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Effect of Dentin Deproteinization Using Sodium Hypochlorite and Bromelain Enzyme on Microtensile Bond Strength of Self-etch Adhesive at Different Dentin Depths: An in-Vitro Study

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Abstract

Background

Dentin bonding remains a clinical challenge in daily dental practice especially bonding to deep dentin due to structural differences compared to superficial dentin. The study aimed to evaluate the effect of dentin deproteinization using sodium hypochlorite (NaOCl) and bromelain enzyme on the microtensile bond strength (μTBS) and failure modes of one-step self-etch adhesive system to superficial and deep dentin.

Methods

Forty-eight sound human third molars were randomly assigned into two main groups according to dentin depth (superficial and deep). Each group was further subdivided into three subgroups (n = 8) according to surface treatment: control (no pretreatment), 10% NaOCl, and 10% bromelain enzyme. After adhesive application and resin composite buildup, specimens were thermoscycled then sectioned into beams to be subjected to μTBS testing. Failure modes were analyzed under stereomicroscopy. Data was analyzed and the significance level was set at ($p \leq 0.05$).

Results

In superficial dentin, the control group showed significantly higher μTBS values (26.78 ± 6.11 MPa) compared with NaOCl (20.55 ± 4.82 MPa) and bromelain (19.55 ± 4.22 MPa), with no significant difference between the two deproteinizing agents. In deep dentin, both NaOCl (23.02 ± 5.35 MPa) and bromelain (20.86 ± 3.61 MPa) significantly improved μTBS compared with control (16.06 ± 3.61 MPa), with NaOCl showed higher mean values compared to bromelain. Failure mode analysis revealed more adhesive failures in deproteinized superficial dentin, while deep dentin treated with NaOCl showed predominating cohesive failures within resin composite.

Conclusions

Deproteinization with sodium hypochlorite or bromelain enhances bonding to deep dentin but jeopardizes bonding to superficial dentin

Background

One of the most challenges that faced the clinician during daily clinical practice is related to the quality of the hybrid layer created by adhesive systems which is directly affected by morphological and structural differences between superficial and deep dentin. More specifically low contents of intertubular dentin and collagen fibrils, which play a critical role in adhesion, as it may complicate the bonding to

deep dentin. Furthermore, the higher number and diameter of dentinal tubules related to deep dentin which is associated with increased dentin's intrinsic wetness which has been considered as another obstacle to efficient bonding to deep dentin [1]. During last decades self-etch adhesives were introduced as a trial to overcome some of the obstacles related to bonding to deep dentin [2]. However, inferior bond strength was recorded as self-etch adhesives have lower demineralization capacity than phosphoric acid etchants, making collagen denaturation more challenging due to the presence of residual dentine mineral phase [3]. Also, the thickness of the hybrid layer generated by the self-etch adhesives is relatively thin, and the resin tag less and shorter. This may be attributed to the weak acidity of the acidic monomer and limited permeability [4]. Recently, literature reported that utilization of deproteinizing agents could enhance the bond strength of self-etch adhesives to dentin [5].

One of the most commonly used deproteinizing agents is sodium hypochlorite (NaOCl) which is considered as the gold standard [6–8]. Unfortunately, according to literature several drawbacks were recorded for such material related to presence of remnants of Sodium hypochlorite and its by-products that may affect the polymerization of dental adhesives. Other disadvantages of using sodium hypochlorite are its toxicity and extreme taste and odour [9]. These disadvantages have resulted in the development of new and different approaches for deproteinizing dentin.

One of these new techniques is using deproteinizing enzymes like collagenase enzyme family as bromelain enzyme. Bromelain enzyme is a proteolytic enzyme obtained from pineapple fruit or stem [10]. It was previously used for its anti-inflammatory, anti-fibrinolytic, and anti-edema properties. It also has antibacterial and anti-metastatic properties, and it is widely used in the medical field. However, there have been few studies on its use in dentistry [11].

Up to our knowledge, there were limited studies that compared the deproteinization effect of sodium hypochlorite in comparison to bromelain enzyme on microtensile bond strength of self-etch adhesives to superficial or deep dentin. Therefore, this study was conducted to compare the deproteinization effect of sodium hypochlorite and bromelain enzyme on microtensile bond strength and mode of failure of self-etch adhesives to superficial and deep dentin.

METHODS

Study Design and Ethical Approval

This in-vitro study was conducted after obtaining ethical approval from Research Ethics Committee of Faculty of Dentistry, Suez Canal University (Approval number: 557/2022).

Teeth Selection

Forty-eight sound permanent third molars were extracted from patients in the age range 20 to 30 years for orthodontic or periodontal reasons. All selected teeth were intact, free of caries, cracks or dental

anomalies. Teeth were carefully cleaned under running water to remove blood and mucus, scaled to remove calculus and any remaining periodontal ligament tissue. Finally, they were polished using fine pumice and soft rubber cups at a conventional speed. Afterwards, the collected teeth were stored in distilled water having 0.5% chloramine-T antiseptic solution at room temperature for no more than three months before being used.[12] A cylindrical teflon mold, with a corresponding metal ring with two opposing screws at its top was used for teeth fixation in acrylic resin blocks [13]. After teeth fixation in the molds, a random number from (1 to 48) using a randomizer website (www.random.org/sequences/) was given for each tooth and written using permanent marker at the base of each mold.

Teeth preparation

All teeth were randomly allocated into two main groups (n = 24) according to the prepared dentin depth (D). Preparation of the superficial dentin group (D1) was performed through exposing of the dentin surface by trimming the occlusal enamel just beneath the central groove using diamond disc under water cooling. Meanwhile, preparation of deep dentin group (D2) was performed through using size 2 round carbide bur which was utilized for making an indentation of 2mm depth from superficial dentin. The depth of the indentation was guided using a rubber stopper mounted to the shaft of the round bur. Deep dentin was reached by removal of the occlusal surface with the same diamond disc under water cooling till the indentation disappeared [14].

Restorative Procedure

Restoration of both superficial and deep dentin groups was performed through subgrouping of each one into three subgroups according to the selected treatment modality of the exposed dentin surface (T). Treatment of the control subgroup (T0) was performed without any previous treatment of the samples' dentin surface. Then, the bonding procedure of this subgroup was performed according to the manufacture instructions as the following: the one-step self-etch adhesive system (Palfique Bond, Tokuyama, Tokyo, Japan) was applied in one coat on dentin surface of each sample by using a microbrush. Then, the bond was left for 10 seconds and mild air flow for five seconds was applied followed by light curing for 10 seconds using a LED light curing device (Radii Plus, SDI, Bayswater, Australia) with a light intensity of 1500 mW/cm2. After that, teeth were restored with 4 mm nanohybrid resin composite (Palfique LX5, Tokuyama, Tokyo, Japan). Resin composite was applied in two increments using the Teflon mold especially constructed for this thickness (8mm length x 8mm width x 4mm height). A celluloid strip was pressed over the composite surface before curing the final increment to create a smooth and non-porous surface. Each increment was polymerized for 20 seconds using the same light curing unit according to manufacturer's instructions [15]. Meanwhile samples of sodium hypochlorite subgroup (T1) were prepared through application of 10% Sodium hypochlorite solution (Biochem, Cairo, Egypt) that was applied by using a microbrush with a gentle rubbing for 1 minute, followed by 1 minute of distilled water rinsing and five seconds of gentle air drying [16]. After that, bonding procedure and resin composite build up were done as mentioned before in the control

subgroup. Finally, the samples of bromelain enzyme (Sigma Aldrich, St.Louis, MO, USA) subgroup (T2) were treated with 10% Bromelain enzyme solution. This solution was prepared through dissolving 10 grams of Bromelain enzyme powder in 100 ml distilled water. Then, dentin surface of each sample in this subgroup was rubbed gently by using a microbrush for 1 minute, followed by 1 minute of distilled water rinsing and five seconds of gentle air drying [17]. After that, bonding procedure and resin composite build up were also done as mentioned before in the control group.

Aging and Testing

All samples were stored for 24 hours at room temperature in distilled water. Samples were subjected to thermocycling procedure that was performed for all samples through 1000 cycle in a thermocycling machine (THE- 1100SD Mechatronic Thermocycler, Germany). The procedure was performed through cold water bath immersion of samples for 30 seconds at 5 degrees followed by hot water bath immersion for 30 seconds at 55 degrees with Dwell time 10 seconds to mimic the thermal fluctuations that occur in oral environment [18].

Microtensile bond strength testing

The restored teeth were sectioned longitudinally to obtain composite dentin beams. Two different colors of permanent ink were used to mark the central and peripheral beams in order to eliminate substrate regional variability. The peripheral beams were excluded and five central beams from each sample were selected for microtensile bond strength testing [13]. Beams of similar length and thickness were selected and were checked using a digital caliper, the resultant beams were 0.9 ± 0.1 mm in thickness and about 6 ± 1 mm in length. Each obtained central beam was mounted on a Geraldeli's jig. Finally, beams on Geraldeli's jig were mounted into a universal testing machine (Instron, MA, USA), with a load cell of 500 N. Tensile load was applied at a crosshead speed of 0.5 mm/min, until failure of the beam occurred.

Failure mode analysis

The modes of failure of central beams' fragments were examined by stereomicroscope (NexiusZoom, Euromex, Netherlands) with magnification 40X. Modes of failure were classified into four types; cohesive failure in resin composite when the failure occurred in the resin composite material, cohesive failure in dentin when the failure occurred in dentin, adhesive failure when the failure occurred at the substrate / restorative material interface, and mixed failure.

Statistical Analysis

The obtained data of microtensile bond strength was recorded and tabulated for statistical analysis using the following tests: A normality test (Shapiro-Wilk) was done to check the normal distribution of

the samples. Descriptive statistics were calculated in the form of Mean ± Standard deviation (SD). The independent sample T test was used to compare between two different groups (superficial group and deep group). One way ANOVA (Analysis of variance) was used to compare between different groups. Bonferroni post hoc test was used for pair wise comparisons among the groups. The Chi-square test was used to test significance of association between categories of failure mode analysis. Statistical analysis was performed using the computer program SPSS software for windows version 26.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp) at significant levels < 0.05 (P- Value).

Results

I. Microtensile bond strength results

Data in Table (1) shows the results of one-way ANOVA analysis of the deproteinizing effect of sodium hypochlorite and bromelain enzyme solutions on the microtensile bond strength of self-etch adhesive system to superficial and deep dentin. The statistical analysis of superficial dentin results revealed that the highest mean value was recorded in the control subgroup (26.78 ± 6.11) followed by sodium hypochlorite subgroup (20.55 ± 4.82). Meanwhile, the lowest mean value was found in bromelain enzyme subgroup (19.55 ± 4.22). A significant difference between control and sodium hypochlorite subgroups on one side and between control and bromelain enzyme subgroups on the other side was recorded (P < 0.001). On the contrary, there was a non-significant difference between sodium hypochlorite and bromelain enzyme subgroups. Regarding the statistical analysis of the current deep dentin data, the results revealed that the highest mean value was recorded in the Sodium hypochlorite subgroup (23.02 ± 5.35) followed by bromelain enzyme subgroup (20.86 ± 4.29) while, the lowest mean value was found in control subgroup (16.06 ± 3.61) and significant difference was recorded between all these tested subgroups (P < 0.001). Concerning the evaluation of the effect of the treatment modality on the microtensile bond strength results of either superficial or deep dentin the results showed that utilization of self-etch adhesive without any preceding treatment modality of the dentin substrate (control subgroup) recorded higher microtensile bond strength values for superficial dentin (26.78 ± 6.11) in comparison to deep dentin (16.06 ± 4.29) with a significant difference between them (P < 0.001). It is worth noting that utilization of sodium hypochlorite as a deproteinizing material showed higher microtensile bond strength values when utilized for deep dentin (23.02 ± 5.35) in comparison to superficial dentin (20.55 ± 4.82) with significant difference between them (P value = 0.033). Surprisingly, utilization of bromelain enzyme as a deproteinizing material showed no significant difference between microtensile bond strength values either when utilized for superficial dentin (19.55 ± 4.22) or for deep dentin (20.86 ± 3.61) (p value = 0.138).

II. Failure mode analysis

Data in Table (2) represents different failure mode results in all tested groups. Concerning the superficial dentin group, failure mode analysis revealed different patterns among the subgroups. In the control subgroup, the predominant failure type was cohesive failure at composite (62.5%), followed by adhesive

failure (17.5%) and dentin cohesive failure (15%), while mixed failure was the least common (5%). In contrast, both deproteinized subgroups showed a shift toward adhesive failure as the most frequent mode accounting for 42.5% in the Sodium hypochlorite subgroup and 50% in the Bromelain enzyme subgroup. However, composite cohesive failure decreased markedly in these subgroups (30% and 27.5%) respectively, while dentin cohesive failure was relatively low (12.5% and 7.5%) respectively. Mixed failure related to deproteinized subgroups (15%) showed an increase compared to the control one. The statistical analysis revealed significant differences within all subgroups ($p < 0.001$, $p = 0.0244$, and $p < 0.001$ for control, sodium hypochlorite, and bromelain subgroups) respectively, and overall comparison among the three subgroups was also statistically significant ($p = 0.008$). Regarding the deep dentin group, failure mode analysis revealed that in the control subgroup, the predominant failure type was adhesive failure (60%), followed by mixed failure (17.5%), followed by composite cohesive failure (12.5%), meanwhile, dentin cohesive failure 10%. In the Sodium hypochlorite subgroup, the most frequent failure mode was composite cohesive failure (60%), while adhesive failure occurred in 20%, dentin cohesive failure in 15%, and mixed failure in 5% of samples. Also, In the Bromelain enzyme subgroup, the highest percentage was observed for adhesive failure (50%), followed by mixed failure (27.5%), composite cohesive failure (15%), and dentin cohesive failure (7.5%). The statistical analysis revealed significant differences within all subgroups ($p < 0.001$), and overall comparison among the three subgroups was also highly significant ($p < 0.001$).

Discussion

Dentin consists of dentinal tubules extending from pulp to dentino-enamel junction, which are surrounded by hyper-mineralized peritubular dentin. Mineral density, tubular diameter and collagen composition varies according to dentin depth. The number and width of dentinal tubules increase with dentin depth, also the intrinsic wetness of dentinal tubules contain most of dentin's water content. While the intertubular dentin decreases with dentin depth, which has been proven to have an important role during hybrid layer formation. So, the effectiveness of adhesive systems varies according to the dentin depth even when the same adhesive system is used [19]. Most of one-step self-etch adhesives come in single bottles with all the necessary ingredients. However, these adhesive polymers exhibit the properties of semi-permeable membranes, permitting water to pass through the adhesive layer even after polymerization, leading to a decrease in mechanical properties [20]. One step self-etch adhesives cannot remove dentin smear layer totally due to their mild acidity which leads to formation of hybridized smear layer that becomes a weak connection at the resin dentin interface [21]. Also, the mineral phase in dentin smear layer can be removed by self-etch adhesives, but not the organic phase.

Dentin deproteinization is a procedure that attempts to dissolve the organic phase of the dentin smear layer and the unsupported collagen fibrils in dentin to enhance the mineral/organic ratio to be similar to enamel and change dentin's surface energy. Some researchers describe this process as a "reverse hybrid layer" as the collagen is not invaded by resin monomers, but the resin monomers infiltrate the original collagen spaces which resulted in higher infiltration of resin monomer into dentin, as well as a better dentin bond strength [22, 23].

The most commonly deproteinizing agent used by many researchers is sodium hypochlorite (NaOCl) as it has a nonspecific proteolytic effect that can dissolve organic components like collagen fibril [6, 8]. Using 10% of NaOCl on dentin produces wide open dentinal tubules, exposes dentin lateral branches, increases the surface roughness and forming a porous dentin with multiple irregularities that increase the mechanical retention. Moreover, hypochlorite may raise the free energy of dentin, so improving dentin wettability and monomer impregnation [5]. It was found that increasing sodium hypochlorite concentration leads to increase in bond strength, but the bond strength was found to be stable at concentration of 10% applied for 1 minute [8]. Therefore, 10% sodium hypochlorite was used in this study and applied for 1 minute. However, there are several disadvantages of sodium hypochlorite, including the development of a fragility zone, cytotoxicity, in addition to its unpleasant taste and odour. Moreover, sodium hypochlorite has a high reactivity with amino acids in collagen, so it is difficult to be washed away [16].

In order to find an alternatives to sodium hypochlorite, multiple techniques and materials were suggested among them the collagenase enzymes [11]. One of these enzymes is bromelain which is a proteolytic enzyme that is extracted from the fruit or stem of the pineapple. It is claimed to promote the transformation of proteins into amino acids and dissolve the unsupported collagen fibrils in dentin that increase penetration of resin monomers and improve the dentin bond strength [17]. It was found that deproteinization using a concentration of 10% bromelain enzyme increases the bond strength compared to other concentration of bromelain enzyme [24]. Also, it was found that applying bromelain enzyme for 1 minute removes unsupported collagen fibrils and improves bond strength while increasing the application time may be harmful to the dentin nature [25]. Therefore, application of 10% of bromelain enzyme for 1 minute was used in the current study.

Concerning the effect of dentin deproteinization on microtensile bond strength of superficial dentin, the results of the current study revealed that both sodium hypochlorite and bromelain enzyme subgroups were significantly lower than control subgroup in which no deproteinizing protocol was performed for dentin substrate. The superior results of control subgroup could be attributed to that superficial dentin contains a lower density of dentinal tubules and a higher proportion of intertubular dentin, which provides a greater surface area for resin infiltration and hybrid layer formation. In the absence of deproteinizing agents, the smear layer remains partially intact in the control subgroup, serving as a protective intermediary that reduces dentin permeability and prevents collapse of the collagen network. This scenario results in a more favorable substrate for adhesive penetration and micromechanical interlocking in case of the control subgroup [26]. Also, the inferior results recorded by sodium hypochlorite subgroup in comparison to the control subgroup could be attributed to that normally superficial dentin offers superior adhesion in comparison to deep dentin because of its high proportion of intertubular dentin and the collagen-rich part between tubules that support a well-formed hybrid layer [27]. However, NaOCl alters this balance by removing collagen fibrils, eliminating the conventional collagen-based hybrid layer in superficial dentin, and generating oxidative byproducts that interfere with resin polymerization, disproportionately compromising the bond strength of superficial dentin [28]. Also, bromelain enzyme subgroup showed lower mean microtensile bond strength values than control

subgroup. This could be related to the self-etch adhesive systems, the collagen scaffold at superficial dentin normally plays a supportive role by allowing simultaneous demineralization and resin infiltration, forming a hybrid layer. However, bromelain application may remove much of superficial collagen that by its turn results in formation of a thinner hybrid layer and less mechanically interlocked, since intertubular dentin (the main site of micromechanical retention) is partially lost. Moreover, superficial dentin that was treated by bromelain has smaller and fewer tubules, which limits resin tag formation by the subsequently applied self-etch adhesive [10].

On the other side, it is worth noting that there was no significant difference between sodium hypochlorite and bromelain enzyme subgroups. This may be attributed to deproteinization with either sodium hypochlorite or bromelain enzyme produces a similar bonding substrate in superficial dentin. The result in both cases is a reduction of exposed collagen fibrils available for resin infiltration; when that endpoint is similar, bond strengths measured at short term can also be similar [29]. Concerning the effect of dentin deproteinization on microtensile bond strength of deep dentin, the results of the current study revealed that sodium hypochlorite and bromelain enzyme subgroups were significantly higher than control subgroups. The superior results recorded by sodium hypochlorite to deep dentin in comparison to the control subgroup could be attributed to that deep dentin with its larger tubules and higher porosity may benefit from sodium hypochlorite treatment because adhesive resins can penetrate more easily, forming resin tags and even a “reverse hybrid layer,” where infiltration occurs into mineralized dentin rather than collagen [30]. Similarly, bromelain enzyme subgroup also showed higher mean microtensile bond strength values than control subgroup. This result may be attributed to that in deep dentin, where dentinal tubules are larger, more numerous, and surrounded by less intertubular collagen. Deproteinization has a more pronounced effect as the removal of collagen exposes wide tubule orifices and underlying minerals, allowing resin monomers to infiltrate more effectively and form stronger resin tags [31]. Concerning the results of failure mode analysis of superficial dentin, there was a statistically significant difference among the subgroups. The results showed that the control subgroup has the highest percentage of composite cohesive failures (62.5%). Such behavior can be attributed to the structural characteristics of superficial dentin, including its thicker intertubular dentin and lower tubular density, which favor stronger micromechanical retention and more uniform resin infiltration even without surface pretreatment[32]. In contrast both deproteinized subgroups showed a shift toward adhesive failure as the most frequent mode, accounting for (42.5%) in the sodium hypochlorite subgroup and (50%) in the bromelain enzyme subgroup. This may be attributed to that removal of the organic matrix alters the micromechanical interlocking and resin infiltration, thereby weakening the resin–dentin interface as mentioned before[31]. Regarding the results of failure mode analysis of deep dentin, the predominant failure type was adhesive failure (60%) in the control. This may be attributed to that inferior bond strength related to deep untreated dentin [20]. Also, bromelain enzyme subgroup recorded high adhesive failure (50%), which may be attributed to that bromelain enzyme treatment may expose tubule orifices even further, allowing more fluid seepage. This moisture interferes with adhesive penetration and polymerization, contributing to interfacial weakness and adhesive failure [25]. In contrast, the predominant failure type at sodium hypochlorite subgroup was composite cohesive failure (60%). This

may be attributed to that sodium hypochlorite acts as a strong deproteinizing, removing exposed collagen fibrils from the demineralized dentin surface and leaving behind a substrate that is richer in hydroxyapatite. Additionally, in deep dentin where the collagen network is already thinner and more vulnerable, this removal minimizes the weak organic zone and allows adhesive monomers to penetrate more effectively into the mineral phase, producing a stronger and more stable resin–dentin interface [33].

Limitation of the Study

The current study's limitations were that it only focused on comparing the effects of Sodium hypochlorite and Bromelain enzyme on superficial and deep dentin, without evaluating other possible pre-treatments or concentrations that could influence the results. Furthermore, the study evaluated the effect of these deproteinizing agents only in a controlled laboratory setting, which does not completely replicate the complex oral environment, including factors such as saliva, temperature variations, and masticatory forces. In Addition, the applied aging protocol doesn't simulate a long-term aging. Finally, the methodology for assessing the measured outcomes, although standardized in research, may still present variability due to sample preparation and testing conditions, which can influence stress distribution and recorded values.

Conclusions

Within the limitations of the current study, the following could be concluded:

1. Pretreatment with Sodium hypochlorite or Bromelain enzyme may positively influences the microtensile bond strength of deep dentin; however, this effect is not evident when applied to superficial dentin.
2. Deproteinization of deep dentin using Sodium hypochlorite has remarkable effect compared to bromelain enzyme.

Declarations

Ethics approval and consent to participate

This in-vitro study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from Research Ethics Committee of Faculty of Dentistry, Suez Canal University (Approval number: 557/2022). All participants from whom the sound permanent third molars were extracted were in the age range of 20 to 30 years. Informed consent was obtained from all participants (aged 20 to 30 years) for the use of their extracted teeth for research purposes prior to their inclusion in the study.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

BA – Conducted the study and wrote the paper

MZ and BH – Analyzed and discuss the data

RS- Analyzed data and revised the paper

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