Influence of diode laser treatment and casein phosphopeptide-amorphous calcium phosphate paste on eroded root dentin

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Abstract

Introduction: It is still unclear whether the pretreatment for dentin hypersensitivity can interfere with the adhesive–dentin bonding strength. This study aimed to evaluate in vitro the effect of pretreatment with a casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) paste and diode laser on the shear bond strength of the etch-and-rinse adhesive system in the adhesive–dentin interface.

Materials and Methods: Seventy-five bovine root specimens were randomly divided into five experimental groups (n = 15). Samples were eroded by six cycles of immersion in hydrochloric acid solution (0.01M; pH = 1.2) for 20 s each, at 45 min intervals, and treated with: G2 – no treatment, G3 – CPP-ACP, G4 – diode laser (970 nm), and G5 – CPP-ACP + Diode laser. After treatments, the morphology of the specimens was analyzed.

Results: The fracture mode of each group was evaluated after restoration with composite resins in the treated area. Data were analyzed by the one-way ANOVA and Tukey’s test (P < 0.05). G3 showed the highest values for shear bond strength; there was no significant difference among G1, G3, and G5 (P > 0.05). The adhesive fracture was predominant for all groups.

Conclusion: The use of desensitizers containing CPP-ACP, associated or not with a diode laser, increased the bond strength of the etch-and-rinse adhesive system on eroded root dentin.

Keywords: Confocal microscopy; dentin; erosion; fracture; surface treatment

INTRODUCTION

Since the number of preventive health dental programs has increased along with the knowledge about oral care, the vitality of teeth within the oral cavity continues to grow.[1] As a result of the reduction of tooth loss, noncarious cervical lesions have become more frequent nowadays.[2,3] One of the complications of this condition is a common complaint among adults and represents one of the most critical and persistent problems, dentin hypersensitivity (DH).[4,6]

DH is an exaggerated response to sensorial stimuli that generally would not disturb a normal and healthy tooth.[6,7] It is described as an acute, nonspontaneous, short, or long-lasting sharp pain caused by the exposure of dentin to thermal, mechanical, chemical, evaporative, tactile, or osmotic stimuli, which cannot be attributed to any other dental defect or pathology.[6,8-10] The prevalence of this condition is variable (8%–57%),[11-13] being much more frequent (72%–98%) in patients with periodontal disease due to the exposure of the root surfaces.[1]

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is a milk protein derivative. This complex remineralizes dentin by buffering free calcium and...
phosphate ions on the dentin surface, protects the dental structure from demineralization, and simulates natural desensitizing processes by occluding dentinal tubules and forming dentin-like minerals.\textsuperscript{[11,14,15]}

Low- or high-power laser has become a successful alternative treatment option to DH.\textsuperscript{[8]} The irradiance of dentin with diode laser provides low-energy emission with little temperature increase, stimulating circulation and cellular activity, and promoting positive responses such as anti-inflammatory, analgesic, vascular, and tissue healing.\textsuperscript{[8,10,16]}

Generally, patients suffer from DH present dental wear, and in some cases, a simple desensitizing treatment becomes insufficient to attend patient’s needs.\textsuperscript{[15]} Thus, when the desensitizing treatment is not enough, the esthetic restorative procedure with composite resin is performed to avoid discomfort and evolution of the noncarious cervical lesion. However, it is still unclear whether the pretreatment with desensitizer agents and laser irradiation can interfere with the adhesive–dentin bonding strength or not, compromising the longevity of the restoration.\textsuperscript{[3,15,17]}

This study aimed to evaluate in vitro the influence of a CPP-ACP desensitizing paste (MI Paste, USA) and low-power diode laser (Sirona Dental Systems, Germany) on the bond strength of the adhesive–dentin interface, the fracture mode after the shear bond test, and the surface morphology after treatment on the eroded root dentin.

**MATERIALS AND METHODS**

**Experimental design**

Seventy-five bovine root dentin specimens were randomly assigned (one factor – desensitizer treatment in five levels) into five groups (n = 15): sound dentin (no treatment), eroded dentin (no treatment), eroded dentin treated with CPP-ACP (MI Paste, GC America, Alsip, IL), diode laser (Sirona Dental Systems, Bensheim, Germany), or CPP-ACP + diode laser [Table 1]. The quantitative variable was bond strength to dentin in megapascals (MPa), obtained from a shear strength test performed in each specimen. The qualitative variables were evaluated by the prevalent fracture mode and the morphology of the dentin surfaces after treatment.

**Specimen preparation**

Fresh extracted bovine incisors were sectioned at the cementoenamel junction, separating root from crowns. Seventy-five specimens of bovine root dentin were obtained (4 mm × 4 mm × 2 mm). The specimens were flattened with #400 sandpaper. In the sequence, the smear layer was prepared with #600 sandpaper for 30 s (DP-9U2, Struers A/S, Copenhagen, Denmark). Fifteen specimens were randomly separated to compose the control group (G1 – sound dentin). The other specimens had their surfaces individually immersed in 5 mL of a hydrochloric acid solution (0.01M and pH = 1.2) inside a container placed on an orbital shaker (CT155, Cientec, Piracicaba, Brazil) for 20 s. This cycle was repeated six times at 45 min intervals. During these intervals, the specimens were rinsed with deionized water and individually stored in 5 mL of artificial saliva.\textsuperscript{[10]}

**Desensitizer treatments**

The specimens were randomly divided into the treatment groups:

- **G1** – Control group without erosion-sound dentin;
- **G2** – Control group eroded: specimens were eroded and did not receive any pretreatment;
- **G3** – Casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACP): specimens were pretreated with CPP-ACP according to the manufacturer’s instructions and applied with a microbrush. After 5 min, the paste was carefully removed;
- **G4** – Diode laser: specimens were irradiated with a wavelength of 970 nm, on contact mode, perpendicular to the surface, with a power of 0.5 W for 20 s, with a fiber of 400 mm in diameter (energy density – 396.82W/cm²);
- **G5** – CPP-ACP + diode laser: specimens were pretreated with CPP-ACP for 5 min and simultaneously irradiated with laser on contact mode (same parameters as G4).

After treatment, the specimens were stored in artificial saliva at 37°C for 24 h.\textsuperscript{[18]} Then, each group was analyzed and restored.

**Morphology analysis**

Five specimens were analyzed with a LEXT OLS4000® confocal laser microscope (LEXT, 3D Measuring Laser Microscope OLS 4000, Olympus Corporation) after desensitizer treatment (2137x magnification).

| Table 1: Material and devices used in this study |
|-----------------------------------------------|
| **Material** | **Composition** | **Manufacturer** |
| MI Paste | Casein Phosphopeptide - Amorphous Calcium Phosphate | GC America, Alsip, IL |
| Diode Laser | 970 nm; 0.5 W; 20 s; energy density - 396.82 W/cm² | Sirona Dental Systems, Bensheim, HE, Germany |
| Adper™ Single Bond | Ethanol, Bis-GMA, silane treated with silica filler, HEMA, glycerol 1,3 dimethacrylate, Bis-GMA, UDMA, TEGDMA, PEGDMA, bis-EMA (b) resins | 3M/ESPE, St. Paul, MN, EUA |
| Bond 2™ | acrylic and itaconic acid copolymer, and diurethane dimethacrylate | 3M ESPE, St. Paul, MN, USA |
| Filtek™ Z350 | Bis-GMA, UDMA, TEGDMA, PEGDMA, bis-EMA (b) resins | 3M ESPE, St. Paul, MN, USA |

HEMA: 2-hydroxyethyl methacrylate
Restorative procedure
After 24 h of treatment, the specimens were analyzed by laser confocal microscopy (×107 magnification).

Subsequently, the specimens were etched with 37% phosphoric acid for 15 s, rinsed for more than 15 s, and excess humidity was removed by an absorbent paper. Then, two layers of the adhesive system (Adper™ Single Bond 2, 3M/ESPE, St Paul, EUA) were applied, followed by photopolymerization for 20 s (Kavo Poly Wireless; Kavo do Brasil, Joinville, SC, Brazil). Next, a split polytetrafluoroethylene jig was placed over the specimen surface to guide the insertion of the composite resin. The composite resin (Filtek™ Z350; 3M ESPE, St. Paul, MN, USA) was inserted over the surface in 2 mm increments, photopolymerization for 20 s, forming the shape of a cylinder (4 mm high, 2 mm in diameter).

Fracture mode evaluation
The specimens were stored for 24 h in distilled water at 37°C. Subsequently, the specimens were tested in an EMIC DL-2000 electromechanical testing machine (EMIC Ltda, São José dos Pinhais, Brazil) set at 0.5 mm/min with a 50 kgf load cell. Bond strengths data were recorded in units of kilogram-force per square centimeter and converted to megapascals.

Fracture mode was evaluated by laser confocal microscopy, classified as adhesive (A), fracture developed between the dentin and the adhesive system; cohesive (C), fracture occurred in the composite resin and/or in dentin; and mixed (M), when both fracture patterns happened.

Data analysis
After checking the normality and homogeneity of the results, data were analyzed by the one-way ANOVA and Tukey’s test \( (P<0.05) \). For morphological evaluation, comparative and descriptive analyses were performed.

RESULTS
The data analysis presented a significant difference between the groups [Table 2]. Eroded surfaces treated with CPP-ACP had the highest values of shear strength compared with the other groups \((P<0.05)\). The similarity was observed among the groups G1, G3, and G5 \((P<0.05)\), suggesting a similar behavior between the sound dentin and the eroded after treatment with CPP-ACP. In addition, the eroded groups that did not receive treatment with CPP-ACP, G2 e G4, presented the lowest values for shear strength.

As for the fracture mode [Figure 1], no differences were found between the groups, and the adhesive fracture was predominant for all groups.

Confocal microscopy images showed different morphological aspects. G1 (sound dentin) presented a smear layer on the surface and G2 (eroded dentin) showed open dentinal tubules and demineralized intertubular dentin. Compared to the other eroded groups, groups treated with CPP-ACP (G3 e G5) revealed some dentinal tubules with smaller diameters or occluded. As for the diode laser group (G4), specimens showed an irregular dentin surface and open tubules [Figure 2].

DISCUSSION
DH is a chronic dental problem. Despite the available self-care and professional treatments, DH still has an uncertain prognosis, \([8]\) and sometimes restorative treatment is the most plausible option. \([3]\) The present in vitro study evaluated the effect of at-home and in-office treatments on the shear bond strength of eroded root dentin. Results showed an increase in bond strength in all the treatments; however, the groups treated with the CPP-ACP exhibited better results. Diode laser was effective in raising the bond strength, even better than the eroded group without treatment, but presented inferior results compared to the sound dentin.

The positive behavior of CPP-ACP corroborates with the results of Yang et al., \([15]\) where the pretreatment with this desensitizing agent increased the immediate bonding strength of the etch-and-rinse adhesive system. These can be explained by the enhancement of the dentin surface wettability caused by the microsurface roughness. These findings indicated that CPP-ACP leads to a wetter surface with lower contact angles, favoring the mechanical

![Figure 1](attachment:image.png)

**Figure 1:** Percentage failure standard (A: Adhesive at the interface, M: Mixed, CR: Cohesive composite resin failure, and CD: Cohesive dentin failure)
interlocking and adhesion.\textsuperscript{15} In addition, it is documented that CPP-ACP can also increase the mechanical properties of the dentin surface by the mineralization of the surface,\textsuperscript{17} which can be observed in the morphological images after the treatment of the eroded dentin with the CPP-ACP paste.

Low-power lasers act by photobiomodulation and superficial interaction in dental structure, favoring adhesive penetration by increasing the bond strength of the adhesive system.\textsuperscript{12,17} This condition can be a consequence of the reduction of exposed dentinal tubules diameter caused by the interaction between the laser light and dental surface, which interacts with the different minerals present and intensifies the production of tertiary dentin.\textsuperscript{2} In addition, it also promotes a photobiomodulation effect and generates analgesia, anti-inflammatory effects, and a state of normality in the tissues.\textsuperscript{2,16} However, the laser group alone was not effective as CPP-ACP. Therefore, the association of desensitizing treatments is desirable since it is described as a synergistic effect.\textsuperscript{11}

The eroded group that receives no treatment presented the lowest results for bond strength. That could be explained by the higher contact angle descendant by the veering of wettability, making it difficult for the bonding agents to wet and spread over the surface.\textsuperscript{19} Consequently, the bonding strength of the restorative material is compromised by dental substrates.

Another relevant factor in the longevity of adhesive restorations is collagen.\textsuperscript{20} The adhesive system applied should involve all the networks of collagen exposed by the dentin demineralization; however, not all collagen fibers are in the hybrid layer; therefore, the adhesive system does not reach them all.\textsuperscript{28} In addition, the remaining collagen fibers at the base of the hybrid layer are susceptible to hydrolytic degradation and the action of collagenolytic enzymes. This results in infiltration and other failures in the restorative materials.\textsuperscript{20} Therefore, the degradation promoted by the enzyme matrix metalloproteinases (MMPs) sabotages the clinical longevity of the adhesive restorations. The subtypes of this enzyme found in human and bovine dentin are activated by an acid pH, heat, and the progression of caries, which can be present in procedures involving adhesive systems.\textsuperscript{20} For that, the pretreatment of dentin with synthetic MMP inhibitors seems necessary to improve bond stability, resulting in enhancing restoration longevity. Remineralization can be a valid option as an MMP inhibitor since the presence of mineral tissue around collagen fibers and its protection by the hybrid layer act as a barrier, protecting collagen against degradation.\textsuperscript{20,21} Thus, the use of CPP-ACP as a pretreatment before adhesive restorations could prolong its clinical longevity due to the mineral tissue formed by calcium and phosphate ions and the electrostatic attraction between calcium phosphate and collagen.\textsuperscript{20,21}

**CONCLUSION**

The use of a desensitizer containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) improved the bond strength of the etch-and-rinse adhesive system on eroded root dentin associated with or not to diode laser. The group with diode laser alone was less effective than CPP-ACP, but the combination of both agents resulted in a satisfactory arrangement.

**Acknowledgments**

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo-FAPESP (grant number 2012/10263-6; 2011/12901-7).
Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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