Evaluating a novel bioactive glass orthodontic bonding agent and its effects on enamel demineralization

John Delplanche, D.M.D.

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Evaluating a novel bioactive glass orthodontic bonding agent and its effects on enamel demineralization

John Delplanche, D.M.D.

Master of Science in Orthodontics Research Advisory Committee:

Signature: [redacted] Date: 16 Dec 2010

John C. Mitchell, Ph.D.
Associate Professor
Department of Restorative Dentistry, Division of Biomaterials & Biomechanics
Oregon Health & Science University

Signature: [redacted] Date: 16 Dec 2010

David A. Covell, Jr., Ph.D., D.D.S.
Associate Professor, Chair
Department of Orthodontics
Oregon Health & Science University

Signatur[redacted] Date: 12·16·10

Jennifer Crowe, D.D.S., M.S.
Assistant Professor
Department of Orthodontics
Oregon Health & Science University

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Authors:

John F. Delplanche[a], Jennifer J. Crowe[a], David A. Covell, Jr.[a], John C. Mitchell[b]

[a]Department of Orthodontics, School of Dentistry, Oregon Health & Science University,

[b]Department of Restorative Dentistry, Division of Biomaterials and Biomechanics, School of Dentistry, Oregon Health & Science University

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Corresponding Author:

John C. Mitchell

611 SW Campus Drive

Portland, OR 97239

Telephone: (503) 494-8223

Fax: 503-494-8260

E-mail: mitchelj@ohsu.edu

Declaration of Interests

All authors express no conflict of interested in the study presented, financial, or otherwise.

Abstract

The formation of white spot lesions (WSL's) and enamel decalcification is a potential risk associated with orthodontic treatment, and therefore ion-releasing bonding agents have been proposed as a means to reduce enamel decalcification for orthodontic patients. This in-vitro study used extracted human third molars, and compared the enamel microhardness adjacent to orthodontic brackets bonded with three different bonding agents (n=10/group): a resin bonding agent (TB-XT) (Transbond XT, 3M Unitek, Monrovia, Calif); a glass ionomer bonding agent (FO-LC) (Fuji Ortho LC, GC America, Chicago, Ill); and a novel bonding agent containing bioactive glass and glass ionomer (BAG-GIC). After 19 days of pH cycling, the teeth were visually evaluated to qualitatively examine the resulting lesions prior to being embedded and sectioned coronally. Microhardness measurements adjacent to the brackets were compared using 2-way ANOVA, comparing treatment against location, with $p \leq .05$. Visually, teeth bonded with a TB-XT showed the most apparent WSL's, and teeth bonded with BAG-GIC showed the least apparent WSL's. At 25 µm and 75 µm beneath the surface, teeth bonded with BAG-GIC had significantly less reduction in hardness than those bonded with the other adhesives ($p<.05$). Teeth bonded with TB-XT showed significantly greater reduction in hardness at all depths up to 100 µm compared to the other adhesives ($p<.05$). Bonding materials that incorporate bioactive glass may help prevent demineralization and the eventual development of WSL's adjacent to orthodontic brackets.

Introduction

Enamel decalcification and formation of white spot lesions (WSL's) is a common problem associated with orthodontic treatment, with a reported prevalence ranging from 2-97% of orthodontic patients [Mitchell, 1992; Boersma et al., 2005]. WSL's may create an esthetic problem post-orthodontic treatment, as they occur most commonly on the buccal surfaces of maxillary incisors [Gorelick et al., 1982; Chapman et al., 2010], and lesions are often clinically apparent five years after removing orthodontic appliances [Øogard, 1989].

Fixed orthodontic appliances amplify the incidence of WSL's by increasing the amount of plaque retention and food accumulation on the smooth surfaces of teeth which are not normally predisposed to demineralization [Øgaard et al., 1988a]. One method used to prevent the development of WSL's during orthodontic treatment is the introduction of topical fluoride. Fluoride aids in remineralization by substituting for hydroxyl ions within the hydroxyapatite crystal, creating a more acid-resistant enamel surface [Featherstone 2000]. Although using a fluoride regimen has been shown to reduce the prevalence of caries during orthodontic treatment [O'Reilley and Featherstone, 1987; Øgaard et al., 1988b] fluoride-containing mouthrinses and dentifrices are subject to unpredictable compliance [Øgaard et al., 1988b]. Also, the beneficial effects are not usually localized to the areas with the greatest demineralization such as orthodontic bracket margins.

Enamel adjacent to orthodontic brackets is continuously subjected to cyclic demineralization and remineralization. When the localized environment shifts to prolonged periods of demineralization, as is the case with food or plaque retention, acids fermented from bacteria dissolve the enamel mineral phases [Feathersone, 1984], causing calcium and phosphate to be lost from the tooth. The demineralization process can be reversed by the presence of calcium, phosphate, and fluoride ions in large enough local concentrations. During remineralization, these ions will deposit directly onto the enamel crystals and create a more acid resistant enamel surface [Featherstone, 2004]. Since the greatest area of plaque retention around orthodontic brackets is immediately adjacent to the bracket [O'Reilley and Featherstone, 1987], a bonding agent may serve as a suitable reservoir for calcium, phosphate, and fluoride ions for both enamel remineralization and prevention of demineralization.

Several commercially available bonding agents have been designed to release ions to aid in enamel remineralization. The first glass ionomer cements were introduced in the early 1970's [Wilson and Kent, 1972], and their ability to release fluoride has been well-documented [Benelli et al., 1993; Hallgren et al.,1992; Hallgren et al.,1993]. Resin-modified glass ionomer cements have been introduced more recently with greater adhesive strength than conventional glass ionomer cements [Silverman et al., 1995] while still providing localized fluoride ion release [Shammaa et al., 1999; Coups-Smith et al., 2002]. Although they demonstrate the ability to release fluoride, commercially available glass ionomer cements do not exhibit the ability to release significant amounts calcium and phosphate, which are also important ions associated with the remineralization process [Featherstone, 2004].

Bioactive glass containing bonding agents, however, have demonstrated the ability to release calcium and phosphate ions in significantly greater quantities than glass ionomer at a low pH [Crowe et al., 2008], as an apatite layer containing calcium and phosphate forms on the surface of bioactive glass [Mitchell, 2003; Forsback et al., 2004]. Since a bonding agent which contains bioactive glass provides a reservoir of crucial ions, we hypothesized that this would result in less reduction in hardness adjacent to orthodontic brackets. Thus, the aim of this study was to evaluate changes in microhardness of enamel adjacent to orthodontic brackets, following pH cycling, which were bonded with an adhesive containing bioactive glass.

Materials and Methods

Three orthodontic bonding agents were compared: Transbond XT (TB-XT) (3M Unitek, Monrovia, Calif); Fuji Ortho LC (FO-LC) (GC America, Chicago, Ill), and a novel bonding agent composed of a mixture of Fuji Ortho LC and bioactive glass (BAG-GIC).

Preparation of Bioactive Glass Bonding Agent

The bioactive glass (BAG) used in this study was prepared in our laboratory by mixing alkoxides to a final formulation of 75 mol% SiO_2, 21 mol% CaO, 4 mol% P_2O_5. BAG adhesive was prepared by mixing Fuji Ortho LC (GIC) powder with BAG at a 50:50 by volume ratio. Fuji Ortho LC liquid was then added to the BAG-GIC powder at an 11 drops:6 scoops ratio. Once the liquid was added, the mixture was hand-mixed according to manufacturer's instructions for Fuji Ortho LC, prior to being used for orthodontic bracket bonding.

Specimen Preparation

Thirty freshly extracted human third molar teeth were evenly divided between the experimental groups (n=10 for each bonding agent). The teeth were collected from local private practices and stored in 0.5% chloramines T solution for less than six months prior to experimental procedures. All teeth were debrided to remove loose tissue, and examined to be visually caries-free and to have intact enamel surfaces.

The enamel bonding site for each tooth was based upon the best visual adaptation to the bracket pad. Adhesive tape was used to isolate the bonding site prior to bracket bonding, leaving an exposed window the size of the bracket pad. Orthodontic brackets (Avex, Opal Orthodontics, South Jordan, Utah) were then bonded to each tooth on the exposed enamel surface with one of the bonding agents. Modified manufacturer's instructions were followed for the TB-XT and FO-LC groups, and the same instructions were followed for the FO-LC and BAG-GIC groups. Adhesive primer was used for TB-XT samples, while no conditioner was used with the FO-LC and BAG-GIC groups. To ensure adequate curing, all brackets were light cured from the mesial, distal, occlusal, and gingival surfaces for 10 seconds each direction using an Ortholux LED curing light (3M Unitek, Monrovia, Calif).

Once the bracket was bonded, the tape was removed and excess bonding agent was removed with a scalpel blade. Acid-resistant varnish was then painted on the entire tooth surface up to 1.2 mm of enamel from the bracket pad. This created an exposed window of enamel around the bracket which was subjected to demineralization and remineralization. To prevent accidental bracket debonding during the subsequent pH cycling, .010" steel orthodontic ligature wire was tied circumferentially around each tooth through the bracket slot. All teeth were stored overnight in reverse osmosis water prior to commencing pH cycling.

pH Cycling

The pH cycling procedure used was an adaptation from a previously described protocol [Featherstone et al., 1986] The demineralization solution consisted of an acetate buffer, 0.075 mol/liter acetate, with calcium and phosphate each 2.0 mmol/liter, and the pH was adjusted to

4.5. The remineralization solution consisted of 1.5 mmol/liter calcium, 0.9 mmol/liter phosphate, 150 mmol/liter potassium chloride, 20 mmol/liter cacodylate, and the pH was adjusted to 7.0 [ten Cate and Duijsters, 1982].

The pH cycling consisted of immersing each tooth individually into 40 mL of demineralization solution at 37°C for a period of 6 hours. After the demineralization phase, each tooth was immersed in 20 mL of remineralization solution at 37°C for 18 hours. [Featherstone et al., 1986]. This cycle was repeated for a total of 19 cycling days, excluding weekends when the teeth were immersed in remineralizing solution, and fresh solutions were used weekly. Immediately following the pH cycling procedure, each specimen was visually examined and qualitative notations were made regarding the appearance appearance of WSL's.

Microhardness Measurements

After visual examination, each specimen was individually embedded in clear epoxy resin. The teeth were sectioned with a diamond wafering blade perpendicular to the long axis, through the center of the bracket slot, and serially polished, ending with 4000 grit silicon carbide polishing paper. For each specimen, either the mesial or distal surface was chosen at random for cross-sectional microhardness testing.

A microhardness tester (Duramin 5, Struers Inc., Westlake, Ohio) with a Knoop diamond under a 25 gram load for 5 seconds was used for the microhardness analysis. 25 grams was chosen to avoid enamel fracture at superficial depths, and measurements were taken to the mesial or distal side of the bracket based upon random assignment. A total of 36 indentations were made at predetermined depths across the lesion as shown in Figure 1. Indentations were created at distances ranging from 200 μm to 1000 μm from the bonding agent, with indentation columns separated by 100 μm. An alternating step-wise pattern was used, starting at 25 or 50 μm from the surface, and extending to 175 or 200 μm from the surface. In this way, four indentations were made at each 100 μm distance away from the bracket adhesive.

Also, indentations were made in the isolated enamel directly beneath the bracket at depths of 25 μm to 200 μm, replicating the same alternating stepwise pattern of indentations seen within the lesion. These indentations were made to create a baseline microhardness measurement for each specific tooth at different depths from the enamel surface. The indentations taken within the lesion were normalized by dividing their Knoop Hardness Number by the corresponding Knoop Hardness number measured beneath the bracket. This normalized value is expressed as a ratio of change in microhardness.

Following the microhardness measurements, thin sections of samples were observed with an environmental scanning electron microscope (FEI Quanta 200 ESEM), in backscattered electron imaging mode without coating.

Statistical Analysis

For each specimen, indentations made within the lesion were expressed as a ratio of change in microhardness. Two-way ANOVAs (SAS for Windows, version 9.1) were used to compare pooled results at specific indentation sites between groups and pooled indentations all from the same depth between experimental groups. Statistical significance was set at $p \leq .05$.

Results

Visual observation prior to embedding the samples revealed that teeth bonded with a TB-XT showed the most apparent WSL's, and teeth bonded with BAG-GIC showed the least apparent WSL's.

Figure 2(a-d), shows the pooled mean normalized microhardness value for each experimental group at the recorded location across the lesion. Note the scale differences used to aid in visualizing differences.

When comparing individual microhardness measurements, TB-XT showed significantly more demineralization than BAG-GIC and FO-LC at a depth of 25 or 50 µm for all distances from the bonding agent ($p<0.05$). TB-XT was also the most demineralized at a depth of 75 µm, at all distances from the bracket adhesive ($p<0.05$) except 1000 µm. No significant differences in micronardness measurements were found at a depth of 100 µm.

FO-LC showed significantly more demineralization than BAG-GIC at 25 µm beneath the enamel surface, at all distances from the bonding adhesive ($p<0.05$) except 400 µm. FO-LC also showed significantly more demineralization than BAG-GIC at 50 µm from the surface, only at 900 µm away from the adhesive ($p=0.0193$) and at 75 µm from the surface, only at 1000 µm away from the adhesive ($p=0.0076$).

When comparing pooled measurements taken at the same depth across the lesion, the following patterns of demineralization are apparent (Table 1). TB-XT had the most demineralization of all groups at depths of 25, 50, and 75 µm from the surface ($p<0.0001$). FO-LC had significantly more demineralization than BAG-GIC at 25 µm and 75 µm from the surface ($p<0.0001$). All other pooled depths showed statistically similar demineralization.

Figure 3 shows backscattered electron imaging of a representative sample, demonstrating an intact enamel surface with subsurface zones of demineralization.

Discussion

Indentations were taken starting at 200 μm from the adhesive because the adhesive primer from the TB-XT samples occasionally flowed beneath the adhesive tape. Therefore, accidental coverage of the enamel surface precluded microhardness measurements at these distances. Also, indentations made at depths greater than 100 μm from the enamel surface showed statistically similar results to measurements taken at 100 μm, so they are omitted from the discussion. All indentations taken at the same distance from the adhesive were not pooled together for statistical analysis. This is because the greatest decrease in microhardness was observed at superficial depths, and a large range of microhardness changes is observed when pooling together superficial and deeper measurements.

The present study measured relative changes in microhardness, which has been shown to be proportional to enamel mineral content [Featherstone et al., 1983]. Since WSL's form in areas of demineralization and low enamel mineral content [Featherstone, 2004], microhardness measurements provide a reliable indication for potential WSL lesion development. WSL's form in superficial enamel, as superficial calcium and phosphate ions are the first to diffuse out of the enamel during the process of demineralization [Featherstone, 2004]. Figure 2 shows that TB-XT allowed significantly greater decrease in microhardness at nearly all measurements within 75 μm of the surface, but showed no significant difference at a depth of 100 μm. This explains why the samples bonded with TB-XT had the most superficial demineralization and visually showed the most apparent WSL's. Conversely, this also explains why the samples bonded with BAG-GIC had the least superficial demineralization, while visually showing the least apparent WSL's.

It has been demonstrated that innate fluoride release from orthodontic bonding agents reduces demineralization adjacent to orthodontic appliances [Donly et al., 1995; Benelli et al., 1993]. Figure 2 shows how samples bonded with fluoride-releasing FO-LC showed significantly less demineralization than TB-XT at nearly all indentations within 75 μm of the surface. It should be noted, however, that FO-LC showed similar demineralization to TB-XT at 1000 μm from the adhesive, 75 μm from the surface. At this distance from the adhesive, FO-LC shows increased demineralization and the protective effect of fluoride release appears to be less effective.

Although fluoride release reduces demineralization, it would be more beneficial for the bonding agent to concurrently release calcium and phosphate, since these ions are intimately involved in the remineralization process [Featherstone, 2000]. This explains why BAG-GIC, with its ability to additionally release calcium, and phosphate, resulted in the least superficial demineralization as well as some areas which appeared to be more mineralized than the control enamel. The measurements which showed a relative increase in microhardness were at the locations closest to the BAG-GIC adhesive, and therefore closest to the reservoir of available calcium and phosphate ions. When looking at more distant measurements, BAG-GIC also had the least demineralization at slightly deeper depths when approaching 1 mm from the adhesive. This suggests that the addition of calcium and phosphate may provide a further-reaching protective effect for enamel adjacent to orthodontic appliances.

The pooled results from the same depth (Table 1) showed that BAG-GIC had significantly less demineralization than all other groups at depths of 25 and 75 μm. These results further demonstrate the importance of calcium and phosphate release, and correlate well with another study [Uysal et al., 2010], which showed significantly less superficial demineralization with a calcium and phosphate-releasing orthodontic composite at superficial depths. The biggest difference when compared to the present study was that Uysal et al studied a bonding agent containing amorphous calcium phosphate (ACP) instead of BAG.

The ideal bonding agent for orthodontics should have sustained ion release to aid in the protection against demineralization while demonstrating sufficient bond strength. The recognized beneficial effects of calcium and phosphate release have led to the development of bonding agents containing ACP, which have the ability to release calcium and phosphate. However, ACP composites may have mechanical instability and inferior strength when compared to glass-reinforced composites [Skrtic et al., 2004]. Several studies evaluating the shear bond strength of brackets bonded with ACP composite have been performed. One study suggested that the use of ACP would result in a greater tendency for early bracket debonds [Foster et al., 2008], and another study suggested that ACP had insufficient bond strength to withstand normal orthodontic forces [Dunn, 2007]. Also, ACP has also shown a significant decrease in bond strength when used as a lingual retainer adhesive [Uysal et al., 2009]. Thus, ACP appears to have favorable ion-release, but insufficient bond strength. Conversely, BAG has a demonstrated ability to release calcium and phosphate selectively at a low pH [Crowe et al., 2008], while providing acceptable shear bond strength [Personal correspondence]. With these important characteristics, BAG-containing adhesives show potential for orthodontic applications.

pH cycling to evaluate carious lesion development has been extensively performed [ten Cate and Duijsters, 1982; O'Reilley and Featherstone, 1987]. We selected this method to evaluate the novel BAG-GIC bonding agents under development in our laboratory as it has been shown to correlate well with conditions associated with 1 month of fixed orthodontic appliance use in vivo [Featherstone et al., 1986]. Six hours of daily exposure to a demineralization solution is intended to simulate the average amount of time a patient experiences an acid challenge, which creates enamel dissolution and ultimately the formation of WSL's adjacent to orthodontic appliances.

An alternating step-wise pattern of microhardness indentations was chosen as the most appropriate method, as we anticipated observing a two-dimensional gradient of change in microhardness. Vertically, the most superficial enamel was expected to show the most demineralization. Horizontally, the ion-release from BAG-GIC and FO-LC created a gradient with the greatest localized ion concentration adjacent to the bonding agent. Therefore, for BAG-GIC and FO-LC, we expected to observe differences in microhardness changes which were dependent on the distance away from the ion-releasing adhesives.

Of the bonding agents tested, only BAG-GIC and FO-LC have the capacity for significant ion release [Cooley et al., 1989; Crowe et al., 2008], and this undoubtedly related to why they both showed much less demineralization than TB-XT. Since the design of this study did not include an additional daily fluoride regimen, it can be assumed that the most readily available ions to prevent demineralization originated from the bonding agent. For this reason, the use of BAG-GIC may be most advantageous in patients with poor oral hygiene, as they may show unpredictable compliance with topical fluoride or fluoride mouth rinses [Øgaard et al., 1988b] and therefore would benefit from the intrinsic ion release.

In summary, we conclude:

1- TB-XT was associated with significantly greater superficial demineralization than FO-LC and BAG-GIC in vitro.

2- FO-LC and TB-XT were associated with significantly greater superficial demineralization, and significantly more superficial demineralization at distances approaching 1 mm from the adhesive, than BAG-GIC in vitro.

3- Patients with poor oral hygiene may benefit from orthodontic bonding agents that release calcium, phosphate, and fluoride.

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Legends/Headings:

Figure 1: Microhardness measurement pattern. The measurement locations consisted of nine columns, each with four indentations. Indentation points started at a distance of 200 μm from the adhesive, extending to 1000 μm away from the bonding adhesive, and were separated by 100 μm increments. The most superficial indentation at 200 μm from the adhesive began at 25 μm from the enamel surface, with three subsequent indentations each extending 50 μm deeper into the enamel until a final depth of 175 μm was measured. The adjacent column at 300 μm away from the adhesive began at 50 μm from the enamel surface, with each subsequent indentation extending 50 μm deeper into the enamel until a final depth of 200 μm was measured. This alternating step-wise pattern was repeated until the furthest distance of 1000 μm was measured.

Figure 2: Mean normalized microhardness of indentations taken at depths of 25 μm (a), 50 μm (b), 75 μm (c), and 100 μm (d) from the enamel surface. Standard error bars are shown. Note the scale differences used to aid in visualizing differences.

Figure 3: Scanning electron micrograph image of a representative lesion, demonstrating intact enamel surface with darker regions of subsurface demineralization. Microhardness indentations are visible as faint horizontal lines.

Table 1: Comparison of all pooled measurements taken at the same depth, ranging from 25 μm to 100 μm from the enamel surface.

Figures:

Figure 1

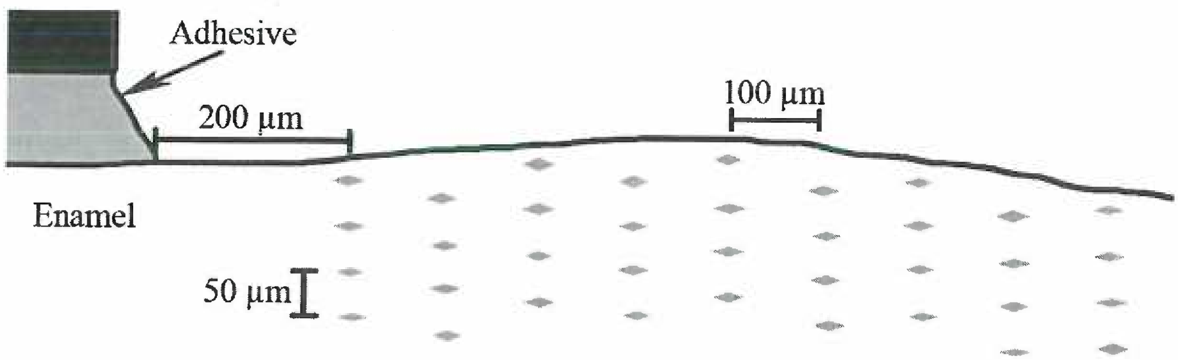
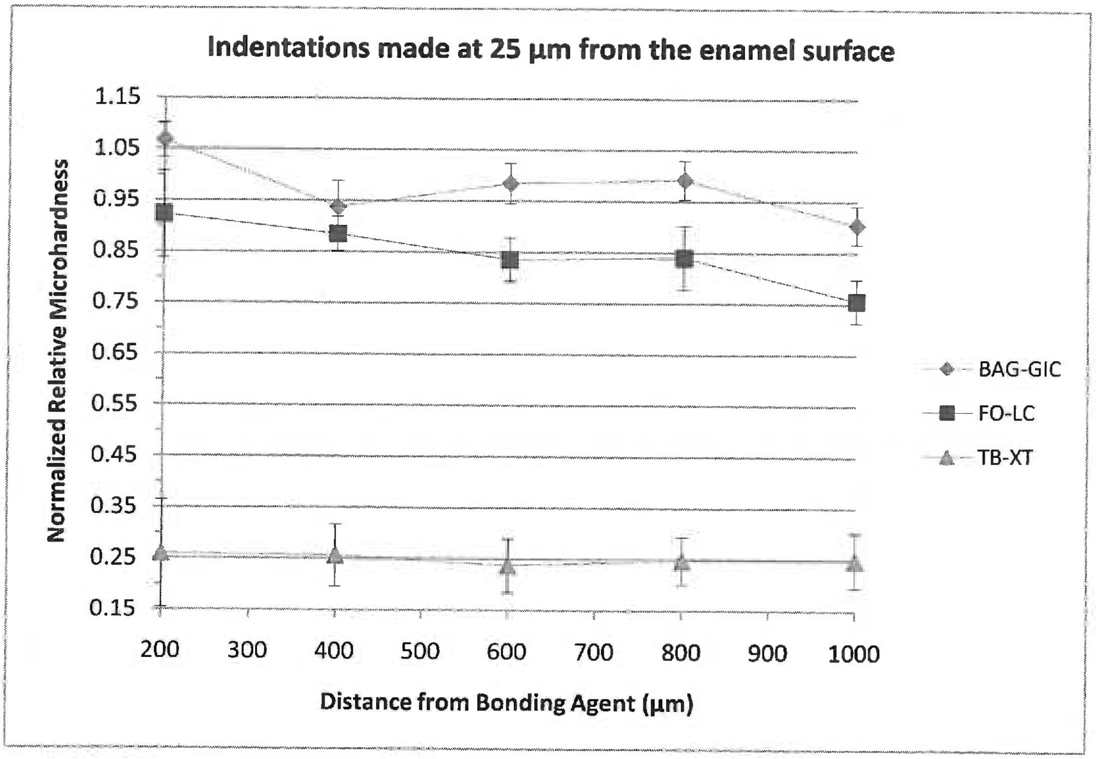


Figure 2

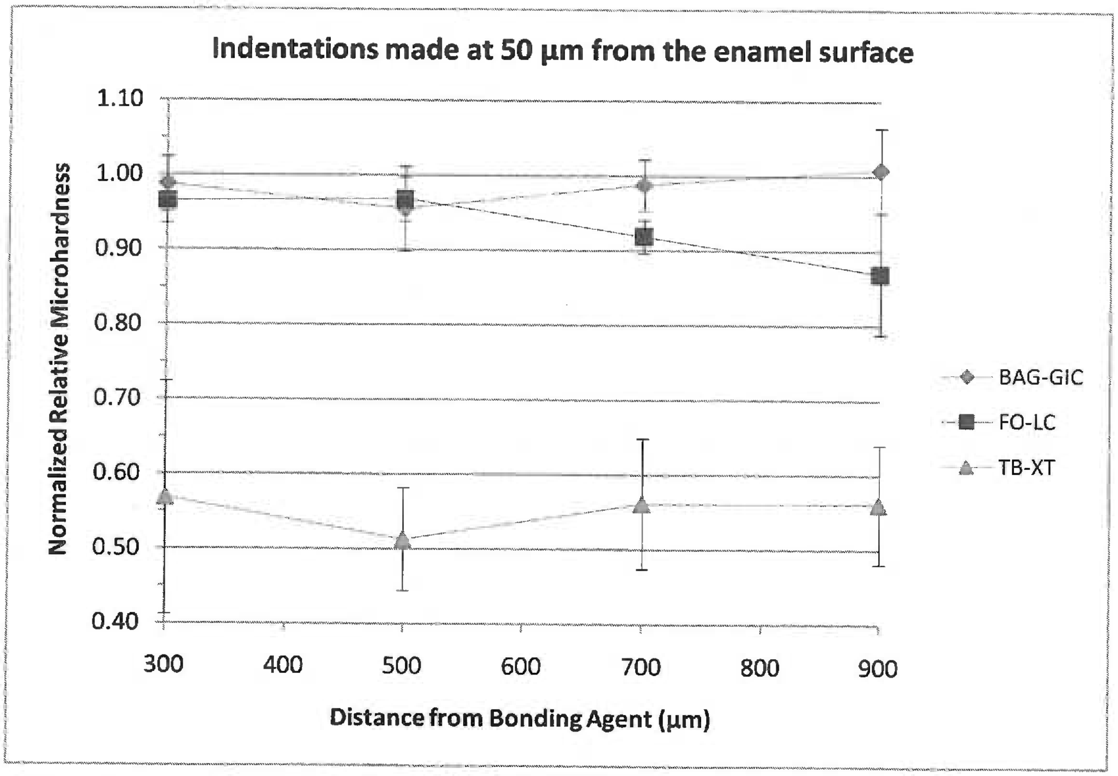
a



Indentations made at 25 µm from the enamel surface
Normalized Relative Microhardness
1.15
1.05
0.95
0.85
0.75
0.65
0.55
0.45
0.35
0.25
0.15
200
300
400
500
600
700
800
900
1000
Distance from Bonding Agent (µm)
BAG-GIC
FO-LC
TB-XT

Figure 2

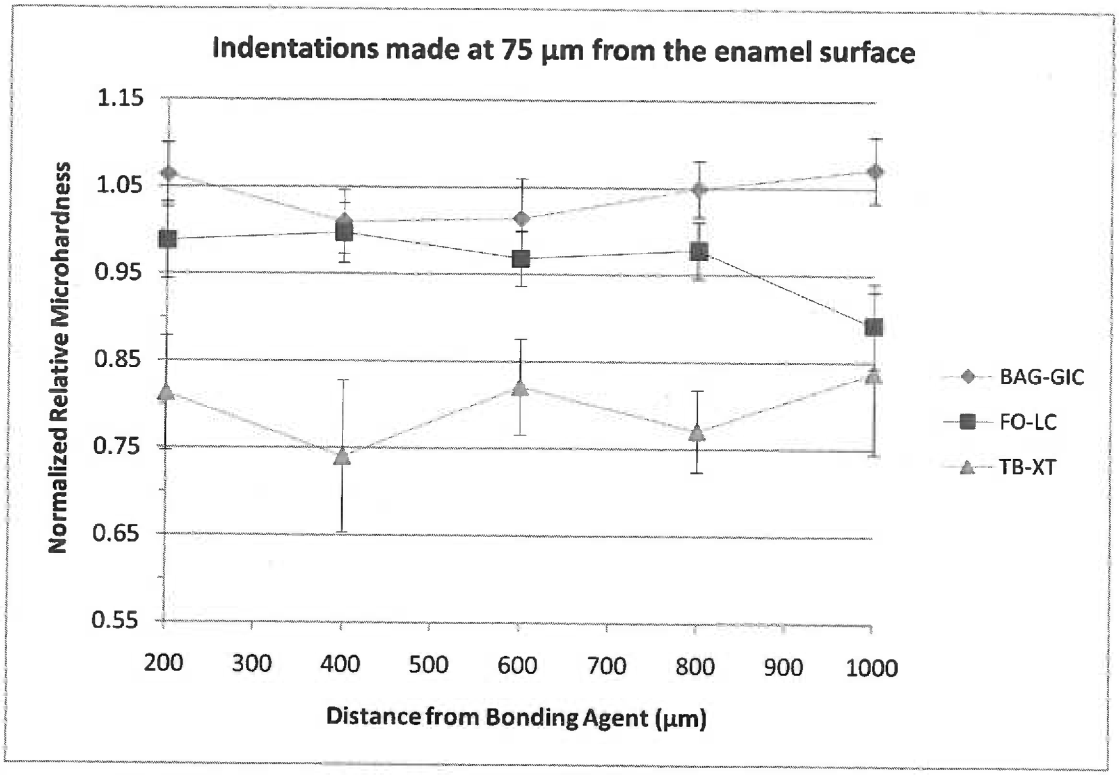
b



Indentations made at 50 µm from the enamel surface
Normalized Relative Microhardness
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
300
400
500
600
700
800
900
Distance from Bonding Agent (µm)
BAG-GIC
FO-LC
TB-XT

Figure 2

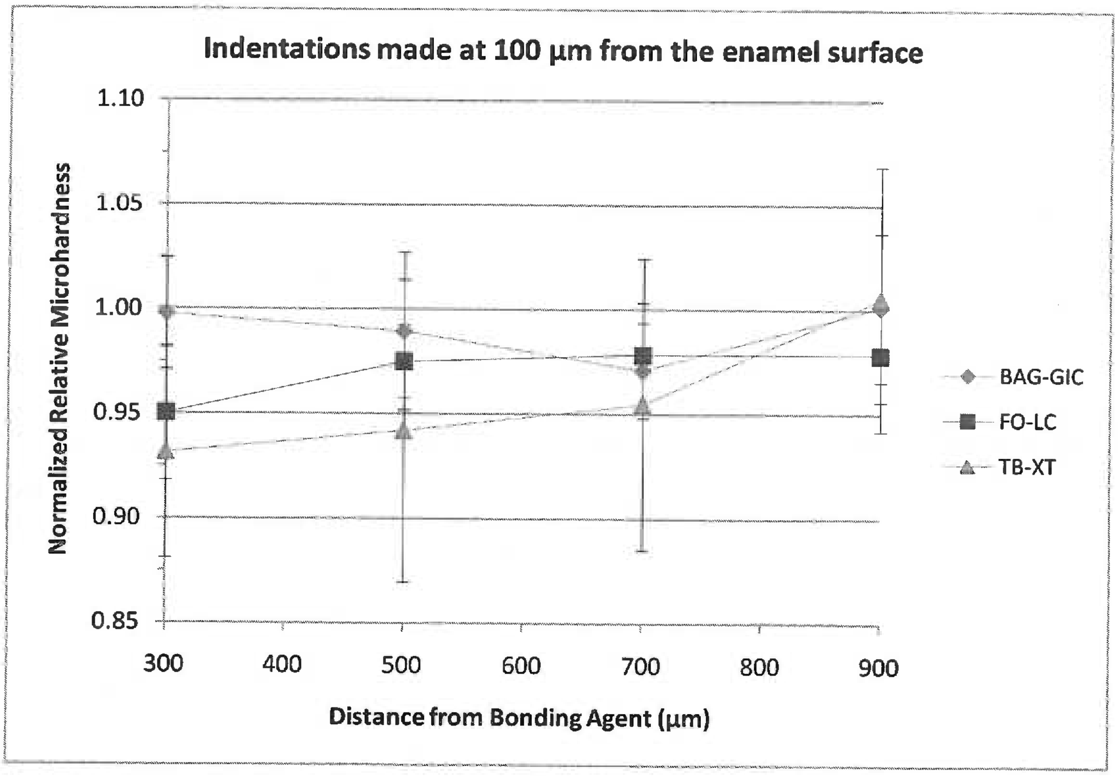
c



Indentations made at 75 µm from the enamel surface
1.15
1.05
0.95
0.85
0.75
0.65
0.55
Normalized Relative Microhardness
200
300
400
500
600
700
800
900
1000
Distance from Bonding Agent (µm)
BAG-GIC
FO-LC
TB-XT

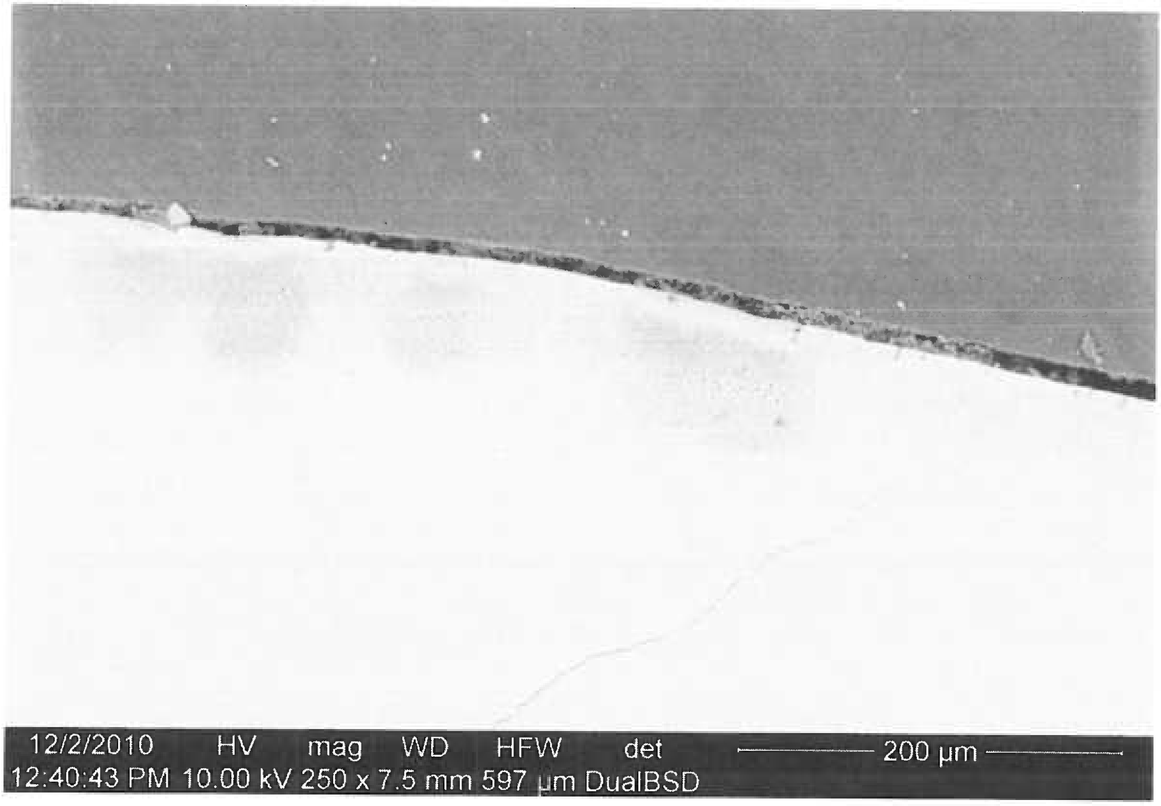
Figure 2

d



Indentations made at 100 µm from the enamel surface
Normalized Relative Microhardness
1.10
1.05
1.00
0.95
0.90
0.85
300
400
500
600
700
800
900
Distance from Bonding Agent (µm)
BAG-GIC
FO-LC
TB-XT

Figure 3



12/2/2010 HV mag WD HFW det
12:40:43 PM 10.00 kV 250 x 7.5 mm 597 μm DualBSD
200 μm

Tables

Table 1

Bonding Agent 1	Bonding Agent 2	Depth (µm)	Observed Demineralization	P -value
BAG-GIC	FO-LC	25	BAG-GIC < FO-LC	<.0001
BAG-GIC	TB-XT	25	BAG-GIC < TB-XT	<.0001
FO-LC	TB-XT	25	FO-LC < TB-XT	<.0001
BAG-GIC	FO-LC	50	BAG-GIC = FO-LC	0.2770
BAG-GIC	TB-XT	50	BAG-GIC < TB-XT	<.0001
FO-LC	TB-XT	50	FO-LC < TB-XT	<.0001
BAG-GIC	FO-LC	75	BAG-GIC < FO-LC	0.0181
BAG-GIC	TB-XT	75	BAG-GIC < TB-XT	<.0001
FO-LC	TB-XT	75	FO-LC < TB-XT	<.0001
BAG-GIC	FO-LC	100	BAG-GIC = FO-LC	0.3702
BAG-GIC	TB-XT	100	BAG-GIC = TB-XT	0.3510
FO-LC	TB-XT	100	FO-LC = TB-XT	0.9668

Introduction

White spot lesions are a common consequence of orthodontic treatment. They are the first visible sign of the process of demineralization, and the precursor to carious lesions. They are troublesome, as they often occur on the labial surface of maxillary anterior teeth [Gorelick et al., 1982; Mizrahi, 1983]. They may regresss naturally after bracket removal, but rarely disappear altogether [Øgaard and ten Bosch, 1994], leading to esthetic consequences and restorative concerns for the post-orthodontic patient [Øgaard et al., 1989]. Since white spot lesions have potential esthetic consequences, their development should be of great concern to every orthodontist, as patients seeking orthodontic treatment often desire esthetics results.

Prevalence of WSL's

Early studies investigated the overall caries experience of orthodontic patients and found that although the total number of carious lesions did not differ significantly from an untreated population, a greater proportion of buccal and lingual lesions were detected [Wisth and Nord, 1977]. Since then, many studies have been performed, providing a wide range of prevalence from 2-96%. This large variation is due to several factors, including: whether idiopathic enamel lucencies were included or excluded, the variety of methods used to assess and score the presence of decalicification, and the use or otherwise of a fluoride regime during treatment [Mitchell, 1992]. Some studies are longitudinal, and some studies are cross sectional. In a cross sectional study design (post-orthodontic patients compared to patients who have not had orthodontics), the prevalence quoted is artificially increased since it is difficult to distinguish between idiopathic white spots and decalicification [Mitchell, 1992]. Using this example, it is easy to see how study design can greatly influence the reported prevalence of white spot lesions.

The teeth which are cited as being most commonly affected vary between studies. Early studies involving patients whose treatment involved bonded appliances (as opposed to full banded appliances) showed that maxillary incisors and mandibular first molars had the highest prevalence [Gorelick et al., 1982]. Another study by Mizrahi, 1983 not only named the most commonly affected teeth, but also the location within those teeth. This study reported that maxillary incisors and first molars were most commonly affected, with opacities found particularly on the cervical and middle thirds of the affected teeth. In contrast, other studies have shown that the first permanent molars in both arches have the highest prevalence [Øgaard et al., 1989], or that the maxillary lateral incisors, canines, and mandibular premolars have the highest prevalence [Geiger et al., 1988]. All of these varying reports within the literature of white spot lesion prevalence and location exist because of a lack of standard detection protocol.

Etiology of WSL's

Oral Hygiene

The carious process depends on the presence of bacteria and sugar in high enough concentrations to be in the immediate proximity to enamel for sufficient amounts of time [Murray, 1989]. Orthodontic patients have several factors which predispose them to demineralization by influencing the conditions listed above. Studies have found that plaque build-up is more common in the presence of orthodontic attachments, as these attachments can make physical plaque disruption more difficult [Ciancio et al., 1985]. This study also found that the plaque build up was most prevalent between the orthodontic attachment and the gingival margin, which correlates with the location of enamel opacities stated by Mizrahi in 1983.

The area between the orthodontic attachment and the gingival margin, which has been described as retentive for plaque [Lundström and Krasse, 1987], creates a localized environment which favors the colonization of bacteria which are associated with the initiation and development of caries, namely lactobacilli and *Streptococcus mutans* [Leverett et al., 1993]. This layer of plaque not only harbors the bacteria, but it also provides a physical barrier, preventing the diffusion of acid away from the enamel and the infusion of calcium and phosphate for remineralization of the enamel [Saloum and Sondhi, 1987]. Since orthodontic patients have more areas which are retentive for plaque, their oral hygiene is even more important for the prevention of white spot lesion development.

Diet

The frequency and total amount of ingested fermentable carbohydrates plays a key role in the development of caries. Frequent consumption of sugary foods or drinks has been shown to be the most damaging, as following sugar intake the pH of plaque drops below the critical level of 5.5 for about 20 minutes [Mitchell 1992]. Fixed appliances restrict the ability of the tongue and saliva to wash away and dislodge food debris, which naturally removes the carbohydrates from the oral environment. As result, patients are advised to consume more fruits, vegetables, and starchy "staple" foods such as grains, bread and potatoes and to minimize their intake of foods and drinks containing sugar to decrease the incidence of caries [Moynihan, 2002].

Appliance Design

It is generally thought that increasing the size of the orthodontic appliance makes it proportionally more difficult for the patient to remove plaque from the remaining tooth enamel. This is not necessarily true, however, for orthodontic bands. If well cemented, orthodontic bands have a protective effect on the enamel and a study by Gorelick et al., 1982 found that there was no difference in white spot prevalence between banded or bonded teeth. Orthodontic bands are only are problematic if the band luting fails, leaving leaky margins which make the enamel beneath the band susceptible to extensive demineralization [Zachrisson, 1975]. It should be noted, however, that orthodontic bands favor plaque accumulation adjacent to the gingival margin [Ciancio et al., 1985], which often results in gingival inflammation [Boyd and Baumrind, 1992], and therefore orthodontic bonding instead of banding appears to be a safer alternative when treating patients with periodontal concerns [Gkantidis et al., 2010].

There is conflicting information published regarding plaque accumulation differences between self-ligating brackets and elastomeric ligatures. Self-ligating brackets or wire ligatures are considered preferable by several studies, as they do not favor plaque accumulation [Pellegrini et al., 2009; Türkkahraman, 2005; Alves de Souza et al., 2008]. However, a comparative clinical study on 100 subjects did not find a significant difference concerning plaque accumulation and periodontal parameters between conventional brackets with elastomeric ligatures and self-ligating brackets [Pandis et al., 2008], and a long-term follow up study using the same patient pool of Pellegrini et al showed no significant differences in measured plaque and bacteria [Buck et al., 2009]. These studies all show that further research needs to be published regarding the difference in plaque accumulation between self-ligating appliances or steel ligatures and elastomeric ligatures.

There are a few other orthodontic appliances factors which may increase the prevalence of white spot lesions. Special consideration regarding the patient's oral hygiene practices and caries risk should be taken into account if using large auxillary appliances or looped archwires, as they contribute to the accumulation of plaque and make removal of food debris more difficult for the patient [Mitchell, 1992]. Lastly, adhesive "flash" around a bonded orthodontic attachment can leave a rough surface which will predispose to plaque accumulation, and therefore any excess should be cleared away before the adhesive cures [Gwinnett and Ceen, 1979].

Prevention of WSL's

Oral Hygiene

Initial orthodontic screenings usually include an evaluation of oral hygiene, and orthodontists often insist that their patients attain a certain level of cleanliness before beginning orthodontic treatment. But, it is foreseeable that some patients will lose motivation over the course of treatment and that home care habits may worsen over time. Oral hygiene instructions throughout the course of treatment is effective in reducing the amount of decalcification [Artun and Brobakken, 1986], and this may also help improve upon any errors in the patient's technique [Lundström et al., 1980], but giving the patient constant instructions can be costly and time-consuming for the practitioner.

Diet

It has been suggested to have patients limit or reduce the frequency of ingesting readily fermentable carbohydrates, or to possibly substitute for them with sweeteners which are non-cariogenic, as this will reduce the likelihood of demineralization [Moynihan, 2002]. This method, along with frequent oral hygiene instruction, relies upon the patient cooperation and will not be successful with poor compliance.

Chlorhexidine

Chlorhexidine may be effective non-toxic rinse for chemical plaque removal, in that it is capable of reducing the oral flora by 99.9% without upsetting the balance of the oral microflora [Hogg, 1990]. Chlorhexidine's effectiveness is due to its absorption into the acquired pellicle, which prolongs its effectiveness intraorally [Hogg, 1990]. But, its biggest disadvantage is the deposition of a brown stain during prolonged use. The staining is easily removed at the end of orthodontic therapy [Brightman et al., 1991], but this often excludes its use in orthodontic patients as they are undergoing long term treatment.

Sealants

Another approach to prevent excessive demineralization adjacent to brackets is to apply a protective coating to the adjacent enamel, thus sheltering it from the external acidic environment. Painting a sealant resin around orthodontic brackets has been suggested, but oxygen inhibition or polymerization limits the enamel protection [Saloum and Sondhi, 1987], as the early unfilled or lightly filled resins were unable to withstand the mechanical and chemical forces of the oral environment. Improvements have been made to the resin sealants, and recently a highly filled sealant (Pro Seal) was shown to exhibit significantly less demineralization than untreated teeth, etched teeth, and teeth treated with an unfilled sealant or a fluoride varnish [Hu and Featherstone, 2005]. This in vitro study shows potential, but Pro Seal needs further assessment in vivo to demonstrate its effectiveness.

Fluoride

The use of fluoride has been well-documented with its ability to aid in enamel remineralization. Fluoride actively substitutes for hydroxyl ions of the enamel crystal during periods of remineralization, forming fluorapatite. Fluorapatite is less soluble than hydroxyapatite, and the incorporated fluoride provides a protective "veneer" on the enamel surface [Featherstone, 2000]. Additionally, fluoride has been shown to directly inhibit bacterial metabolism and glycolysis if in great enough concentrations [ten Cate and Featherstone, 1991;

Levine, 1991]. Knowing these characteristics, researchers have explored several different methods to deliver fluoride intraorally.

The majority of patients will successfully prevent demineralization using fluoridated toothpaste, but daily mouth rinsing with 0.5% sodium fluoride solution has been shown to be effective at reducing the prevalence of decalicification during orthodontic treatment in high risk patients [Zachrisson, 1975; Saloum and Sondhi, 1987]. Carlos [1985] showed that stannous fluoride and acidulated phosphate fluoride are no more effective than sodium fluoride. Still, the proper method of sodium fluoride delivery needs to be studied with further research [Benson et al., 2004], and the major drawback of any fluoride topical or mouthrinse is that it is dependent on patient compliance, which may be unpredictable [Geiger, 1992; Øgaard et al., 1988].

To combat noncompliant patients, fluoride may be applied professionally. There are conflicting reports about the affects on bond strength if fluoride is applied during the bonding procedure. Low et al [1975] found that the bond strength between fissure sealant and resin was reduced following fluoride treatment prior to etching, but later studies found that using the same technique prior to bonding orthodontic appliances had no significant effect on bond strength [Wang and Sheen, 1991; Bishara et al., 1989]. When applied prior to orthodontic banding, one in vivo study has shown that well-fitting bands show significantly less demineralization on the underlying enamel [Adriaens et al., 1990].

Calcium and Phosphate

Enamel is exposed to acidic environments below the critical pH several times daily, as the oral environment is constantly changing between periods of demineralization and remineralization. As pH lowers, the enamel crystals composed of carbonated hydroxyapatite

[LeGeros, 1991] begin to partially dissolve. Calcium and phosphate diffuse out of the tooth, leading eventually to cavitation if the process continues.

If localized in great enough concentrations, calcium and phosphate diffuse into the tooth and are able to deposit onto the surface of the crystal remnants in the non-cavitated lesion. Also, fluoride will be incorporated into the tooth surface if available during this process of remineralization, and the resultant tooth surface will be more acid resistant [Featherstone, 2004]. This is important for several reasons, and a detailed representation of enamel dissolution is presented below:

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

[Dawes, 2003]

First, calcium and phosphate are basic building blocks of the hydroxyapatite crystal. Fluoride may substitute for hydroxyl ions if present, but calcium and phosphate are already innately within the hydroxyapatite structure, and therefore remineralization is directly dependent on their localized concentrations. Secondly, since the oral cavity is in a constant flux between environments suitable for demineralization and remineralization, the process of demineralization occurs many times daily. This shows that a supply of calcium and phosphate is needed constantly, and also explains why it is found in saliva [Featherstone, 2000].

Ion-Releasing Bonding Materials

For orthodontic patients, the tendency to harbor acidogenic bacteria is most prevalent in the surrounding edges of brackets and bands, as this is the area in which there is a rough surface and plaque can accumulate in greater amounts [O'Reilly and Featherstone, 1987]. For this reason, it may be advantageous to use the orthodontic bonding adhesive as a source for the ions utilized in remineralization.

Glass Ionomer

Glass ionomer (GI) cements were originally introduced in the realy 1970's [Wilson and Kent, 1972], and they have a well-documented ability to release fluoride thereby enhancing remineralization [Benelli et al., 1993; Hallgren et al., 1992; Hallgren et al., 1993]. Although an in vitro study indicated that GI cement protected the enamel beneath and also 1 mm around an orthodontic attachment from decalcification [Valk and Davidson, 1987], a recent Cochrane Review [Benson et al., 2004] stated that there was weak evidence that cementing brackets with fluoride-releasing GI can reduce white spot severity. This is likely due to the fact remineralization is concentration dependent, and that GI releases its highest concentration of fluoride on the first day, then sharply decreasing on the second day before gradually decreasing to undetectable levels [Sonis and Snell, 1989; Basdra et al., 1996]. Also, GI has been suggested as more appropriate for banding as opposed to bonding, since it has relatively low bond strength [Saito et al, 1999; Staley et al, 2004; Cook, 1990 all; Cook and Youngson, 1988].

Resin Modified Glass Ionomer

With the addition of 10-20% resin monomers to traditional glass ionomers, resin modified glass ionomer (RMGI) cements have improved physical properties while maintaining fluoride releasing capability [Diaz-Arnold et al., 1999]. Resin monomers within the RMGI are able to penetrate into the rough outer enamel surface, creating a micromechanical bond to the tooth, which is why RMGI cements provide adequate bond strength to withstand orthodontic forces [Lippitz et al., 1998]. However, similarly to GI cements, there has been conflicting results concerning the anticariogenic effects of RMGI's [Gaworski et al., 1999; Papagiannoulis et al., 2002; Chung et al., 1998; Gorton and Featherstone, 2003; Pascotto et al., 2004; Czochrowska et al., 1998].

Bioactive Glass

Bioactive materials are those that are capable of chemically bonding to living tissue. They serve as a scaffold upon which the body can "anchor" new soft tissue, and serve as a source of minerals [Kokubo et al., 2003]. Bioactive glass (BAG) has biomimetic properties and when immersed in body fluids the resulting ionic reactions will lead to the formation of tooth-like hydroxylapatite that can even deposit on organic polymers [Mitchell, 2003; Forsback et al., 2004].

When immersed into a solution, the BAG content releases ions that interact with each other and with the ions present in the surrounding solution ultimately forming surface nucleation sites for Ca and PO_4 [Mitchell, 2003; Forsback et al., 2004]. Previous studies have shown that BAG is capable of releasing calcium and phosphate selectively at a low pH in much greater quantities than GI [Crowe et al., 2008], and this ability to selectively release additional ions gives BAG the possibility of superior prevention against enamel demineralization.

Amorphous Calcium Phosphate

Amorphous calcium phosphate (ACP) shows potential for use in orthodontics since, similarly to BAG, it has the ability to release calcium and phosphate. When comparing mechanical properties, however, ACP composites may have mechanical instability and inferior strength when compared to glass-reinforced composites [Skrtic et al., 2004], and evaluations of ACP composite shear bond strength have been conflicting. Two recent studies showed that ACP has significantly less shear bond strength than resin based (non-GI) adhesives. One study concluded that ACP had a "low, but satisfactory bond strength" since its bond strength was similar to a third RMGI adhesive [Foster, 2008]. A second study only compared ACP to a resin based (non-GI) adhesive and stated that brackets bonded with ACP failed at significantly lower levels [Dunn, 2007]. Also, ACP has also shown a significant decrease in bond strength when used as a lingual retainer adhesive [Uysal et al., 2009]. Finally, ACP has shown the ability to allow significantly less superficial demineralization than a conventional composite resin in vitro [Uysal et al., 2010]. These studies show that ACP may have future uses in orthodontics, but further studies need to be conducted to fully evaluate its mechanical properties.

pH Cycling

The legitimacy of pH cycling to evaluate carious lesion development has been amply demonstrated in previous studies [ten Cate and Duijsters, 1982; O'Reilley and Featherstone, 1987]. A pH cycling regimen including subjecting samples to 6 hours of demineralization followed by 18 hours of remineralization has also been shown to correlate well with conditions associated with 1 month of fixed orthodontic appliance use in vivo [Featherstone et al., 1986]. Six hours of daily exposure to a demineralization solution is intended to simulate the average amount of time a patient experiences an acid challenge, which creates enamel dissolution and the formation of WSL's adjacent to orthodontic appliances. This cycling method is preferred over simply immersing the samples into an acidic environment for a prolonged period of time because it provides the samples time to remineralize, and more closely replicates in vivo conditions [Featherstone et al., 1986].

Methods to Assess Demineralization

There are several different methods commonly used to evaluate enamel demineralization as it penetrates into enamel adjacent to orthodontic brackets. The following is a brief outline of three different methods which may be utilized.

Polarized Light

Polarized light is a visual method which measures differential light diffraction, which may be caused by either porosity change [Featherstone 1992] or by demineralization with subsequent alteration of enamel crystalline structure. It can be used as a measure of lesion depth and area of the zones of demineralization. However, the relative mineral change which results in the appearance of demineralization has not been well defined, making this is a good quantitative, but not qualitative evaluation [Featherstone 1992]. Several studies have used this method to measure the area or depth of lesion penetration into enamel [Vorhies et al., 1998; VanMiller and Donly, 2003; Donly et al., 1995], making this an effective visual method to analyze demineralization.

Transverse Microradiography

Transverse microradiography (TMR) is another visual method which can be used on either thin sections or specimen blocks. The primary parameter needed will be DELTA Z, or the volume percent mineral before and after treatment. Reference points for sound enamel and dentin will be needed in published reports, as well as detailed information to make the data the most meaningful (e.g. how many scans across how many lesions and at what position) [Featherstone 1992]. This method is similar to polarized light, in that they both are imaging methods which do not affect the sample in any way.

Cross Sectional Microhardness

Cross sectional microhardness (CSMH) can be used in the same way as TMR, and is well established for enamel studies. It can be used on enamel blocks as well as thin sections. Once again, all conditions used must be sated in the paper (e.g. load and duration), and indentations need to have reference points in sound enamel. [Featherstone 1992]. CSMH is not adequate to accurately define the exact margin of a lesion, as the measurement increments are too large (e.g. 25 microns). If this is desired, polarized light micrographs would be more appropriate.

Future Research

The BAG hybrid bonding agent formulation used in this study has been the subject of several recent studies, with the following results:

- Crowe et al [2008] demonstrated that it exhibits significantly more selective calcium and phosphate ion release at low pH than resin modified glass ionomer
- This BAG formulation shows no statistically significant difference in shear bond strength than a resin modified glass ionomer [Tüfekçi, personal correspondence]. This thesis will be completed in the near future and submitted for publication.

Further research involving this BAG formulation would likely involve in vivo clinical trials. Brackets may be bonded in vivo, and several study designs may be used (e.g. split-mouth), possibly looking for development of WSL's post-orthodontic treatment and evaluating the brackets for bond failure rates. Also, this formulation may have clinical application if examined as a band luting agent. Since band luting is a common use for glass ionomers in orthodontics, this may be a logical application for the BAG material used in this study.

Similar BAG-containing agents are also currently being studied at Oregon Health & Science University. Currently, BAG is being incorporated directly into a resin adhesive and studied for ion-release in a similar study design to Crowe, et al. The same BAG-resin adhesive is also being studied for potential changes in enamel microhardness, with a similar study design as used in this thesis. After it is tested for shear bond strength, this other formulation of BAG may prove to be valuable for bonding orthodontic brackets. But, clinical trials will need to be performed for the resin formulation of BAG as well.

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