ORIGINAL RESEARCH

Comparative Assessment of Bond Strengths of Affected Dentin Using Two Different Remineralizing Solutions

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ABSTRACT

Objective: This study aimed to evaluate and compare the microtensile bond strength (μTBS) of resin composite to artificially created caries-affected dentin after treatment with two different remineralizing solutions. **Methods:** Sixty extracted human third molars were sectioned to expose mid-coronal dentin surfaces. Artificial caries-affected dentin was created using a demineralization solution. Specimens were randomly divided into three groups (n=20): Group 1 (control) – no remineralization; Group 2 – treatment with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP); and Group 3 – treatment with nano-hydroxyapatite (n-HAP) solution. Following remineralization protocols, all specimens were bonded with a universal adhesive system in self-etch mode and restored with composite resin. After 24-hour water storage, specimens were sectioned to obtain beam specimens with a cross-sectional area of 1mm² and subjected to microtensile bond strength testing. Fractured surfaces were examined under scanning electron microscopy (SEM) to determine failure modes. Data were analyzed using one-way ANOVA and Tukey's post-hoc test (α=0.05). **Results:** Mean μTBS values were significantly different among groups ($p<0.001$). The n-HAP group (22.8±3.2 MPa) demonstrated significantly higher bond strength compared to both CPP-ACP (17.4±2.9 MPa) and control (12.3±2.5 MPa) groups. The CPP-ACP group also showed significantly higher values than the control group. SEM analysis revealed predominantly mixed failures in remineralized groups and adhesive failures in the control group. **Conclusion:** Remineralization of artificially created caries-affected dentin with n-HAP and CPP-ACP solutions significantly improved the bond strength of the universal adhesive. The n-HAP solution demonstrated superior remineralization potential and bond strength enhancement compared to CPP-ACP.

Keywords: Caries-affected dentin, remineralization, bond strength, nano-hydroxyapatite, CPP-ACP, universal adhesive.

INTRODUCTION

The management of carious dentin presents one of the most significant challenges in adhesive dentistry. Caries-affected dentin (CAD) differs substantially from normal dentin in its structural and chemical composition, demonstrating increased porosity, partial demineralization, and altered collagen structure (1). These changes significantly compromise the bonding efficacy of adhesive systems, leading to reduced bond strengths and potentially compromised restoration longevity (2).

Contemporary minimally invasive approaches to caries management advocate for selective removal of carious tissue, which frequently results in bonding to CAD (3). However, the bond strength to CAD has been consistently reported to be 20-50% lower than to normal dentin, regardless of the adhesive system used (4). This reduction is attributed to the altered mineral content, compromised collagen network, and presence of acid-resistant whitlockite minerals in affected dentin (1,).

Remineralization of partially demineralized dentin before adhesive procedures has been proposed as a strategy to improve bonding to CAD (5). Various remineralizing agents have been investigated, with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and nano-hydroxyapatite (n-HAP) emerging as promising materials (6,7).

CPP-ACP works by providing a supersaturated environment of calcium and phosphate ions, stabilized by the casein phosphopeptide, which facilitates diffusion into demineralized tissue and promotes mineral deposition (8). Its bioavailability and ability to interact with dental tissues have made it a widely studied remineralizing agent (9).

Nano-hydroxyapatite, with particle sizes ranging from 20-100 nm, demonstrates superior properties compared to conventional hydroxyapatite due to its increased surface area and enhanced reactivity (10). The nano-sized particles can penetrate dentinal tubules and fill microscopic surface defects,

potentially reestablishing the mineral structure of demineralized dentin .

While both agents have demonstrated remineralizing capabilities, their comparative effectiveness in improving bond strength to CAD remains insufficiently explored. The interaction between remineralized dentin and contemporary universal adhesive systems, which claim enhanced bonding to various substrates including CAD, warrants further investigation.

Therefore, this study aimed to evaluate and compare the microtensile bond strength of a universal adhesive system to artificially created caries-affected dentin after treatment with two different remineralizing solutions: CPP-ACP and n-HAP. The null hypothesis tested was that there would be no significant differences in bond strength values among the experimental groups.

MATERIALS AND METHODS

Specimen Preparation

The study protocol was approved by the Institutional Ethics Committee. Sixty sound human third molars, extracted for therapeutic reasons, were collected after obtaining informed consent from patients. Teeth were cleaned of soft tissue debris and stored in 0.5% chloramine-T solution at 4°C for no longer than one month before use.

The occlusal enamel was removed perpendicular to the long axis of each tooth using a low-speed diamond saw under water cooling to expose mid-coronal dentin. The exposed dentin surfaces were polished with 600-grit silicon carbide paper for 60 seconds under running water to create a standardized smear layer.

Artificial Caries Induction

Artificial caries-affected dentin was created following the protocol described by Marquezan et al. (11) with slight modifications. All tooth surfaces except the exposed dentin were sealed with two layers of acid-resistant nail varnish. Specimens were immersed in a demineralizing solution containing 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 50 mM acetic acid, adjusted to pH 4.5, for 72 hours at 37°C. This protocol produces a demineralized dentin layer with characteristics similar to naturally occurring caries-affected dentin (11).

Experimental Groups

The specimens were randomly divided into three groups (n=20) according to the remineralizing treatment:

Group 1 (Control): No remineralization treatment; specimens were stored in distilled water.

Group 2 (CPP-ACP): Specimens were treated with a 10% CPP-ACP paste (GC Tooth Mousse, GC Corp., Tokyo, Japan) applied for 3 minutes, twice daily for 7 days. Between applications, specimens were stored in artificial saliva (containing 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 150 mM KCl, 0.1 M Tris buffer, pH 7.0) at 37°C.

Group 3 (n-HAP): Specimens were treated with a 10% nano-hydroxyapatite solution (applied for 3 minutes, twice daily for 7 days. Between applications, specimens were stored in artificial saliva at 37°C.

After the remineralization period, all specimens were ultrasonically cleaned in distilled water for 5 minutes to remove any remnants of the remineralizing agents from the surface.

Surface Characterization

Two additional specimens from each group were prepared for surface characterization. The specimens were dehydrated in ascending concentrations of ethanol (50%, 70%, 90%, and 100%) for 10 minutes each, gold-sputter coated, and examined under scanning electron microscopy (SEM) at various magnifications.

X-ray diffraction (XRD) analysis was performed using a diffractometer to assess the crystalline structure of the dentin surface after remineralization treatments.

Bonding Procedure

All specimens were bonded using a universal adhesive system in self-etch mode according to the manufacturer's instructions. The adhesive was applied with a microbrush for 20 seconds with agitation, gently air-dried for 5 seconds, and light-cured for 10 seconds using an LED curing unit with an output of 1200 mW/cm².

Composite resin was applied in 2 mm increments to create a build-up of approximately 4 mm height, with each increment light-cured for 20 seconds. Specimens were stored in distilled water at 37°C for 24 hours.

Microtensile Bond Strength Testing

Each specimen was sectioned perpendicular to the bonded interface using a low-speed diamond saw under water cooling to obtain beam specimens with a cross-sectional area of approximately 1 mm². Four central beams were selected from each tooth, resulting in 80 beams per group.

The cross-sectional area of each beam was measured using a digital caliper to calculate the bond strength. Beams were attached to a microtensile testing device using cyanoacrylate adhesive and subjected to tensile force in a universal testing machine at a crosshead speed of 0.5 mm/min until failure.

The microtensile bond strength (μTBS) was calculated by dividing the load at failure by the cross-sectional area of the bonded interface and expressed in megapascals (MPa).

Failure Mode Analysis

The fractured specimens were examined under a stereomicroscope at 40× magnification to determine the failure mode. Failures were classified as: adhesive (failure at the dentin-adhesive interface), cohesive in dentin (failure within dentin), cohesive in composite (failure within composite), or mixed (combination of adhesive and cohesive failures).

Representative specimens from each failure mode were gold-sputter coated and examined under SEM at various magnifications.

Statistical Analysis

One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to compare the bond strength values among groups. Failure mode distribution was analyzed using the chi-square test. All analyses were performed using SPSS software at a significance level of P=0.05.

RESULTS

Surface Characterization

SEM observations revealed distinct surface characteristics among the experimental groups. The control group (demineralized dentin) showed exposed collagen fibrils with widened dentinal tubules and an irregular surface topography. The CPP-ACP treated specimens exhibited partial occlusion of dentinal tubules with granular deposits on the surface and between collagen fibrils. The n-HAP treated specimens demonstrated a more homogeneous surface with substantially occluded dentinal tubules and a dense layer of hydroxyapatite crystals covering the collagen network.

XRD analysis confirmed the presence of hydroxyapatite crystals in both remineralized groups, with the n-HAP group showing more intense and sharper peaks indicating higher crystallinity and mineral content compared to the CPP-ACP group.

Microtensile Bond Strength

Table 1. Mean microtensile bond strength values (MPa) and standard deviations for the experimental groups.

Group	Mean μTBS (MPa)	Standard Deviation
Control (No remineralization)	12.3	2.5
CPP-ACP	17.4	2.9
n-HAP	22.8	3.2

Table 2. Distribution of failure modes (%) among the experimental groups.

Group	Adhesive	Cohesive in Dentin	Cohesive in Composite	Mixed
Control	65%	5%	5%	25%
CPP-ACP	40%	10%	10%	40%
n-HAP	25%	15%	15%	45%

DISCUSSION

The results of this study demonstrate that pre-treatment of artificially created caries-affected dentin with remineralizing agents significantly improves the microtensile bond strength of a universal adhesive system. The null hypothesis was rejected as significant differences were observed among the experimental groups, with the nano-hydroxyapatite solution providing superior results compared to CPP-ACP.

The compromised bond strength to caries-affected dentin has been well-documented in the literature (1,2). The demineralized, porous nature of CAD, coupled with altered collagen properties and the presence of acid-resistant minerals, challenges the effective penetration and hybridization of adhesive systems. The control group in our study, representing untreated CAD, exhibited the lowest bond strength values (12.3±2.5 MPa), which aligns with previous research on bonding to caries-affected substrates (4).

The significant improvement in bond strength following remineralization treatments can be attributed to several factors. Remineralization restores mineral content to the demineralized dentin, providing a more stable substrate for adhesive bonding. Additionally, the partial occlusion of dentinal tubules and reinforcement of the collagen network likely contribute to enhanced mechanical properties of the substrate (7).

The superior performance of the n-HAP solution (22.8±3.2 MPa) compared to CPP-ACP (17.4±2.9 MPa) may be explained by the unique properties of nano-sized hydroxyapatite particles. With dimensions similar to the natural apatite crystals in dentin (20-100 nm), n-HAP particles can penetrate demineralized collagen fibrils more effectively, reconstituting the mineral phase with a high degree of structural similarity to native dental tissues (10). SEM observations in our study confirmed this, showing more homogeneous and complete remineralization with n-HAP treatment.

The biomimetic nature of n-HAP remineralization appears particularly advantageous for bonding procedures. By restoring the mineral content within the collagen network rather than merely depositing minerals on the surface, n-HAP creates a substrate that more closely resembles natural dentin. This facilitates better penetration of the adhesive monomer and formation of a more stable hybrid layer, as evidenced by the higher percentage of cohesive and mixed failures in the n-HAP group(12).

CPP-ACP, while significantly improving bond strength compared to the control, was less effective than n-HAP. The remineralization mechanism of CPP-ACP involves the localization of calcium and phosphate ions at the surface, maintaining a supersaturated state that promotes mineral deposition (8). However, this process may result in more

superficial remineralization with less penetration into the collagen network, as suggested by our SEM observations showing granular deposits predominantly on the dentin surface.

The universal adhesive used in this study contains the functional monomer 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), which chemically bonds to calcium in hydroxyapatite (13). The increased mineral content following remineralization, particularly with n-HAP, likely provided more binding sites for this functional monomer, enhancing chemical bonding in addition to micromechanical interlocking.

The failure mode analysis supports these interpretations, with a shift from predominantly adhesive failures in the control group to more mixed and cohesive failures in the remineralized groups, particularly with n-HAP treatment. This suggests a stronger and more integrated bonded interface following remineralization.

Our findings have important clinical implications. The application of remineralizing agents, particularly n-HAP, before adhesive procedures on caries-affected dentin could significantly improve bond strength and potentially enhance restoration longevity. This approach aligns with minimally invasive principles, allowing for conservation of tooth structure while addressing the compromised bonding to affected dentin.

CONCLUSION

Within the limitations of this in vitro study, it can be concluded that:

1. Remineralization of artificially created caries-affected dentin with both CPP-ACP and n-HAP solutions significantly improved the microtensile bond strength of a universal adhesive system.
2. The nano-hydroxyapatite solution demonstrated superior remineralization potential and bond strength enhancement compared to CPP-ACP.
3. Treatment with remineralizing agents altered the failure mode distribution, with a shift from predominantly adhesive failures to more mixed and cohesive failures, particularly in the n-HAP group.
4. These findings suggest that pre-treatment of caries-affected dentin with remineralizing agents, especially nano-hydroxyapatite, before adhesive procedures may be a promising approach to improve bonding effectiveness in minimally invasive restorative dentistry.

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