ORIGINAL ARTICLE

Microtensile Bond Strength of Composite Resin Following the Use of Bromelain and Papain as Deproteinizing Agents on Etched Dentin: An *In Vitro* Study

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Abstract

Aim and objectives: The aim of this study was to evaluate and compare the deproteinizing effect of sodium hypochlorite, bromelain, and papain on microtensile bond strength of composite resin to etched dentin.

Materials and methods: Eighty freshly extracted permanent molars were wet grounded into a flat surface using a diamond disk to expose the superficial dentinal surface. Teeth were etched with 37% phosphoric acid for 15 seconds and rinsed with water and blot dried. Teeth were divided into four groups ($n = 20$) based on the method of dentin deproteinization. Group I: only etching; group II: deproteinized with 5.25% sodium hypochlorite for 1 minute; group III: deproteinized with 8% bromelain enzyme for 1 minute; and group IV: deproteinized with 8% papain enzyme for 1 minute. All the samples were washed off with distilled water to remove deproteinizing agents. Sample surfaces were blot dried and bonding of the dentin surface was performed and restored with light cure bulk fill composite. Samples were stored in distilled water (37°C/24 hours) and thermocycled. Then, the teeth were longitudinally sectioned and individually fixed to a sectioning block using acrylic resin. The block was mounted on hard tissue microtome and sectioned to get one to three slabs of 1 mm thick sections. The beam was then attached to a custom-made jig using screws subjected to the Instron universal testing machine. A tensile load was applied at a crosshead speed of 0.5 mm/minute until the beam fractured.

Results: Higher mean bond strength was recorded in group IV followed by group III, group II, and group I, respectively. Group III presented a statistically significant highest mean score compared to other study groups with group I and group II ($p < 0.001$), followed by group IV having significantly higher mean score compared to group I and group II ($p < 0.001$) and finally a significant difference was observed between group II and group I ($p < 0.001$). However, the mean microtensile bond strength score did not differ significantly between group III and group IV ($p = 0.20$).

Conclusion: Within the limitations of this present *in vitro* study, the following conclusions were drawn. The microtensile bond strength of dentine tested in various deproteinizing agents is as follows: 8% bromelain > 8% papain > 5.25% NaOCl > control group. Naturally occurring deproteinizing agents, such as bromelain and papain, used in this study have resulted in greater bond strength values when compared to that of traditionally used chemical agent such as NaOCl.

Keywords: Adhesion, Bromelain, Papain.

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Introduction

The essential precept of adhesion to enamel is based totally upon an exchange technique by using which inorganic tooth material is exchanged for artificial resin.[1] The sturdiness of the adhesive–dentin interface is related to the capacity of adhesive monomers to occupy the spaces created with the aid of the elimination of apatite mineral through acid etching and to envelop the exposed collagen fibrils.[2] In etch-and-rinse adhesives, phosphoric acid eliminates the mineral content material of the dentin tissue to show the collagen mesh. Eventually, the dentin is saved wet for bonding to prevent the disintegrate of the collagen mesh and to permit for the penetration of the adhesive resin.[3,4]

Loss of bond energy of etch-and-rinse adhesives has been demonstrated in some research. A zone of collagen is created at the bottom of the hybrid layer by partial infiltration of the resin monomer into unsupported collagen fibrils, which is due to the acid etching with strong acid. This leaves the collagen fibrils exposed to biodegradation by using matrix metalloproteinases (MMPs) present in dentin and compromise the bond strength.[5]

This biodegradation can be prevented by means of two methods, MMP inhibitors or deproteinizing materials. Matrix metalloproteinases are present as latent form in the dentin substrate.[6,7] The pH adjustments can modulate the activation and expression of MMPs that bring about biodegradation of the collagen fiber.[5] Particular inhibitors like chlorhexidine may want to inhibit

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MMPs, however it turned into now not capable of absolutely prevent the lower in bond strength.[8]

Dentin deproteinization is amendment of the dentin substrate using proteolytic materials. Deproteinizing materials do away with the unsupported collagen network and reduce the discrepancy between the depth of acid etching accomplished and enhance the bond strength.[7] Sodium hypochlorite (NaOCl) solution has been used as a deproteinizing agent to enhance the steadiness of the hybrid layer.[9] But NaOCl has several drawbacks.[10] To overcome these, newer agents have been investigated.

Bromelain is a deproteinization agent obtained from the pineapple fruit stem. It catalyzes hydrolysis of protein into amino acid.[11] In intact dentin, monomer's diffusion potential is enhanced and in acid etch dentin removal of the collagen network by bromelain. One-minute application of bromelain on acid etch dentin showed better collagen removal and better shear bond strength than sodium hypochlorite when used as a deproteinizing enzyme.[7,8]

Papain is similar to human pepsin. It belongs to the Caricaceae family (papaya). Papain is a proteolytic enzyme that is successfully (Papacárie, Brazil) used to remove degraded organic matrix in carious lesion.[12] The carious development formed "mantle" fibrin, which is eliminated by papain without harming the intact collagen fibrils.[13] The use of the deproteinizing agent before bonding orthodontic band on etched enamel has shown an increase in the shear bond strength.[14]

Hence, this study is carried out to compare the deproteinizing effect of sodium hypochlorite, bromelain, and papain on microtensile bond strength of composite resin to etched dentin.

Materials and Methods

Eighty periodontally compromised, intact molars were collected and stored. The occlusal surface of all the test samples was wet grounded into a flat surface using a diamond disk to expose the superficial dentinal surface. In total, 37% phosphoric acid was applied for 15 seconds and cleaned with water and dried.

Teeth were then divided into four groups ($n = 20$) depending on the deproteinization method of dentin.

Group I: Only etching;

Group II: Deproteinized with 5.25% sodium hypochlorite for 1 minute;

Group III: Deproteinized with 8% bromelain enzyme for 1 minute;

Group IV: Deproteinized with 8% papain enzyme for 1 minute.

All the samples were cleansed with distilled water. Bonding of the dentin surface was performed, and the samples were then restored with light cure bulk fill composite. The samples were thermocycled for 500 cycles between 5°C and 55°C with a dwell time of 30 seconds to simulate the oral environment. Then, the teeth were longitudinally sectioned into two halves with the diamond disk using a low-speed handpiece, and the intact half of the sample was selected for the study. The samples were then cropped to a dumbbell shaped (Fig. 1) using ultrafine diamond bur operated in a high-speed handpiece with ample water spray.

Microtensile Bond Strength

The sample was fixed individually using acrylic resin to a sectioning block. The block was subjected to hard-tissue microtome and sectioned to get one to three slabs of 1-mm-thick sections. The slices had been then trimmed and formed to form a mild curve along the adhesive interface with superfine diamond burs to gain a rectangular cross-sectional shape with the surface area of approximately 1 mm × 1 mm.

The beam was then connected to a custom-made jig and has been subjected to the Instron universal testing machine (Fig. 2). A tensile load was applied at the pace of 0.5 mm/minute till the beam fractured, and the load at which the fracture befell changed into was recorded (Fig. 3). The microtensile bond strength values were recorded in units of megapascals (MPa).

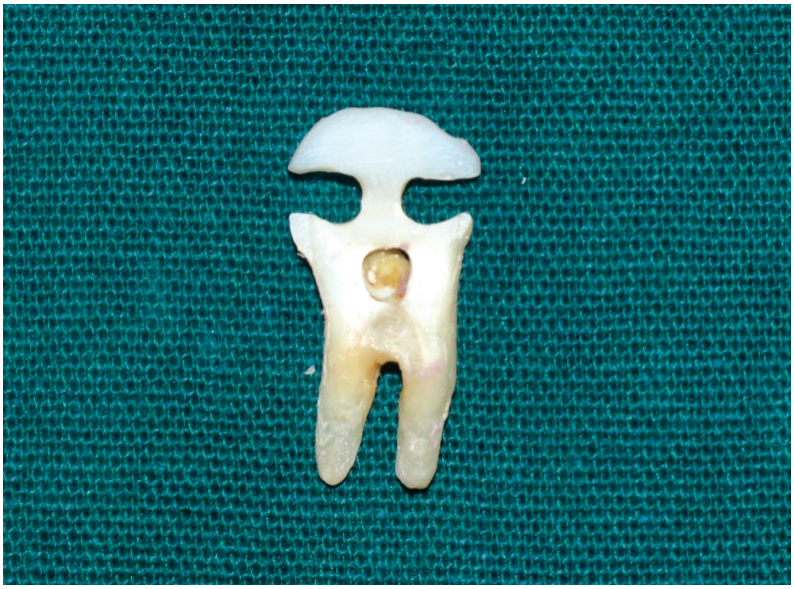

Fig. 1: Dumbbell-shaped specimen

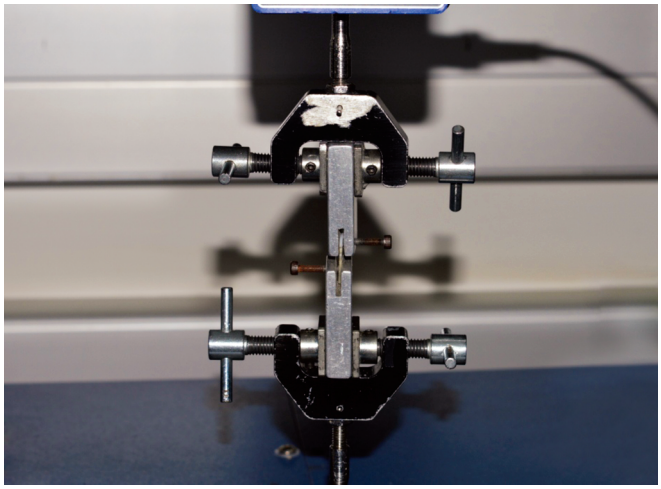

Fig. 2: Universal testing machine

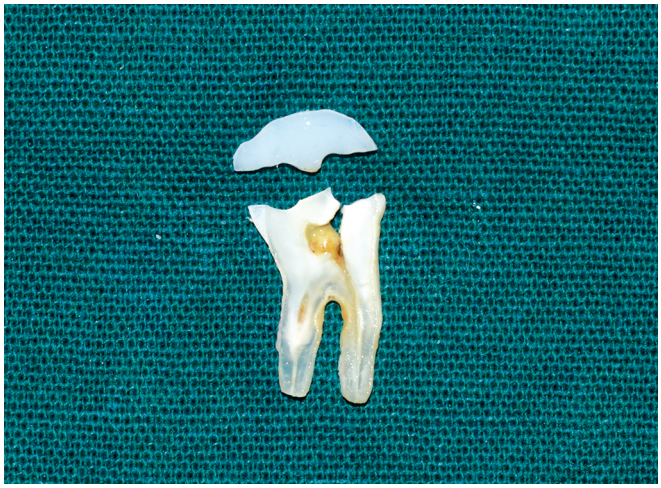

Fig. 3: Fractured specimen

Table 1: Comparison of mean microtensile bond strength between the four study groups using one-way ANOVA test

Groups	*N*	*Mean*	*SD*	*Standard error*	*Minimum*	*Maximum*	*F*	*p value*
Group I	20	21.94	1.86	0.42	18.65	24.74	52.221	<0.001*
Group II	20	25.79	1.79	0.40	23.23	28.95		
Group III	20	31.65	2.71	0.61	26.9	34.9		
Group IV	20	29.96	3.89	0.87	24.21	34.74		

*Statistically significant; SD, standard deviation; ANOVA, analysis of variance

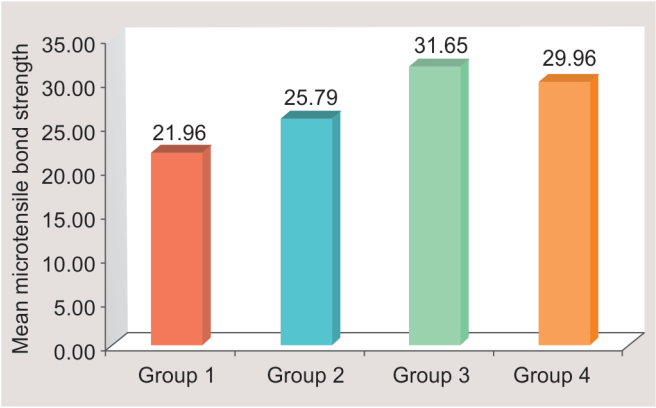

Fig. 4: Comparison of mean microtensile bond strength between the four study groups

Results

The application of bromelain enzyme substantially influenced the bond strength ($p < 0.05$) as shown in Table 1 and Figure 4. Statistically significant differences were not demonstrated in bromelain- and papain-treated group as shown in Table 2. Group III represents the highest bond strength compared with others groups.

The test results demonstrated that group I exhibited a mean score of 21.94 ± 1.86, group II with 25.79 ± 1.79, group III with 31.65 ± 2.71, and group IV with 29.96 ± 3.89. There was a statistically significant distinction within the mean microtensile bond strength between the four study groups at $p < 0.001$.

Multiple comparisons between the groups using Tukey's honest significant difference (HSD) *post hoc* analysis revealed (Table 2) that group III presented a highest mean score that was statistically significant followed by group IV having higher mean score compared with group I and group II, and that was significantly different. However, the mean microtensile bond strength score did not differ significantly between group III and group IV ($p = 0.20$).

Discussion

An application of resin-dentin bonding agent changes the properties of dentin from hydrophilic to more hydrophobic, crystalline to organic, relatively impermeable to highly permeable, and acid-labile surface to acid-resistant surface.[15]

Smear layer containing microcrystalline debris with the thickness up to 1 to 2 µm.[15] It can be removed by 37% phosphoric acid application for 15 seconds which leads to the collagen fibril exposure.[16] An adhesive comonomers infiltrate in-between the space of collagen fibrils forming hybrid layers.[17]

Bonding of composite resin to dentin varies with the quality of the dentin substrate. There is enough evidence in the literature that bonding of composite resin is much less with carious dentin when compared with sound dentin.[18] There have also been many studies showing composite dentin bond strength is greater in superficial dentin when compared with deep dentin.[19] Hence, in the present study, human non-carious, superficial dentin were used.

Nanoleakage most commonly occurs in the total-etch procedure due to the variation between dentin demineralization depth and monomer penetration which leads to the collagen degradation and causes nanoleakage.[20] Studies have shown that the removal of damaged collagen with deproteinizing agent can be an appropriate approach to overcome this hassle. Because it alters the composition of the dentin surface, it turns into similar to etched enamel that is greater predictable and hydrophilic substrate for bonding.[6]

Table 2: Multiple comparison for mean differences in microtensile bond strength between the four study groups

Group	*G1 vs G2*	*G1 vs G3*	*G1 vs G4*	*G2 vs G3*	*G2 vs G4*	*G3 vs G4*
Mean difference	−3.85	−9.72	−8.02	−5.86	−4.17	1.69
p value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.20

*Statistically significant

Sodium hypochlorite is a deproteinizing agent when used after acid etching dissolves the exposed collagen fibrils and alters ultramorphology of the dentin surface.[21] Since it has certain drawbacks associated, newer agents have been investigated.

Bromelain is a complex mixture of proteinases that acts on gelatin and degrades it into oligo peptides and amino acids. It also has collagenase activity.[11] Studies have reported that 1-minute application of the bromelain enzyme on etched dentin significantly decreased nanoleakage by removal of the collagen fibrils.[6] Studies have shown that the proteolytic action of bromelain is greater liable to spontaneous inactivation in water while the bromelain concentration is dilute (less than 6%).[22] Hence, in this study, 8% bromelain was used as a deproteinizing agent.

Papain (Papacárie) is a proteolytic enzyme that is successfully used to remove degraded organic matrix in carious lesion.[15] Pithon et al. reported that papain deproteinization action escalated as the concentration of papain increased to 8 and 10% that causes bond strength enhancement at 8 and 10%.[14] Hence, in the present study, 8% papain was used as a deproteinizing agent.

The bonding agent used in this current study is (Prime and Bond NT; Dentsply, Germany) acetone based and requires a moist dentin surface to produce adequate bonding.[23] Duarte et al. found that nanoleakage was absent in deproteinized specimens where Prime and Bond NT was used as a bonding agent. This is due to the presence of acetone, which has water shifting potential and resulted in improved contact of Prime and Bond NT with the deproteinized dentin, thus reducing the nanoleakage.[24]

The specimens were restored with composite, SureFil smart dentin replacement or shrinkage decreased resin (SDR), after deproteinization and before subjecting them to bond strength test. SureFil flow contains a polymerization regulator, chemically embedded within the middle of the polymerizable resin spine of the SDR monomer, to bring low the polymerization shrinkage and high mechanical property.[25,26]

Restored samples were subjected to microtensile bond strength test. All the experimental groups tested in this study showed high bond strength values confirming that deproteinizing agents used in this study had a significant effect when compared with the controlled group, confirming the positive effect. This could be because of their deproteinizing action, which eliminates the peripheral unstable collagen layer and the subsurface remnants from the etched dentin surfaces and multiplied wettability and permeability of the dentin substrate because of the expansion of dentinal tubules near the outer dentin floor improving the spreading and diffusion of adhesive monomers through dentin.[22,27] The diffusion of the monomers into the dentin and dentinal tubules occurs due to hydroxyapatite high surface energy when compared with the collagen.[27]

The various groups tested in this study showed that group III had higher microtensile bond strength values followed by group IV, group II, and group I. However, the difference between group III and group IV was found to be statistically not significant.

In total, 5.25% NaOCl-treated (group II) group had a significantly lower bond strength compared with bromelain and papain group. This is because NaOCl application can cause organic matrix mutilation.[23] In addition, incomplete polymerization occurs due to the free oxygen which is liberated during NaOCl breakdown.[10,28]

In group III, when bromelain was used on etched dentin, it significantly increased the bond strength. This is because of the ability of bromelain enzyme to wipe out the deteriorate collagen network from acid etched dentin precisely. Disruption of the degraded collagen can cause increase in the bond strength by increasing diffusion potential.[6] Also, it has been shown in the previous study that bromelain enzyme has better effectiveness in removing unsupported collagen matrix when compared with NaOCl and showed lower nanoleakage.[7]

Papain (group IV) had significantly higher bond strength than group I and group II but comparable with bromelain (group III).[13] Papain has been used as a chemo-mechanical agent for caries removal (Papacárie).[14] It acts with the aid of solely breaking down the partially disrupted collagen molecules and contributing to the degradation and removal of the fibrin "mantle" formed via the carious process, without destructive intact collagen fibrils.[13]

In the present study, papain group (group IV) showed marginally lower bond strength values than the bromelain group (group III) that was not statistically significant.

To summarize the data analysis, the deproteinizing agents bromelain and papain alter the dentin substrate that increases the bond strength of composite resin to etched dentin. Bond strength after the use of bromelain and papain seems to be higher than when NaOCl was used.

Furthermore, the effect of these agents in various concentrations and time periods together with the ultrastructural changes on dentin can bring more helpful information on the optimal effect of their deproteinizing action. More long-term clinical studies will ultimately prove the feasibility and the long-term success of these agents in clinical practice.

Conclusion

The microtensile bond strength of dentin tested with various deproteinizing agents is as follows: 8% bromelain > 8% papain > 5.25% NaOCl > control group.

Bond strength was higher in all experimental groups confirming that deproteinizing agent used in this study had a significant effect.

Naturally occurring deproteinizing agents used in this study, bromelain and papain, resulted in higher bond strength values compared with NaOCl.

Bromelain and papain can be considered as an effective alternative deproteinizing agent on account of their collagenase activity.

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