A novel biomimetic orthodontic bonding agent for prevention of white spot lesions: An *in vitro* study of surface microhardness changes adjacent to orthodontic brackets

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A novel biomimetic orthodontic bonding agent for prevention of white spot lesions: An *in vitro* study of surface microhardness changes adjacent to orthodontic brackets

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Abstract

Objective: To compare changes in enamel microhardness adjacent to orthodontic brackets after using bonding agents containing various compositions of bioactive glass compared to a traditional resin, and following a simulated caries challenge.

Materials and Methods: Extracted human third molars (n=10 per group) had orthodontic brackets bonded using one of four novel bioactive glass (BAG)-containing orthodontic bonding agents (BAG-Bonds) or commercially available Transbond XT. The four new adhesives consisted of BAG in varying percentages incorporated into a traditional resin monomer. The teeth were cycled through low-pH demineralizing and physiologic-pH remineralizing solutions over a period of 14 days. Microhardness was measured on longitudinal sections of the teeth 100, 200, and 300 μm from the adhesive edge and beneath the brackets, at depths of 25 to 200 μm from the enamel surface. Normalized hardness values were compared using three-way analysis of variance.

Results: Significantly less reduction in enamel microhardness was found with the experimental adhesives at depths of 25 and 50 μm at all distances from the adhesive edge. In all groups there were no significant changes in enamel microhardness past 125 μm depth. Results varied with the different BAG-Bonds with 81BAG-Bond showing the smallest decrease in enamel microhardness.

Conclusions: The BAG-Bonds tested in this study showed a reduction in the amount of superficial enamel softening surrounding orthodontic brackets compared to a traditional bonding agent. The results suggest that clinically BAG-Bonds may aid in maintaining enamel surface hardness, therefore helping prevent white spot lesions adjacent to orthodontic brackets.

Introduction

Formation of incipient caries, commonly called white spot lesions (WSLs), is an unaesthetic, common side effect of orthodontic treatment with fixed appliances.[1] The WSL has been defined as a clinically detectable manifestation of subsurface enamel demineralization, representing the first stage of caries formation.[2] The lesion can demonstrate up to 50% reduction in enamel mineral loss.[1] This mineral loss changes the hardness and refractive index of the enamel, causing a scattering of light and giving the enamel a chalky, opaque appearance.[3] WSLs are both an esthetic concern and a disconcerting visible sign of enamel demineralization secondary to orthodontic treatment.

The prevalence of WSLs during orthodontic treatment is due to a variety of factors. First, fixed orthodontic appliances make oral hygiene more difficult, predisposing patients to an increase in plaque build-up on tooth surfaces at the gingival margin, and adjacent to attachments.[4] Second, the addition of orthodontic appliances within the mouth creates a rapid increase in bacterial flora, predominantly *Streptococcus mutans* and *Lactobacilli*.[5] This reduces the pH at the plaque/enamel interface, causing calcium and phosphate ions to migrate from enamel apatite into solution resulting in loss of mineral.[6] These factors create an environment that favors demineralization of enamel, compromising the esthetic result, and in severe cases, require restorative treatment.[7,8]

Preventing the development of WSLs during orthodontic treatment has been attempted through various approaches. Methods involving fluoride-containing mouth rinses, gels, varnishes and dentrifices, have been shown to reduce the prevalence of caries during orthodontic treatment, but compliance is unpredictable and the ability to supply the fluoride to areas where it is needed presents challenges.[9-11]

Conceptually, fluoride-releasing bonding agents have great potential to minimize decalcification around orthodontic brackets.[12] A limitation has been glass-ionomer cements (GICs) and resin-modified glass ionomer cements (RMGICs) having bond strengths that are substantially lower than those of conventional resins.[13,14] Moreover, with recently introduced amorphous calcium phosphate (ACP)-based remineralization materials, such as MI paste, clinical trials found insufficient evidence to make a recommendation regarding their long-term effectiveness.[15] A noncompliance-based material with sustained ion release near the brackets should be an ideal preventive solution for WSL formation.

Bioactive glass (BAG) materials have recently been incorporated into the field of dentistry, and are surface-active materials known to successfully release ions in simulated body fluid.[16] Sol-gel BAG is a three-dimensional cross-linked matrix of hydrolyzed alkoxides of SiO_2, CaO, and P_2O_5, that can leach ions such as calcium, phosphate, and fluoride.[17,18] This release of ions into surrounding solution is a process that has the potential for preventing demineralization of enamel, and thereby preventing WSLs surrounding orthodontic brackets.

Previous *in vitro* investigations of ion release from the novel BAG-containing orthodontic resin bonding agents (BAG-Bonds) used in this study demonstrated significantly higher calcium and phosphate ion levels in the media under acidic (cariogenic) conditions than from a conventional resin adhesive control.[19] The capacity of BAG-Bonds to release ions and buffer an acidic environment may help to prevent demineralization surrounding orthodontic brackets.

The aims of this study were (1) to evaluate the *in-vitro* ability of novel BAG-Bonds to inhibit superficial enamel demineralization surrounding orthodontic brackets after being exposed to an artificial caries challenge, and (2) to test the hypothesis that these novel adhesives will

demonstrate less demineralization surrounding orthodontic brackets compared to a conventional bonding resin.

Materials and Methods

Preparation of BAG-Bond

Four BAG-Bonds (62BAG-Bond, 65BAG-Bond, 81BAG-Bond, and 85BAG-Bond) were developed in our laboratory (Table 1). The BAG samples were prepared by mixing two resin monomers with compositions of bioactive glass, an accelerator and an amine, as previously described.[19] BAGs were synthesized by sol-gel methods,[17] and were added to the monomer mixture until the workability of each product was similar to the control material, Transbond XT (3M Unitek, Monrovia, CA), as judged by an experienced clinician.

Sample Preparation

Fifty extracted, caries-free human 3rd molar teeth were collected and stored in 0.5% chloramine-T solution at 4°C. The experimental teeth were examined to verify they were free of white spot lesions or significant enamel defects. All teeth were rinsed with deionized water and randomly assigned to one of five groups (n = 10 for each group), as shown in Table 1.

The enamel was cleaned with a rubber prophylaxis cup at slow speed using a mixture of non-fluoridated pumice and water. For each tooth, the enamel bonding site was based on the best visual adaptation of the bracket pad. To cover and protect adjacent enamel surfaces during the etching procedure, tape with a window the size of the bracket base removed was applied to each tooth.[12]

The window region was etched with 37% phosphoric acid gel (3M Unitek) for 30 seconds, and copiously rinsed with deionized water. The tape was removed and the tooth was dried with compressed air.

After application of primer (Transbond XT Primer, 3M Unitek), enough of the selected resin adhesive to cover the entire bracket base surface was applied to the mesh pad of each

bracket (Victory Series™, 3M Unitek). Brackets were oriented perpendicular to a tooth's long axis, 4 mm from the cusp tip to the middle of the bracket slot, and excess primer and resin was removed with a sharp scaler. The adhesive was light-cured for 20 seconds from the mesial and distal sides with an Ortholux LED curing light (3M Unitek). Acid-resistant varnish was applied to each tooth, leaving a 1mm-rim of exposed enamel surrounding the bracket. All teeth were stored overnight in distilled water prior to pH cycling.

In vitro pH Cycling

A 14 day *in-vitro* caries challenge was followed, based on a modification of a pH cycling protocol originally described by Toda and Featherstone.[20] Each individual tooth was immersed in 40 mL of artificial saliva, pH 7.0 (40 mL of 1.5mmol/L Ca, 0.9 mmol/L PO4, 0.1 5mol/L KCl, and 20 mmol/L cacodylate buffer) for 18 hours, followed by 6 hours in 40 mL of a buffered artificial caries challenge solution, pH 4.4 (40 mL of 2.0 mmol/L Ca, 2.0 mmol/L PO_4, 0.075 mol/L acetate). Fresh solutions were prepared each week. Between each fluid change, teeth were rinsed with deionized water. The cycle was repeated 5 days a week, with teeth remaining in mineralizing solution during weekends.

Assessment of Demineralization

Each specimen was individually embedded in clear epoxy resin (Epoxicure, Buehler, Lakebluff, IL), and sectioned parallel to the long axis, buccolingually through the brackets using a water-cooled diamond wafering blade on a low speed rotary saw, producing one 2mm section for each tooth (Accutom-5, Struers Inc., Westlake, OH). Sections were serially polished through 4000 grit silicon carbide polishing paper.

Knoop microhardness testing (Duramin 5, Struers Inc., Westlake, OH) was used for the microhardness analysis (25p/5sec). For each tooth, 48 indentations were made occlusal and cervical to the adhesive—located 100, 200, and 300 μm from the adhesive edge, and at 8 depths from the external surface of the enamel: 25, 50, 75, 100, 125, 150, 175, and 200 μm. (Figure 1). Three sets of indentations were also made in the isolated enamel directly beneath the bracket at the same 8 depths, in order to create a baseline microhardness measurement at each depth. For each tooth, the three Knoop Hardness values (KHN) obtained at each depth beneath the bracket were averaged and used to normalize the other data points by dividing them by the corresponding average KHN at each depth. Data are presented as a percent change in microhardness.

Statistical Analysis

Three-way analysis of variance (ANOVA) with Tukey post-hoc test (SAS, SAS, Cary, NC) compared the percentage change in microhardness versus distance and depth, (α=0.05.)

Results

Locations that showed significant change in enamel microhardness for each adhesive are shown in Figure 2. Enamel with 81BAG-Bond group was significantly softened at a depth of 25 μm from the surface at all 3 distances from the adhesive edge, and at a depth of 50 μm at distances of 100 and 300 μm. The 62BAG-Bond and 85BAG-Bond groups both showed similar results, with significant reductions in hardness occurring to 75μm from the surface at all 3 distances, as well as to 100 μm deep, 100 μm from the adhesive. 65BAG-Bond and Transbond XT groups had reductions in enamel hardness that extended the deepest, with significant softening occurring at depths between 100 and 150 μm .

Comparison of changes in microhardness values for each group at the recorded location across the WSL are shown in Figure 3 and Table 2. At depths of 25 and 50 μm, all four BAG-Bond adhesives showed significantly less reduction in hardness than Transbond XT at all distances from the adhesive edge ($p<0.05$).

Results varied for the intermediate depths up to 125 μm. Beyond 125 μm depth and at all distances, no significant differences in enamel microhardness were found among the five adhesive groups.

Results comparing the four different BAG-Bond adhesive groups to each other, at all 3 distances and at depths up to 125μm are shown in Table 3. At 100 μm from the adhesive edge and at depths of 25, 50, and 75 μm, 81BAG-Bond demonstrated less reduction in enamel microhardness than 62BAG-Bond and 65BAG-Bond.

Discussion

WSLs form in superficial enamel due to outward diffusion of calcium and phosphate ions, eventually leading to cavitation if the process continues.[22] Our bioactive glass-containing adhesives have previously shown calcium ion release into surrounding solution.[19] This study *in-vitro* evaluated the potential use of these novel BAG-containing orthodontic bonding agents for the prevention of WSLs. Four different BAG-Bond adhesive resins were used to bond orthodontic brackets to extracted human teeth, and were pH cycled through an artificial caries challenge that has been shown to correlate well with conditions associated with 1 month of fixed orthodontic appliance use *in-vivo*.[23] Comparisons were made to the popular, commercially available adhesive, Transbond XT.

Demineralization around orthodontic brackets can be assessed using various methods. In this study, areas of mineral loss due to acidic challenge were evaluated using cross-sectional microhardness values. This method can be correlated to caries because there exists a strong correlation ($r = 0.91$) between enamel microhardness and the percentage of mineral in the caries lesion.[21] Since WSLs are areas of low mineral content, microhardness measurements provide a reliable indication of enamel demineralization and potential WSL development.

This study used an *in-vitro* pH cycling model for the evaluation of demineralization surrounding novel BAG-containing adhesives. This widely used protocol for exposing enamel to combinations of demineralization and remineralization is designed to mimic the dynamics of mineral loss and gain involved in caries formation.[24] However, this *in-vitro* protocol has important limitations, as it is not able to completely simulate the complex intraoral conditions leading to caries development such as bacterial biofilms and saliva, nor is it able to simulate clearance of the products from the oral cavity.[25] These limitations should be kept in mind when evaluating the results of this study. Nonetheless, previous *in-vitro* investigations of an

experimental glass ionomer cement containing BAG have found the bonding agent inhibited growth of the cariogenic bacteria, *Streptococcus mutans*.[26] Thus this anticariogenic effect may also contribute to BAG's potential for preventing WSLs.

Teeth in the 81BAG-Bond group were significantly demineralized only to depths of 25 and 50 μm. 81BAG-Bond contains the highest surface area of bioactive glass at 320 m^2/g (Table 1). Previous studies have shown that bioactive glass materials with high-specific surface area and pore volume contribute to a high release of ions.[27] BAG's ability to be a reservoir of available ions held by ionic rather than covalent bonds allows for easier release of calcium to the surroundings than from the tooth surface. In addition to its higher surface area, 81BAG-Bond also contains fluoride. Released fluoride ions may become incorporated in the calcium-phosphate precipitate or tooth mineral as a precursor to highly insoluble fluoroapatite.[19] In the current study, 81BAG-Bond demonstrated a superior ability to prevent demineralization, perhaps due to an increase release of ions and a higher bioactivity rate when compared to the other BAG-Bond resins.

Teeth bonded with 62BAG-Bond and with 85BAG-Bond displayed significant demineralization from 25 to 75 μm deep at all 3 distances from the adhesive edge, and at 100 μm deep at 100 μm distant from the adhesive edge. 62BAG-Bond contains fluoride and 31% mol calcium, yet has the lowest surface area of bioactive glass ($75m^2/g$). 85BAG-Bond contains no fluoride and only 11% mol calcium, yet has the second highest surface area of BAG ($268m^2/g$). Seemingly, these two BAG-Bond resins do not have the combination of ideal properties to allow for maximal demineralization prevention—as seen with 81BAG-Bond. While ion release increases with increased surface area, the incorporation of fluoride in the BAG-Bond may also be valuable for maximal WSL prevention.

Teeth in both the 65BAG-Bond and Transbond XT groups demonstrated significant demineralization from depths of 25 to 125 μm at all distances. Possible explanations for these findings are that although 65BAG-Bond has a high content of calcium, it contains no fluoride and has the second lowest surface area of all the BAG-Bond resins. Transbond XT is a non ion-releasing conventional composite resin with no detectable fluoride release, and in the absence of daily fluoride exposure, it has been associated with extensive erosive enamel lesions.[28] Thus combining the results of all groups indicates that key factors for combating demineralization include the presence of fluoride and increased BAG-Bond surface area, whereas varying the calcium content has less impact.

Microhardness tests for the five adhesives revealed that the greatest decrease in enamel microhardness occurred at the most superficial depths of 25 and 50 μm. The 4 BAG-Bond adhesives demonstrated significantly less demineralization than the Transbond XT control at depths of 25 and 50 μm for all distances from the adhesive. This result is encouraging as research has shown that preventative measures are most effective when the WSL formation is in earliest stages as remineralization can take place if the lesions are less than 65 μm in depth.[29] Investigators have also demonstrated that a WSL after 3 months *in-vivo* typically extends to around 100 μm deep from the enamel surface.[1] Since the novel BAG-containing adhesives in this study allowed for decreased demineralization at these superficial depths, these adhesives hold potential for helping to precent WSL formation within the boundaries of what occurs *in-vivo*.

At the middle depths of 75, 100, and 125 μm from the enamel surface, results varied. At 75 μm depth and 100 and 200 μm away from the adhesive, and 125 μm depth at 200 and 300 μm from the adhesive, all teeth within the BAG-Bond groups had less demineralization than the

control. These results indicate that the WSLs created by the pH cycling protocol were most likely shallow lesions in their initial stages.

At 125 μm depth and deeper, all 5 groups showed no significant difference. These results indicate that the enamel at these depths remained largely unaffected by the demineralization challenge, most likely due to the brevity of the acidic challenge.

Brown et al[19] investigated the ion release profiles of each of the novel BAG-Bonds tested in this study. The results indicated that BAG-Bonds have the potential for decreasing the rate of enamel demineralization by increasing the pH in the milieu adjacent to the bracket/tooth interface, as well as by releasing a large amount of calcium ions into solution.[19] These results, consistent with the current findings, suggest that incorporating BAG into resin adhesives provides a reservoir of calcium, phosphate, and even fluoride ions, that may help to prevent enamel demineralization surrounding orthodontic brackets during treatment.

Conclusions

All BAG-Bond novel adhesives outperformed Transbond XT at maintaining superficial enamel hardness surrounding orthodontic brackets.

81BAG-Bond, a formulation that contained fluoride and had relatively high BAG surface area, had the smallest change in enamel microhardness values compared to the other novel adhesives. Combining an ideal amount of bioactive glass into resin adhesives may help to reduce the amount of superficial enamel softening surrounding orthodontic brackets compared to a conventional resin adhesive system.

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Figure Legends

Figure 1. Location of microhardness indentations demonstrating distances from adhesive edge and depths into enamel. "UB" indicates the 3 series of indents taken under the bracket, where the results served as a normalization control for data measurements from each tooth.

Figure 2. Square symbols indicate locations where significant reductions in enamel microhardness were found relative to microhardess measured under the bracket bases ($p \leq .01$).

Figure 3. Mean and standard error of percent change in enamel microhardness for the five resin groups measured at 100 μm, 200 μm, and 300 μm from the adhesive edge. ($p \leq .05$).

Figure 1.

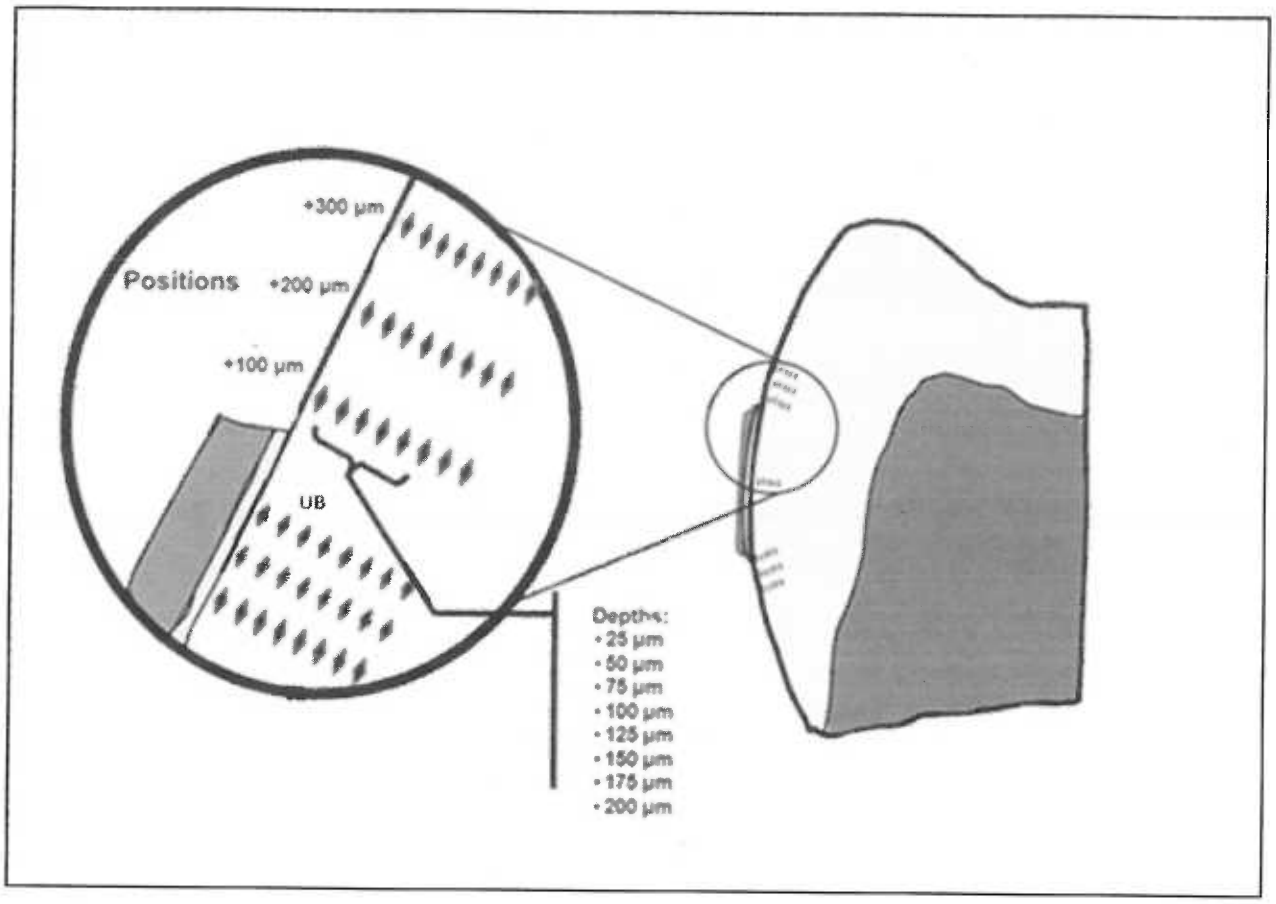
+300 µm
Positions
+200 µm
+100 µm
UB
Depths:
• 25 µm
• 50 µm
• 75 µm
• 100 µm
• 125 µm
• 150 µm
• 175 µm
• 200 µm

Figure 2.

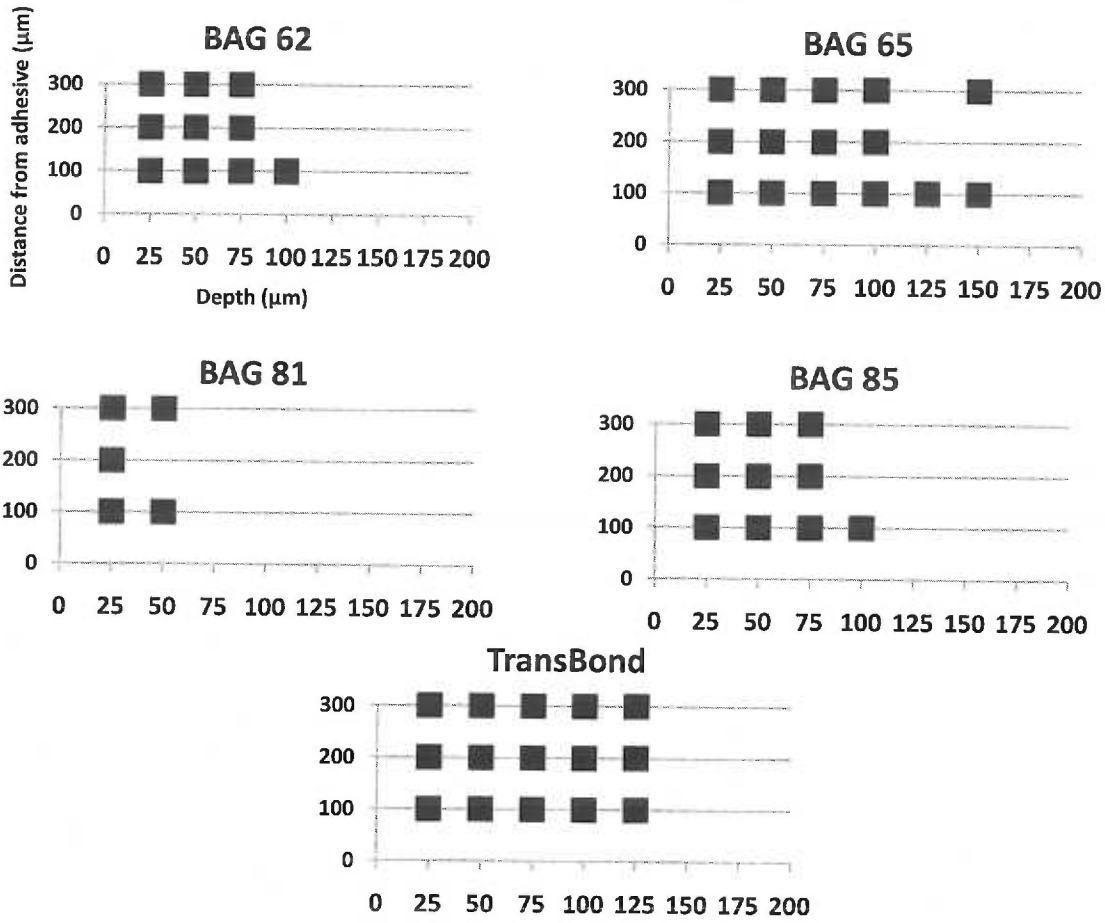
BAG 62
Distance from adhesive (µm)
300
200
100
0
0 25 50 75 100 125 150 175 200
Depth (µm)
BAG 65
300
200
100
0
0 25 50 75 100 125 150 175 200
BAG 81
300
200
100
0
0 25 50 75 100 125 150 175 200
BAG 85
300
200
100
0
0 25 50 75 100 125 150 175 200
TransBond
300
200
100
0
0 25 50 75 100 125 150 175 200

Figure 3.

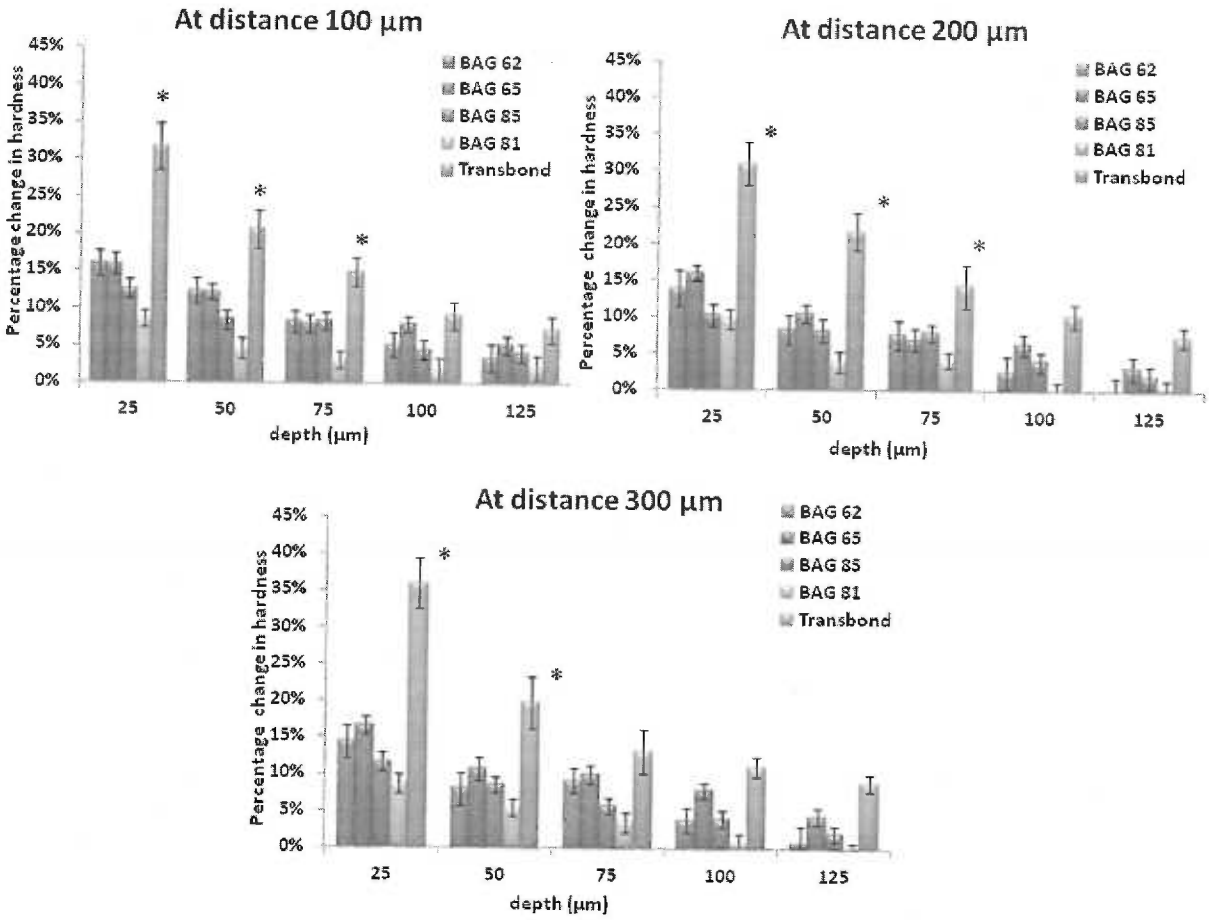
At distance 100 µm
Percentage change in hardness
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
25
50
75
100
125
depth (µm)
BAG 62
BAG 65
BAG 85
BAG 81
Transbond
*
At distance 200 µm
Percentage change in hardness
depth (µm)
At distance 300 µm
Percentage change in hardness
depth (µm)

Table 1. **BAG Composition: Molar %, Surface Area and Monomer ratio**

Adhesive Name	mol% SiO_2	mol% CaO	mol% P_2O_5	mol% B_2O_3	mol% F	Surface Area of BAG (m^2/g)	BAG:Monomer ratio in bond (by weight)
62BAG-Bond	62	31	4	1	3	75	58:100
65BAG-Bond	65	31	4	0	0	144	49:100
81BAG-Bond	81	11	4	1	3	320	37:100
85BAG-Bond	85	11	4	0	0	268	33:100
Transbond XT	--	--	--	--	--	n/a	n/a

Table 2. **Comparison of significant decreases in microharndess (p≤0.05 for BAG-Bond groups compared to the Transbond XT (TB) group at depths up to 125 μm.**

Distance from Adhesive Edge(μm)	Depth from Enamel Surface (μm)	Observed Enamel Softening
100	25	All BAGs < TB
	50	All BAGs < TB
	75	All BAGs < TB
	100	81, 85BAG < TB
	125	81BAG < TB
200	25	All BAGs < TB
	50	All BAGs < TB
	75	All BAGs < TB
	100	62, 81, 85BAG < TB
	125	All BAGs < TB
300	25	All BAGs < TB
	50	All BAGs < TB
	75	81, 85 BAG < TB
	100	62, 81, 85BAG < TB
	125	All BAGs < TB

Table 3. **Comparison of significant changes in microhardness ($p \leq 0.05$) among BAG-Bond groups at depths up to 125 µm.**

Distance from Adhesive Edge (µm)	Depth from Enamel Surface (µm)	Observed Enamel Softening
100	25	81BAG < 62, 65BAG
	50	81BAG < 62, 65BAG
	75	81BAG < 62, 65, 85BAG
	100	81BAG < 65BAG
	125	No significant difference
200	25	81, 85BAG < 65BAG
	50	81BAG < 65BAG
	75	No significant difference
	100	81BAG < 65BAG
	125	No significant difference
300	25	81BAG < 65BAG
	50	No significant difference
	75	81BAG < 62, 65BAG
	100	81, 85BAG < 65BAG
	125	81BAG < 65BAG

Literature Review

WHITE SPOT LESIONS

Background

Orthodontic treatment is an esthetic service provided to patients who wish for straighter teeth and a more pleasing smile. Anything that detracts from an esthetic end result is both troublesome and frustrating for the orthodontic practitioner. A very common consequence of orthodontic treatment is the development of white spot lesions (WSLs).

White spot lesions are clinically detectable manifestations of subsurface enamel demineralization.[1] It has been found that nearly half of all patients who receive fixed orthodontic appliance therapy experience WSLs in some capacity.[2] These incipient caries can appear in as few as four weeks following the placement of fixed orthodontic appliances.[1,2,3,4,5]

White spot lesions are due to poor oral hygiene and plaque retention around orthodontic appliances.[6] A combination of factors such as a diet high in fermentable carbohydrates, suboptimal oral hygiene, long intervals between appointments, and poor patient compliance with the use of fluoride toothpastes and mouth rinses contribute to the development of WSLs. A wide range of reported prevalence is due to several factors including whether idiopathic enamel lucencies were included, the variety of methods use to assess the presence of decalcification, and the use of a fluoride regime during treatment.[7] With reported prevalence of WSLs in orthodontic patients reaching as high as 95%,[3] a method for preventing WSLs without the need for compliance is imperative.

Previous research has shown that WSL mineral content can be reduced by as much as 10-50% compared to intact enamel apatite.[4,8] This mineral loss is attributed to the correlation between fixed orthodontic appliances, an increase in plaque accumulation at the enamel/bracket interface and an increase in cariogenic bacteria.[9,10,11] These cariogenic bacteria will then

metabolize carbohydrates from food breakdown, and will begin to release acid by-products. These by-products then reduce the pH at the plaque/enamel interface around the bracket, causing calcium and phosphate ions to diffuse out from enamel apatite into solution resulting in the loss of mineral content.[12]

A study that evaluated artificially induced white spot lesions in extracted premolars demonstrated that WSLs are subsurface enamel demineralization with an intact superficial layer.[13] The calcium and phosphate at the surface come from subsurface enamel layers. As the lesion forms, the outer surface of enamel remains mineral-rich, however its morphology is slightly more porous than sound unaffected enamel.[14] This pattern of demineralization creates an optical phenomenon,[3] which is why the lesion appears white to the eye. Demineralized enamel causes greater light refraction because it is more porous than intact enamel—hence the term white spot lesion.[15]

WSLs in orthodontic patients can be prevented by good compliance with a fluoride toothpaste in combination with a 0.05% sodium fluoride mouthrinse.[16] The key with all of these prevention measures is compliance, and without it, the tool is a weak preventive adjunct at best. A study which asked patients to decrease their caries risk with a daily fluoride mouth rinse showed a compliance rate of only 13%.[16] Among the subjects that did comply with a fluoride rinse at least every other day, they showed a reduction in carious lesions by 21% compared with those who were rinsing less frequently.[16]

Although WSLs may regress following bracket removal, they often persist, causing esthetic concerns.[3,17,18] White spot lesions may regress naturally after bracket removal, but rarely disappear altogether.[19] If left untreated, WSLs may progress into serious cavitations, requiring restoration. Because a high incidence of WSLs occurs on maxillary anterior teeth,[2,16,20] the

optimal esthetic result from orthodontic treatment may be largely compromised. Mizrahi's study in 1983 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. WSLs play a significant role in affecting the orthodontic end result, and often times is disappointing for both the patient and doctor alike.

Etiology

Multiple pathologic and protective factors can affect the rate and amount enamel demineralization. These factors include saliva, diet, plaque, type of enamel apatite, local ion concentrations and pH levels.[12] It is the delicate interplay between all of these factors that renders enamel at risk for demineralization. When the plaque at the plaque/enamel interface becomes under-saturated with ions present in enamel, apatite demineralization ensues.[21,22] Unfortunately, the practitioner cannot easily predict which patients will be at increased risk for development of white spot lesions. It has been shown that previous caries experience before orthodontic treatment is not a reliable indicator as to the susceptibility of individual patients to demineralization.[6]

Plaque

Research has shown that fixed orthodontic appliances predispose patients to an increase in plaque build-up on tooth surfaces at the gingival margin, around the attachments, and between attachments.[23] These appliances prevent the tongue from naturally and effectively removing material alba from around the teeth and also make it challenging for orthodontic patients to clean sufficiently around the appliances.[1] As a result of this, retained carbohydrates at the enamel surface and poor attention to plaque removal by the patient results in prolonged exposure to acid and encourages growth of aciduric bacteria.[1] The carious process depends on the presence of

bacteria and sugar in high enough concentrations to be in the immediate proximity to enamel for a sufficient amount of time.[24]

The area between the orthodontic attachment and the gingival margin, which has been described as retentive for plaque,[25] creates a localized environment which favors the colonization of bacteria that are associated with the initiation and development of caries, namely *Lactobacilli* and *Streptococcus mutans.*[26,27] High levels of *Streptococcus mutans* and/or *Lactobacilli* are associated with the initiation and development of enamel demineralization.[12,25] In conjunction with increased levels of plaque and increased cariogenic bacteria, the plaque in orthodontic patients has been found to have a lower resting pH than that in nonorthodontic subjects.[28] Since orthodontic patients have more areas that are retentive for plaque, their oral hygiene is even more important for the prevention of white spot lesions. An orthodontic bonding agent that is antimicrobial with the ability to buffer the acidic environment at the plaque/enamel interface would be beneficial.

Diet

Enamel demineralization and caries formation is directly related to the frequency and the amount of carbohydrates consumed. Oral bacteria, such as *Strep mutans* and *Lactobacilli*, exposed to fermentable carbohydrates are able to metabolize sugars into acids such as lactate or acetate. These acids can then decrease local pH at the enamel/plaque interface to levels below critical pH for prolonged periods of time.[29] If not mechanically removed, this cariogenic plaque in contact with the enamel surface results in enamel demineralization.[30,31]

Saliva

Saliva is one of the most important factors influencing enamel demineralization and remineralization.[1] Both the rate of salivary flow as well as the salivary contents themselves

influence the balance between mineral loss and gain at the enamel surface. Higher salivary flow rates increase saliva's buffering capacity and promote better clearance of bacteria and bacterial substrates,[1] due to the protective factors in saliva including minerals, proteins, and antibacterial components.[12] Subsequently, demineralization is more commonly seen where there is lower salivary flow rates and higher exposure to carbohydrates, such as is around the maxillary anterior teeth.[2]

Saliva contains key minerals, calcium and phosphate, which play a role in maintaining and protecting enamel. These ions influence the driving force for the precipitation or dissolution of enamel.[22] Anderson et al found that the amount of calcium in the saliva of children and adults differs significantly.[22] Compared to adults, children typically have less calcium in their saliva both at rest and during stimulation. Consequently, oral pH in children does not have to drop as much as in adults before demineralization occurs. In addition, the lack of calcium in children's saliva results in an increased driving force toward demineralization. These two factors put children at an increased risk for enamel demineralization compared to adults.[22] Since the majority of orthodontic patients are adolescents, these findings indicate that a product used during orthodontic treatment that releases calcium ions at the enamel/plaque interface would be particularly advantageous in this specific population of patients.

Enamel

Enamel is a carbonated hydroxylapatite represented by the following simplified formula: $Ca_{10-x}(Na)_x(PO_4)_{6-y}(CO_3)_z(OH)_{2-u}(F)_u$.[12, 32] As enamel is formed during tooth development, impurities such as carbonate and fluoride are commonly substituted into the hydroxylapatite crystal lattice. This incorporation of carbonate ions creates disruptions in the crystal lattice making it less stable than pure hydroxylapatite. There is a direct relationship between the

amount of carbonated hydroxylapatite in enamel and the rate of apatite dissolution.[33,34] Conversely, the replacement of hydroxide ions with fluoride ions results in a more stable, less soluble form of apatite, known as flurapatite.[12]

Intraoral Calcium and Phosphate

The precipitation and dissolution of hydroxylapatite can be described by the following reaction:[21]

$$\underset{\text{Solid}}{Ca_{10}(PO_4)_6(OH)_2} \rightleftarrows \underset{\text{Solution}}{10Ca^{2+} + 6PO_4^{3-} + 2OH^-}$$

Precipitation ⇆ Dissolution

The rate of demineralization is affected by pH, the concentration and type of acid present and the degree of saturation of ions in the demineralizing solution.[35] An increase in concentrations of calcium and phosphate ions drives the above reaction to the left, favoring precipitation or remineralization of hydroxylapatite. On the contrary, a drop in local calcium and phosphate concentrations drives the reaction towards dissolution or demineralization of enamel apatite. Very small differences in plaque ion concentrations may result in large differences in the enamel demineralization rates due to local ion concentration imbalances.[35] It should be noted that the surface layer must remain intact for remineralization to take place, therefore the resistance of the lesion to mechanical deformations is an important parameter.[36] As long as the surface layer remains intact and saliva is saturated with calcium and phosphate ions, acid buffering agents such as bicarbonate and phosphate ions can diffuse into plaque, neutralize acids, and remineralize the enamel lesions.[37] An orthodontic bonding agent that would provide bioavailable calcium and phosphate ions at the plaque/enamel interface to drive the reaction towards remineralization, such as the proposed bioactive glass, should help prevent enamel dissolution in cariogenic environments.

Critical pH

The thermodynamic driving force for demineralization and remineralization is also a function of pH. The "critical pH" is the pH at which the enamel is just saturated with respect to hydroxylapatite. A pH of 5.5 is the accepted critical pH for hydroxylapatite.[38] As pH decreases, the enamel crystals composed of carbonated hydroxylapatite begin to partially dissolve.[39] Since the oral environment is constantly changing, enamel is exposed to acidic environments below the critical pH several times daily. A decrease in pH below the critical pH results in undersaturation of calcium and phosphate triggering dissolution of enamel minerals until saturation is re-established in solution. Critical pH is not a fixed value because it depends on other factors such as the concentration of calcium and phosphate in the local environment adjacent to enamel, not just in free saliva at large.[22] A higher local concentration of calcium and phosphate ions will lower the critical pH, meaning oral pH values have to decrease further before enamel dissolves.[21] An orthodontic bonding agent that provided a source of calcium and phosphate ions around the bracket/enamel/plaque interface to decrease critical pH would provide a driving force toward enamel precipitation.

Prevention

WSL prevention methods have been aimed at patient education, oral hygiene instruction and fluoride regimens, plaque factors, appliance design, bonding techniques and tooth enamel.[1,7] The success of most of these preventive aims depends on patient compliance and unfortunately, with the lack of patient compliance and motivation throughout a 2 year treatment course, there is a high prevalence of WSLs in orthodontic patients.[2,6,3,16,20] It has been suggested to have patients limit or reduce the frequency of ingesting readily fermentable carbohydrates, or to possibly

substitute them with sweeteners which are non-cariogenic, as this will reduce the likelihood of demineralization.[31]

The effect of topical fluoride on preventing enamel demineralization is well known and the use of specific fluoride regimens during orthodontic treatment may prevent or minimize white spot lesion formation.[12,30,40] However, it has been reported that regular use of fluoridated toothpaste by itself during fixed orthodontic appliances is not sufficient for prevention of WSLs.[5] Therefore, supplemental regimens have been recommended for reduction of caries during orthodontic treatment,[5,16,4,41] such as fluoride-containing mouthrinses, gels, and varnishes.[40,42,43] Unfortunately, the effectiveness of fluoride by topical application or home rinse programs has been limited by the challenge of localizing the fluoride to the region where it is needed, the specific product's substantivity, and by the unpredictability of patient compliance.[4,41]

Dental materials that release fluoride, calcium, and phosphate independent of patient compliance are appealing. Several fluoride-releasing orthodontic bonding agents and auxiliaries are currently on the market, but research regarding their anticariogenic effects is conflicting.[44-49] An optimal orthodontic bonding agent would release calcium and phosphate in addition to fluoride to maximize its preventive potential.

ORTHODONTIC BONDING MATERIALS

Cements

Glass-ionomer cements (GICs) and resin-modified glass ionomer cements (RMGICs) are fluoride-releasing orthodontic cements provide a non-compliance based means for prevention of white spot lesions. Studies show these bonding agents are able to release fluoride *in vivo*, but their bond strengths have been shown to be substantially lower than those of conventional resins,[44,50-56] and there have been mixed results concerning their anticariogenic effects.[44,45]

Although GICs and RMGICs are commercially available, there is currently no bonding agent on the market that provides a biomimetic approach whereby calcium and phosphate ion release near the brackets would prevent enamel demineralization.

Resins

Resin composites are composed of an organic polymer matrix, inert filler particles, a coupling agent, and an initiator-accelerator system.[57] The matrix polymerizes via a chemical activation, operator-controlled light activation, or dual-cured with both light and chemical activation.[21,58] Resins have some advantages over cements—they achieve their optimal physical properties faster, are less brittle and more fracture resistant, and have higher bond strengths. However, because resins are easily contaminated with debris or moisture, they can be clinically technique sensitive.[57] Currently, there are several commercially available fluoride-containing resins. However, the concentration of fluoride leached from composites is generally lower when compared to GICs and RMGICs.[59,60]

BIOACTIVE GLASS

Bioactive glasses were discovered in 1969 and provided for the first time an alternative, second generation, interfacial bonding of an implant with host tissues.[61] Bioactive glass (BAG) is a "soluble" surface-active biomaterial that has been shown to successfully release ions in simulated body fluid.[62,63] Bioactive materials serve as scaffolds upon which the body can anchor new soft tissue, and provide a source of minerals.[63]

Bioactive glass can be produced by either a melt-derived or a sol-gel derived method.[64] Currently, most commercially available BAG products contain melt-derived BAG—these have applications in tooth and bone tissue regeneration following injury or surgical procedures, such as placement into fresh extraction sites to maintain alveolar bone levels, pulp capping, sinus

obliteration, and treatment of dentinal hypersensitivity.[61] Sol-gel bioactive glass is a three-dimensional cross-linked matrix of hydrolyzed alkoxides of SiO_2, CaO, and P_2O_5.[61] The sol-gel method allows for higher purity and homogeneity of the glass. It also results in BAGs with greater surface areas and greater bioactivity relative to the melt-derived method of preparation.[64]

BAG has biomimetic properties when immersed in simulated body fluid (SBF), which lead to the formation of tooth-like hydroxylapatite that can even deposit on organic polymers.[65,66] Under these conditions, BAGs release ions that interact with each other as well as ions present in the surrounding solution, ultimately forming surface nucleation sites for Ca and PO_4. [65,66] This is the key to BAG's potential for apatite growth and the potential for prevention of demineralized enamel.[65,66] As the solution becomes supersaturated, a Ca-P precipitate is deposited onto the glass surface. Researchers have combined various bioactive glasses with RMGIC to create a restorative material capable of remineralizing damaged dentin or enamel in addition to releasing fluoride. The experimental groups with added BAG demonstrated increased calcium and fluoride concentrations in SBF over time, and a Ca-P-like precipitate was observed after 336 hours.[67] Research by Crowe et al, 2008 (unpublished) is the first to demonstrate BAGs potential as a filler in resin composites, providing bioavailable calcium and phosphate ions when incorporated into both GICs and RMGICs. Brown et al[74] investigated the development and ion-releasing capabilities of an orthodontic composite resin containing sol-gel derived BAG—aptly named BAG-Bonds. When comparing the developed BAG-Bonds to Transbond XT, the novel BAG-Bonds demonstrated the capacity for buffering acidic oral environments, having significant release of calcium ions into their surrounding environment, and holding the potential to be a biomimetic bonding agent that may reduce or eliminate white spot lesions.[74]

DEMINERALIZATION

pH Cycling

The comparative effects of different fluoride agents have been examined in dynamic models with alternating periods of de- and remineralization.[68] The dynamic cycles of de- and remineralization are simulated by sequentially immersing enamel specimens in acidic (demineralizing) and supersaturated (remineralizing) buffer solutions.[5] A pH cycling regimen that includes subjecting cycles to 6 hours of demineralization followed by 18 hours of remineralization has been shown to correlate well with conditions associated with 1 month of fixed orthodontic appliance use in vivo.[5] In vivo, high and low pH alternate periodically depending on a variety of factors. A series of cycles to demineralize and remineralize enamel is present in the true intraoral environment, and a pH cycling model can closely simulate the conditions encountered *in vivo* within a carefully controlled environment.

METHODS TO ASSESS DEMINERALIZATION

Polarized Light

Polarized light is a visual method that measures differential light diffraction, which may be caused by either porosite change[69] 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 a good quantitative, but not qualitative evalution.[69]

Transverse Microradiography

Transverse microradiography is another visual method which can be used on either thin sections or specimen blocks. Using a primary parameter, Delta Z, the volume percent mineral

before and after treatment is measured. This method is similar to polarized light, in that they both are imaging methods which do not affect the sample in any way.[69]

Cross Sectional Microhardness

Demineralization caused by frequent ion loss can be detected by a decrease in enamel microhardness.[70] It has been demonstrated there is a linear relationship between the square root of Knoop microhardness and the mineral content of dental tissues; therefore the demineralization caused by frequent ionic losses could be detected by the reduction of the enamel microhardness.[71] Despite this linearity, each hardness calculation of mineral content carries with it possible error relative to the actual mineral density (i.e. the 'spread' around each determined point).[68] The Knoop microhardness is obtained through the measure of the length of the major diagonal left by the penetration of the diamond, and calculated with the standard formula for Knoop microhardness.[72] Load and duration must be stated, and indentations need to have reference points in sound enamel.

PROPOSED RESEARCH

Based on previous research, bioactive glass has been incorporated into a resin monomer and has demonstrated significant ion release.[73,74] This capacity for buffering acidic oral environments could potentially reduce or eliminate white spot lesions. This purpose of this research project is to assess 4 different BAG-Bonds' potential to affect the process of demineralization surrounding a bracket *in vitro,* compared to the conventional Transbond XT adhesive. Teeth will have brackets bonded using a BAG-Bond resin or the control resin, and will then be subjected to pH cycling, simulating the intraoral acidic challenge. The cross-sectional microhardness of each sample will then be measured, as to quantify BAG-Bond's potential for the prevention of demineralized enamel.

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APPENDIX 1: Modifications and Future Research

The results from this project indicate the promising potential of incorporating sol-gel bioactive glass into a resin for bonding orthodontic brackets. As discussed, the 4 different formulations of BAG-Bond demonstrated significantly less demineralization at superficial depths from the enamel surface than did the control Transbond XT adhesive.

Within this study, only 3 distances were tested—100, 200, and 300 μm from the adhesive edge, both cervical and occlusal to the bracket. In addition, 8 depths were tested, with depths greater than 125 μm proving to show no difference in enamel demineralization. This is to be expected, as the short caries challenge would not allow for demineralization to develop deep into the enamel. To get a true idea of the trend and shape of the white spot lesion, it would have been helpful to test *less* depths (perhaps ranging from 0-100 μm) and *more* distances (perhaps ranging from 100-900μm from the adhesive edge). These measurements might allow for more discussion as to exactly what is happening with the BAG-Bond—how ions are being released, where their effect is being seen, and help determine the shape of the entire WSL.

Within this study, 4 different BAG-Bond formulations were compared to the control of Transbond XT. A next step might be to compare the best BAG-Bond (so in this case might be 81BAG-Bond) to not only Transbond XT, but to more variables such as an amorphous calcium phosphate (ACP)-filled composite resin that has been shown to release calcium phosphate ions as well. Testing BAG-Bond's ability to prevent demineralization more than other marketed agents would be a logical step towards clinical viability.

At this point, several projects have been done evaluating 4 different BAG-Bonds and their potential as a feasible orthodontic bonding agent. With Dr. Melissa Brown's 2010 study on ion release, Dr. Cole Johnson's 2011 study on bond strength, and now my 2011 study on

prevention of demineralization, it would be interesting to combine the results of all 3 studies and review the implications. Does ion release correlate to the demineralization seen? Do these two variables correlate to BAG-Bonds' ability to withstand bracket forces, thereby demonstrating workable bond strength for clinical use? A review of the 3 projects would continue to shed light on the novel resins' *in vitro* potential.

The BAG-Bond resins used in this project, as they were formulated, demonstrated sub-par viscosity and workability at the lab bench. Even if these resins could prevent any and all demineralization surrounding a bracket, their clinical workability must be as good as or better than the commonly used bonding agents *in vivo*. At this stage, these BAG-Bond resins exhibit a stringiness and tackiness that would not be ideal chairside. Further research should be attempted to rework the chemical makeup of the resin, so that the viscosity would allow for easy and quick bracket bonding. Once this viscosity is ideal, the BAG-Bond's inhibition of enamel demineralization should then be tested.

This project used a simulated caries challenge, without the incorporation of any cultured bacteria. A future project could be performed that would include typical caries-causing bacteria such as *Strep mutans* and *Lactobacilli* within the pH cycling solutions. This may shed light on the "bigger picture" of white spot lesion formation, and BAG-Bond's ability to prevent demineralization.

Finally, the project performed here was an *in vitro* experiment, with a methodology meant to simulate a one-month *in vivo* caries challenge. Future projects could be done to simulate a longer caries challenge (i.e. 3 months, 6 months, etc) to further evaluate the novel resins' potential as a WSL prevention method.