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Efficacy of Orthodontic Bonding Agents in Preventing Demineralization Around Brackets

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Dentistry at Virginia Commonwealth University.

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Abstract

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By Morris Lewis Poole, D.D.S.

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Enamel demineralization is a concern in orthodontic patients with poor oral hygiene. To curtail this problem, amorphous calcium phosphate (ACP) containing adhesives have been developed. The purpose of this *in vitro* study was to evaluate the cariostatic potential of an ACP containing orthodontic bonding agent adjacent to brackets.

Sixty human molars were randomly distributed into: ACP adhesive, resin modified glass ionomer cement (RMGIC), and conventional composite resin groups (N=20 each). Brackets were bonded following the manufacturer's instructions. Tooth enamel through a 2mm window around the brackets was cycled in demineralization (6 hrs) and remineralization (18 hrs) solutions. After 14 days, teeth embedded in resin and were sectioned. Knoop indentations were performed to determine enamel hardness.

There were no statistically significant differences between the control and experimental groups. However, both Fuji Ortho LC (RMGIC), and Aegis Ortho (ACP)

showed a trend toward a reduction in demineralization. In addition, it was also shown that the initial acid etching of the enamel significantly reduces enamel hardness.

Introduction

The advantages of fixed orthodontic therapy include improvements in esthetics, oral function, health, and social well being.[1,2] However, enamel decalcification, usually in the form of white spot lesions (WSLs), may be a concern concurrent with orthodontic treatment if the oral hygiene is poor.[3] The formation of these enamel lesions may compromise the esthetics and in severe cases may necessitate extensive restorative dental treatment.

Dental enamel is composed primarily of hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$, but it also contains several impurities such as carbonate and fluoride. The proportions of these impurities vary from person to person, and from tooth to tooth. Enamel solubility is not fixed and shows a slight variation due to these impurities.[4]

When HA is in contact with water, the following reaction occurs:

$$\begin{array}{rcl} \text{Precipitation} & \leftrightarrow & \text{Dissolution} \\ Ca_{10}(PO_4)_6(OH)_2 & \leftrightarrow & 10Ca^{2+} + 6PO_4^{3-} + 2OH^- \\ \text{Solid} & \leftrightarrow & \text{Solution} \end{array}$$

Upon a small amount of HA dissolution; calcium, phosphate, and hydroxyl ions are released. This process continues until the water is saturated with respect to HA or until the pH rises. At that point, the rate of the forward reaction (mineral dissolution) is equal to the rate of the reverse reaction (mineral precipitation).[5]

Acidogenic bacteria, mainly *Steptococcus mutans* and lactobacilli, are the main pathogens in the caries process as they produce acids while metabolizing fermentable carbohydrates. The oral pH decreases as hydrogen ions are released, thus the acids

diffuse through the plaque into the enamel. Consequently, the enamel solubility reaches the critical pH beginning around 5.5 and demineralization occurs on the enamel surface. As the acidic pH decreases the hydroxyapatite lattice becomes more soluble and the ions leach out.[6] The diffusion of calcium and phosphate ions out of enamel produces a chalky white spot lesion or opacity, and may progress to cavitations if demineralization continues.[7]

Due to difficulty in cleaning around the brackets and auxiliary attachments, orthodontic patients may develop significantly more WSLs than non-orthodontic patients if the proper oral hygiene measures are not carried out.[8,9] In addition to plaque retention on the surfaces usually not susceptible to caries, the formation of a thicker biofilm in the presence of fixed appliances provides a protective shield for the acidogenic bacteria. Increased levels of *S. mutans* and lactobacilli after the bonding of orthodontic appliances have been reported in previous studies.[10] Since plaque accumulates around the brackets, these lesions typically form adjacent to brackets usually between the gingiva and bracket on the buccal surfaces of teeth.[7,11,12] Although it usually takes 6 months for the caries to develop in non-orthodontic patients, WSL can become noticeable around the brackets within 1 month of the bracket placement in orthodontic patients.[13]

Prevention of demineralization begins with patient education, oral hygiene instruction, and regular professional oral hygiene visits. When patients understand the detrimental effects of enamel demineralization along with periodic maintenance, they may focus more attention on dental care. Proper tooth brushing technique and periodic reinforcement is reported to reduce plaque accumulation.[14] For the prevention of WSLs,

the everyday dental care is crucial in maintaining acceptable levels of oral hygiene. However, it has been reported that patient cooperation, for example with fluoride daily rinsing, was less than 15% in orthodontic patients.[15]

In order to reduce the need for patient compliance, fluoride releasing composite resins and resin modified glass ionomer cements (RMGICs) with fluoride releasing ability have been developed for bonding brackets.[15-17] Since fluoride releasing glass ionomer cements (GICs) have a potential to minimize demineralization and lessen the need for patient compliance, they have been used in orthodontics as a luting agent for cementing bands. However, studies have shown that the amount of fluoride released decreases to undetectable levels within a few days.[17] Even though GICs can be recharged with the use of a fluoride containing dentifrice or topical fluoride agent, they are shown to exhibit a wide range of rechargeability and anticariogenic ability.[18-20] Therefore, it would be more beneficial for orthodontic patients if these adhesives could sustain a more continuous and long-term fluoride release.[20] Nevertheless, GICs have been shown to reduce demineralization around orthodontic appliances.[21] Despite offering advantages such as eliminating the need for a dry working field and enamel surface preparation with acid etching as well as fluoride release, these adhesives are not appropriate for direct bracket bonding due to their relatively low bond strength.[22-24]

Since fluoride release from an orthodontic bonding agent would be beneficial in decreasing demineralization adjacent to the brackets, hybrid glass ionomer cements or resin modified glass ionomer cements (RMGICs) have been developed for bracket bonding in orthodontics by combining the bond strength of composite resin with the

fluoride releasing ability of glass ionomer cement.[25] Studies on RMGICs have shown that these adhesives are able to release fluoride while providing adequate bond strength to withstand orthodontic forces. It should be noted that their bond strength values, although within the required range of 5-7 MPa, are substantially lower compared to those of conventional resins.[26-28]

Calcium and phosphate are the critical ions in the remineralization process and are present in saliva. Since dissolution and precipitation of these minerals depend on the pH and the concentrations of ions in saliva, amorphous calcium phosphate (ACP) is thought to play an important role in preventing demineralization and promoting remineralization. Therefore, it has properties of both a preventive and restorative material.[29] ACP is one of the most reactive and soluble calcium phosphate bio-available compounds.[30] Once it gets incorporated into plaque, ACP serves as an ion reservoir in the saliva by rapidly releasing supersaturating levels of calcium and phosphate ions in proportions favorable for the solution formation of hydroxyapatite.[31] Therefore, ACP may act as an enhanced delivery system of necessary ions to significantly prevent demineralization.[32] Despite a cariostatic potential, there is a concern regarding the use of ACP containing orthodontic adhesives as it has been shown that this product does not have as strong a bond strength as resin based composites when used as a lingual retainer adhesive.[33] However, a recent research study has shown the bond strength of an ACP orthodontic cement to be within the acceptable range for orthodontic appliances.[34]

The cariostatic property of a RMGIC, Fuji Ortho LC (GC America, Alsip, IL), around the brackets has been previously reported.[35-37] It became a standard practice to use this adhesive as a control in demineralization studies.

In the literature, there are only a few studies on the cariostatic potential of the ACP containing adhesives.[29,32,38] In addition, the techniques used in determining the presence of WSLs are controversial.[39-41] A recent study by Uysal et al.[32] reported that ACP containing composite resin was as effective as Fuji Ortho LC in preventing demineralization. However, in their study DIAGNOdent was used as the method of WSL detection. Unfortunately, this instrument has received mixed reviews on its ability for determining demineralization.[39,40]

It is probably due to the lack of standardized clinical examination methods that the reported prevalence of WSLs in orthodontically treated patients exhibits such a wide range.[7,42] Gorelick et al.[41] reported that 50% of the patients had at least one white spot lesion at the end of orthodontic treatment. However, a recent study using visual examination of pictures before and after orthodontic treatment reported demineralization of maxillary anterior teeth to be roughly 77%.[43] In another study, the number of patients having WSLs was reported as 97%.[42]

In the literature, visual inspection has initially been the principal method of examination to detect demineralization. However, this technique is subjective and the criteria may vary greatly from study to study. Other methods have included comparison of photos or slides, quantitative light-induced fluorescence (QLF), polarized light microscopy, microradiographs, or microhardness testing.

To study demineralization *in vivo*, QLF has been successfully used since this method offers a more objective approach to detect caries. This technique uses light fluorescence to determine enamel decalcification and is able to show a closer correlation with mineral content.[44] Pretty et al.[45] were able to demonstrate that QLF successfully detected subclinical lesions as well as monitored remineralization and demineralization. QLF works by light illuminating the tooth surface and, with the help of special filters a digital fluorescent image, is captured by a camera. The image is then transferred to a computer and displayed on a monitor where the carious lesions have a darker appearance compared to sound enamel. Demineralization or mineral loss from caries is detected and measured as a decrease in fluorescence. QLF has been demonstrated to be a worthy instrument in the detection of white spot lesions. However, taking into consideration the time and equipment needed for such an analysis is extensive and expensive. Furthermore with QLF, selection of the tooth area on the images can be difficult, and the shape can vary depending on the border selection tool used.[46]

Recently, the DIAGNOdent has been used widely both *in vivo* and *in vitro* for detecting enamel demineralization. This portable hand held instrument also uses fluorescence to distinguish between the carious and sound tooth structure. Although it has been previously reported that its detection methods were sensitive for early demineralization and caries, recent studies concluded that the readings were more likely to be because of bacteria and not actual demineralization.[39,47,48] Also, Frentzen et al.[40] showed significant differences in readings with DIAGNOdent after the polishing and

calibration procedures. In light of these studies, it was suggested that this instrument should be used as an adjunct to conventional methods in detecting caries.[49,50]

Since enamel hardness is thought to be affected by its mineral content, the microhardness test is widely used in in-vitro studies to investigate enamel demineralization.[51,52] Previous studies have shown that microhardness is a reliable technique because it is simple, quantitative, and reproducible.[51-54] Featherstone reported a direct relationship between the hardness values and mineral content of the enamel.[54] Kielbassa et al.[55] also determined a reliable correlation between microradiographic and microhardness data, strengthening the validity behind microhardness testing.

The purpose of this in-vitro study was to determine the cariostatic potential of an amorphous calcium phosphate containing adhesive and a resin modified glass ionomer cement using the microhardness test.

Materials and Methods

In this study, extracted human molars were collected from the Virginia Commonwealth University Oral and Maxillofacial Surgery Clinic and stored in 10% formalin at 25°C until prepared. Sixty, defect free teeth were randomly distributed into the following three groups (N = 20): 1) control (Transbond XT, 3M Unitek, Monrovia, CA), 2) resin modified glass ionomer cement (Fuji Ortho LC, GC America Inc, Alsip, IL), and 3) amorphous calcium phosphate containing adhesive (Aegis Ortho, Bosworth Co, Skokie, IL).

Table I: Bonding agents tested

Group		n	Etchant	Primer	Adhesive
1	Transbond	20	Etching Gel	Transbond XT Primer	Transbond XT
2	Fuji Ortho	20	Ortho Conditioner	-	Fuji Ortho LC
3	Aegis Ortho	20	Acid Etch	Aqua Bond	Aegis Ortho

All of the bonding procedures were carried out by the same clinician. Prior to bonding, each tooth surface was polished with a non-fluoridated flower of pumice for 10 seconds. Subsequently, teeth were rinsed and dried with oil and moisture free air for another 10 seconds. Orthodontic metal brackets (Victory Series, 3M Unitek, Monrovia, CA) were bonded on the tooth surfaces following each of the manufacturer's instructions (Table I).

Teeth in the control group were etched with a conventional 35% phosphoric acid (Etching Gel, 3M Unitek, Monrovia, CA) for 15 seconds followed by rinsing and drying

with air thoroughly. After enamel surface preparation, a thin uniform coat of Transbond XT Primer (3M Unitek, Monrovia, CA) was applied to the enamel surface. A small amount of Transbond XT adhesive was then applied to the bracket base, and the bracket was placed onto the tooth surface. After the removal of adhesive flash, an Ortholux XT light curing unit (3M Unitek, Monrovia, CA) was used to cure the cement for five seconds on the mesial, distal, occlusal and gingival aspects of the bracket for a total of 20 seconds according to the manufacturer's recommendation.

Teeth in the Fuji Ortho LC group were prepared by applying a thin coat of GC Fuji Ortho Conditioner (GC America Inc, Alsip, IL), 10% polyacrylic acid for 20 seconds, followed by rinsing. Fuji Ortho LC cement was prepared using a 3:1 powder to liquid ratio. First the powder was divided into two equal parts. After mixing the first part with all of the liquid for 10 seconds, the remaining powder was incorporated and mixed thoroughly for an additional 10-15 seconds. The bracket base was then coated with the adhesive, and a moist cotton roll was wiped on the enamel surface just prior to placing the bracket. After the removal of the excess cement, brackets were light cured for 10 seconds from the mesial, distal, occlusal and gingival aspects for a total of 40 seconds.

Teeth in the Aegis Ortho ACP group were etched with 35% phosphoric acid (Acid Etch, Bosworth Co, Skokie, IL) for 30 seconds. Teeth were then rinsed and dried to show a white, frosty appearance. A drop of a primer (Aqua Bond, Bosworth Co, Skokie, IL) was rubbed on the surface with a microbrush for 10 seconds and then dried with air. Aqua Bond was also applied to the bracket base as recommended by the

manufacturer. The Aegis Ortho ACP adhesive was then placed onto the bracket pad that was subsequently bonded onto the prepared enamel surface. After the removal of the excess adhesive, brackets were cured for 10 seconds on the mesial, distal, occlusal and gingival aspects for a total of 40 seconds.

Each tooth surface was painted with an acid-resistant varnish (Xtreme Wear, Sally Hansen, New York, NY) creating a window of enamel measuring 2x2 mm around the bracket base so that only this area would be exposed to the demineralization and remineralization solutions. Each group was painted with a different color to aid with identification during the cycling. The teeth were then left to dry over night (Figure 1).

Figure 1: Teeth with acid-resistant varnish being applied.

Subsequently, teeth were immersed in 600 mL of demineralization solution for 6 hours at 37°C. Each group of teeth was placed in a color coordinated, sealed container to simulate the oral environment and maintain the titrated pH. The demineralization solution consisted of an acetate buffer, 2.0 mmol/liter calcium, 2.0 mmol/liter phosphate, and 0.075 mol/liter acetate adjusted to pH 4.5. They were removed from the solution and thoroughly rinsed with water to remove any of the previous solution. The samples were then immersed in 600 mL of remineralization solution for nearly 18 hours at 37°C. This solution consisted of 1.5 mmol/liter calcium, 0.9 mmol/liter phosphate, 150 mmol/liter potassium chloride, and cacodylate (20 mmol/liter) with the pH adjusted to 7.0. The demineralization / remineralization cycle was performed for 14 days, with fresh solutions after 7 days, to mimic the demineralization and remineralization phases in the caries process (Figure 2).[3] Every other day the pH of each solution was measured using pH indicators (JT Baker, Phillipsburg, NJ) to check that the pH of each solution was at the correct level.

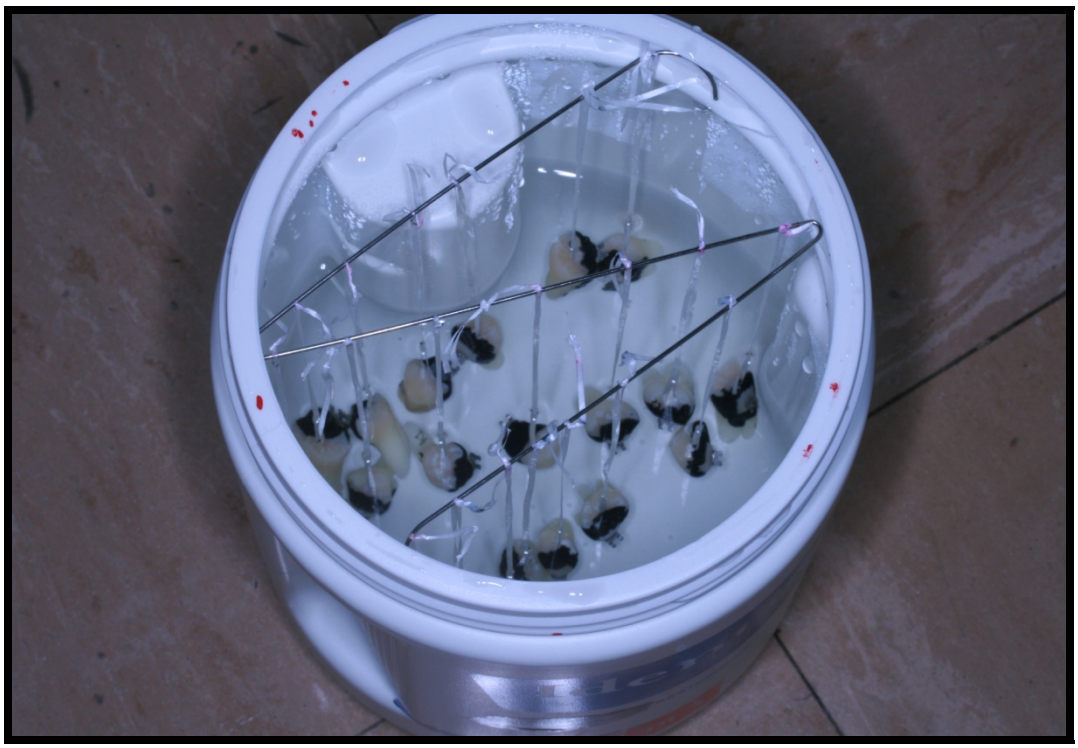

Figure 2: Group of teeth in the demineralization / remineralization solution.

At the end of 14 days, teeth were removed and rinsed carefully to eliminate all of the solution. Following careful bracket removal, the teeth were embedded in resin (EpoxiCure Resin, Buehler, Lake Bluff, IL) and allowed to cure overnight. They were then sectioned longitudinally with a low speed saw (Accutom-5, Struers Inc., Westlake, OH) using a low concentration diamond blade. Samples were polished using 4000 grit laboratory grade silicon carbide wet grinding paper with non-adhesive back (Struers Inc., Westlake, OH). The hardness of the enamel surrounding the brackets was evaluated by using a microhardness tester (Duramin-5 Hardness Tester, Struers Inc., Westlake, OH). Indentations were made with the long axis of the diamond parallel to the outer enamel surface (Figure 3). The five distance locations were: underneath the bracket, 50 μm, 200

μm, 500 μm, and 1500 μm away from the bracket base toward the varnish laterally. For each depth location, the first indentation was made with a 200 gram force load, 10 second press time at 25-35 μm deep from the enamel surface. The next mark was at 50 μm, and subsequent indentations were done in 25 μm steps toward the dentin up to 200 μm, for a total of eight depth indentations.

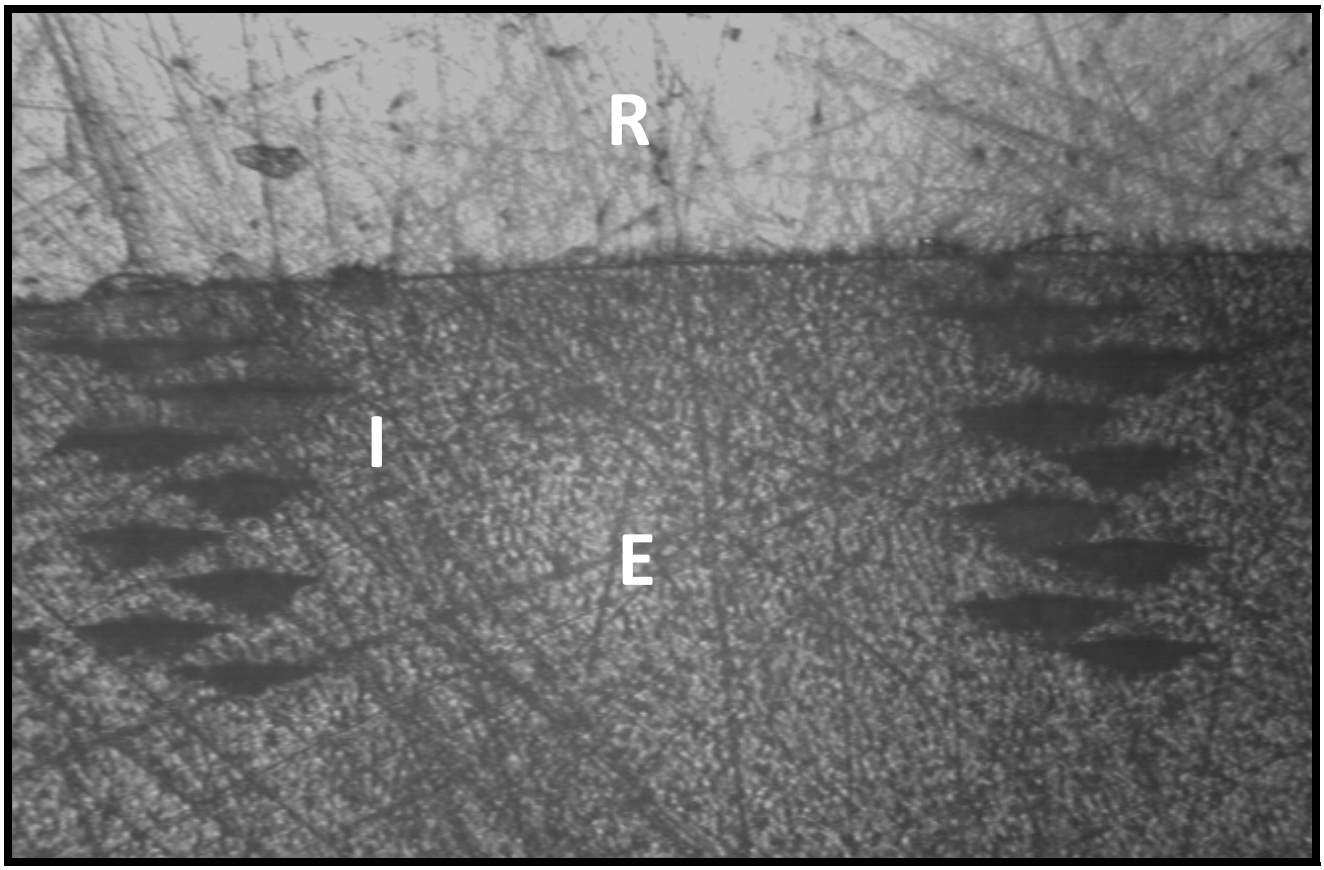

Figure 3: Knoop hardness number indentation sequence at 10x magnification. First indentation was made at 25-35 μm deep from the outer enamel surface. *E*, enamel; *I*, indentation row; *R*, resin.

The length of the indentations was used to determine the Knoop hardness number (KHN) for each sample. The Knoop hardness was calculated using the following formula: KHN = 14229L / d^2, where the load L is in grams force and the long diagonal *d* is in μm. The KHN of the eight depth indentations was then reported in kgf / μm^2 unit for each of the five distance sections. The mean of the last three indentations underneath the bracket was chosen as the KHN of the sound enamel for that specific tooth. The matrix of the remaining four section's site measurements (50 μm, 200 μm, 500 μm, and 1500 μm) away from the bracket edge toward the varnish, and 8 depth indentations (25-35 μm, 50 μm, 75, μm, 100 μm, 125 μm, 150 μm, 175 μm, and 200 μm) were then converted to percent hardness of the sound enamel as previously determined to reduce any discrepancies of enamel hardness amongst different samples.

Statistical Analyses

The three groups were evaluated for differences in percent demineralization calculated from the Knoop hardness tests. Other variables included distance from the bracket toward the acid resistant nail polish (50 µm, 200 µm, 500 µm, and 1500 µm), as well as depth below the enamel surface toward the dentin (25-35 µm, 50 µm, 75, µm, 100 µm, 125 µm, 150 µm, 175 µm, and 200 µm). Therefore, three-way ANOVA was used first to study the differences between and within these variables. Then, two-way ANOVA was performed to analyze differences among group and depths. Post-hoc pair-wise comparisons with Tukey method adjustment were performed to determine statistically significant changes in percent hardness between groups. SAS (version 9.1, SAS Institute Inc., Cary, NC) was used to perform all the analyses with a significance level of $P \leq 0.05$.

Results

The average KHN for Transbond XT, Fuji Ortho LC, and Aegis Ortho at the depth of 25-35 μm was 171.7, 145.2, and 205.8 respectively (Table II). The average percent hardness for the same three materials at 25-35 μm was 58.7%, 48.7%, and 66.2%. At the next test depth of 50 μm, the KHNs (and percent hardness) were: 217.5 (74.6%), 242.8 (81.7%), and 238.9 (77.1%). The distance from the bracket toward the varnish (50 μm, 200 μm, 500 μm, and 1500 μm) demonstrated no statistically significant differences in percent demineralization. Therefore, the analysis of percent hardness was computed by summing the corresponding depth measurements together.

Table II: Mean KHN and percent hardness of sound enamel

	Indentation Depth (μm)							
Group	**25-35**	**50**	**75**	**100**	**125**	**150**	**175**	**200**
Transbond (Control)	171.7 (58.7%)	217.5 (74.6%)*	242.5 (82.9%)	271.2 (93.0%)	277.4 (95.0%)	287.1 (98.3%)	294.1 (100.6%)	291.4 (99.6%)
Fuji Ortho	145.2 (48.7%)	242.8 (81.7%)*	275.2 (92.4%)	280.3 (94.2%)	289.9 (97.3%)	298.8 (100.2%)	299.8 (100.7%)	309.7 (104.0%)
Aegis Ortho	205.8 (66.2%)	238.9 (77.1%)	276.1 (89.1%)*	287.0 (92.6%)	294.4 (94.9%)	310.3 (100.2%)	305.4 (98.7%)	307.0 (99.1%)

* Statistically significant difference from group's previous hardness percentage ($P < 0.05$)

A statistically significant difference was found between Fuji Ortho LC and Aegis Ortho materials at a depth of 25-35 microns. Initially, Aegis Ortho demonstrated the least amount of demineralization, followed by Transbond XT, and then Fuji Ortho LC

(Figure 4). There was no statistically significant difference between the materials from 50 down to 200 microns below the enamel surface. However, Aegis Ortho and Fuji Ortho LC showed a trend toward less demineralization than Transbond XT at 50 and 75 µm. The remaining hardness measurements showed no discrepancy among the three orthodontic adhesives, and reached a plateau starting at 150 microns.

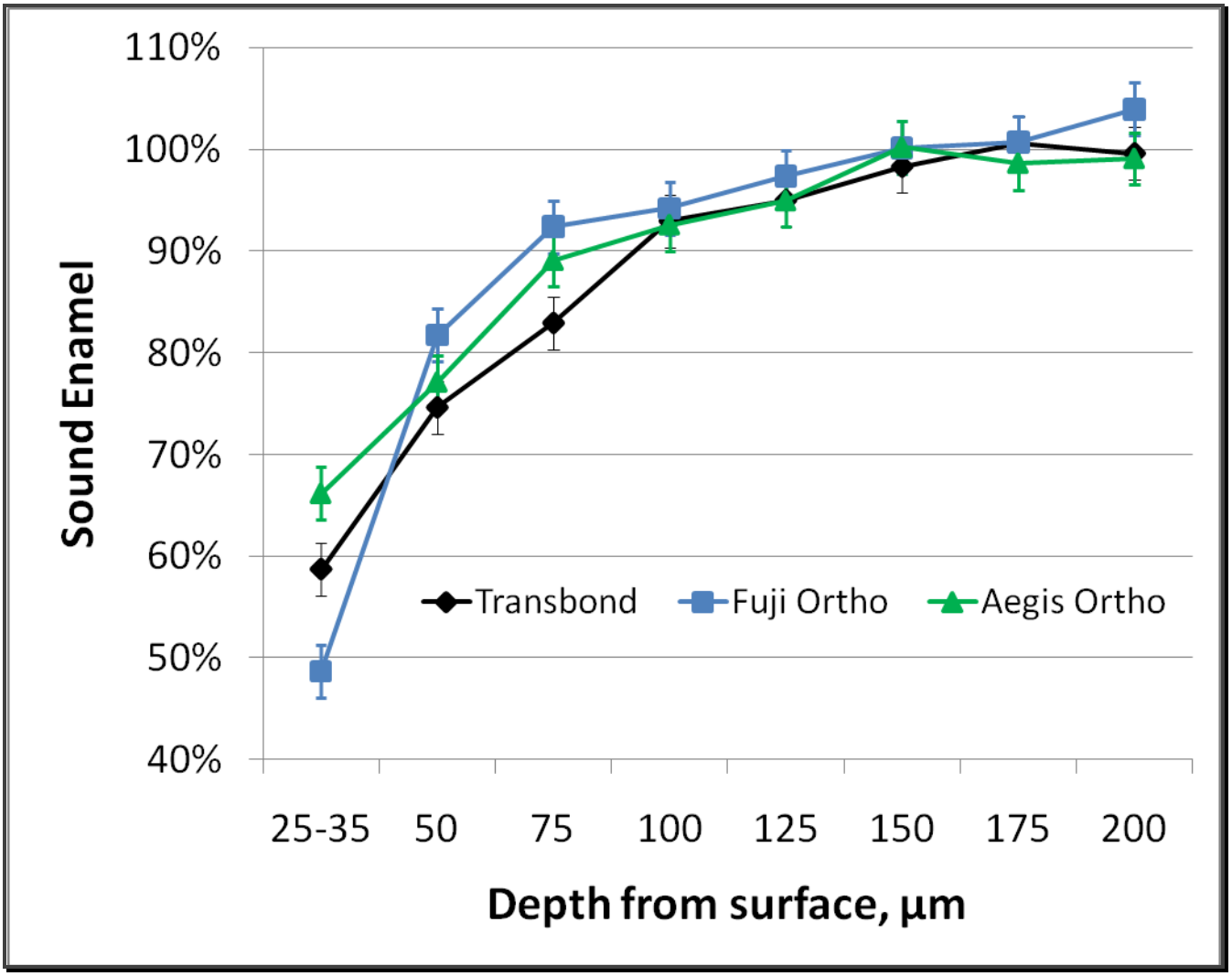

Figure 4: Illustration of relative mineral loss. Each point represents the mean of all teeth tested in each group.

Significant changes in percent demineralization were demonstrated among the more superficial depths. There was significant change in demineralization with the Transbond and Fuji Ortho from 25-35 μm down to the 50 μm measurements, and there was a significant difference between the 50 μm and 75 μm measurements of the Aegis Ortho.

Evaluation of the demineralization directly under the bracket due to the initial acid etch was also conducted. Overall, the more superficial microhardness measurements were substantially reduced when compared to the deeper measurements (Table III). The percent hardness at 25-35 μm for all three adhesives was 20-25% less than the sound enamel. The next depth (50 μm) demonstrated 10-15% demineralization of the original enamel. There was evidence of notable demineralization up to 75 μm deep. However, the enamel showed a trend of increased mineralization once the measurements were taken at deeper levels, as shown in Figure 5.

Table III: Percent hardness of sound enamel directly underneath the bracket.

	Indentation Depth (μm)							
Group	**25-35**	**50**	**75**	**100**	**125**	**150**	**175**	**200**
Transbond	74.9%	84.2%	86.5%	95.7%	93.1%	96.9%	99.7%	103.5%
Fuji Ortho	78.8%	89.0%	93.4%	96.9%	99.3%	98.8%	96.4%	104.8%
Aegis Ortho	79.7%	84.1%	91.6%	94.7%	99.1%	98.9%	99.9%	101.2%

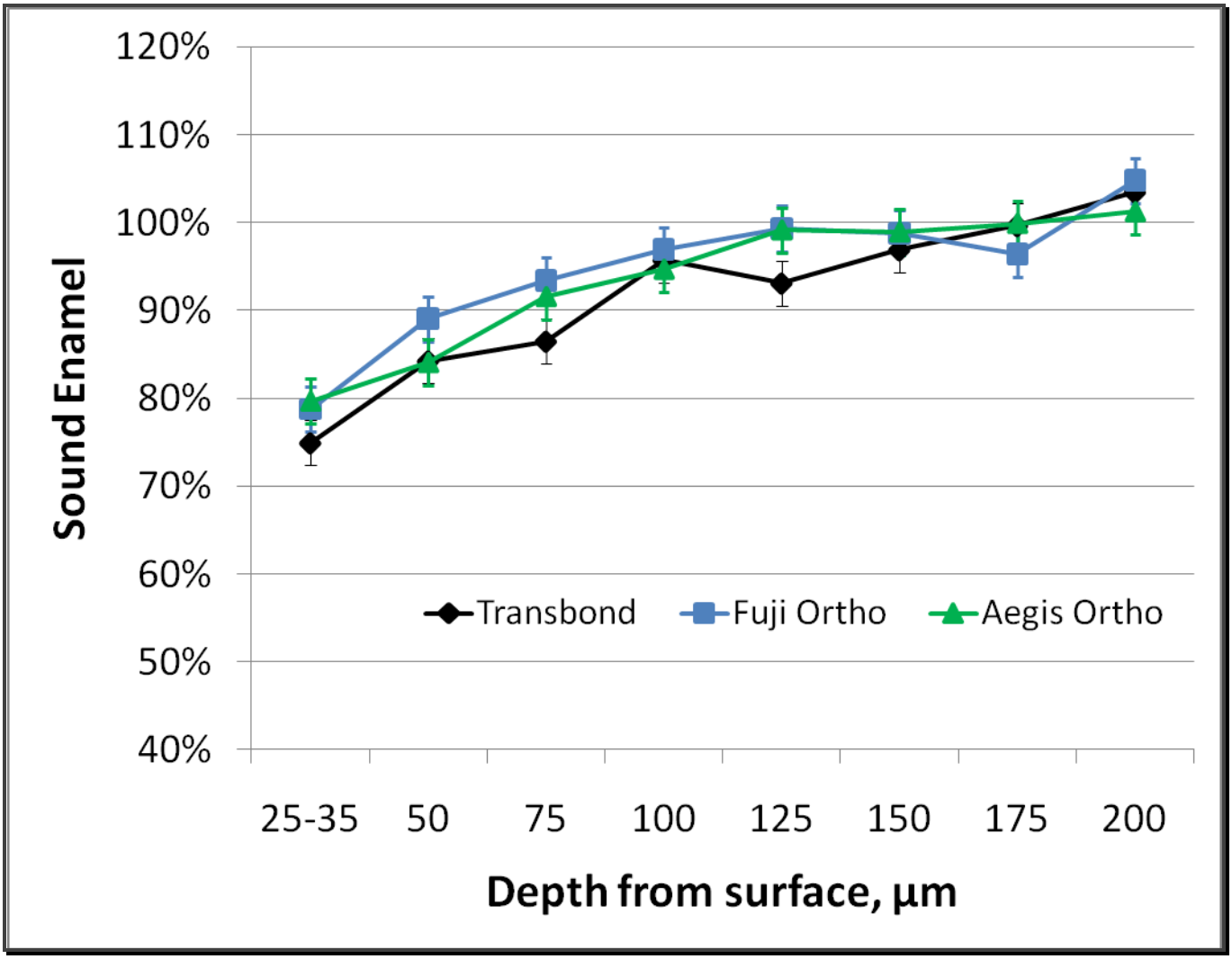

Figure 5: Illustration of relative mineral loss directly underneath the bracket.

Discussion

Due to poor oral hygiene during orthodontic treatment, enamel decalcification may occur. It has been previously shown that orthodontic patients develop significantly more white spot lesions than non-orthodontic patients.[9] This demineralization of enamel is due to the decrease in oral pH because acidogenic bacteria in the dental plaque produce acids while metabolizing carbohydrates. These white spot lesions typically form around the brackets due to plaque accumulation in the adjacent area.[11,12] A recent study reported that 38% of orthodontic patients had at least one lesion at 6 months into treatment.[56] While other studies reported the prevalence of WSLs in orthodontic patients at debonding as high as 97%.[42] Therefore, it is important for clinicians to develop preventive measures as soon as appliances are placed.[36]

In the present study, two commercially available orthodontic adhesives were compared with a non-fluoridated resin for their ability to inhibit demineralization. Previous studies have shown that these fluoride or ACP containing materials have a potential to minimize demineralization.

One surprising finding in this study was that teeth bonded with Transbond XT did not exhibit significant demineralization when compared to those bonded with Fuji Ortho LC and Aegis Ortho. This was somewhat not expected as Transbond XT does not contain fluoride or any other elements in its structure that could prevent demineralization.[37] Failure to create significant demineralization on the surfaces of the control teeth may be attributed to the length (14 days) of the experiment, or remineralization / demineralization solutions that were not adequate. It has been

previously reported that WSLs could be created *in vitro* within 14 days; however, in some of these studies an aggressive acidic solution rather than demineralization / remineralization solutions were used. It has been shown that in the oral environment, plaque pH can drop below a critical level after food consumption, in which enamel has an increased susceptibility to demineralization from the acidic plaque.[57] Therefore, in this study, the use of a pH-cycling model that simulates a cariogenic challenge supports the determination of a dose-material response relationship similar to what is found in a clinical setting.

In light of the results of the current study, it is suggested that demineralization / remineralization cycles should be run longer than 14 days, and preferably for 30 days. In fact, O'Reilly and Featherstone[13] as well Øgaard et al.[12] were able to observe white spot lesions *in vivo* when teeth were extracted after 4 weeks of fixed orthodontic therapy.

As mentioned previously, the experimental design of this study was developed to simulate the oral environment by creating a cariogenic challenge with a demineralization / remineralization solution on multiple teeth. *In vitro* studies are useful because using human subjects is not always feasible and may pose some ethical problems. However, it should be kept in mind that the ability to replicate the complex oral environment is quite difficult.

In this study, teeth were exposed to a 600 ml solution in sealable containers instead of being placed in individual vials. In this manner interactions between the adjacent teeth and adhesive were similar to the oral environment. In the literature, both large and small volume solutions have been previously used in demineralization

studies.[36,37] Both volumes were found to be adequate to create lesions on control teeth showing viability of either method.

Since visual inspection is a subjective method to determine demineralization, in this study Knoop microindentation was used as a quantitative measurement method. The KHN test was chosen because it is less sensitive to elastic recovery and measurement errors compared to other microindentation tests. Moreover, the Knoop hardness test is better for testing enamel because it is more ideal for hard, brittle materials as well as very thin sections.[58]

One concern with any hardness test is operator reliability in measuring the indentation lengths. Proper magnification and a smooth sample surface is necessary for a good view of the field to make accurate measurements. During hardness measurements, unexpected problems were encountered as cracks or other lines on the sample surface made it difficult to determine the exact distance of the long diagonal indentation (Figure 6). In addition, it was necessary to place teeth completely perpendicular to the indenter to ensure a uniform, symmetric indentation. In this study, initially indentations were made every 25 microns as described in the study by Featherstone et al.[54] However, this protocol was modified as indentations were too close to each other and at times overlapped, causing sample surfaces to crack. This could be because we used an indenter load of 200 gram force versus Featherstone's variable load of 15, 25, or 50 grams. Their protocol operated a load of 15 grams toward the outer edge to avoid cracking and then 50 gram force for more sound enamel. Therefore, in the present study indentations were performed in a staggered mode yielding a zig-zag pattern.

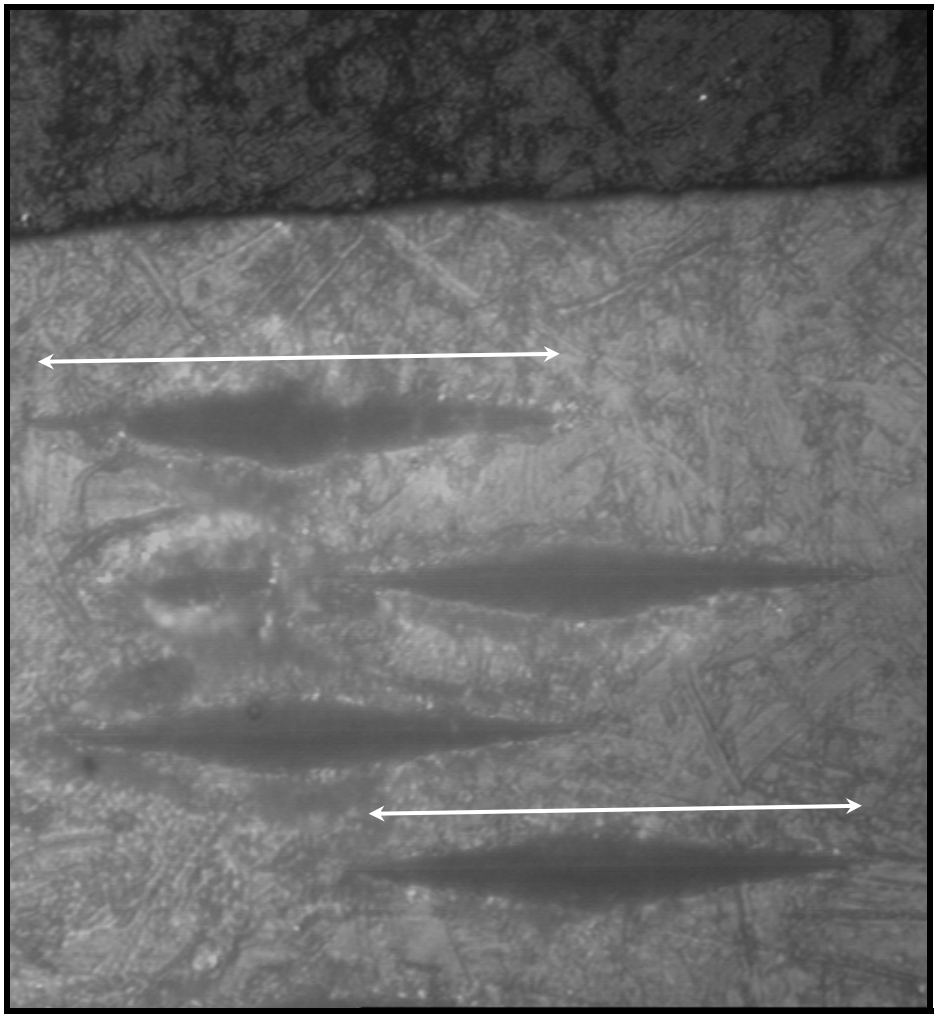

Figure 6: Knoop microhardness indentation under 40x magnification. Lines show long diagonal measurement.

Based on these observations, it may be concluded that the experimental design and tooth preparation protocol for microhardness testing can be technique sensitive.

It has been previously shown that enamel is an inhomogeneous material and its hardness changes from the enamel surface to the dentinoenamel junction. Hydroxyapatite crystals are more densely packed in the outer enamel than the inner enamel. Also, it has been reported that for every 2 fluoride ions, 10 calcium and 6

phosphate ions are required to remineralize a pre-existing WSL and to form 1 unit cell of fluoroapatite. Sudjalim et al.[35] reported that lesions on the labial aspect of maxillary anterior teeth are often calcium-limited and therefore remineralization is less effective in these areas. In addition, another study suggested that the microhardness would show variation because of the inhomogeneous fluoride incorporation into the enamel structure of the enamel adjacent to bonding agent / bracket area. He et al.[59] reported that Knoop hardness and hydroxyapatite density were significantly higher in the outer-layer than in the middle - or inner-layers of enamel. In the same study, it was also shown that the KHNs were significantly lower on the lingual than on the buccal sides.[59] The KHN of sound human enamel is reported to be 355-431.[60] Therefore, one may conclude that in addition to the indentation location, the wide range in the KHN values may be attributed to differences in the hydroxyapatite and fluorapatite ratios within the enamel structure of different individuals.

In order to take into account the differences in the KHN within the same tooth because of the location and how much fluoride has been incorporated to the enamel, demineralization was calculated by comparing the value of KHN at a specific measurement point to that of the sound enamel. Therefore, in this study demineralization was reported as percent KHN of the sound enamel.

In this study, the KHN of the sound enamel ranging from 287 to 310 was lower than the reported range of 355-431.[60] However, the KHN for softened enamel was in agreement as previous established, where the values ranged from 149 to 179.[60] In this study, enamel depths of 150 μm or greater had KHN of the sound enamel as previously

reported by Hu et al.[3] Directly under the bracket at 25-35 μm where the enamel was prepared for bonding via etch, but not exposed to the solution cycles, the KHN of enamel was 220-246. This supports the notion that initial acid etch significantly reduces sound enamel hardness, as determined in our study to range from 287 to 310.

In the literature, a linear relationship between KHN and mineral content has been shown.[54,55] In this study, the most superficial indentation at 25 μm showed a 20-25% decrease in percent hardness under the bracket due to the initial acid etching in preparation for bonding. Hu et al.[3] showed that etched teeth had 5% to 10% less mineral content than non-etched enamel after their demineralization / remineralization cycling, while Davidson et al.[51] found a 50% reduction in the hardness by etching for three minutes. Because enamel with less mineral content would predispose the tooth to caries, care should be taken so that only the area where the bracket is to be bonded should be etched. Otherwise, applying a sealant to the etched area should be accomplished as a precautionary measure to minimize any iatrogenic demineralization. Another alternative would be to create a template that is placed on the teeth to isolate the etch only to the area of the ideal bracket base location. This protocol may be possible *in vitro*, however it is not clinically feasible.[25]

The distance tested was up to 1.5 mm away from the bracket base toward the varnish because this area has been reported as being the most susceptible to demineralization.[11,12] In this study, there were no statistically significant differences in percent demineralization among the various distances away from the bracket base (50 μm

– 1500 μm). These finding appear to validate that if a bonding material has the ability to minimize demineralization, they are effective in the zone most at risk.

Although there were no statistically significant differences between the control and experimental groups, both Fuji Ortho LC and Aegis Ortho orthodontic adhesives showed a trend toward a reduction in demineralization at the 50 μm and 75 μm depths of enamel. This is in accordance with results from a previous study.[32]

Conclusions

Fuji Ortho LC and Aegis Ortho showed trend toward reducing demineralization. However, the failure to create visible WSLs on teeth that were bonded with a fluoride free resin warrants future research with longer demineralization / remineralization cycling times to show significant differences between the control and experimental groups.

Due to its technique sensitivity, microhardness testing requires better experimental technique to be a suitable method to determine demineralization. When similar experiments are repeated the following improvements in hardness measurement techniques should be considered: reduce the indentation force to 25-100 grams with appropriate increase in magnification, improve polishing procedure to provide smoother scratch free surface, consider a vacuum deposited thin coating of a metal like gold to better define the indentation outline. Also, the demineralization and remineralization solutions should be reevaluated for the proper clinical balance.

Since the hardness of the etched enamel showed a significant reduction, special attention should be observed while applying acid to enamel prior to bonding to prevent superfluous demineralization.

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