ABSTRACT

Background: Enamel demineralization is an event which is always an integral part of fixed orthodontic treatment due to which fluoride releasing bonding agents are considered to be the most effective but have lower bond strength. Thus, this in vitro study has compared the degree of demineralization and bond strength of conventional and fluoridated bonding agents.

Materials and Methods: One hundred and five extracted human premolars divided into Group I evaluated to study demineralization and Group II to evaluate bond strength. Group I was subgrouped into (A, B, C, and D and Group II was subgrouped into A, B, and C (n = 15 in each subgroup). All samples were bonded with metal brackets using Transbond Plus™, Discover LC orthodontic adhesive™, and Transbond XT™; the first two being fluoride releasing and the third being a conventional bonding composite. Group I samples were followed by sectioning and studied for mean depth of demineralization at the margins of the brackets using polarized microscopy. Group II samples were evaluated only for shear bond strength. Statistical analysis was done using ANOVA and Tukey’s multiple comparison tests.

Observation and Results: The mean depth of enamel demineralization and standard deviation was compared between subgroups A and C and B and C and the P value obtained was 0.02 in each group, suggestive of a considerably lesser degree of demineralization in fluoride releasing composites compared to conventional composite. Similarly, when shear bond strength was compared between subgroups A and C and B and C the P value obtained was 0.04 and 0.00, respectively. Thus, the shear bond strength of the fluoride releasing composites was lesser than that of the conventional composite but well within the clinically acceptable range.

Conclusion: Fluoride releasing composites can be used to avoid demineralization around the brackets.

Key words: Demineralization, fluoride releasing bonding agents, in vitro, polarizing microscopy

INTRODUCTION

Bonding of orthodontic attachments has been the universal norm for bracket placement since the 1970s.[1-2] An ideal bonding system should successfully attach orthodontic appliances to teeth, allow time for accurate placement, withstand a load of masticatory forces, have biocompatibility, and prevent degradation of tissues in the oral cavity. Contemporary systems available to clinicians possess most of these requirements.

Placing a fixed orthodontic appliance in the mouth for a long period favors the growth of dental plaque, makes oral hygiene very difficult, restricts the salivary self-cleaning properties, and so creates an environment that favors the onset of caries.[3-4]

This suggests the need for a preventive program to reduce the incidence of peri-bracket demineralization. The chief...
Demineralization with fluoride releasing and conventional bonding agents

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motivation for patients to undergo orthodontic treatment is the perfect smile and improved esthetics it promises, but these demineralized zones of enamel often undermine treatment results that are otherwise excellent. The vast research being undertaken with goals to eliminate these demineralized/white spots signifies the magnitude of the issue and the concern it generates in the orthodontic profession.

In a study by Borzabadi-Farahani et al. in 2014, nanoparticles have been used to prevent microbial adhesion and enamel demineralization in orthodontic therapy. The previous studies investigated the antimicrobial or physical characteristic over a short time span, i.e., 24 h to a few weeks. Long-term studies are lacking.\(^5\)

Fluoride was introduced into dentistry over 70 years ago and is now recognized as the prime factor responsible for the dramatic decline in caries prevalence that has been observed worldwide.\(^6\) Fluoride reduces caries by helping to prevent demineralization and by remineralizing early carious lesions. In the 1980s, it was established that fluoride controls caries mainly through its topical effect. The benefits of fluoride in inhibition of carious lesion development and enhancement of remineralization of white spot lesions are well-documented. The previous studies proved the effectiveness of fluoride regimens such as fluoride varnish, gel, rinse, and dentifrice.\(^7\) These methods rely on patient compliance and provide only limited benefit in preventing demineralization.

Vahid-Dastjerdi et al. in 2012 compared the in vitro weekly cumulative fluoride release (WCFR) of three Glass Ionomer Cements (GICs) Granitech, Bandtite, and Ariadent used for orthodontic bonding. Specimens were immersed in 5 ml of deionized water, and the WCFR was measured at weekly intervals, on days 1, 8, 15, 22, and 29, using the potentiometry device and single junction saturated calomel electrode technique. The study concluded that Bandtite followed by Granitech showed higher WCFR compared to Ariadent.\(^9\)

Chambers et al. in 2015 conducted a study and concluded that coatings could be applied to brackets and wires, which prevent bacterial adhesion to prevent demineralization of tooth enamel and formation of white spot lesion.\(^10\)

To overcome these drawbacks, there have been many innovations in providing topical fluoride reservoirs. Examples of these are fluoride incorporated in bonding cements, composites, and certain fluoride releasing brackets and modules.\(^11\) Among these, bonding adhesives are located at the center of labial enamel and hence an obvious choice as fluoride reservoir. Fluoride-releasing ability can therefore be perceived as a desirable property of an orthodontic bonding agent. Researchers have tried to convert the bonding resin into a reservoir for fluoride which can provide protection against demineralization.\(^12-17\) However, the incorporation of fluoride in the bonding composite should not compromise its primary function of providing adequate shear bond strength that is below the accepted threshold in clinical practice.\(^18,19\) Orthodontic adhesives that contain fluoride-releasing agents are now available but are relatively new. Very few commercially available orthodontic bonding composites contain fluoride and are hence not widely used.

Therefore, there is a need to determine their efficacy in reducing demineralization and also to ascertain that their bond strength is sufficient to meet clinical needs. There is a paucity of studies investigating the effect of fluoride releasing bonding agents.

The present study was designed considering all the above-mentioned criteria of importance in clinical practice. The study not only evaluated the depth of demineralization around brackets in fluoride releasing and nonfluoride releasing groups but also evaluated the shear bond strength of the two.

**MATERIALS AND METHODS**

Patients attending Oral Surgery Department (OPD) of D. Y. Patil, School of Dentistry, Nerul, Navi Mumbai, indicated for extractions of premolars were randomly included in the study. The discarded extracted teeth were collected and preserved in fixative. This study does not contain any studies with human participants or animals. In addition, patient’s identity was not revealed. Therefore, ethical approval was not considered and taken.

The samples for the present study consisted of 105 healthy premolars of patients undergoing extraction for orthodontic treatment. Teeth affected by caries, fluorosis, stains, and deformity, etc., were excluded from the study.

The teeth were cleaned and stored in normal saline. These were divided into two groups, Group I (n = 60) and Group II (n = 45), to study the demineralization of enamel around brackets and to compare the bond strength of the bonding adhesives, respectively.

The bonding adhesives used were 2 fluoride releasing bonding adhesives, Transbond Plus™, Discover light cure orthodontic adhesive™, and a conventional bonding adhesive Transbond XT™ designated as adhesives X, Y, and Z, respectively.

Group I was subdivided into subgroups A, B, C, and D (n = 15 in each subgroup). Group II was subdivided into subgroups A, B, and C (n = 15 in each subgroup).
All subgroups A, B, and C of both Groups I and II were bonded with adhesives X, Y, and Z, respectively. Stainless steel, PEA premolar brackets manufactured by Modern Orthodontics®, having uniform base morphology and base surface area of 9.3 mm² was used for bonding all samples. Bonding procedure remained same for all samples, but the material used was different according to the subgroups they belonged to.

Group I Subgroup D was only etched with 37% phosphoric acid for 30 s and served as a control having baseline demineralization [Consort Diagram 1].

Evaluation of demineralization under polarization microscope (Group I)

Twenty-four h after bonding, the samples of Subgroup A, B, and C were placed in an acidic medium in individual bottles to produce artificial carious lesions. The acidic medium used was a solution of methylcellulose buffered with acetic acid to a pH of 4.5, the critical pH for hydroxyapatite (HA) dissolution. After 21 days of immersion, the samples were rinsed and dried in increasing concentration of alcohol (50%, 70%, 80%, and 90%).

The samples were debonded and demineralization was identified clinically as chalky white areas. No clinical grading of white spot lesion was performed.

All these samples of subgroups A, B, and C along with the acid etched samples of Subgroup D were mounted in acrylic resin for sectioning [Figure 1]. The teeth were sectioned using a mechanized hard tissue microtome (LEICA SP 1600) [Figures 2 and 3] to obtain buccolingual longitudinal midsections of 100 micron thickness at “Shriram Chandra Dental College and Hospital, Chennai.”

Microscopic examination was done in the Department of Oral Pathology and Microbiology of the same institute. The sections were oriented and fixed longitudinally on glass slides and observed under a polarized microscope by two observers using image analysis software (Leica application suite, LES core version 3.8) of Leica research microscope (Model No. DM1000 LED, Leica Microsystems GmbH Ernst-Leitz-Straße 17-37 | 35578 Wetzlar (Germany)) [Figures 4-6].

Evaluation of shear bond strength (Group II)

Bonded samples were mounted on acrylic blocks and were tested only for the shear bond strength of bonding materials used in each subgroup by using Instron Universal testing machine with a load cell of 50 KN and a crosshead speed of 3 mm/min as per procedure given by Cristina Rastelli et al.[21] They were not treated with acidic medium and therefore not examined for demineralization under a polarized microscope. Debonding force for each sample
Observation and results

Statistical analysis was carried out, and the readings were tabulated for each of the groups, and the mean and standard deviations were calculated for the following:

a. Depth of demineralization in micrometer
b. Force in Mega Pascal required for the bond failure

The observations showed that the highest mean depth of demineralization in Subgroup C-184.15 µm followed by Subgroup D-118.94 µm, followed by Subgroup A-110.60 µm, and the least mean depth was noted in Subgroup B-109.10 µm (Table 1).

| Subgroup  | Mean (µm) | SD (µm) |
|-----------|-----------|---------|
| Subgroup A| 110.60    | 27.39   |
| Subgroup B| 109.10    | 60.36   |
| Subgroup C| 184.15    | 104.83  |
| Subgroup D| 118.94    | 43.84   |

SD=Standard deviation

When the depth of demineralization compared between Subgroups B and C, the mean and standard deviation of Subgroup B were 109.10 and 60.36, respectively. The mean and standard deviation of Subgroup C were 184.94 and 104.83, respectively. On comparison of mean and standard deviation between Subgroup B and Subgroup C, P value was found to be 0.02. Since P value for the Subgroup B and Subgroup C is less than that of 0.05 indicates a significant difference between Subgroup B and Subgroup C (Table 2).

The highest mean shear bond strength was found in Subgroup C 14.99 Mpa followed by Subgroup A 11.88 Mpa. Subgroup B was found to have least shear bond strength in this study with a mean of 7.68 Mpa (Table 4).

The mean and standard deviation of bond strength of Subgroup A were 11.88 and 4.37, respectively. The mean
and standard deviation of Subgroup C is 14.99 and 3.44, respectively. On comparison of mean and standard deviation between subgroup A and Subgroup C, \( P \) value was found to be 0.04. Since \( P \) value for the Subgroup A and Subgroup C is less than that of 0.05, it indicates significant difference between Subgroup A and Subgroup C [Table 5].

The mean and standard deviation of bond strength of Subgroup B were 7.68 and 2.06, respectively. The mean and standard deviation of Subgroup C were 14.99 and 3.44, respectively. On comparison of mean and standard deviation between Subgroup B and Subgroup C, \( P \) value was found to be 0.00. Since \( P \) value for the Subgroup B and Subgroup C is less than that of 0.05, it indicates a significant difference between Subgroup B and Subgroup C [Table 6].

**DISCUSSION**

The prevalence of White spot lesions around the bonded brackets ranges between 2% and 96% and remain an adverse effect accompanying orthodontic treatment; something that has not been avoidable in clinical practice until now.\(^4\)

Several causes have been attributed to the occurrence of demineralization around brackets.\(^2\) (1) Orthodontic attachments make tooth cleaning difficult and predisposes to plaque accumulation on the tooth surface around the attachment. (2) There is a significant influence of diet on demineralization during orthodontic treatment, particularly the frequency of carbohydrate consumption. (3) The key feature is a dietary carbohydrate-induced enrichment of the plaque microorganisms such as mutans streptococci and lactobacilli which cause a decrease in the pH value. (4) Demineralization occurs predominantly at sites in the dentition characterized by diminished salivary effects. A good example of this effect is the observation that during fixed orthodontic therapy, the site with the highest incidence of demineralization is the maxillary anterior teeth.

Various researchers have tried numerous methods to control demineralization such as fluoride therapy, fluoride-releasing sealer, and laser therapy.\(^2\)

![Figure 5: Demineralization zone of enamel observed under polarizing light microscope (area marked by arrow)](image)

![Figure 6: Measurement of demineralization](image)

**Table 2:** Comparison of mean demineralization depths and standard deviation between Group I subgroups A and C (µm)

| Subgroup   | Mean (µm) | SD (µm) | \( P \) |
|------------|-----------|---------|--------|
| Subgroup A | 110.60    | 27.39   | 0.02   |
| Subgroup C | 184.15    | 104.83  |        |

\( SD \) = Standard deviation

**Table 3:** Comparison of mean demineralization depths and standard deviation between Group I subgroups B and C (µm)

| Subgroup   | Mean (µm) | SD (µm) | \( P \) |
|------------|-----------|---------|--------|
| Subgroup B | 109.10    | 60.36   | 0.02   |
| Subgroup C | 184.94    | 104.83  |        |

\( SD \) = Standard deviation

**Table 4:** Mean and standard deviation of the shear bond strength in Group II (Mpa)

| Subgroup   | Mean (Mpa) | SD (Mpa) |
|------------|------------|----------|
| Subgroup A | 11.88      | 4.37     |
| Subgroup B | 7.68       | 2.06     |
| Subgroup C | 14.99      | 3.44     |

\( SD \) = Standard deviation

**Table 5:** Comparison of mean and standard deviation of shear bond strength between Group II subgroups A and C (Mpa)

| Subgroup   | Mean (Mpa) | SD (Mpa) | \( P \) |
|------------|------------|----------|--------|
| Subgroup A | 11.88      | 4.37     | 0.04   |
| Subgroup C | 14.99      | 3.44     |        |

\( SD \) = Standard deviation

**Table 6:** Comparison of mean and standard deviation of shear bond strength between Group II subgroups B and C (Mpa)

| Subgroup   | Mean (Mpa) | SD (Mpa) | \( P \) |
|------------|------------|----------|--------|
| Subgroup B | 7.68       | 2.06     | 0.00   |
| Subgroup C | 14.99      | 3.44     |        |

\( SD \) = Standard deviation

The studies conducted by Cury and Tenuta\(^2\) and Arnold\(^2\) concluded that mineral loss (demineralization) or gain (remineralization) at enamel surface is a dynamic physicochemical process occurring when oral bacteria form a biofilm on the enamel surface and this biofilm is exposed to fermentable dietary carbohydrates and a critically low pH for tooth dissolution is maintained for a certain time. However, if any fluoride is present in the biofilm fluid,
and the pH is not lower than 4.5; HA is dissolved at the same time as fluorapatite (FA) is formed. The net result is a decrease in enamel dissolution. This mineral gain as FA during the pH drop has not been termed as remineralization but rather as a decrease in demineralization because the mineral redeposited is different from that lost. This is an indirect effect of fluoride reducing enamel demineralization at an acidic pH not below 4.5.

The current study tried to approximate in vivo environmental acidic conditions as far as possible. Extracted human teeth were used to increase the accuracy of the study. The sample size of 15 per subgroup was deemed adequate when compared to other similar studies. The samples were immersed in methylcellulose solution buffered with acetic acid to a pH 4.5 which is below the critical Ph of HA to ensure sufficient measurable demineralization. The time frame of 21 days chosen for immersion of samples was considered sufficient when compared to immersion time in many other studies. Each sample was separately immersed in individual bottles to increase the accuracy of the study. Furthermore, this study had a control group (Group I Subgroup D) in which analysis of enamel demineralization caused by etching of enamel was also considered as a baseline. Further demineralization under acidic medium was studied in samples of Group I Subgroups A, B, and C. The etching demineralization was common to all four subgroups.

This study was able to contrast demineralization consequent to acidic immersion with baseline demineralization because of acid etching common to all bonding procedures and thereby demineralization that was additional to etching process was put in perspective, and so was the effect of fluoride releasing composite.

The polarizing light microscopy chosen to observe the depth of demineralization is considered a superior tool for precise quantitative measurements of enamel sections than stereomicroscopy used in several similar studies cited elsewhere.

This study not only measured demineralization using fluoride-releasing and conventional composites but also compared their shear bond strengths. There are many studies that have compared one or the other parameter but not both. On this account, the scope of the present study was more exhaustive.

The results of this study show that in Group I the mean demineralization depths of Subgroups A (110.60 µm) and B (109.10 µm) that used fluoride releasing composites are significantly lesser compared to the mean demineralization depth of Subgroup C (184.15 µm) that used conventional composites and marginally lesser compared to that of Subgroup D (118.94 µm). The study also shows that enamel gained/saved in samples of Group I subgroups A and B was approximately 7–8 µm as compared to samples of Subgroup D. It could be summarized that in these two fluoride releasing subgroups, there has been some amount of FA remineralization of acid etched enamel when immersed in acidic solution at pH 4.5. This critical pH of 4.5 is readily achieved with sugary foods and beverages several times a day. In this study, even though immersion was complete and constant for 3 weeks, justifiable comparison with in vivo conditions can still be made as the FA gained is resistant to further demineralization at this pH.

According to the results of this study, in Group II, the mean bond strengths of Subgroups A (11.88 Mpa) and B (7.68 Mpa) that used fluoride releasing composites are lesser compared to the mean bond strength of Subgroup C (14.99 Mpa) but well within clinically acceptable limits. The comparison of the bond strength is very relevant since a fluoride releasing composite with clinically unacceptable bond strengths cannot be used as a bonding adhesive.

The results and analysis of the current study shows that there is decreased enamel demineralization when fluoride releasing composites are used compared to conventional composites and fluoride releasing composites have marginally lesser shear bond strength but within clinically acceptable bond strength limits.

This study indicates that fluoride releasing composites might be a good choice for orthodontic bonding since samples with fluoride releasing bonding composites exhibit fewer degree of demineralization compared to conventional bonding composite. These may also have some capacity to remineralize enamel that has been demineralized after acid etching with phosphoric acid. The fluoride releasing composites also exhibit adequate shear bond strength, hence making these good options for orthodontic bonding.

**CONCLUSION**

Based on the results of this in vitro study, it can be concluded that Transbond Plus™ and Discover light cure orthodontic adhesive™ exhibit significant inhibition of demineralization compared to the conventional bonding agents. This quality makes either bonding agent a potential improvement for use as an adjunctive preventive dentistry measure with shear bond strength even though low but within clinically acceptable limits.

Future clinical (in vivo) experiments are essential to satisfactorily and comprehensively evaluate fluoridated bonding agents for their remineralizing properties, as well as the adequacy of their bond strength.

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Conflicts of interest
There are no conflicts of interest.

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