Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 18 July 2026
Revised 21 Aug 2026
Accepted 05 Sep 2026

Natural Resources for Human Health 2026, Vol. 6 (21s): 487–497

KEYWORDS:
Dentin Deproteinization, Microtensile bond strength, Bromelain, Sodium hypochlorite

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

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ABSTRACT:

Background: Dentin bonding is challenging due to structural differences between superficial and deep dentin. The study aimed to evaluate the effect of dentin deproteinization using sodium hypochlorite (NaOCl) and bromelain enzyme on the microtensile bond strength (μTBS) of self-etch adhesive to superficial and deep dentin.

Materials and Methods: Forty-eight sound human third molars were randomly assigned into two main groups according to dentin depth. 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 thermocycled then sectioned into beams to be subjected to μTBS testing.

Results: In superficial dentin, the control group showed significantly higher μTBS values compared with NaOCl and bromelain, with no significant difference between the two deproteinizing agents. In deep dentin, both NaOCl and bromelain significantly improved μTBS compared with control with NaOCl showed higher mean values compared to bromelain. **Conclusions:** Deproteinization with sodium hypochlorite or bromelain enhances bonding to deep dentin but decreased bonding to superficial dentin

BACKGROUND

Quality of the hybrid layer created by adhesive systems 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. This may be 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 shorter resin tags. 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 the Research Ethics Committee of the Faculty of Dentistry, Suez Canal University (Approval number: 557/2022). All methods and experimental protocols were performed in accordance with the relevant guidelines and regulations of the ethical committee and the Declaration of Helsinki. Informed consent was waived by the Research Ethics Committee because the study utilized anonymized, discarded extracted human teeth obtained following orthodontic or periodontal treatment.

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. Informed consent was obtained from all individual participants prior to tooth extraction and collection for research purposes according to the guidelines of the Research Ethics Committee of the Faculty of Dentistry, Suez Canal University. 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 until 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 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. Beams that fractured during preparation or being attached in the testing jig were classified as pretest failures (PTFs). For statistical analysis, each PTF was assigned the lowest bond strength value measured within its respective experimental group.

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).

Table (1): Mean and standard deviation (SD) values of microtensile bond strength test of all tested groups.

Subgroups	Main groups				T test	P value
	Superficial		Deep			
	mean	SD	mean	SD		
Control (T_0)	26.78^{aA}	6.11	16.06^{cB}	4.29	9.08	<0.001**
Sodium hypochlorite (T_1)	20.55^{bB}	4.82	23.02^{aA}	5.35	2.17	0.033**
Bromelain (T_2)	19.55^{bA}	4.22	20.86^{bA}	3.61	1.50	0.138 ns
F test	23.49		25.35			
P value	<0.001**		<0.001**			
****; means significant difference, (ns): means non-significant difference.**						

II. Failure Mode Analysis

Regarding 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].

One-step self-etch adhesives act as 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 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 [8]. The selection of 10% NaOCl, rather than the lower concentrations (1%–5%), used in some deproteinization protocols showed that increasing NaOCl concentration leads to greater deproteinization efficacy, but that bond strength reaches a stable plateau at 10%, applied for one minute. The 10% concentration has been directly compared against 5% NaOCl confirmed that 10% NaOCl consistently achieves greater collagen removal [39]. 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].

The usage of 10% NaOCl on deep dentin in the current study although its confirmed cytotoxicity could be attributed to the in-vitro model used in the current work where no pulpal tissue could be affected therefore bond strength could be accurately tested. Moreover, the application protocol was limited to 1 minute application, consistent with protocols reported to achieve

proper deproteinizing effect without a proportional alteration in dentin substrate. Additionally, specimens were rinsed immediately as step reported to remove unreacted hypochlorite and its reactive by-products from the dentin surface [9]. The bond strength testing on non-vital dentin cannot itself establish pulpal biocompatibility, and that extrapolating the present protocol to deep clinical cavities approximating the pulp would require dedicated in-vivo or ex-vivo pulp chamber cytotoxicity data before 10% NaOCl deproteinization of deep dentin could be recommended as a clinical procedure; this point has been added to the limitations.

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. As bonded restorations are subjected to different temperature during function within the oral cavity, thermocycling was employed in this study. According to ISO 11405, the use of 500 thermal cycles between 5°C and 55°C is considered to be suitable to simulate short-term aging of dental materials. In this study the number of thermocycles was 1000 cycles representing thermally induced stresses, which are equivalent to 1.2 month of clinical use to simulate thermal fluctuations that occur in the oral environment. Moreover, thermocycling was performed at the macro-specimen before longitudinal sectioning into beams. Pre-sectioned beams were selected to maximize water exposure along the interface that increases the risk of pre-test failures due to edge-stress concentration during thermal changes. Furthermore, thermocycling of intact block better preserves the clinical reality of macro-volumetric shrinkage and thermal expansion stresses across the dentin-composite interface [13]. The results of the current study revealed that concerning the effect of dentin deproteinization at superficial dentin on microtensile bond strength, there was no significant difference between Sodium Hypochlorite and Bromelain. Both showed significantly lower mean microtensile bond strength values than control group. The superior results of control group 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 group, serving as a protective intermediary that reduces dentin permeability and prevents collapse of the collagen network. Which results in a more favorable substrate for adhesive penetration and micromechanical interlocking [26]. Regarding Sodium Hypochlorite group, it showed lower mean microtensile bond strength value than control group. This could be attributed to the surfaces treated with NaOCl appear to be free of loose collagen fibrils. The lack of bare collagen fibrils raises the possibility that the polymer created when adhesives are light-cured could degrade during testing. The current adhesives contain hydrophilic monomers that are very likely to absorb water. Moreover, oxidation of NaOCl lead to the presence of reactive residual free-radicals that may interfere with the propagation of vinyl free-radicals formed during light-activation of the adhesive, resulting into incomplete polymerization impairing the mechanical performance of obtained bonding interfaces. Additionally, inability to eliminate all residual water retained in the deepest area of demineralized and deproteinized dentin leading to formation of poorly polymerized polymer chains that would be weaker and less stable than formed in water-free area. Another explanation for these results could be because the application of Sodium Hypochlorite altered the dentin's chemical and physical characteristics decreasing the dentin's flexural strength and elastic modulus [27].

On the other side, Bromelain group showed lower mean microtensile bond strength values than control group. This could be related to that higher concentration of Bromelain that may cause damage to the dentin collagen fibers at this concentration of the enzyme. Also, it may have an adverse impact on dentin's mechanical properties through destroying its organic content due to its increased proteolytic activity. As a result, the bond strength may be weakened because organic adhesive monomers might not be able to sufficiently penetrate the demineralized dentin [28]. The above-mentioned result comes in agreement Sharafeddin and Mauroufi,[17] who showed that control (non-treated) group revealed higher bond strength than deproteinized groups. Additionally, results also come in agreement with Nima et al.,[16] who reported that NaOCl did not improve bond strength of deproteinized superficial dentin. Finally, the results agree with Geng Vivanco et al.,[24] who reported that pretreatment with Bromelain has no effect on bond strength of superficial dentin.

Meanwhile, this result comes in disagreement with Yarmohammadi et al.,[29] who showed that deproteinization using Bromelain enzyme increased the bond strength between resin composite and superficial dentin compared to control group. The conflict might be due to the difference in Bromelain concentration as they used 25 % concentration and different adhesive protocol were applied as they used etch and rinse adhesives. Moreover, Mahdan,[30] showed that NaOCl pre treatment showed higher bond strength with superficial dentin than control group, this conflict may be due to using total-etch adhesive protocol. Additionally, the Siqueira et al.,[31] showed that using NaOCl may maintain a long-term stability of a resin/dentin interface formed by etch-and-rinse and self-etch adhesives after three years of water storage. This conflict may be due to the difference in type of tested dentin as they used eroded dentin and different concentration of NaOCl as they used 5.2% Sodium Hypochlorite. In addition, the results disagree with Kusumasari et al.,[32] who showed that using 6% Sodium Hypochlorite could improve the μTBS of self-etch adhesives. Additionally, Sharafeddin and Haghbin, [33] showed that Bromelain enzyme was effective in increasing the bond strength to superficial dentin. This conflict from obtained results may be due to the difference in Bromelain concentration as they used 3% Bromelain and different adhesive protocol used. Finally, Khatib et al.,[18] showed that naturally occurring deproteinizing agents like Bromelain and Papain produce high bond strength.

Concerning the effect of dentin deproteinization at deep dentin on microtensile bond strength, results showed a significant difference between different tested groups (control, Sodium Hypochlorite and Bromelain). The Sodium Hypochlorite group showed the highest mean value followed by Bromelain, while the lowest mean value was found in control group. Concerning the Sodium Hypochlorite group, it exhibited the highest microtensile bond strength. This may be attributed to the fact that NaOCl is considered as a non-specific deproteinizing agent that can dissolve the organic phase of the smear layer of dentin surface. This can reduce the thickness of the smear layer and raise the mineral to the organic ratio on the bonding surface, which may make it easier for resin monomers to penetrate. Consequently, improve the μTBS to deep dentin [34]. Regarding the Bromelain group, it showed higher microtensile bond strength than control group. This may be attributed to the ability of Bromelain enzyme to remove collagen fibrils enhancing the bond strength [35]. Meanwhile, the control group showed the lowest bond strength at deep dentin. This may be attributed to that self-etch adhesives depending on interaction between functional monomers and hydroxyapatite. In deep dentin, the residual hydroxyapatite and smear layer components decrease, so this reduces the ability of self-etch adhesives' functional monomers to chemically bond and form a durable hybrid interface [36]. This result comes in agreement with Yuan et al, [34] and Khan et al.,[35] who found that after NaOCl dentin deproteinization, the bond strength to deep dentin was increased. They attributed that to the removal of the unsupported collagen fibrils from deep dentin through the usage of Bromelain enzyme may improve the bond strength. However, this result comes in disagreement with Bedir and Telatar, [37] who showed that deproteinization using NaOCl at deep dentin with self-etch adhesives results in a lower bond strength compared to control group without deproteinization. This conflict might be due to the difference in NaOCl concentration as they used 5.25%. Also, the teeth were submitted to 5000 thermo cycles while teeth in this study were submitted to 1000 cycles. Also, this result comes in disagreement with Sharafeddin et al.,[25] who showed that 10% Bromelain enzyme has no effect on bond strength and the control group had the highest bond strength. This conflict might be due to using different adhesive protocols. Finally, this result comes in disagreement with Ayman et al [8], who showed that the control group had the highest bond strength with deep dentin compared to bromelain group. This conflict might be due to using different adhesive protocol as they used etch and rinse adhesives. The intergroup comparison of deproteinization effect on superficial and deep dentin showed a significant difference between subgroups. In control groups, there was a significant difference between superficial and deep layers where superficial dentin showed higher mean microtensile bond strength values than deep dentin. This may be attributed to untreated tissues having underlying biological differences. Because they are more exposed to external stimuli, superficial dentin frequently has higher levels of cellular activity or resilient structures than deeper dentin [38]. Also, superficial dentin is characterized by a lower density and smaller diameter of dentinal tubules, providing a higher proportion of intertubular dentin. The main region where micromechanical retention occurs through resin infiltration and hybrid layer formation. The high density of intertubular dentin facilitates adhesive monomer penetration into the demineralized collagen matrix, as well as more effective polymerization, resulting in a denser and more uniform hybrid layer. Furthermore, superficial dentin exhibits reduced dentinal fluid flow compared to deeper layers, minimizing the risk of monomer dilution and phase separation within the adhesive layer [39]. In contrast, Deep Dentin possesses higher density of tubules with larger diameters, leaving less intertubular dentin available for hybridization. The larger lumens and increased permeability promote pulpal fluid transudation, which may compete with adhesive monomers for access within the demineralized collagen mesh. This increased moisture content can compromise monomer infiltration, lower the degree of

conversion, and promote void formation within the hybrid layer [38]. Additionally, the higher organic content and reduced mineralization in deep dentin may further prevent the formation of strong resin tags and stable micromechanical interlocking. Taken together, these structural and physiological differences between superficial and deep dentin explain the reduced bonding performance of untreated deep dentin observed in the control group [2]. These results come in agreement with Kominami et al.,[38] who reported that microtensile bond strength is decreased by increasing dentin depth. It is worth noting that Sodium Hypochlorite groups showed a reversed trend, with deeper tissue values showed a higher statistically significant difference than superficial layer. This may be attributed to thicker demineralized collagen layers at deep dentin which are more difficult for resin monomers to penetrate. So, NaOCl deproteinization removes these unsupported collagen fibrils, exposing a mineral-rich surface that facilitates monomer infiltration directly into mineralized dentin rather than through a thick collagen mesh. In addition, superficial dentin, being more mineralized, might resist chemical effects of NaOCl but remain prone to collagen degradation and resin-polymerization interference which amplifies bond-strength reduction [7]. This comes in agreement with Abuhaimed and Neel [7]. Meanwhile, Bromelain groups showed no significant difference between superficial and deep dentin. This may be attributed to its enzymatic mechanism promoting uniform debridement without layer-specific aggression. This comes in agreement with Khan et al., [35] and Khatib et al., [18] who showed that Bromelain was effective in removing unsupported collagen fibers, enhancing resin infiltration without adversely affecting the dentin structure regardless of dentin depths.

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. Finally, this in-vitro model tested bond strength on non-vital, extracted teeth and did not include an assessment of the pulpal biocompatibility of 10% NaOCl on deep dentin; consequently, the present findings support the bonding rationale for this concentration and exposure time but should not, on their own, be extrapolated to justify direct clinical application of 10% NaOCl deproteinization in deep cavities approximating the pulp without further in-vivo or ex-vivo cytotoxicity evaluation.

Clinical Relevance

By eliminating unsupported collagen fibres for facilitating deep monomer infiltration, a deproteinising agent greatly increases microtensile bond strength in deep cavities. On the other hand, deproteinizing agents decreases bond strength in superficial cavities by breaking down the intact intertubular dentin which has no clinical benefit.

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.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Ethics Statement

This in-vitro study was conducted after obtaining ethical approval from the Research Ethics Committee of the Faculty of Dentistry, Suez Canal University (Approval number: 557/2022). All methods and experimental protocols were performed in accordance with the relevant guidelines and regulations of the institutional committee and the Declaration of Helsinki. Informed consent was obtained from all individual participants prior to tooth extraction and collection for research purposes

FUNDING

This research was self-funded

Author's contributions

BA, conducted the study, recorded the results and wrote the manuscript.

Natural Resources for Human Health
eISSN: 2583-1194

RS, study director and approval of final manuscript.

BH and MZ, data interpretation, review and editing.