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Original Research Article

To evaluate the effect of papain gel and bromekain gel on the shear bond strength of orthodontic brackets: An in-vitro study

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Abstract

Introduction: Contemporary orthodontic treatments depend on a strong clinical bond between the orthodontic bracket and the tooth surface to effectively resist the mechanical stresses and thermal variations present in the oral environment. The bond strength between the enamel-adhesive interface will be compromised by the presence of salivary pellicle during bonding. Since bonding is an essential aspect of all orthodontic patients, prior to bonding, enamel conditioning of teeth is required.

Aims & Objectives: The current study is to verify the hypothesis that the use of papain gel and bromelain gel, prior to orthodontic bracket bonding would increase the shear bond strength.

Materials and Methods: 75 extracted premolars were divided in to three groups, Group A – control, Group B - Deproteinization with 10% papain gel, Group C – Deproteinization with 5% bromelain gel.

Results: The results of the shear bond strength test demonstrated that the highest bond strength values were attained in the group B followed by group C, which did not differ statistically from another group ($p < 0.05$). The control group had the lowest value in which papain and bromelain gel were not used.

Conclusion: enamel deproteinization using 10 % papain gel showed that there is increase in bond strength compared to 5 % bromelain gel and control group (without enamel deproteinizing). But there was no statistically significant increase in bond strength in all the three groups.

Keywords: Papain, Bromelain, Pellicle, 10% papain gel, 5% Bromelain gel

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1. Introduction

Modern orthodontic treatment necessitates an effective clinical bond between the orthodontic bracket and the tooth surface to endure the mechanical and thermal influences of the oral environment.[1] In 1955, Buonocore pioneered the application of micromechanical bonding between a dental material and the enamel surface by applying 85% phosphoric acid to the enamel surface.The prism structure's enamel crystals are differently dissolved by acid-etching, forming a porous enamel surface layer with a depth of 5 to 50 µm². This results in a roughened surface that can be retained by micromechanical means.[1] Newman[2] in 1965 introduced first bonding in orthodontics. He bonded plastic orthodontic attachments to the tooth surface using epoxy resins, either in addition to or instead of the tooth's metal banding.. Since then, there has been a major research drive to increase bond strength between dental material and dental hard tissue.[2]

In addition, the presence of the acquired salivary pellicle during bonding may weaken the bond between orthodontic glue and enamel.By preventing direct contact between acid and the tooth surface, the protein covering enamel acts as a selective permeability membrane or diffusion barrier, slowing the pace at which dental hard tissue dissolves. Therefore, the organic ingredient that prevents effective enamel etching should be removed by treating the enamel. The layer of external organic matter,

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according to studies[3,] is what keeps the acid from efficiently etching the tooth surface, producing an uneven etching pattern and an unreliable area for orthodontic bonding.[3]

The initial suggestion for deproteinizing the enamel surface prior to bonding brackets was made by Justus et al.[4] This process entails the extraction of organic materials, specifically proteins, from the enamel surface. The research indicates that sodium hypochlorite (NaOCl) effectively removes the organic material on the enamel surface through a dissolving action. Among various agents that possess similar deproteinization effects, papain stands out.[4] Papain is an enzyme derived from the latex of Carica papaya fruits, which are part of the Caricaceae family, commonly known as papaya. Because of the enzyme's selectivity, this cysteine protease may remove debris from tissues without harming them. It also has antibacterial and anti-inflammatory qualities. In 2003, a Brazilian product named “Papacarie” (Formula e Ação, São Paulo, Brazil) was launched, marking the introduction of papain into the field of dentistry. This product is employed for the chemical removal of dental caries. When papain is applied to contaminated dentin, it displays proteolytic, chlorinating, and oxidizing activities on the damaged collagen, without acting on the healthy dentin. Pithon et al.[7] recently recommended using 10% papain as a deproteinizing agent prior to acid etching and confirmed that this increased bond strength was caused by the elimination of organic components.[7]

A proteolytic enzyme of the cysteine proteinase group, bromelain was isolated from the pineapple, Ananas comosus, a tropical plant belonging to the Bromeliaceae family. In addition to aiding in wound debridement, its qualities include anti-inflammatory, anti-edematous, anticoagulant, antimicrobial, and antimetastatic actions. Because of its anti-inflammatory properties, bromelain has been utilized in dentistry since the 1960s, especially in minor oral surgery. This material has more recently been utilized as the primary component of dentifrices to remove stains.[8]

Since bonding is an essential aspect of all orthodontic patients, prior to bonding, enamel conditioning of teeth is required. The current study is to verify the hypothesis that the use of papain gel and bromelain gel, prior to orthodontic bracket bonding would increase the shear bond strength. Thus the aims of the study are:

1. To evaluate the effectiveness of papain gel on shear bond strength of orthodontic bracket by prior application of the gel.
2. To evaluate the effectiveness of bromelain gel on shear bond strength orthodontic bracket by prior application of gel.
3. To compare the effects of papain gel and bromelain gel as deproteinizing agent prior to bracket positioning.

2. Materials and Methods

75 therapeutically removed, non-carious, non-fluorosed premolars were used in the study. They were obtained for orthodontic purposes from the department of oral and maxillofacial surgery and kept in a 0.1% thymol solution. When choosing the teeth, care was taken to make sure the buccal enamel was intact, the teeth were free of cavities and/or repair, hypoplasia, attrition, abrasion, erosion, or fracture, and they hadn't received any prior chemical treatment.

2.1. Materials used

1. 75 Non-carious, Non-Fluorosed, Extracted Premolars.
2. Metal Brackets - 0.022 x 0.028 slot (3M).
3. Papain gel and Bromelain gel.
4. 37% Phosphoric Acid Gel – Prime Etch (Epse scotch bond universal etchant syringe).
5. Transbond XT light cure adhesive (3M, Unitek, Monrovia, California).
6. Primer – Transbond XT (3M, Unitek, Monrovia, California)

2.2. Equipment used

1. Instron universal testing machine (MCSTNE-2.5T, India).
2. Light cure unit (Dentsply Spectrum).

Figure 1; Metal bracket .022”.028 slot (3M)

Figure 2: Transbond XT (3M)

Figure 3; 37% Phosphoric acid (Epsescotchbond universal etchant

Figure 4: TransbondXT Primer (3M)

Figure 5: 10% Papain gel

Figure 6: 5% Bromelain gel

Figure 7: Extracted Premolars

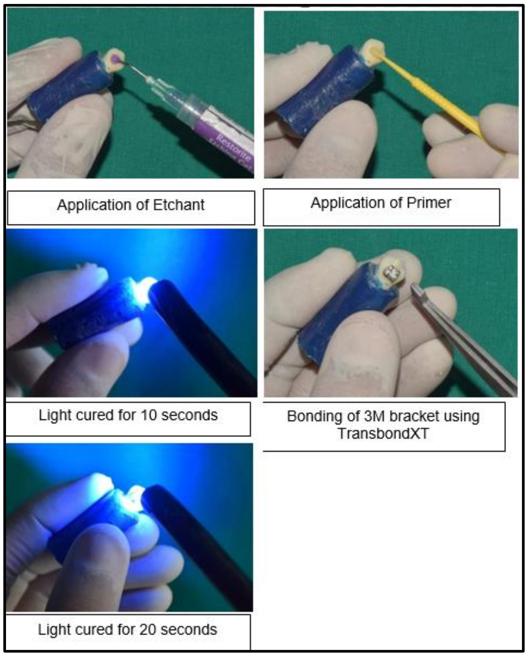


Figure 8: Group A bonding procedure

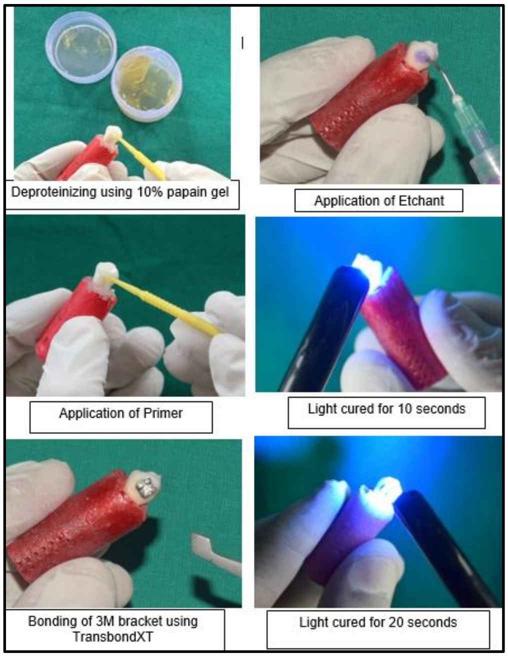


Figure 9: Group B bonding procedure

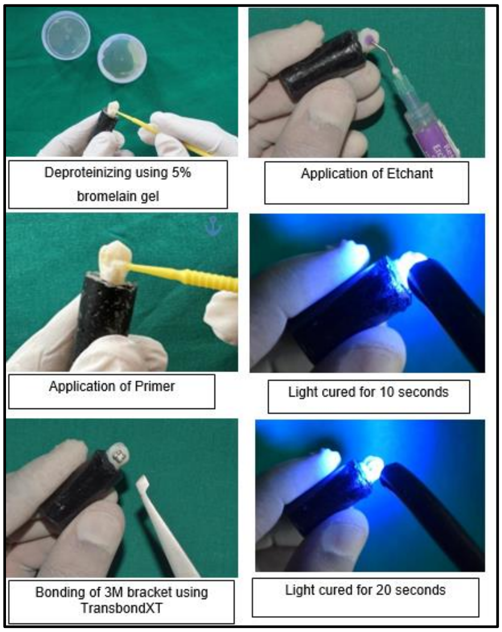
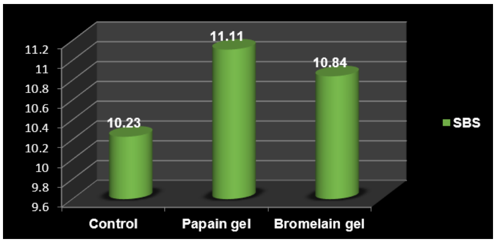


Figure 10: Group C bonding procedure



Graph 1; Mean comparison of shear bond strength (SBS) between groups

2.3. Preparation of the teeth

Prior to testing, every tooth was treated with care to ensure that the enamel was unharmed chemically or physically. All teeth underwent a 15-second prophylactic treatment with a pumice stone and water, followed by an equal amount of time spent washing and drying. Only the crown section of the teeth was visible since they were positioned vertically on blocks of color-coded acrylic (methyl methacrylate self-cure resin foundation).

2.4. Brackets used in the study

In the study, 3M metal brackets with stainless steel premolar brackets and an MBT 0.022" slot were utilized. According to the makers' information, the bracket base's surface area was 9.806 mm².

2.5. Gels used in the study

1. Papain gel
2. Bromelain gel

2.6. Procedure

Three groups of teeth were randomly selected and treated as follows.

1. **Group A**: primer application and bracket attachment using Transbond XT resin (3M Unitek, CA, USA) after 15 seconds of etching with 37% phosphoric acid
2. **Group B**: primer application and bracket attachment using Transbond XT resin (3M Unitek, CA, USA) after deproteinization with 10% papain gel and 37% phosphoric acid for 15 seconds.
3. **Group C**: Deproteinization for 15 seconds using 37% phosphoric acid and 5% bromelain gel, followed by primer application and bracket attachment using Transbond XT resin (3M Unitek, CA, USA).

Group A: The group consisted of twenty-five teeth which were etched with 37% phosphoric acid for 15 seconds, followed by thorough washing and drying until frosty white appearance is seen on enamel. Thenprimer is applied on the etched enamel in a thin flim and light cured for 10 seconds with light curing unit (Dentsply).After priming, Transbond XT adhesive was applied on the bracket base and positioned near the center of facial surface of the tooth with sufficient pressure to express excess adhesive flash, which was removed from the margins of the bracket base using bracket positioner and cured for 20 seconds with the use of light cure unit.

Group B: The group consisted of twenty-five teeth which were deproteinization with 10% papain gel for 60 seconds, followed by etching with 37% phosphoric acid for 15 seconds, followed by thorough washing and drying until frosty white appearance is seen on enamel. Then primer is applied on the etched enamel in a thin flim and light cured for 10 seconds with light curing unit (Dentsply). After priming, Transbond XT adhesive was applied on the bracket base and positioned close to the tooth's center of the facial surface. The bracket positioner was used to remove any excess adhesive flash from the bracket base's edges, and a light cure unit was used to light cure the adhesive for 20 seconds.

Group C: The group consisted of twenty-five teeth which were deproteinization with 5% bromelain gel for 60 seconds, followed by etching with 37% phosphoric acid for 15 seconds, followed by thorough washing and drying until frosty white appearance is seen on enamel. After priming, the bracket base had been coated with Transbond XT adhesive, which was then positioned close to the tooth's center of the facial surface with enough pressure to express excess adhesive flash. The bracket positioner was used to remove the excess adhesive flash from the bracket base's edges, and a light cure unit was used to light cure the bracket base for 20 seconds.

3. Results

The present study was done to evaluate the effectiveness of papain gel and bromelain gel on shear bond strength of orthodontic bracket with prior application of gels during bonding procedure (i.e. before etching with 37 % phosphoric acid).

Seventy-five human premolars were used for this study. Which were further divided in three groups, Group A (control group), Group B (papain gel group), Group C (bromelain gel group). Deproteinization was not done in group A before to the bonding process (that is, prior to etching with a 37% phosphoric acid concentration). In group B, deprotenization was done using 10% papain gel before to the bonding process (that is, prior to etching with 37% phosphoric acid). Before the bonding process (i.e., before etching with 37% phosphoric acid), group C underwent deproteinization using 5% bromelain gel.

The shear bond strength test findings showed that group B (10% papain gel) achieved the highest bond strength values, followed by group C (5% bromelain gel), which did not differ statistically from another group ($p < 0.05$). The control group, which did not utilize papain or bromelain gel, had the lowest levels. There were no statistically significant variations between group B and group C's shear bond strength data when evaluated statistically. Group A's shear bond strength was 2.43 MPa at its lowest and 21 MPa at its highest; group B's and C's respective shear bond strengths were 5.82 MPa and 19.06 MPa and 5.66 MPa and 21.15 MPa, respectively (**Table 1**).

Table 1: Mean comparison of shear bond strength (SBS) between groups

Groups	n	Mean	SD	Minimum	maximum	Test statistic	P value
Group A (control)	25	10.2328	5.03103	2.43	21	0.575	0.750
Group B (Papain gel)	25	11.1152	4.25669	5.82	19.06		
Group C (Bromelain gel)	25	10.8492	4.07977	5.66	21.15		

Kruskal Wallis test; $p \leq 0.05$ considered statistically significan

Table 2: Pair wise comparison – post hoc analysis

Comparison between		P value
Group A (control)	Group B (Papain gel)	0.479
	Group C (Bromelain gel)	0.560
Group B (Papain gel)	Group C (Bromelain gel)	0.900

4. Discussion

The practice of directly bonding orthodontic brackets has been recommended since the 1960s.[2] Enamel is made up of 96% inorganic material by weight, while the remaining 4% comprises 3% water and 1% organic materials (including proteins and lipids). The protein content found in normal enamel ranges from 0.04% to 0.7%. These organic components pose challenges for adhesive substances to bond effectively to the tooth enamel surface, leading to a reduction in shear bond strength. Enamel contains proteins such as amelogenins, enamelins, and other proteins like ameloblastin and sheathlin.

All solid surfaces in the oral cavity are coated with a protein-based layer known as the acquired salivary pellicle. The bond strength between orthodontic adhesives and enamel might be affected by the presence of the pellicle during the bonding process. Functionally, it contributes to maintaining mineral balance in tooth enamel. This structure is created by the selective binding of proteins, peptides, and various molecules found in oral fluids. It is an organic film that is free from bacterial colonization, covering both hard and soft oral tissues, consisting of proteins, glycoproteins, enzymes, and mucins or their derivatives. Numerous proteins have elevated proline levels ranging from 35% to 40%, leading to their classification as proline-rich proteins (PRPs), which account for nearly 70% of the overall protein composition in human parotid saliva. The presence of these proteins on the enamel acts as a barrier to diffusion or a membrane with selective permeability, preventing acids from directly contacting the tooth surface and thereby decreasing the rate of dissolution of dental hard tissues.

Consequently, it is important to condition the enamel surface to remove the organic materials that interfere with proper enamel etching. However, it's crucial to note that the organic layer cannot be completely eliminated without taking into account the proteins that are present in the enamel crystals. Research has indicated that this layer of external organic substances inhibits the acid from adequately etching the surface, leading to inconsistent etching patterns and an unreliable area for orthodontic bonding.

Using 37% phosphoric acid to etch enamel after removing organic materials from the enamel surface results in longer adhesive tags that penetrate deeper into the enamel. It is crucial to understand that phosphoric acid primarily affects the mineralized tissues, or inorganic components, of the enamel. Unfortunately, this acid does not remove organic materials, as evidenced by the "collagen network" that remains after the phosphoric acid demineralizes the dentin while leaving the collagen fibers intact. The presence of these longer tags significantly enhances the mechanical retention of adhesives to the enamel. Consequently, removing organic substances from the enamel surface prior to acid etching improves the resistance against the debonding of orthodontic brackets by creating a more effective acid etching pattern on the enamel.

One major disadvantage of orthodontic bonding is the risk of enamel demineralization, which occurs due to plaque buildup around the brackets and bands. This may result from inadequate oral hygiene practices and the presence of retentive areas near the orthodontic attachments, all of which contribute to the emergence of white spot lesions. The occurrence of enamel demineralization following the application of a fixed orthodontic appliance can affect as many as 50% of patients. Enamel demineralization is acknowledged as a potential side effect of bonding orthodontic brackets using composite resins.

In orthodontics, the formation of white spot lesions and marginal gingivitis near fixed orthodontic devices results from the accumulation of bacterial biofilm. (21) Bishara and Ostly(32) have noted that decalcification represents a significant consequence of orthodontic treatment on tooth enamel. The bond strength between orthodontic components and the enamel can be weakened by the presence of an acquired pellicle.

In order to reduce and prevent the formation of white spot lesions, there is a growing awareness of the benefits of new fluoride-releasing materials. Among the various options available, glass ionomer cements stand out as they provide chemical bonding to enamel, dentin, and other surfaces while also releasing fluoride. However, these cements exhibit lower bond strength to enamel surfaces compared to orthodontic composites. To address this, resin-modified glass ionomer cements were created to combine the key attributes of both materials, offering shear bond strength and fluoride release without diminishing the bond strength to the tooth surface.[7]

The strength of the bonding between orthodontic attachments and the enamel surface is compromised by the existence of acquired pellicle, which covers the soft and hard tissue in the oral region, especially the surface of tooth enamel. The most prevalent components of this membrane, which is a biofilm devoid of bacterial colonization, include proteins, glycoproteins, enzymes, and mucin or its derivatives. These organic components reduce the composite's shear bond strength by making it harder for it to stick to the surface of the tooth enamel.[5] Therefore, the goal of enamel surface prophylaxis is to get rid of organic material that could make enamel etching less successful. Consequently, it has been suggested that the extra step of deproteinizing the enamel surface using a proteolytic agent prior to acid etching may be advantageous.

With the purpose of eliminating the influence of the organic matrix on the adhesion of composites to the enamel surface, Justus et al suggested the use of 5.25% sodium hypochlorite for 60 seconds as enamel conditioning agent before etching with 37% phosphoric acid, showed good results. There are various substances with the characteristics of deproteinizing surfaces, among these are papain and bromelain.[7]

Pithon and collaborators proposed the use of papain, an enzyme present in papaya fruit, and previously achieved statistically significant results related to the shear bond strength of orthodontic bracket. Bromelain is a proteolytic enzyme with the similar characteristics to that so papain and is extracted from Ananas comosus, which belongs to the Bromeliaceae family. This enzyme may be used in association with papain for the purpose of removing protein from enamel surface prior to orthodontic bracket bonding(8).

Roberto Justus et al[4] conducted a study to test the shear bond strength of Transbond XT and RMGIC bonded on the buccal surface of the teeth which underwent deproteinization using sodium hypochloride (NaOCl). It is conducted in a sample size of seventy-six extracted human premolars teeth. They were randomly divided in four groups, two control group and two experimental group. In group 1(experimental) and group 2 (control) brackets were bonded using Transbond XT and group 3 (experimental) and group 4 (control) brackets were bonded using RMGIC. The buccal surface of human premolars was deproteinized using 5.25% sodium hypochloride for 60 seconds followed by acid etching for 30 seconds and orthodontic brackets were bonded either by Transbond XT or RMGIC in experimental group (1 and 3 group). Whereas, in control group (2 and 4 group) except deproteinization the same procedure was followed. The study concluded that there was no significant difference in Transbond XT groups, whereas there was significant difference in between RMGIC groups. The SBS for Transbond XT group with 5.25% NaOCl was 9.41±4.46 MPa, the present study showed a greater SBS of 11.11 ± 4.25 MPa when deproteinization was carried with 10 % papain gel. While the bromelian gel group showed SBS of 10.84 ± 4.07 MPa which is also comparatively greater than Transbond XT with NaOCl. The control groups in both studies showed lower SBS compared to groups which used enamel deproteinizing agents, that is,Transbond XT without NaOCl showed SBS of 8.12 ±3.10 MPa, also the present group showed SBS of 10.23 ± 5.03 MPa without use of any deproteinizing agent. Thereby the current study supports the use of deproteinizing agents prior to bonding procedure. The Roberto Justus study and present study concluded that the use of enamel deproteinizing agent will slightly increase the shear bond strength compared to the group were enamel deproteinizing is not carried out. When compared between SBS of NaOCl, papain gel, bromelain gel, NaOCl showed lower bond strength.

Matheus Melo Pithon et al (2012)[7] conducted a study on one hundred and twenty bovine incisors, divided into eight groups, each group was treated with different combinations of Transbond XT, or RMGIC, with and without papain gel as deproteinizing agent and with and without 37% phosphoric acid as acid etching agent, and concluded that there was significant increase in the shear bond strength with 10% papain gel, irrespective of the etching agent. The current study, though not statistically significant, showed similar results as Matheus et al reported that Transbond XT deproteinized with 10% papain gel showed highest bond strength (80.8± 50.88 MPa), while the present study showed a highest bond strength of 11.11 ±4.25 MPa using papain geland control group with SBS of 10.23 ± 5.03 MPa. The group with 10% papain gel used prior to bonding showed a difference in bond strength in both studies, this difference in shear bond strength between the Matheus Pithon study and present study may be due to the different arrangement of prism and interprism between the bovine enamel and human enamel (Cadwell and Johanson).

Matheus M Pithon et al (2013)[5] conducted a study on one hundred and eighty bovine mandibular permanent incisors, which were divided into six groups, in each group, brackets were bonded with resin-modified glass ionomer cement (RMGIC) after enamel deproteinization with different concentration of papain gel (2%, 4%, 6%, 8% and 10%) respectively. The study showed highest shear bond strength in groups with 8% and 10% papain gel used for enamel deproteinization prior bonding with RMGIC. The bond strength of group with 10 % papain gel showed SBS of 10.3±7.0 Mpa, which differed statistically from other groups ($p<0.05$). Similar results were obtained in the present study, 10 % papain gel showed SBS 11.11 ±4.25 MPa, control group showed SBS 10.23 ± 5.03 MPa. Though not statistically significant from other groups, 10% papain gel showed similar shear bond strength values to the study done by Pithon et al, thereby validating 10% papain gel to be effective composition for superior deproteinizing property.

Matheus M Pithon et al (2016)[8] studied efficiency of different concentrations of 3% and 6% bromelain gel in association with 10% papain gel on enamel deproteinization before bracket bonding on bovine teeth. One hundred and ninety-five bovine incisors were involved in the study which were further divided into thirteen groups. Prior to orthodontic bracket bonding using Transbond XT or RMGIC, enamel deproteinizing agents, either 3% or 6% bromelain gel in combination with 10% papain gel was used. The study concluded that deproteinization with 3% and 6% bromelain plus 10% papain gel significantly increased the shear bond strength when acid etching was performed with 37% phosphoric acid followed by primer application and bonding using Transbond XT compared to RMGIC, therefore Transbond XT was preferred in the present study. Among 3% and 6 % concentrations of bromelain gel, 6% bromelain gel plus 10% papain gel showed highest SBS (28.4±18.2 MPa), followed by 3% bromelain plus 10% papain gel (25.3±11.7MPa). The current study applied bromelain gel and papain gel individually in two different groups since both compounds shown similar properties and an increased SBS was shown in both the groups, papain gel (11.11 ±4.25 MPa), bromelain gel (10.84 ± 4.07 MPa). The difference in SBS in the previously conducted study and the current study may be due the variation in the prism structure of bovine and human teeth.

Ravi M Agarwal et al (2015)(33)suggested papacarie as a new deproteinizing agent, therefore compared it with 10% papain gel, before and after acid etching which might enhance the quality of shear bond strength between enamel surface and composite resin complex. Around one hundred and twenty-five extracted human premolars were used and divided into five groups- control, papacarie before acid etching, papacarie after acid etching, 10% papain gel before acid etching, 10% papain gel after acid etching. The study outcome showed higher SBS in group with both papacarie before acid etching (35.13±7.23MPa) and 10% papain gel before acid etching (36.88±7.96 MPa), than after acid etching or with the control group, which aligns with results of present study

Enhancing the enamel's retentive qualities for optimal adherence was the shared goal of all these studies. Since the marginal sealing at the base of the attachment with the enamel surface has improved, deproteinization is a wise step to include. A potential tactic to maximize adhesion is to utilize a 10% papain and 5% bromelain solution as a deproteinizing agent to eliminate organic components of the acquired pellicle and the enamel structure prior to acid etching.

The process of etching has ever been consistent for giving a reasonably good bond strength by allowing the formation of resin tags in the enamel, with a necessity to overcome the compromised bond strength due to presence of organic element on the enamel surface during bonding. Enamel deproteinizing agent showed a considerable increase in bond strengths. Enamel conditioning agents like NaOCl and papain gel have been under study for their effectiveness. The current study involved the use of papain gel and bromelain gel as enamel deproteinizing agents, which clearly showed higher SBS than control group, but the results of the study are not statistically significant.

This investigation supports the idea that the shear bond strength is increased by deproteinizing using a 10% papain solution prior to acid etching. Matheus M. Pithon et al.'s tests on the efficiency and necessary concentrations of deproteinization using 10% papain gel revealed a clinically significant increase in shear bond strength. For improved bond strength, 10% papain gel can be applied to enamel conditioning before to acid etching. The results reported here are preliminary findings from an in vitro study, however deproteinization with 5% bromelain gel shown a favorable but statistically insignificant increase in shear bond strength when compared to the group without deproteinization.

5. Conclusion

Considering the results of the study, enamel deproteinization using 10 % papain gel showed that there is increase in bond strength compared to 5 % bromelain gel and control group (without enamel deproteinizing). But there was no statistically significant increase in bond strength in all the three groups.

6. Source of Funding

None.

7. Conflict of Indian.

None.

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