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Research Article

Effect of Ficin and Sodium Hypochlorite on Enamel Deproteinization Prior to Orthodontic Brackets Bonding : A Comparative Study

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Abstract

Objective: This study aimed to assess and compare the impact of using ficin and two different concentrations of sodium hypochlorite (NaOCl) (0.5% and 5.25%) as deproteinizing agents on enamel surface before the application of phosphoric acid (H3PO4) etching, with a specific focus on their impact on the shear strength of orthodontic brackets.

Materials and Methods: TA total of 120 freshly extracted human premolars were randomly divided into four groups : group 1 (control) was treated with H3PO4, group 2 was treated with ficin followed by H3PO4, group 3 was treated with 0.5% NaOCl followed by H3PO4, and group 4 was treated with 5,25% NaOCl followed by H3PO4. The orthodontic brackets were then bonded with RELIANCE® LIGHT BOND™, and all samples were subjected to shear bond strength evaluation using the EZ20 universal testing machine at a speed of 2 mm/min. The mode of failure was analyzed using the Adhesive Remnent Index (ARI). Statistical analysis was performed using one-way ANOVA followed by Tukey's Post Hoc multiple comparison test for shear bond strength.

Results: The mean shear resistance of groups 1,2,3 and 4 were 12,21 ± 1,94 MPa ; 18,11 ± 2,14 MPa ; 14,96 ± 2,27 MPa and 15,28 ± 3,13 MPa, respectively.

Conclusions: It can be recommended to add a deproteinization step of the enamel surface prior to acid etching in the overall procedure for bonding orthodontic attachments, using ficin or 0.5% sodium hypochlorite, which have proven to be new allies in orthodontic bonding.

Keywords: Ficin; Sodium Hypochlorite; Deproteinization; Orthodontic Brackets; Shear Bond Strength; Adhesif Remnant Index

Introduction

The initial stage of orthodontic treatment involves the bonding process, which is performed in both conventional treatment, involving the attachment of brackets, buttons, and tubes, and aligner treatment, which includes the bonding of dental attachments. Mastering this clinical step is a key link in the technical chain necessary for reliable orthodontic treatment [1,2].

The bonding strength in orthodontics should be adequate to withstand the forces exerted during treatment and masticatory pressures. However, it should not be excessive to ensure the easy removal of the orthodontic brackets at the end of the treatment.

Bracket debonding during the treatment is one of the most prevalent issues in orthodontic practice. The consequences include increased treatment time with all the impacts that this implies, such as additional material costs, additional visits and increased risk of root resorption and periodontal problems [3-5].

Thus, several techniques have been suggested to enhance orthodontic bonding, and these include the preparation of tooth surfaces using methods such as pumice powder, air abrasion, or LASER [6-8].

Another method that has been widely studied in the literature is to remove organic substances from the enamel surface prior to acid etching in order to increase the shear strength of orthodontic attachments by providing a better acid etching pattern on enamel. The concept of deproteinizing the enamel surface was intially introduced by Justus [9], who utilized 5.25% sodium hypochlorite (NaOCl) for this purpose.

Among substances with similar properties, ficin is remarkable. It is a proteolytic cysteine enzyme extracted from the latex of the fig tree (ficus carica). Traditionally used in the food industry for meat tenderization, milk coagulation and cheese making, it also has modern uses such as the production of bioactive peptides and antibody fragments, in addition to its interesting antibacterial effect.

However, despite all these promising properties, the studies that have focused on using ficin in dentistry are limited to a single study that was previously conducted by us [10]. This has prompted further exploration of this enzyme, including its role in deproteinization and its effects on the adhesive strength of orthodontic brackets, as well as a comparison with other deproteinizing agents.

In this context, our work aims to evaluate and compare the influence of ficin application and 0.5% and 5.25% sodium hypochlorite (NaOCl) as enamel deproteinizing agents before acid etching with 37% orthophosphorique acid on the shear strength of orthodontic brackets.

Materials and Methods

Collection, Preparation and storage of the samples:

One hundred twenty human premolars, freshly extracted for orthodontic reasons, were collected for this study from patients aged 12 to 25 years. Teeth with enamel cracks, fractures, dental pathologies, malformations or restorations, were excluded from the study (Figure 1).

The collected teeth were cleaned under running water immediately after extraction to remove blood and periodontal tissue adhering to their roots, and were subjected to scaling and polishing (Figure 2).

Afterwards, in order to secure the specimens during the tests, all the teeth were mounted in silicone molds using self-polymerizing acrylic resin (MAJOR ORTHO, Italy), keeping the crown exposed (Figure 3).

Figure 1: Study population.

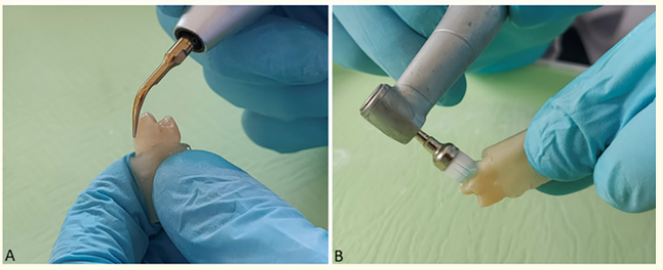

Figure 2: Scaling with an ultrasonic scaler (A) and polishing with a conter angle mounted brush using pumice paste (B).

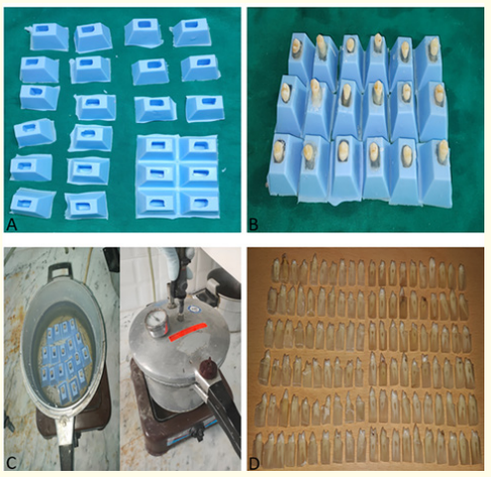

Figure 3: Embedding teeth in acrylic resin blocks.

The specimens were then stored at room temperature in saline solution for several weeks to maintain the characteristics of the tooth enamel surface *in vivo*. The saline solution was changed weekly to prevent the growth of microorganisms.

Extraction of ficin

Ficin was extracted from fresh fig latex according to the following procedure

- Collection of the latex by manually incising the immature fruits' stalks and young stems of the fig tree (Figure 4).
- The latex was diluted with distilled water (1: 0.5) and mixed well.
- Centrifugation was performed at 5000 rpm for 15 min at 4°C to separate the enzyme extract from gums and other debris (Figure 5).
- Recovery of the intermediate layer containing the enzymatic system. The ficine obtained is a light brown, viscous solution with a pronounced fruity smell. The recovery yield of the enzyme system, in the crude state, from the fig tree latex is 70.25% (56.2mL of ficin for 80mL of latex) (Figure 6).
- The ficin solution was Conserved at -20°C until use.

Figure 4: Latex collection.

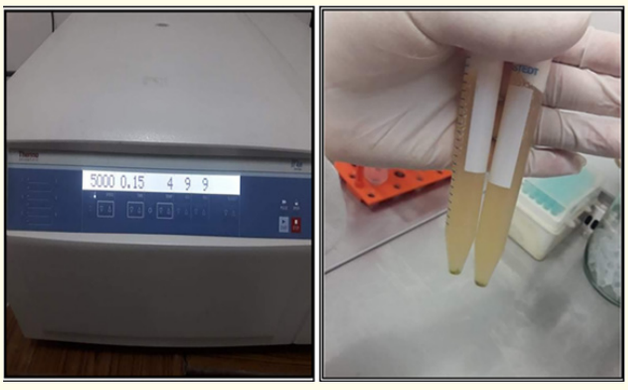

Figure 5: Centrifugation set at 5000 rpm for 15 minutes at 4°C.

Sampling and brackets bonding

Samples were randomly divided into four different enamel preparation groups and treated as follows

- Group 1 (control group) was etched with 37% orthophosphoric acid for 30 seconds. No deproteinization was performed.

Figure 6: The intermediate layer containing the enzymatic system.

- Group 2 was deproteinized with ficin for 30 seconds, washed with water, and air-dried. The treated surface was then etched with 37% orthophosphoric acid for 30 seconds.
- Group 3 was deproteinized with 0.5% sodium hypochlorite for 30 seconds, washed with water and air-dried. The treated surface was then etched with 37% orthophosphoric acid for 30 seconds.
- Group 4 was deproteinized with 5.25% sodium hypochlorite for 30 seconds, washed with water and air-dried. The treated surface was then etched with 37% orthophosphoric acid for 30 seconds.

The ficin, 0.5% and 5.25% sodium hypochlorite were all applied on the vestibular surface of the enamel with an applicator brush using circular friction movement.

Then, orthodontic brackets Thin Arch mini (GAC™) were fixed in the center of the crown using LIGHT BOND™ adhesive resin (RELIANCE®, USA) witch contains fluoride. Subsequently, each bracket was light-cured with an LED appliance (iLed, Guilin Woodpecker, china-2500 mW/cm^2) for 30 seconds.

Once the bonding step was completed, brackets were ligated using a 0.25 mm orthodontic ligature wire, and the specimens were stored in saline solution at room temperature for 48 hours before being subjected for shear bond strength testing (Figure 7).

The shear bond strength (SBS) tests

The shear bond strength tests were conducted using the universal testing machine EZ20. Traction was applied parallel to the tooth surface in ordre to simulate a shear force at a constant rate of 2 mm/min until bracket debonding occured (Figure 8).

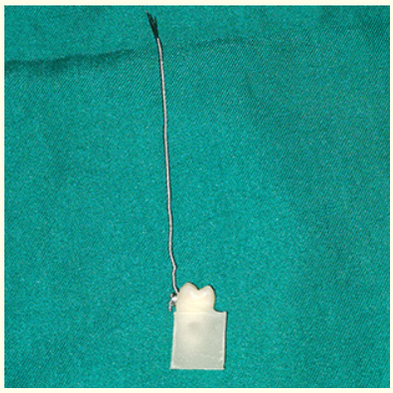

Figure 7: A sample ready for shear testing.

Figure 8: EZ 20 Testing Machine.

The shear load was recorded at the point of failure in Newtons (N) and then converted to megapascals (MPa = N.mm-2) according to the ratio SBS = N/S, where SBS is the shear bond strength (MPa), N is the load at debonding (Newton), and S is the bracket base surface area (10.45 mm2, as specified by the company.

Adhesive Remnant Index (ARI)

The bracket bases and enamel surfaces of all test samples were then examined under a stereomicroscope at 20x magnification to determine the amount of adhesive resin remaining on the enamel surface. This assessment was made in ordre to classify the specimens according to the adhesive remnant index (ARI) and to assess the mode of debonding.

The ARI scores were arranged according to the criteria given by Artun and Bergland [11], with scores ranging from 0 to 3. A score of 0 indicating no adhesive remaining on the tooth surface, 1 indicated less than half of the enamel bonding site was covered with adhesive, 2 indicated more than half of the enamel bonding site was covered with adhesive, and 3 indicated that the entire adhesive remained on the tooth surface.

Statical analysis

The data thus obtained were subjected to statistical analysis using SPSS version 21.0 software for Windows. Descriptive statistics, such as mean, standard deviation, median, minimum, and maximum values of shear bond strength, were calculated for all four groups.Analysis of variance (ANOVA) was applied to determine whether there were significant differences between the groups, followed by Tukey's Post Hoc multiple comparison test. All the tests were performed at a 95% confidence level with a significance level set at 0.05.

Results

The mean shear resistance of groups 1,2,3 and 4 were 12,21 ± 1,94 MPa ; 18,11 ± 2,14 MPa ; 14,96 ± 2,27 MPa and 15,28 ± 3,13 MPa, respectively (Table 1, Figure 9).

	Group 1 "Control" (MPa)	Group 2 "Ficin" (MPa)	Group 3 "NaOCl 0.5%" (MPa)	Group 4 "NaOCl 5.25%" (MPa)
N	30	30	30	30
Minimum	8.42	12.06	8.80	6.98
Maximum	15.31	21.05	18.85	19.52
Median	11.72	18.47	15.31	16.07
Mean	12.21	18.11	14.96	15.28
Standard-deviation	1.94	2.14	2.27	3.13
Variance	3.76	4.58	5.14	9.80

Table 1: Descriptive statistics of the shear force variation for the 4 experimental groups.

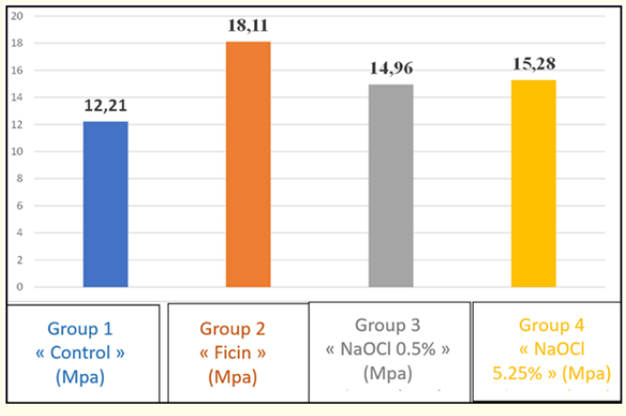

Figure 9: Mean shear resistance of the different groups measured in megapascals.

A statistically significant difference ($P < 0.001$) was observed between the groups when the analysis of variance test was applied. Pairwise comparison of the different groups using Tukey's post hoc test established that the difference was statistically significant ($p < 0.05$), with the exception of group 3 and group 4 (NaOCl groups) where the difference was not significant between them ($p = 0.955 > 0.05$).

Regarding the ARI scores, the groups in which enamel was treated with deproteinizing agents (Groups 2,3 and 4) presented a high frequency of ARI scores 2 and 3. Whereas for the control group (group 1), a high frequency of ARI scores 0 and 1 was observed (Table 2, Figure 10).

	Indice 0	Indice 1	Indice 2	Indice 3
Group 1	8 teeth	13 teeth	5 teeth	4 teeth
Group 2	0 teeth	4 teeth	18 teeth	8 teeth
Group 3	1 tooth	3 teeth	16 teeth	10 teeth
Group 4	0 teeth	4 teeth	17 teeth	9 teeth

Table 2: Distribution of Adhesive Remnant Index (ARI) for each group.

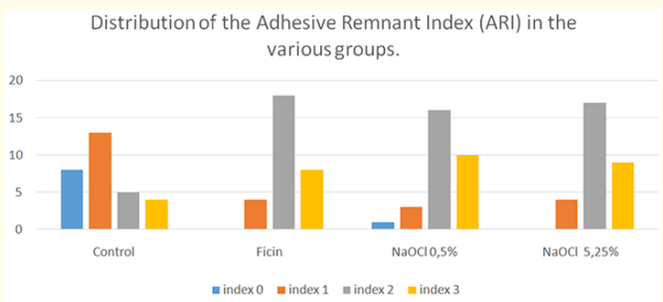

Figure 10: Schematic representation of the Adhesive Remnant Index (ARI) distribution in the various groups.

Discussion

Despite all the advancements in orthodontics, a fundamental problem has not yet been completely solved: the increased risk of enamel decalcification around orthodontic brackets. This phenomenon, often associated with poor oral hygiene habits, leads to the formation of white spots and marginal gingivitis adjacent to orthodontic brackets [12,13].

In order to minimize and prevent the occurrence of these lesions, the clinical approach with the greatest potential for efficacy, as described in the literature, is bonding brackets with fluoride-releasing materials. Among all the materials available on the market, resin-modified glass ionomer cements and fluoride-releasing composite resins are noteworthy [14-17]. However, it's important to note that these materials have a lower bond strength to the enamel surface compared to conventional composite resins [18].

In order to combine the important biological characteristics of these adhesives with good enamel adhesion, a new method of enamel surface conditioning had to be developed to increase the shear bond strength. This method consists of eliminating the influence of the acquired pellicle that persists after prophylaxis of the tooth surface, on one hand, and the organic matrix forming the enamel surface, on the other hand, on composite adhesion to the enamel surface.

Justus [9] was the first to suggest the use of 5.25% NaOCl for 60 s as a surface deproteinizing agent prior to acid etching. This approach was based from techniques successfully used in endodontics, where sodium hypochlorite is used to disinfect and remove debris and organic material from root canals. The results obtained showed a clear improvement in adhesive strength and shear strength for the group subjected to NaOCl deprteinization.

Consistent with these results, our study revealed that the mean shear bond strength in the NaOCl groups (groups 3 and 4) was 14.96 MPa and 15.28 MPa, respectively. These values were notably higher and statistically significant compared to the control group (group 1), which exhibited an average shear bond strength of 12.21 MPa.

However, sodium hypochlorite is a strong oxidizing agent that can cause undesirable reactions on oral soft tissues if not used with caution, especially in young, uncooperative children, in addition to its unpleasant chlorine odor andbad taste [19]. These multiple drawbacks have led to the search for an alternative material.

In the environment, there are various substances that have considerable deproteinization characteristics, other than sodium hypochlorite. Among these, papain and bromelain can be mentioned, which are alkaloid enzymes extracted respectively from papaya latex (carica papaya) and pineapple stem latex (ananas comosus). These enzymes have been the subject of numerous previous studies, that have tested their deproteinizing power and their effects on the retention of orthodontic brackets, showing good results [20-24]. Another enzyme belonging to the family of cysteine-proteases, whose properties are similar to those of papain and bromelain, is called ficin (or ficain), it is a plant-derived proteolytic enzyme extracted from the latex of the fig tree.

The results of this study indicate that the group deproteinized with ficin had the highest shear bond strength, with a statistically significant difference compared to the groups deproteinized with

sodium hypochlorite. This demonstrates that ficin is a more potent and effective deproteinizing agent than sodium hypochlorite in terms of improving adhesive power and retention of orthodontic brackets.

In literature, different enzymes from the same family have been evaluated. Pithon [25] tested the effect of 10% papain gel on enamel deproteinization before the bonding procedure. The results obtained indicate that the group subjected to papain deproteinization, followed by phosphoric acid etching and bonding with Transbond XT, achieved the highest shear bond strength compared to the group in which Transbond XT was used without prior deproteinization of the enamel surface.

Another very interesting result found in our study is related to groups 3 and 4, deproteinized with 0.5% and 5.25% sodium hypochlorite. These groups showed no statistical difference between them and obtained better shear bond strength results compared to the control group.

In the literature, no previous study has compared sodium hypochlorite at these two concentrations, 0.5% and 5.25%. Most of the existing studies have compared NaOCl at 5% and 10%.

Therefore, considering its toxicity, especially at high concentrations, and the fact that there was no significant difference in terms of improving the retention of orthodontic attachments when its concentration varied, it is recommended to use 0.5% sodium hypochlorite as a surface deproteinizing agent to minimize potential adverse effects.

Regarding the adhesive remnant index (ARI), the control group exhibited a statistically significant difference when compared to the groups treated with deproteinizing agents (groups 2, 3, and 4). However, there was no significant difference between the latter groups. In the control group, most specimens had scores of 0 and 1, indicating that little adhesive remained on the enamel after bracket debonding. In the test groups (groups 2, 3, and 4), most specimens had a score of 2 or 3, indicating that more adhesive remained on the enamel when deproteinizing agents were used. These results are in agreement with the studies by Justus., *et al*. [9]. and Pithon., *et al*. [24].

These results can be considered favorable as lower IRA scores (score 0 and 1) indicate that the mode of failure is closer to the enamel/adhesive interface, which increases the risk of enamel fracture during debonding of attachments. Additionally, this type of failure indicates poor bonding between the enamel and the bonding material, which means that bracket debonding is likely to occur more frequently during treatment, disrupting chair time and therefore prolonging the overall duration of orthodontic treatment.

On the other hand, higher IRA scores (scores 2 and 3) can be attributed to better adhesion of the composite resin to the surface of the deproteinized enamel. The more adhesive remains on the tooth surface, the safer the debonding is, as the location of the fracture shifts towards the adhesive/bracket interface, thus preventing excessive force transfer to the enamel and avoiding any damage to its surface [23,26].

Conclusion

At the end of our experimentation, it can be recommended to add a deproteinization step of the enamel surface prior to acid etching in the overall procedure for bonding orthodontic attachments, using ficin or 0.5% sodium hypochlorite, which have proven to be new allies in orthodontic bonding.

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