



## EVALUATION OF MICROTENSILE BOND STRENGTH OF COMPOSITE RESIN WITH DIFFERENT BONDING AGENTS ON ETCHED DENTIN TREATED WITH BROMELAIN AND PAPAINE: AN INVITRO STUDY

### Dentistry

**Dr. Prasangi Vijaya Bhanu\*** Post Graduate, Dept. Of Conservative Dentistry And Endodontics, Gsl Dental College And Hospital, Rajahmundry, India \*Corresponding Author

**Dr. Kanumuri Ramakrishna Raju** Head Of The Department, Dept. Of Conservative Dentistry And Endodontics, Gsl Dental College And Hospital, Rajahmundry

**Dr. Chinta Saipriyanka** Assistant Professor, Dept. Of Conservative Dentistry And Endodontics, Gsl Dental College And Hospital, Rajahmundry

**Dr. Jolling Diksha** Post Graduate, Dept. Of Conservative Dentistry And Endodontics, Gsl Dental College And Hospital, Rajahmundry, India

### ABSTRACT

**Aim:** Using various bonding substances, the purpose of this research is to evaluate as well as contrast the effects of 10% bromelain and papain on the microtensile durability produced by composite resin when applied to etched dentin. **Material and methodology:** Current research was conducted on 30 human permanent mandibular molar teeth. Disto-occlusal & Mesio-occlusal conservative cavities were prepared, etchant application was done for 15 seconds and rinsed with water and blot dried. Prepared samples randomly split into 2 groups/categories (n=15) depending on the deproteinization agent used. (G1):10% Bromelain for 1 min. (G2):10% Papain (1 min) and then washed off. MO cavities treated using Prime Bond NT bonding agent & DO cavities treated using Adper Single-Bond 2 bonding agent and restored with bulkfill composite (Tetric-N-Ceram) and the samples subjected to thermocycling. Samples then sectioned vertically into 1mm<sup>2</sup> thick slabs by using hard tissue microtome and were subjected to universal testing machine for analysis of the durability of the microtensile bond. **Statistical analysis:** The software SPSS version 20 (IBM, SPSS, Armonk, NY, USA) was used to conduct the statistical analysis. Data evaluation techniques include descriptive statistics and a single direction assessment of deviation using Tukey's post-hoc testing. **Results:** With a microtensile bond strength of 58.14±1.97 MPa, Group 1 (Bromelain-MO) showed highest microtensile bond strength and Group 4 (Papain-DO) 21.92±2.86 MPa has the least microtensile bond strength. The microtensile bond strength varied between the two groups in a statistically significant. Every pairwise comparison shown to be statistically significant by Tukey's post hoc testing. **Conclusion:** The mean microtensile bond strength values of bromelain treated with Prime bond NT and Adper single bond 2 was significantly high in comparison to papain treated with Prime bond NT and Adper single bond 2.

### KEYWORDS

Adhesion, Bromelain, Etch and rinse, Papain, Smear layer

### INTRODUCTION

The hybrid layer and degree of polymerized resin infiltration into demineralized dentin determine the longevity of restorations and the strength of their bond. A bonding process called hybridization causes a hybrid layer to grow on the surface of the dentin. Sharafeddin F, et al. study showed that prior to dentin surface bonding, the smear layer should be removed for improved hybrid layer formation. (Matrix metalloproteinases) MMP's, which are present in saliva and etched dentin, break down the unsupported collagen fibrils inside the hybrid layer. MMP's are quiescent within the dentin extracellular matrix until they are needed<sup>[1]</sup>.

The acidic environment created by biological carious process or by various chemical interactions with bonding agents activates various dental MMP's. So as to inhibit this process various matrix metalloproteinases inhibitors and deproteinizing agents are present. These inhibitors, which include chlorhexidine, having the property that helps in destruction of unsupported collagen fibrils within hybrid layers, which helps to sustain the strata of hybrids<sup>[2]</sup>. Duarte, et al. used different irrigation solutions. Among these sodium hypochlorite (NaOCL) does have deproteinizing impact, but it also has time-dependent effect. Utilizing sodium hypochlorite to deproteinize acid-etched dentin has the drawback of toxicity, extreme taste and odour had led to opening up different perspective to deproteinize the dentin<sup>[3]</sup>.

Several deproteinizing agents, both chemical and natural are commercially available. Examples of natural deproteinizing agents are bromelain and papain. Bromelain is a mixture of endopeptidases and it is a fibrinolytic and proteolytic enzyme economically taken from pineapple's fruit stem. Khan, et al. study shown that withdrawal of delicate collagen fiber with bromelain enzyme, after etchant, increases bond strength and decreases leakage of adhesive restoration<sup>[4]</sup>.

The papaya fruit, *Carica papaya*, a member of Caricaceae family, from where papain is extracted. Because of specificity of cysteine enzymatic protein that exhibits antibacterial activity, anti-inflammatory qualities and functions as an agent for debris clearance without having an adverse effect on tissues. According to Pal, et al. study the shear bond

strength has increased when deproteinizing solution have used initially to bonding an orthodontic band to etched enamel<sup>[5]</sup>.

Maintaining the chemical equilibrium between the hydrophilic and hydrophobic functional structures of dentin bonding agents has improved both their physical and functional qualities. However, dentin bonding agent wettability significantly increases after acid etching and deproteinization procedure. Regarding using different bonding agents after using various deproteinizing agents, to assess the microtensile bond strength of composite resin is not well documented.

### MATERIALS AND METHODS

In this investigation, thirty complete mandibular molar teeth were used, which are extracted due to compromised periodontium, obtained from Oral and Maxillofacial Surgery Department. The total sample size is 30 with 15 in each group. 30 extracted human permanent molar teeth collected and cleaned of debris and calculus. Preoperative radiographs are taken and microscopic evaluation is done to evaluate internal resorption and other defects of the teeth. Disinfection is done with 0.5% chloramine-T solution. All the samples were stored in 0.1 % thymol solution till usage. Metallic moulds with dimensions of 2cms acrylic resin (DPI, India) is used to mount sample tooth.

After complete setting of the acrylic resin Disto-occlusal & Mesio-occlusal conservative preparations done using hand piece and 269 bur (Brasseler USA). All the cavities were made with standardized depth and width i.e., 3x2.5x2mm dimensions. Prepared (Mesio-occlusal)MO & (Disto-occlusal)DO cavities were air dried and Etchant is applied (37% phosphoric acid) in compliance with the manufacturer's guidelines and then washed off. Sample pairs were divided randomly (n=15) depending on type of deproteinization agent used.

GROUP 1 (G1): Deproteinized with 10% Bromelain (1 minute) and then flushed off with water. GROUP 2 (G2): Deproteinized with 10% (1 minute) and then flushed off with water. 5th generation bonding agent (Etch and rinse adhesive) is applied and cured with Dentsply spectrum curing light. (Mesio-occlusal)MO cavities for each group treated with Prime & Bond NT bonding agent and & (Disto-

occlusal)DO cavities treated with Adper Single Bond 2 bonding agent. Tetric N Ceram bulk-fill posterior restorative composite is placed as a single increment into the prepared cavities and were subjected to light cure. Then samples were subjected to thermocycling (500 cycles at 5°C ± 2°C-55°C ± 2°C with 30 s dwell time and 5 s transfer time) for simulating oral environment. The completed samples were adhered to the sectioning block individually using acrylic resin. After that, the block is cut into slabs that are between 0.7 and 1.0 mm thick using a hard-tissue microtome (Figure 1). With each slab being roughly 1 mm by 1 mm (Figure 2).



Fig. 1 : During sectioning



Fig. 2 : Sectioned slab

Fig. 3 : Universal testing machine

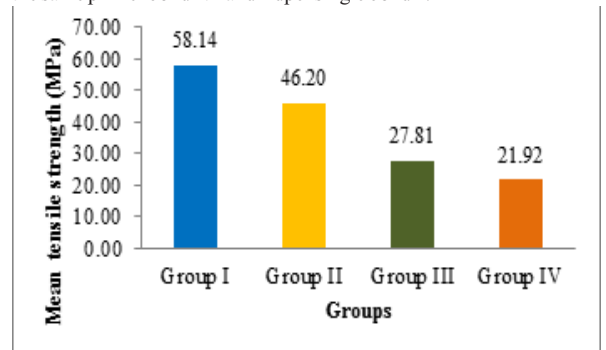
The separated beams were then tested using the Instron international checking equipment (Figure 3). The beam was subjected to a tensile force of 0.5 mm/minute until it cracked, and the load at which fracture happened was noted. Furthermore, Megapascals (MPa) has been employed to determine the microtensile endurance of bonds.

**RESULTS**

Graph 1 and Table 1 depicts, the mean microtensile bond strength of group 1 is 58.14±1.97 MPa, minimum and maximum microtensile bond strengths are 54.12 MPa and 61.75 MPa respectively. In Group 2 the mean microtensile bond strength is 46.20±1.61MPa, minimum and maximum microtensile bond strengths are 43.36 MPa and 48.96 MPa respectively. In Group 3 the mean microtensile bond strength is 27.81±1.99 MPa, minimum and maximum microtensile bond strengths are 24.18 MPa and 30.92 MPa respectively. In Group 4 the mean microtensile bond strength is 21.92±2.86, minimum and maximum microtensile bond strengths are 15.23 MPa and 26.44 MPa respectively. The intra group comparison for all groups showed p value of <0.001 which indicates significant difference within the group.

The average readings for microtensile bond strength of bromelain treated with Prime bond NT and Adper single bond 2 are statistically

significant and substantially higher than those of papain treated with the same primer bond NT and Adper single bond 2.



Graph 1: Microtensile bondstrength between different groups

Table 1: Comparison Of Mean Tensile Stress At Tensile Strength [mpa] Among The Four Groups

Groups	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum	F value	P value
Group I	15	58.14	1.97	0.51	54.12	61.75	894.616	<0.001*
Group II	15	46.20	1.61	0.42	43.36	48.96		
Group III	15	27.81	1.99	0.51	24.18	30.92		
Group IV	15	21.92	2.86	0.74	15.23	26.44		

**DISCUSSION**

According to Dayem, et al. the longevity of composite restorations depends on several factors, one of such factor is the junction between tooth structure and filling materials. At this junction there will be presence of smear layer. This smear layer can be removed by 37% phosphoric acid applied for about 15 seconds which leads to the collagen fibril exposure. An adhesive comonomers infiltrate in-between these spaces of collagen fibrils forming hybrid layers<sup>[6]</sup>.

Gwinnett, et al. demonstrated that gap formation can be successfully avoided by establishing an area of interdiffusion zone between demineralized dentin and resin. In the end, an incomplete penetration of this zone weakens the bond<sup>[7]</sup>. Taldoeano, et al. showed that matrix metalloproteinases (MMP's) and cysteine cathepsins are also found in dentin. Only after being exposed to acid, they become activated, which weakens the collagen fibrils and causes the resin-dentin bond to break. The bonding strength is weakened as a result. Several strategies, including the use of matrix metalloproteinases (MMP's) inhibitors, have been employed to prevent these biodegradations<sup>[8]</sup>.

Following acid etching to improve the restorative interface integrity, few studies showed that some of the deproteinizing agents were used to dissolve and remove the collagen fibers; this procedure is known as deproteinization<sup>[9-11]</sup>.

According to some studies sodium hypochlorite (NaOCL) has a bad taste and odour, which makes it a strong oxidising agent that can damage soft tissues in the mouth if not handled cautiously<sup>[12-14]</sup>. As a result, people are looking for alternatives. Because of this, employing a natural deproteinizing agent, which appears to function as a potential replacement for sodium hypochlorite, might be beneficial<sup>[15-17]</sup>.

Among the many natural enzymes observed, papain and bromelain are two examples<sup>[18]</sup>. Papain is alkaloid element derived from latex and an exterior covering of ripen papaya fruit. It also contains proteolytic activity, anti-bacterial and antiinflammatory property which improved subsequent enamel bonding<sup>[19]</sup>. According to Bussadori, et al. Papain is also available under gel form under brand name Papacarie which is different from product used in present study, which is not produced from pure papain<sup>[20]</sup>.

A class of enzymes called bromelain is extracted from the pineapple fruit, Ananas comosus, which belongs to the order Bromeliaceae. Since the pineapple stem has the highest concentration, extraction is possible and economical because the stem is a byproduct. When the enzyme bromelain is applied to conditioned dentin, leakage scores are

greatly decreased, and the global leakage scores are at their lowest. Because the collagen structure in dentin that has been acid-engraved may be successfully removed by the enzyme known as bromelain, monomer diffusion potential to undamaged dentin is increased while nanoleakage is minimised.

The impact of various types of concentration of papain on orthodontic bonding of bracket were also consequently studied by Pithon, et al. 2012 to confirm hypothesis that deproteinization of enamel with papain concentration of different percentage such as 2, 4, 6, 8, and 10 increases SBS with different concentrations. The conclusion was that the deproteinization with 8 percent and 10 percent gel of papain strengthens the shear bond strength (SBS) of brackets bonded with resin modified glass ionomer cement (RMGIC). Similarly 10% bromelain gel was found to be ideal concentration by Farahnaz, et al. in their study. Taking into consideration results of these studies concentration of 10% was found ideal and was used for present study. The current study findings demonstrated that bromelain has a stronger bond strength than papain. This is because the weakened dentin may be precisely removed by the activity of the enzyme. Furthermore, the breakdown of the desecrated collagen may improve bonding capacity through improving diffusion prospective, even though papain acts with the aid of solely breaking down the partially disrupted collagen molecules and contributing to the degradation and elimination of the filamentous mantle developed using the carious procedure, without harming unchanged collagen fibrils. This finding is statistically significant and results similar to study conducted by Khatib et al 2020<sup>[21]</sup>.

Buonocore first proposed acid-etched enamel adhesion in 1955. According to Buonocore as there is difference in bond strength between different total etch adhesives based on this in present study comparison between Prime bond NT and Adper single bond 2 have been compared.

In present study total-etch adhesive bonding agents ethanol are used as a backup solvent. Moreover, the elevated reactivity as well as permeability of ethanol aid in the displacement of water and the penetration of monomers into the opened dentinal tubules. Another study conducted by Knobloch LA, et al. revealed that total etch adhesives had significantly higher microtensile bond strength (MTBS) and reviewed similar results with present study<sup>[22]</sup>.

Increasing demand for aesthetic dentistry resulted in fabrication of resin composites for enhanced toughness, appearance as well as rigidity over the last few decades. Abuelenain DA, et al. showed that nanofilled materials offers excellent polish retention, lustrous appearance, resistance, tensile strength and ultimate aesthetics<sup>[23]</sup>. Tetric N-Ceram composite material with nanosized filler particles was used in this study. According to Borges FT, et al. shear bond strength (SBS) is also higher in Tetric N-Ceram comparing to other bulk-fill composites. Explained by absence of TEGDMA in the material's composition, which has roughly half the molecular weight of other monomers<sup>[24]</sup>.

In addition, Tetric N-Ceram composite material also includes new photo-initiator system (Ivocerin<sup>TM</sup> - dibenzoyl germanium compound). It takes in visible light over broader wavelength range of 370 to 460nm. Reactivity is increased with appropriate formulations; greater depths of cure can achieve. In this present study thermocycler (Mechatronik CS3. 4) carried out 500 cycles at 5°C ± 2°C - 55°C ± 2°C, 30 s dwell time, 5s transfer time which was kept same for all the sample teeth to avoid confounding factor. In this present study, the dimensions of the cavities were standardized depth and width of the cavity as follows: 3x2.5x2mm dimensions. A microtensile cohesiveness experiment was employed in this particular research due to its accurate stress dispersion at the precise contact<sup>[25]</sup>.

Present study, comparison between bonding agents it was noticed that among bromelain group bond strength was more in bromelain treated with Prime & Bond NT (PBNT) (61.75 MPa) followed by bromelain treated with Adper single bond 2 (48.96 MPa) and in papain group bond strength was more in papain treated with Prime & Bond NT (PBNT) (30.92 MPa) followed by papain treated with Adper single bond 2 (26.44 MPa) which were statistically significant.

## CONCLUSION

Following conclusions reached within constraints of current in study.

Measured using different deproteinizing substances, the material's microtensile bonding durability and bonding agent are as follows: Bromelain (Prime bond NT) > Bromelain (Adper Single Bond 2) > Papain (Prime bond NT) > Papain (Adper Single Bond 2).

Natural deproteinizing agents used in study, such as bromelain, papain resulted in increased bond strength values when combined with different bonding agents.

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