EFFECT OF TWO DIFFERENT DEPROTEINIZING AGENTS ON MICROTENSILE BOND STRENGTH BETWEEN RESIN COMPOSITE AND DEEP DENTIN USING TWO RESTORATIVE PROTOCOLS. AN IN-VITRO STUDY

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ABSTRACT

The purpose of this study was to evaluate the microtensile bond strength of resin composite to deep dentin using two different restorative protocols.

Materials and Methods: Forty-five sound permanent molars were used to expose deep dentin. Teeth were etched with 37% phosphoric acid then rinsed. Teeth were divided into five equal groups (n=9) according to deproteinizing method. Group 1: only etching (control), Group 2 and 3: deproteinized with 10% sodium hypochlorite Group 4 and 5: deproteinized with 10% bromelain enzyme. Teeth were restored with two restorative protocols either: packable nanohybrid bulk-fill resin composite or bulk-fill flowable and packable bulk-fil nanohybrid resin composite. Teeth were stored in distilled water (37°C/24 hours) then sectioned into beams. The beams were subjected to microtensile bond strength testing using a universal testing machine at a crosshead speed of 0.5mm/min until failure occurred.

Results: There was a statistically significant difference (p<0.05) between microtensile bond strength values of different groups. Group 1 recorded the highest microtensile bond strength with non-statistically significant difference from Group 4 but a statistically significantly higher microtensile bond strength than other groups. Group 5 recorded the lowest mean microtensile bond strength.

Conclusion: Deep Dentin deproteinization, either with 10% sodium hypochlorite solution or 10% bromelain enzyme solution, has no improvement effect on microtensile bond strength with the packable bulk-fill resin composite used in this study. Application of flowable bulk-fill resin composite, as a liner, has a deterioration effect on the microtensile bond strength of deproteinized deep dentin to packable bulk-fill resin composite.

KEYWORDS: Dentin deproteinization, Bromelain enzyme, Sodium hypochlorite, Bond strength.

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INTRODUCTION

Dentin bonding has always been a challenging process to develop a durable strong bond due to its complex structure. The complexity of dentin bonding is related to the differences in biochemistry, morphology and mechanical properties of different dentin types, ages and different depths. Moreover, dentin is modified over time due to physiologic or pathologic conditions[1,2]. There are morphological and structural differences between superficial and deep dentin (DD) affecting the ability of bonding of adhesive systems which is related to the quality of the formed hybrid layer. Bonding to deep dentin is limited due to less intertubular dentin and collagen fibrils[3].

Dentin bonding can be improved through modifications of the physical properties of the bonding agent or enhancing the dentin surface to facilitate the application of the adhesive agent[4]. Proteolytic agents' usage on acid-etched dentin, which known as dentin deproteinization, has been suggested to modify the dentin surface for better bonding. This turns the dentin surface into a surface nearly like etched enamel that has greater predictable and hydrophilic substrate for bonding[2].

Sodium hypochlorite (NaOCl) has been used as a deproteinizing agent. This is due to the non-specific proteolytic action of sodium hypochlorite, which leads to the production of N-chloramines with terminal amine groups and the fragmentation of long peptide chains. Allows the fragmentation of long peptide chains with the formation of N-chloramines with terminal amine groups[5]. It was reported that the bond strength increased with increasing the concertation of NaOCl until a plateau at 10% NaOCl concentration for a 60 second application[6]. However, Sodium hypochlorite has several drawbacks such as the formation of a fragility zone as well as its cytotoxic effect, bad taste and odour[7].

Bromelain enzyme is a proteolytic enzyme extracted from the plant pineapple (Ananas comosus); it is present in fruit and stem. It is mainly acting on the collagen matrix degrading it into oligopeptides and amino acids[8]. Bromelain enzyme has been assessed for its deproteinization effect which revealed bromelain ability to remove the collagen network from acid etched dentin. This should lead to an increased monomer infiltration ability with a more intact dentin substrate[9].

Resin Composite (RC) has developed rapidly since its introduction into the dental market due to the increase in its usage in daily dental practice. To overcome the associated drawbacks with conventional resin composite, a new class of restorative materials called "bulk fill materials" has been introduced. These materials can be applied up to four or five mm in a single increment. This should simplify the restorative procedures in wide and deep cavities saving clinical time. In addition, these materials are claimed to have less shrinkage stresses compared to conventional resin composite. This may be attributed to having stress reliever molecules and polymerization modulators[10,11]. Moreover, application of flowable resin composite liner is beneficial to minimize polymerization shrinkage stresses at the bonded interface and increase the adaptation of resin composite to cavity boundaries[12].

Based on the previous findings, it could be assumed that bromelain enzyme application as a deproteinizing agent may enhance the bond strength of resin composite to etched deep dentin without the side effects associated with sodium hypochlorite. Bromelain enzyme effect on bond strength of acid etched dentin has not been tackled enough in the literature. Therefore, this study was carried out to evaluate the microtensile bond strength of resin composite to deep dentin using two different restorative protocols.

MATERIALS AND METHODS

Sample size calculation

Sample size calculation was done by power analysis used microtensile bond strength as the primary outcome. The effect size f = 0.5695921 was calculated based upon the results of Khatib et al. 2020,[13] and assuming that the standard deviation within each group = 4.5, using alpha level of 5% and Beta level of 80% i.e. power = 80%. The minimum estimated sample size was a total of 45 samples (9 samples per group). Sample size calculation was done using G*Power version 3.1.9.2.

Study design and grouping

A total of 45 extracted, for periodontal reasons, human sound permanent molar at age ranging from 16 to 40 years were collected and stored in saline solution that was changed daily until the beginning of the study. Teeth were embedded in self-cured acrylic resin (Acrostone Dental & Medical Supplies, Cairo, Egypt) 2 mm above cementoenamel junction. Deep dentin level was standardized through removal of occlusal surface of each tooth using an automated diamond saw machine (Isomet 4000, Buehler Ltd., Germany) until removal of enamel and exposure of a flat layer of dentin surface (superficial dentin).

A 2 mm from the flat occlusal surface was measured by a graduated periodontal probe and marked all over the circumference of the tooth Teeth were remounted to the automated diamond saw machine to cut off this 2 mm in order to expose deep dentin.

Experimental Design and Sample Grouping

Teeth were randomly divided into five equal groups (n=9), one control group and four experimental groups. In control group (Group 1): Admira Fusion X-tra resin composite was applied directly after bonding procedures to etched deep dentin. In experimental groups, teeth were divided according to deproteinizing method and the applied restorative protocol. Group 2: was deproteinized by 10% sodium hypochlorite solution followed by application of Admira Fusion X-tra resin composite. Group 3: was deproteinized by 10% sodium hypochlorite solution followed by X-tra base resin composite liner before applying Admira Fusion X-tra resin composite. Group 4: was deproteinized by 10% bromelain enzyme followed by application of Admira Fusion X-tra resin composite. Group 5: was deproteinized by 10% bromelain enzyme followed by X-tra base resin composite liner before applying Admira Fusion X-tra resin composite. The used materials, their composition and manufacture are listed in Table (1).

Fig. (1): Deep dentin level determination; 2 mm from the flattened occlusal surface confirmed with a graduated periodontal probe.

Fig. (2): Tooth after flattening exposing deep dentin.

TABLE (1): The used materials, their composition and manufacture.

Material	Composition	Manufacture
Sodium Hypochlorite Solution	10% Sodium Hypochlorite solution.	Piochem, Cairo, Egypt
Bromelain Powder	Bromelain from pineapple stem - ≥3 units/mg protein, One unit releases 1.0 micromole of P-Nitrophenol from N-Alpha-CBZ-Lysine P-Nitrophenyl Ester per minute.	Sigma-Aldrich Chemie GmbH, Germany
Packable Nanohybrid Bulkfill Resin Composite (Admira Fusion X-tra)	Matrix: Ormocer resin matrix, large and pre-condensed molecules of an inorganic matrix with a high degree of cross-linking. Fillers: (84 wt%) Silicon dioxide nanofillers (20-40 nm) and glass ceramics filler content. Photo initiator: is camphorquinone.	VOCO GmbH Cuxhaven, Germany.
Flowable Bulkfill Resin Composite (X-tra base)	Matrix: is composed of different methacrylate Bis-EMA and aliphatic methacrylate. Inorganic filler particles: (75%w- 58 vol %) Barium aluminosilicate glass, fumed silica and ytterbium fluoride. Photo initiator: is camphorquinone. Amines and an inhibitor butylhydroxytoluene.	VOCO GmbH Cuxhaven, Germany.

Deep Dentin Surface treatments

For all groups, deep dentin was etched by the application of 35% phosphoric acid for 15 seconds then rinsed. In Group 2 and 3, teeth were deproteinized by a ready-made sodium hypochlorite solution, with a concentration of 10%, applied by a disposable microbrush (regular size, Shofu, Kyoto, Japan) with constant agitation for one minute then rinsed with distilled water. While in Group 4 and 5, teeth were deproteinized by a prepared bromelain enzyme. Deproteinizing agent was prepared by dissolving 10 grams of bromelain powder in 100 ml of distilled water for one minute. It was applied by a disposable microbrush with constant agitation for one minute then rinsed with distilled water.

For all groups, Solobond M (VOCO, Cuxhaven, Germany) was applied according to manufacture instructions by a microbrush. It was light cured for 20 seconds using LED light curing device (Bluephase N, Ivoclar Vivadent, Schaan, Liechtenstein). Following adhesive procedure teeth were restored with 4mm resin composite block using bulkfill technique. Teeth in Groups 1,2 and 4 were restored with packable bulk-fill nanohybrid resin applied as a single increment then light cured for 20 seconds. While teeth in Group 3 and 5 were restored with 2

mm of bulk-fill flowable resin composite then light cured for 20 seconds followed by 2 mm of packable bulk-fill nanohybrid resin composite and light cured for 20 seconds. After curing, each tooth was mounted on the cutting machine.

Fig. (3) Confirming 4mm resin composite thickness using a graduated periodontal probe.

Microtensile Bond Strength Testing

Teeth were sectioned into beams measuring 0.9 mm x 0.9 mm (±0.1 mm for both dimensions) and a height of 5.5±1 mm. Obtained beams of each tooth were stored in distilled water at 37ºC temperature for 24 hours then mounted into a universal testing machine (Instron, MA, USA) with a load cell of 500

N. Tensile load was applied at a cross-head speed of 0.5 mm/min until bonding failure occurred.

Statistical analysis

The obtained data of microtensile bond strength was recorded in MegaPascal (MPa) and tabulated. Data were presented as mean and standard deviation (SD) calculated for each group. Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). The data was found to be normally distributed. One-way ANOVA test was used to compare between different groups. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA test is significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows.

Fig. (4) Beam mounted on universal testing machine

RESULTS

Data in Table (2) represents the mean values (in MPa) for microtensile bond strength and standard deviation of all tested groups. Meanwhile Figure (5) shows a bar chart representing mean values (in MPa) for microtensile bond strength of all tested groups. There was statistically significant difference between the microtensile bond strength values of different groups (P-value <0.001, Effect size = 0.704). Pair-wise comparison between groups revealed that Group 1 recorded the highest microtensile bond strength with a non-statistically significant difference from Group 4, but statistically significantly higher than other groups. Group 5 showed the least microtensile bond strength value between all tested groups.

Concerning the effect of deep dentin deproteinization on microtensile bond strength to packable bulk-fill resin composite, results showed that there was no statistically significant difference between Group 2 and Group 4. Both groups showed lower mean microtensile bond strength values than Group 1. Group 2 showed a statistically significant lower mean value than Group 1, while Group 4 showed a non-statically significant lower mean value than Group 1.

Regarding the effect of flowable bulk-fill resin composite liner on microtensile bond strength between deproteinized deep dentin and packable bulk-fill resin composite, results showed that there was no statistically significant difference between Group 3 and Group 5. Both groups showed a statistically significant lower mean microtensile bond strength values than Group 1 (Control). Moreover Group 3 showed a non-statistically significant lower mean microtensile bond strength value than Group 2. On the other side, Group 5 showed a statistically significant lower mean microtensile bond strength value than Group 4.

TABLE (2) Mean values (in MPa) for microtensile bond strength, standard deviation (SD) and results of one-way ANOVA test of all tested groups.

Group (n = 9)	Mean	SD
Group 1	26.5 [A]	4.1
Group 2	20.4 [BC]	3.3
Group 3	17.6 [CD]	1.7
Group 4	23.5 [AB]	3
Group 5	13.8 [D]	2.4
P-value	<0.001*	
Effect size (*Eta Squared*)	0.704	

*: Significant at P ≤ 0.05, Different superscripts indicate statistically significant differences between groups
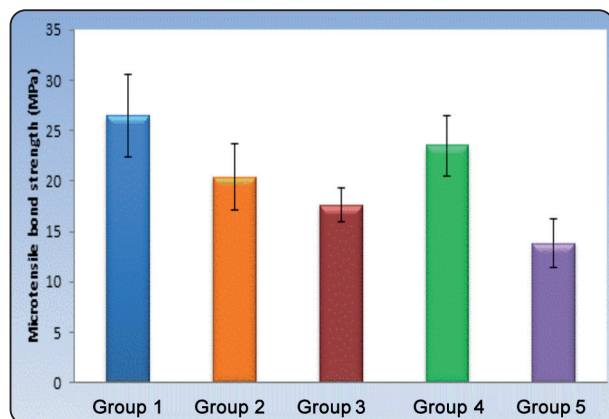

Fig. (5): Bar chart representing mean values (in MPa) and standard deviation for microtensile bond strength of all tested groups.

DISCUSSION

Dentin is a biological structure that is composed of a collagen matrix with carbonate-rich apatite crystals dispersed between collagen fibrils. Dentinal tubules density differs according to dentin depth. Superficial dentin has fewer tubules and more intertubular dentin, while deep dentin is composed of large funnel-shaped dentinal tubules with much less intertubular dentin. The intertubular dentin has been proven to be beneficial during hybrid layer formation. Water content also varies at different dentin levels being lower in superficial dentin than deep dentin[3]. Bonding to deep dentin is reported to be difficult due to its high water content, lower content of intertubular dentin and collagen fibrils[14]. Therefore, bonding to deep dentin represents a challenge. Thus, deep dentin was selected as a substrate to be tested in this study.

Dentin deproteinization is a process that aims to remove the organic part of the dentin smear layer aiming to increase mineral/organic ratio and altering its chemical composition, to be nearly like etched enamel, and changing surface energy of dentin resulting in a more stable interface[15]. Some authors termed this process a "reverse hybrid layer" where the collagen is not infiltrated with resin monomers, but the resin monomers occupy the original spaces of collagen. This showed an increase in infiltration of resin monomer into etched dentin with subsequent increase in the dentin bond strength[14,16].

Sodium hypochlorite has been considered the gold standard for dentin deproteinization by many researchers[6,17]. Sodium hypochlorite has the ability to increase the surface roughness which in turn shows better mechanical retention of resin tags[16]. It can also increase dentin surface energy which improves the penetration and compatibility of hydrophobic monomers to etched dentin. However, sodium hypochlorite deproteinizing effect depends on its concentration and application time. Aguilera et al.,2012,[18] stated that increasing sodium hypochlorite concentration leads to increase in bond strength. However, there is stability in bond strength reached at concentration of 10% applied for 60 seconds. Therefore, 10% sodium hypochlorite applied for one minute on etched deep dentin was used in this study.

However, sodium hypochlorite showed several side effects such as formation of a fragile zone, intolerable taste, unfavourable odour and

cytotoxicity. Moreover, sodium hypochlorite is also difficult to be washed away from dentin surface due to its high reactivity with amino acids located in collagen. All these unfavourable effects rendering usage of sodium hypochlorite, as a dentin deproteinizing agent, not recommended by many researchers and dental practitioners[5,19].

To overcome the associated side effects of sodium hypochlorite, bromelain enzyme was suggested to be an alternative deproteinizing agent. Bromelain enzyme is produced from the tropical fruit pineapple (Ananas comosus L.Family Bromeliacease). It demonstrated the capacity to effortlessly remove unsupported collagen from the etched dentin surface[9,20]. It was reported that the proteolytic action of bromelain may be subjected to inactivation in water if its concentration is less than concentration of 6%[13]. Sharafeddin & Moraveji,2022,[21] found that dentin deproteinization by 10% bromelain enzyme after phosphoric acid etching has a beneficial effect on the used restorative materials in their study. Therefore, 10% bromelain enzyme as deproteinizing agent was used in this study.

Restorative procedures are facilitated with the development of new resin composite called 'bulk fill materials'. Bulk filing technique has become more popular to be applied due to its simple and saves clinical time especially in deep and wide preparations [22]. These materials are thought to have less shrinkage stress. This is attributed to its composition which has stress reliever molecules and polymerization modulators. Bulk-fill resin composite is available either as a packable bulk-fill resin composite designed to restore the entire of the prepared cavities or flowable bulk-fill resin composite designed to be applied as a base or lining material leading to decrease in polymerization contraction stresses[10].

Concerning the effect of deep dentin deproteinization on microtensile bond strength to packable bulk-fill resin composite, results showed that there was no statistically significant difference between deproteinization with sodium hypochlorite and bromelain enzyme. Both groups showed lower mean microtensile bond strength values than control group. Regarding Sodium hypochlorite showed a statistically significant lower mean microtensile bond strength value than control group. This may be attributed to its potent biological oxidant effect that causes formation of superoxide radicals. The free radicals created during the light activation of the adhesive system may be affected by the reactive residual radicals present in dentin. This can lead to early chain termination and insufficient polymerization.[5,23].

On the other side, bromelain enzyme showed a non-statistically significant lower mean microtensile bond strength value than control group. This may be attributed to the spaces created by collagen removal that will be occupied by water. This may lead to increase bond degradation due to hydrolysis with subsequent microleakage. Moreover, the degree of conversion of resin monomers may be negatively affected due to increased hydrophilicity[24]. Although there was no significant difference between deproteinization with sodium hypochlorite or bromelain enzyme, bromelain enzyme showed higher mean microtensile bond strength. This may be due to the ability of bromelain enzyme to wipe out deteriorated collagen fibers from acid-etched dentin and better effectiveness of bromelain enzyme to remove unsupported collagen fibers compared to sodium hypochlorite[13].

Flowable bulk-fill resin composite application as a liner showed a non-statistical significant difference with both deproteinizing agents. This may be attributed to the more stresses created by polymerization contraction at deproteinized deep dentin − X-tra base resin composite interface compared to deproteinized deep dentin − Admira Fusion X-tra resin composite interface. This could be related to the following two reasons:

(1) Difference in filler content of X-tra base RC which is 75% by weight while the filler content of Admira Fusion X-tra RC is 84% by weight. (2) Difference in organic matrix, X-tra base RC is made from different methacrylates (Bis-EMA and aliphatic methacrylate). Methacrylates showed high shrinkage stresses which may led to reduction in bond strength[25,26]. While Admira Fusion X-tra RC is an Ormocer-based resin composite material. Ormocer matrix shows low polymerization shrinkage that may be attributed to its resin system that is constructed from inorganic-organic copolymers replacing classic monomers. The reduced amount of organic resin compared to dimethacrylates based resin composites also results into less polymerization shrinkage[27].

CONCLUSION

Within the limitations and the obtained results of this study, the following were concluded:

1. Deep dentin deproteinization, either with 10% sodium hypochlorite solution or 10% bromelain enzyme solution, has no improvement effect on microtensile bond strength with the packable bulk-fill resin composite.

2. Flowable bulk-fill resin composite application, as a liner, has a deterioration effect on the microtensile bond strength of deproteinized etched deep dentin to packable bulk-fill resin composite.

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