragment and considered the mean value of the specimens.

2.6. Fracture mode evaluation

After the shear bond test, the specimens were dehydrated in colloidal silica for 24 h. The fracture mode was analyzed by using a stereomicroscope (Leica Microsystems, Wetzlar, Germany), at 50× magnification in order to classify the fracture: (AD) adhesive, when the fracture occurred between the dentin and the adhesive system; (C)