Ion release from a novel resin orthodontic bonding agent for prevention of white spot lesions: An in vitro study

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Acknowledgements

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Ion release from a novel orthodontic resin bonding agent for the prevention of white spot lesions: An in vitro study

Abstract

Objective: To measure ion release from four sol-gel bioactive glass-containing orthodontic resin bonding agents (BAG-Bonds) following immersion into simulated body fluid (SBF) at pH 4 and 7.

Materials and Methods: Four BAG-Bonds, two containing fluoride, were developed. Prepared in our laboratory, the BAG-Bonds were composed of a mixture of resin monomers and bioactive glasses (BAGs). Workability of the final BAG-Bonds determined the amount of filler added to each, and this varied according to BAG composition. Commercially available Transbond XT was used as the control. Three disks (10mm x 2mm) of each material were individually suspended in 3.5 mL of SBF at pH4 and 7. SBF was analyzed to measure pH and ions released at 1hr, 10 hrs, and 100hrs. Calcium was measured by atomic absorption analysis, phosphate by UV visible spectrometry, and fluoride by an ion-specific electrode. The data were compared by a 3-way ANOVA with $p \leq 0.05$.

Results: Significant differences in calcium and phosphate ion release were found between the four BAG-Bonds and the control at multiple time-points. Significant changes in pH were also found. There was no measureable release of fluoride from any of the materials.

Conclusions: The BAG-Bonds showed the capacity for buffering acidic oral environments and significant release of calcium ions into their surrounding environment,

and hold the potential to be a biomimetic bonding agent that may reduce or eliminate white spot lesion formation.

Introduction

White spot lesions (WSLs) are clinically detectable manifestations of subsurface enamel demineralization, representing the first stage of caries formation.[1] Nearly half the patients who receive fixed orthodontic appliance therapy develop WSLs due to poor oral hygiene and plaque retention around orthodontic appliances.[2,3] With rapid accumulation of plaque, the incipient caries form as early as four weeks following the placement of fixed orthodontic appliances.[1,2,4-6] WSLs have been found to have a 10-50% reduction in enamel mineral content.[5,7] Although WSLs may regress to a variable extent following bracket removal, they often persist causing esthetic concerns.[4,8,9] Given this ongoing side-effect of orthodontic treatment, advances aimed at preventing WSLs continues to be an area of strong interest in orthodontics.

Previous investigation has shown that regular use of fluoridated toothpaste during fixed orthodontic treatment is often insufficient for prevention of WSLs.[6] A number of supplemental regimens have been recommended,[6,9-11] including fluoride-containing mouth rinses, gels, and varnishes.[12-14] The effectiveness of fluoride as applied through such applications has been limited by several challenges including: localizing the fluoride to areas where it is needed; the substantivity of fluoride-containing products; and the lack of predictability related to patient compliance.[9,10]

Fluoride-releasing orthodontic bonding agents, such as glass-ionomer cements (GICs) and resin-modified glass ionomer cements (RMGICs), were developed in part for prevention of WSLs. Although these bonding agents have been shown to release fluoride *in vivo*, their bond strengths have been substantially lower than those of conventional resins[15-22] and reports have been mixed regarding anticariogenic effects.[19,23] Although

GICs and RMGICs are commercially available, no bonding agent is currently on the market that provides a biomimetic approach whereby calcium (Ca) and phosphate (PO_4) ion release adjacent to brackets would potentially prevent demineralization.

Incorporating bioactive glass (BAG) into a composite resin may result in a biomimetic adhesive. Sol-gel BAG is a three-dimensional cross-linked matrix of hydrolyzed alkoxides of SiO_2, CaO, P_2O_5.[24] BAG has biomimetic properties when immersed in body fluids, leading to the formation of tooth-like hydroxylapatite.[25,26] Under *in-vitro* and *in-vivo* conditions, BAGs release ions that interact with each other as well as ions in the surrounding solution, forming a supersaturated solution and a Ca-P precipitate . This process has the potential for subsequent remineralization of demineralized enamel.[25,26]

Additional, *in vitro* investigations of an experimental GIC containing 30wt% BAG have found the bonding agent inhibited growth of the cariogenic bacteria, Streptococcus mutans.[27] Thus this anticariogenic effect may also contribute to BAG's potential for preventing WSLs.

The purpose of this study was: 1) to develop novel BAG-containing composite resins to be used for bonding of orthodontic brackets; and 2) to test the hypotheses that (a) these novel resins would release ions into simulated body fluid (SBF) at pH4 and 7, and (b) that the release of these ions would result in a change in pH of their surrounding environment.

Materials and Methods

Preparation of BAG-Bond

Four BAG-containing orthodontic resin bonding agents (62BAG-Bond, 65BAG-Bond, 81BAG-Bond, and 85BAG-Bond) were developed in our laboratories (Table 1). These were prepared by mixing (SpeedMixer DAC, Flack Tek, Landrum, SC) two resin monomers with one of four compositions of bioactive-glass (62BAG, 65BAG, 81BAG, or 85BAG), 0.4 wt% camphoroquinone (Polysciences, Warrington, PA) and 0.8 wt% ethyl 4-dimethylaminobenzoate (Research Chemicals Ltd., Heysham, Lancs.). The resin monomers were ethoxylated bisphenol A dimethacrylate (EOBPADMA) and BisGMA (both from Esstech Corp., Essington, PA). The ratio of EOBPADMA to BisGMA used was 2.5:1, selected after a pilot study comparing ratios of 3:1, 2.5:1 and 2:1 showed that 2.5:1 had the highest Knoop hardness and shear bond-strength values.

BAGs were synthesized in our laboratory by sol-gel methods,[25] ball milled, sieved, and micronized (Sturtevant, Hanover, MA). Average particle size ranged from 0.04 to 3.0 μm, as determined by laser particle size measurements (Beckman Coulter LS13 320, Brea, CA). BET method[28] was used to measure the surface areas of each BAG. BAGs were added to the monomer mixture until the workability and viscosity of each product was similar to the control material, Transbond XT (3M Unitek, Monrovia, CA). Viscosity was measured using a DV-III Ultra, rheometer (Brookfield Engineering, Middleboro, MA) while workability of each formulation was evaluated by the same experienced dentist. This resulted in variable BAG to monomer ratios for each BAG-Bond and likely reflected differences in surface area of each BAG.

Sample Preparation

Simulated body fluid (SBF)(Table 2) was freshly prepared[29] and titrated to desired pH levels of 4 (SBF4) and 7 (SBF7) using nitric acid. Cylindrical molds (10mmx2mm) were made with polyvinylsiloxane impression material (Aquasil-Ultra, Dentsply, Milford, DE). Each adhesive was packed into a mold and an end of a piece of non-waxed dental floss was embedded in the material to allow for suspension of the disk in SBF. The filled molds were compressed between glass slides and light cured (Demi, Kerr, Middleton, WI) for 40 seconds from each side. The curing light was measured with a radiometer (EFOS Inc., Williamsville, NY) before, during and after sample preparation to ensure a constant output >1000 mW/cm^2. Disks of Transbond XT prepared in the same manner served as controls.

Each sample (n=3 replicates/time point/SBF solution; total = 18 samples of each composition) was immersed into 3.5 ml of SBF (Figure 1) and continuously agitated on a biaxial orbital shaker (Thermo Fisher, Pittsburgh, PA) at 37°C. At pre-determined time-points (1 h, 10 h, 100 h), samples were removed and the fluid was analyzed.

Sample Analysis

pH

The pH of the SBF samples at each time point was measured and recorded. Afterward, SBF7 samples were acidified to pH 4 using nitric acid to ensure released ions remained in solution.

Ion Analysis

Calcium ion concentrations were measured using atomic absorption (AA) spectrometry (Analyst 300, Perkin Elmer, Waltham, MA). Fluoride ion concentrations were determined by measurements using a fluoride ion-specific electrode (Orion, Thermo Fisher, Beverly, MA) and comparison to a calibration curve. Phosphate ion concentrations were determined by the vanadomolybdophosphoric acid colorimetric method[30] where a spectrophotometer (Ultrospec II, LKB, Cambridge, England) measured the color intensity of each sample at a wavelength of 400 nanometers, and the result was compared to a calibration curve.

Statistical Analysis

For comparison of pH levels and ion release, a 3-way analysis of variance (ANOVA) was run (SAS 9.1, SAS, Cary, NC), with α=0.05.

Results

pH

In SBF4, significant increases ($p < 0.05$) in pH were found with 62BAG-Bond and 65BAG-Bond at all time-points, and with 81BAG-Bond and 85BAG-Bond at 100 hrs. In SBF7, significant increases were found with 62BAG-Bond at 10 and 100 hrs, and with 65BAG-Bond at 100 hrs (Figure 2).

Calcium

In both SBF4 and SBF7, significant increases ($p<0.05$) in calcium concentration were found with 62BAG-Bond and 65BAG-Bond at all time-points, with 81BAG-Bond at 10 and 100 hrs, and with 85BAG-Bond at 100 hrs (Figure 3).

Phosphate

In SBF4, significant decreases ($p<0.05$) in phosphate concentration were found with 62BAG-Bond, 65BAG-Bond, 81BAG-Bond and 85BAG-Bond at 100 hrs only. In SBF7, significant decreases ($p<0.0001$) were found with 62BAG-Bond and 65BAG-Bond at 100 hrs (Figure 4).

Fluoride

With both SBF solutions, no significant change in fluoride concentration was found with any of the BAG-Bonds at any time.

Discussion

This study evaluated the potential use of novel BAG-containing orthodontic resin bonding agents for the prevention of WSLs. In order to measure this potential *in vitro*, samples of the light-cured adhesives were soaked in SBF at either pH4 or 7. The resulting SBF solutions were analyzed over time for changes in pH and ion concentrations. Comparisons were made to a popular, commercially available adhesive, Transbond XT.

The BAG-Bonds tested demonstrated differing buffering capacities (Figure 2). In SBF4, an environment below the critical pH[31,32] for enamel demineralization, the buffering capacity of 62BAG-Bond and 65BAG-Bond was significant within one hour and increased profoundly by 100 hrs. In comparison, 81BAG-Bond and 85BAG-Bond showed significant buffering capacity only at 100 hrs. Differences in the buffering capacity among the BAG-Bonds can be attributed to their respective chemical compositions where 62BAG-Bond and 65BAG-Bond contain more calcium than 81BAG-Bond and 85BAG-Bond. Consequently, the 62BAG-Bond and 65BAG-Bond had the potential to absorb more H^+ ions as calcium was released.

In the non-cariogenic SBF7 environment, a modest increase in pH was seen with the 62BAG and 65BAG resins at 100 hrs, but did not result in an overly basic environment, rising only to a maximum pH of 7.23, likely due to the low thermodynamic driving force[36] for ion exchange at this pH.

The implications of BAG-Bond's ability to buffer cariogenic environments are significant because the rate of enamel demineralization is inversely proportional to the pH of its environment.[35] When plaque pH drops below critical pH, a change in the ion

concentration gradients between the tooth surface and the plaque results in dissolution of enamel.[31,32] Results of this study suggest that BAG-Bonds have the potential for decreasing the rate of enamel demineralization by increasing the pH adjacent to the bracket/tooth interface (Figure 2),or for entirely preventing enamel demineralization by maintaining the environment above the critical pH. This may be particularly applicable in adolescent patients because they have a greater propensity toward WSLs, with their tendency to have a higher critical pH than adults.[32]

Significant increases in calcium levels measured in SBF (Figure 3) were likely due to a calcium concentration gradient established between the SBF and each BAG-Bond. Ions from the SBF are known to displace calcium from the BAG-Bond in both the acidic and neutral environments.[33] The greater amount of calcium released in SBF4 compared to SBF7 environments can be attributed to the higher concentration of H^+ in SBF4. At the lower pH, an under-saturation of calcium and phosphate in the SBF would create a thermodynamic driving force[36] that forces more calcium out of the BAG-Bond relative to the situation occurring in the higher pH. This is consistent with current findings that with increased pH, less calcium had been released into solution.

Enamel demineralization and subsequent white spot lesion formation are a result of the loss of calcium and phosphate from the enamel matrix during local ion concentration imbalances at the enamel surface. Dawes found that "the critical pH below which enamel dissolves is not constant but rather is inversely proportional to the amount of calcium and phosphate in saliva and plaque fluid."[31] With significant release of calcium, BAG-Bonds would have the ability to decrease the critical pH of the surrounding enamel environment. A decrease in critical pH would necessitate a greater

reduction in pH from plaque activity before enamel dissolution could occur,[31] resulting in a net decrease in risk for WSLs.

The significant decreases in phosphate ion levels measured in SBF (Figure 4) can be explained by supersaturation of the solution immediately surrounding the BAG-Bond and incorporation of the ions onto the resin surface by precipitation of CaP. The CaP precipitate is the precursor for hydroxylapatite formation.[34] There is clinical evidence that WSLs can be remineralized if the enamel surface overlying the lesion remains intact.[37] As long as the environment adjacent to the WSL is supersaturated with calcium and phosphate, the calcium and phosphate can pass through the intact enamel layer and remineralize the subsurface lesion.[31] In addition, precipitated CaP could act as a template for remineralization in areas where there has been superficial enamel erosion. Although it has been suggested that enamel erosions do not recalcify because they lack a matrix that supports crystal growth,[31,37] BAG-Bond may provide a biomimetic matrix that is conducive for remineralization.

The loss of phosphate from the surrounding environment may also deny an essential nutritional source for bacteria, reducing plaque formation.[38] During fixed orthodontic appliance therapy increases in Streptococcus mutans and lactobacilli have been found around bracket margins and on the gingival third of teeth, [39-41] thus having an antibacterial component associated with the adhesive would be advantageous for the prevention of WSL's.

Although 62BAG-Bond and 81BAG-Bond contained fluoride, no detectable amount of fluoride was released into solution. This may be attributable to the process of polymerization of the BAG-Bonds where the fluoride becomes incorporated into the

polymer matrix, unable to leach-out into solution, or it may be that it becomes incorporated in the Ca-P precipitate as a precursor to highly insoluble fluoroapatite. Even though fluoride was not detectable within the SBF solution, it would likely be found within the ionic milieu at the surface of the adhesive/tooth interface, and hence be available for integration at the adhesive/enamel interface[6] or for bactericidal activity[42].

Conclusions

Results of this study suggest that combining BAG into resin adhesives may result in a "smart material" that provides a reservoir of crucial ions for remineralization or for the protection of enamel from demineralization in the following ways:

1) BAG-Bonds raise the pH of cariogenic environments and may also decrease critical pH, resulting in decreased mineral loss from teeth, ultimately preventing or reducing the severity of WSLs.
2) The reservoir of calcium and phosphate within BAG-Bond provides an ion source for precipitation of CaP onto the tooth surface, holding the potential for remineralization of demineralized enamel.
3) The removal of phosphate from the environment decreases the resources for bacterial metabolism, and may have antimicrobial effects resulting in a more favorable environment for prevention of WSLs.

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Figure Legends

Figure 1. Sample configuration. Embedded floss suspended the disk to ensure that all surfaces were constantly exposed to the solution.

Figure 2. Mean change in pH over time compared to Transbond XT. A=SBF at pH4, B=SBF at pH7. Note the difference in Y-axis scale. Significant differences ($p<0.05$) are noted by * above columns.

Figure 3. Mean change in calcium concentration over time compared to Transbond XT. A=SBF at pH4, B=SBF at pH7. Significant differences ($p<0.05$) are noted by * above columns.

Figure 4. Mean change in phosphate concentration over time compared to Transbond XT. A=SBF at pH4, B=SBF at pH7. Significant differences ($p<0.05$) are noted by * above columns.

Figure 1.

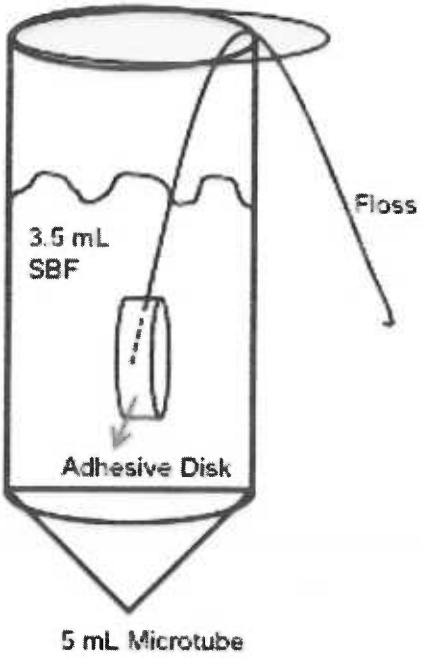
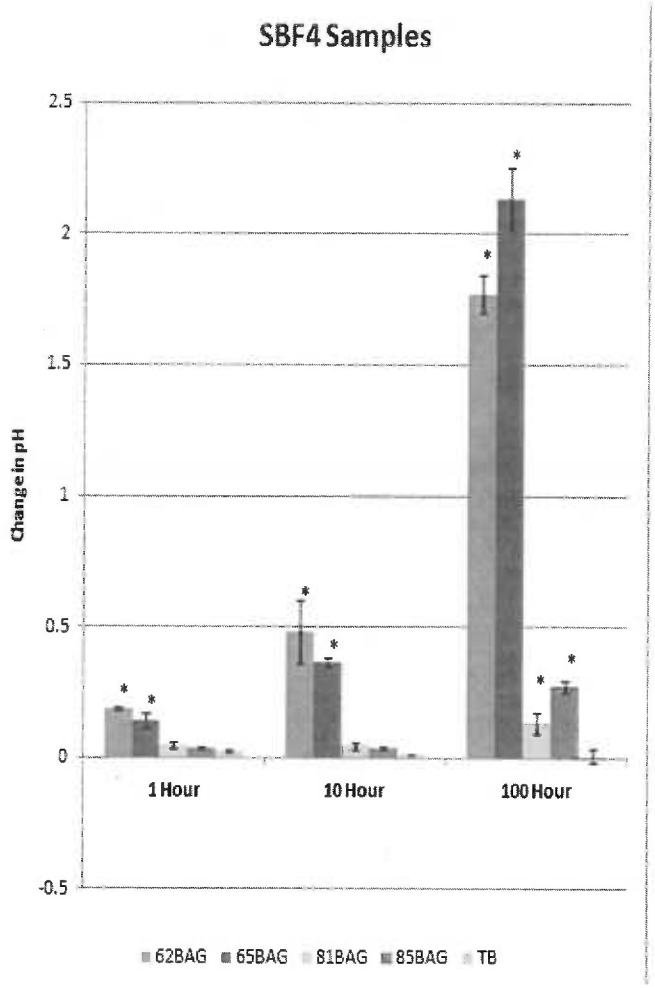


Figure 2.

A.


SBF4 Samples
Change in pH
2.5
2
1.5
1
0.5
0
-0.5
1 Hour
10 Hour
100 Hour
62BAG
65BAG
81BAG
85BAG
TB

B.

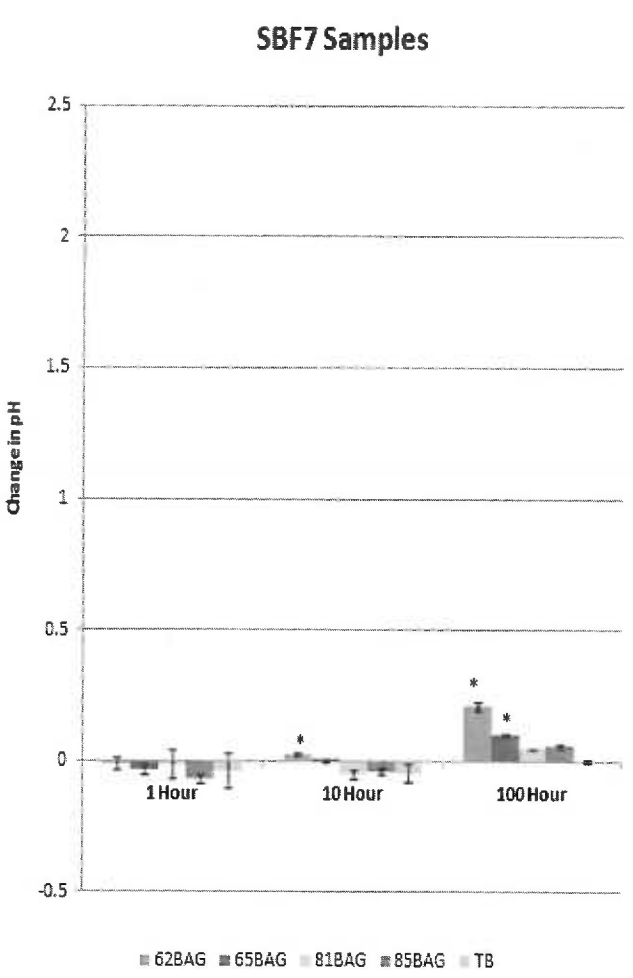
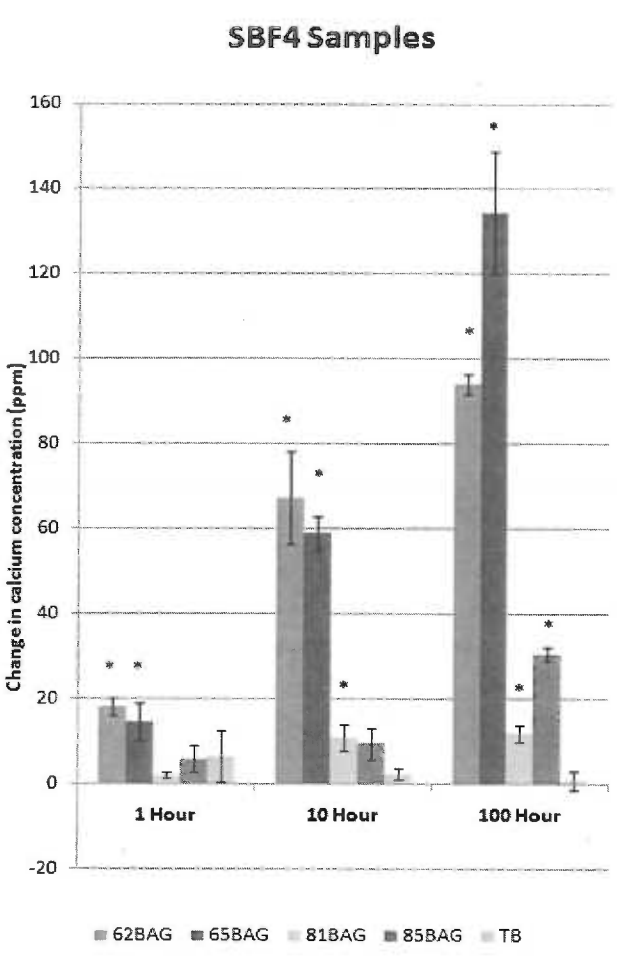

SBF7 Samples
Change in pH
2.5
2
1.5
1
0.5
0
-0.5
1 Hour
10 Hour
100 Hour
62BAG
65BAG
81BAG
85BAG
TB

Figure 3.

A.


SBF4 Samples
160
140
120
100
80
60
40
20
0
-20
Change in calcium concentration (ppm)
1 Hour
10 Hour
100 Hour
62BAG
65BAG
81BAG
85BAG
TB

B.

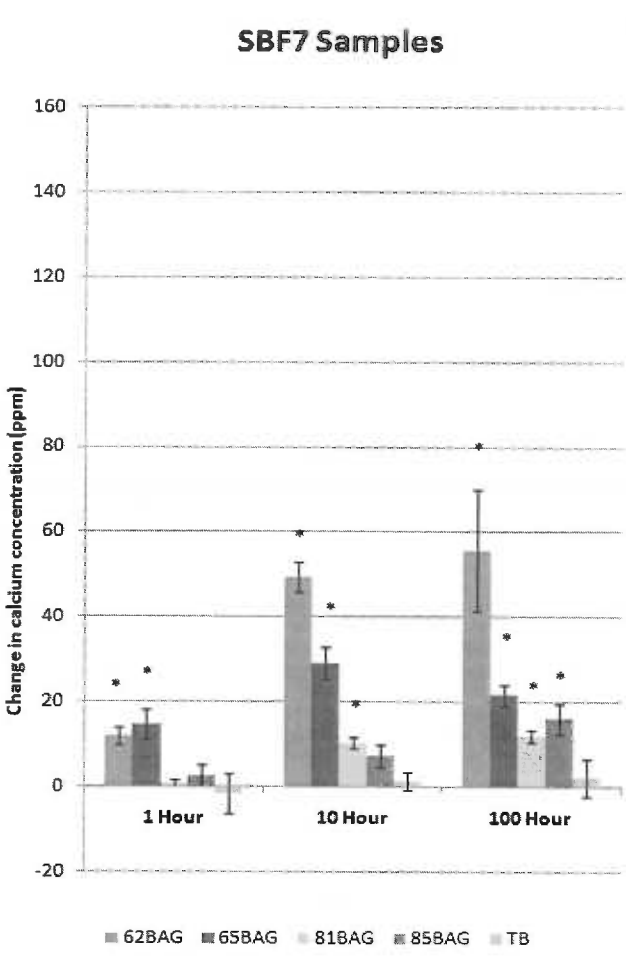
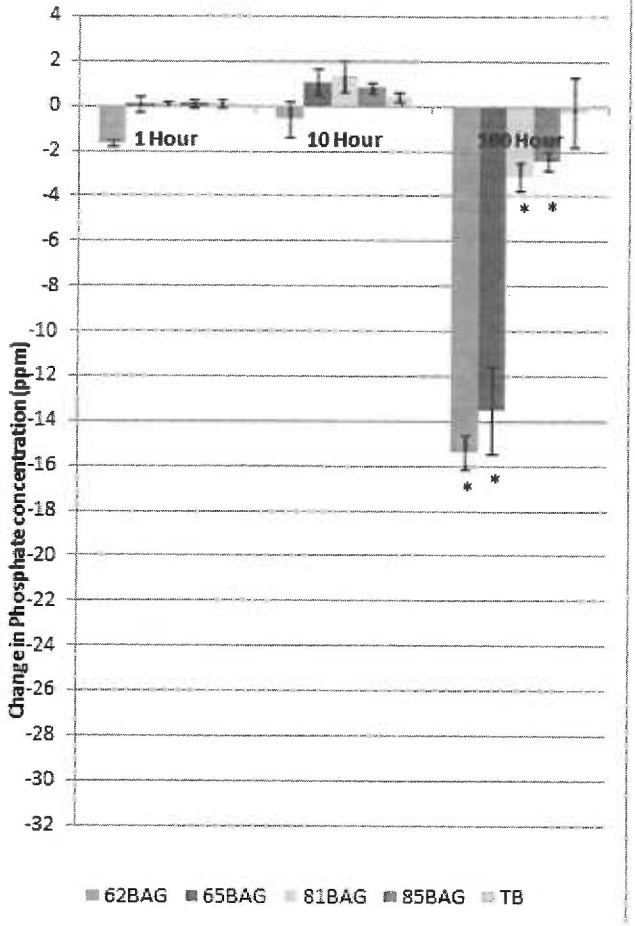

SBF7 Samples
160
140
120
100
80
60
40
20
0
-20
Change in calcium concentration (ppm)
1 Hour
10 Hour
100 Hour
62BAG
65BAG
81BAG
85BAG
TB

Figure 4.

A.


SBF4 Samples
4
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
-22
-24
-26
-28
-30
-32
Change in Phosphate concentration (ppm)
1 Hour
10 Hour
100 Hour
*
*
*
*
62BAG
65BAG
81BAG
85BAG
TB

B.

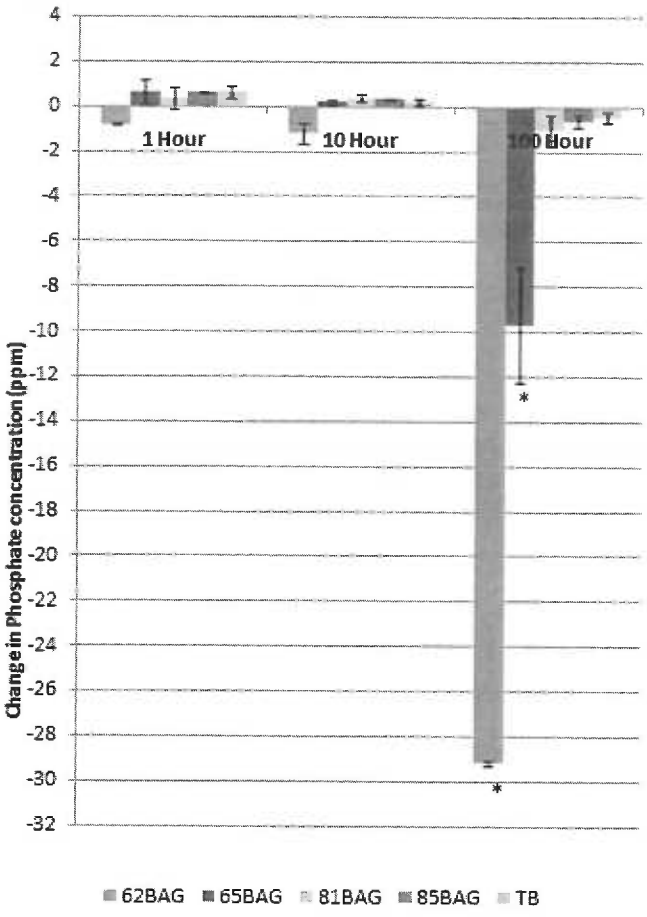

SBF7 Samples
4
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
-22
-24
-26
-28
-30
-32
Change in Phosphate concentration (ppm)
1 Hour
10 Hour
100 Hour
*
*
62BAG
65BAG
81BAG
85BAG
TB

Table 1.

	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	62	31	4	1	3	75	29:50
65BAG	65	31	4	0	0	144	49:100
81BAG	81	11	4	0	4	320	37:100
85BAG	85	11	4	0	0	268	33:100

Table 2.

Ion	Concentration (ppm)
Mg^{2+}	36
Ca^{2+}	100
K^{+}	195
Na^{+}	3266
SO_4^{-}	16
HPO_4^{2-}	31
HCO^{3-}	50
Cl^{-}	5247

Literature Review

WHITE SPOT LESIONS

Background

White spot lesions (WSLs) are clinically detectable manifestations of subsurface enamel demineralization.[1] Nearly half of all patients who receive fixed orthodontic appliance therapy experience WSLs[2] due to poor oral hygiene and plaque retention around orthodontic appliances.[3] These incipient caries can appear in as few as four weeks following the placement of fixed orthodontic appliances.[1,2,4-6] A combination of factors such as diets high in fermentable carbohydrates, suboptimal oral hygiene, long intervals between appointments, and poor patient compliance with the use of fluoride toothpastes and mouthrinses contribute to the development of these WSLs. With prevalence of WSLs in orthodontic patients reaching as high as 95%,[4] a means for preventing WSLs without the need for compliance is necessary.

Studies have shown that WSL mineral content can be reduced by as much as 10-50% compared to intact enamel apatite.[5,7] In orthodontic patients, 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.[8-10] As a result of carbohydrate metabolism by these bacteria, an acid by-product is released. This reduces 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.[11]

A study evaluating artificially induced white spot lesions in extracted human pre-molars demonstrated that WSLs are subsurface enamel demineralization with an intact

superficial layer.[12] It was concluded that the outer surface calcium and phosphate come from subsurface enamel layers. As these lesions form, the outer surface of enamel remains mineral-rich, however its morphology is slightly more porous than sound unaffected enamel.[13] This pattern of demineralization creates an optical phenomenon.[4] Demineralized enamel causes greater light refraction because it is more porous than intact enamel resulting in the appearance of a white spot on the enamel.[14]

Research has shown that WSLs in orthodontic patients can be prevented by good compliance with a fluoride toothpaste in combination with a 0.05% sodium fluoride mouthrinse.[15] A study by Geiger *et al.* in 1992, which asked patients to decrease their caries risk with a daily fluoride mouth rinse, showed a compliance rate of only 13%. Among those 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.[15] These results show that there is an indication for the development of a compliance-free method for preventing WSLs in orthodontics.

Although research has shown that WSLs may regress following bracket removal, they often persist, causing esthetic concerns.[4,16,17] If left untreated, WSLs may progress into more serious cavitations, requiring restoration. Because a high incidence of WSLs occur on maxillary anterior teeth,[2,15,18] the optimal final esthetic result from orthodontic treatment may be compromised. Therefore, it is important that the professional orthodontic community focus on methods for preventing WSLs.

Etiology of WSLs

Multiple localized inter-related pathologic and protective factors can effect enamel demineralization. Such factors include diet, saliva, plaque, type of enamel

apatite, local ion concentrations and pH levels.[11] It is an interplay between all of these factors that renders enamel at risk or not for demineralization. When the plaque at the plaque/enamel interface becomes under-saturated with ions present in enamel, apatite demineralization ensues.[19,20]

Diet

Demineralization and caries formation is directly related to the frequency and the amount of carbohydrates consumed. Oral bacteria exposed to fermentable carbohydrates are able to metabolize the sugars into acids such as lactate or acetate. These acids can decrease local pH at the enamel/plaque interface to levels below critical pH for prolonged periods of time.[21] If not mechanically removed, this cariogenic plaque in contact with the enamel surface results in enamel demineralization.[22,23]

Saliva

One of the most important factors influencing enamel demineralization and remineralization is saliva.[1] Both the rate of salivary flow as well as its contents influence the balance between mineral loss and gain at the enamel surface. 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.[20]

Anderson et al. found that the amount of calcium in the saliva of children and adults differs significantly.[20] 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 before demineralization occurs compared to adults. In addition, the lack of calcium in children's saliva results in a decreased driving force back toward remineralization. These two factors in children put them at an increased risk for enamel

demineralization compared to adults.[20] Considering that the majority of orthodontic patients are adolescents, the results from this study 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.

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.[11] 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] Conversely, the lingual surfaces of mandibular incisors are in close proximity to salivary glands and rarely develop demineralization.[1]

Plaque

Studies have 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.[24] Fixed 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] Retained carbohydrates at the enamel surface as a result of the fixed appliances and poor attention to plaque removal by the patient results in prolonged exposure to acid exposure and encourages growth of aciduric bacteria.[1]

With the increased plaque accumulation around orthodontic appliances, an increase in acidogenic oral bacteria including Streptococcus mutans and lactobacilli during orthodontic treatment has been seen.[8-10,25] One study[26] reported a fivefold

increase in lactobacillus counts in patients undergoing active orthodontic treatment. Following removal of the appliances, the lactobacilli counts dropped back to pre-appliance levels. Therefore, a direct relationship with the placement of the orthodontic appliances and an increase in lactobacilli was reported.[26] In addition to lactobacilli increases, Rosenbloom and Tinanoff[10] evaluated levels of Streptococcus mutans before, during and after orthodontic treatment and found increased levels during active treatment.

High levels of Streptococcus mutans and/or lactobacilli are associated with the initiation and development of enamel demineralization.[11,27] 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] This is another factor contributing to the orthodontic patient's increased risk of enamel demineralization. To decrease this risk, an orthodontic bonding agent that is antimicrobial with the ability to buffer the acidic environment at the plaque/enamel interface would be beneficial.

Enamel apatite

Enamel is a carbonated hydroxylapatite represented by the following simplified formula: $Ca_{10-x}(Na)_x(PO_4)_{6-y}(CO_3)_z(OH)_{2-u}(F)_u$.[11,29] As enamel mineral is laid down during tooth development, impurities such as carbonate and fluoride are frequently substituted into the hydroxylapatite crystal lattice. The incorporation of carbonate ions creates disruptions in the crystal lattice rendering 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.[30,31] On the contrary, the production of

fluorapatite by replacement of hydroxide ions with fluoride ions results in a more stable less soluble form of apatite.[11]

Intraoral Calcium and Phosphate

Demineralization rates are affected by pH, the concentration and type of acid present and the degree of saturation of ions in the demineralizing solution.[32] The precipitation and dissolution of hydroxylapatite can be described by the following reaction:[19]

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

Precipitation ⇆ Dissolution

An increase in local concentrations of calcium and phosphate ions drives the above reaction to the left favoring precipitation, or remineralization, of hydroxylapatite. Conversely, undersaturation of 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.[32] An orthodontic bonding agent that would provide bioavailable calcium and phosphate ions at the plaque/enamel interface to drive the reaction toward remineralization should help prevent enamel dissolution in cariogenic environments.

Critical pH

The thermodynamic driving force for de- and remineralization is also a function of pH. The pH at which the enamel is just saturated with respect to hydroxylapatite is called the 'critical pH'. A pH of 5.5 is the accepted critical pH for hydroxylapaptite.[33] A decrease in pH below this 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.[20] 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.[19] It would be ideal to have an orthodontic bonding agent that provided a source of calcium and phosphate ions around the bracket/enamel/plaque interface to decrease critical pH and provide a driving force toward enamel precipitiation.

Prevention

Current targets for WSL prevention in fixed orthodontic appliance therapy have been aimed at patient education, oral hygiene instructions and fluoride regimens, plaque factors, appliance design, bonding techniques and tooth enamel.[1,21] The success of the majority of these preventive aims depend on patient compliance. The patient is responsible for monitoring his or her diet, thorough oral hygiene practices, the use of adjunct fluoride treatments, and remaining motivated throughout the entire course of treatment, which is typically about 2 years. Unfortunately, with the lack of patient compliance and motivation, there is a high prevalence of WSLs in orthodontic patients.[2-4,15,18]

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.[11,22,34] However, it has been reported that regular use of fluoridated toothpaste by itself during fixed orthodontic appliances is not sufficient for prevention of WSLs.[6] Therefore, supplemental regimens have been

recommended for reduction of caries during orthodontic treatment,[6,15,17,35] such as fluoride-containing mouthrinses, gels, and varnishes.[34,36,37] Unfortunately, the effectiveness of fluoride by topical application or home rinse programs has been limited by challenges of localizing the fluoride to the region where it is needed, the specific product's substantivity, and by the unpredictability of patient compliance.[17,35]

The use of dental materials that release fluoride, calcium and phosphate independent of patient compliance is appealing. Several fluoride-releasing orthodontic bonding agents and auxiliaries (i.e. fluoride-releasing elastomeres) are currently on the market, but research regarding their anticariogenic effects is conflicting.[38-43] An optimal orthodontic bonding agent would release calcium and phosphate in addition to fluoride to maximize its preventive potential.

ORTHODONTIC BONDING MATERIALS

Patients that are most at risk for developing decalcification during orthodontic therapy are often the least compliant in regards to adjunct hygiene instructions. Consequently, bonding agents containing fluoride have been developed to offset poor patient compliance.[41,43,44]

Cements

Glass-ionomer cements (GICs) and resin-modified glass ionomer cements (RMGICs) are fluoride-releasing orthodontic cements that were developed to 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[38,45-51] and there have been mixed results concerning their anticariogenic effects.[38,39]

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. Developing an orthodontic composite resin-bonding agent with this ability would be a valuable tool for prevention of WSLs.

Resins

Resin composites are composed of an organic polymer matrix, inert filler particles, a coupling agent and an initiator-accelerator system.[52] The matrix polymerizes via a chemical activation, operator controlled light activation or dual cured with both light and chemical activation.[19,53] Resins adhere to enamel via mechanical retention. To ensure maximum bond strength, enamel must be acid-etched or roughened, dried and free of contamination.[52]

Resins have some advantages over cements. Resins 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.[52]

Currently, there are several commercially available fluoride-containing resins. However, the concentration of fluoride leached from composites is generally lower compared to conventional and resin-modified glass ionomer cements.[54,55] The clinical efficacy of fluoride-releasing composites with respect to WSL prevention has not been promising.[21]

One resin (Aegis Ortho, The Bosworth Co, Skokie, IL) is currently on the market that releases calcium and phosphate according to the manufacturer. It is an unstable

amorphous calcium phosphate (ACP)-filled composite resin. ACP breaks down under acidic conditions releasing calcium and phosphate (Skrtic et al., 1996 and 2004). Research regarding the potential for ACP to inhibit demineralization is conflicting.[56] Two studies have been published regarding its bond strength, but their results are also conflicting.[48,57]

The release of remineralization ions from resins is unlikely unless the resin contains a soluble glass.[53] Bioactive glass (BAG) is a "soluble" surface-active biomaterial[58] that has been shown to successfully release ions in simulated body fluid (SBF).[59] If BAG is incorporated into an orthodontic bonding resin, it may release ions at the plaque/enamel/bracket interface that would be available for prevention of WSLs.

BIOACTIVE GLASS

Bioactive glass was discovered in 1969. It is classified as a bioactive material because it is capable of chemically bonding to living tissue. Bioactive materials serve as scaffolds upon which the body can "anchor" new soft tissue, and provide a source of minerals.[59]

Bioactive glass can be produced by either a melt-derived or a sol-gel derived method.[60] Currently, most commercially available BAG products contain melt-derived BAG (MBAG). MBAGs have applications in tooth and bone tissue regeneration following injury or surgical procedures, for example placement into fresh extraction sites to maintain alveolar bone levels, pulp capping, sinus obliteration and treatment of dentinal hypersensitivity.[61] Sol-gel derived BAGs are a newer generation of BAGs with great potential.

Sol-gel bioactive glass (BAG) is a three-dimensional cross-linked matrix of hydrolyzed alkoxides of SiO_2, CaO, 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.[60]

Whether melt-derived or sol-gel derived, BAG has biomimetic properties when immersed in simulated body fluid (SBF), which lead to the formation of tooth-like hydroxylapatite.[62,63] Under these conditions, BAGs release ions that interact with each other as well as ions present in the surrounding solution. As the solution becomes supersaturated, a Ca-P precipitate is deposited onto the glass surface. Herein lies the potential for apatite growth and the potential for remineralization of demineralized enamel.[62,63] In addition, investigations of an experimental GIC containing 30wt% MBAG have found it to inhibit growth of the cariogenic bacteria Streptococcus mutans in vitro.[64] This may also contribute to BAG's potential for preventing WSLs and remineralization of enamel if incorporated into an orthodontic resin adhesive.

In view of the known relationship between apatite growth and ion release, two main in vitro methods are used to determine bioactive character of glasses when submerged in SBF.[65] These include measurement of ion release profiles on the glass surface or in SBF and monitoring for apatite formation on the glass surface. Ion release studies have demonstrated an initial increase in calcium ions in solution as calcium is released from the BAG.[63] This is followed by a dramatic decrease in calcium ions in solution as they are precipitated back onto the glass surface. Phosphate concentrations undergo an initial drop when measured from immersion solution because these ions are

bulkier and slower to migrate from the glass. So, the initial apatite layer is formed by phosphate in solution until phosphate is able to leach from the BAG onto the 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. In 2004, Yli-Urpo *et al.* added various concentrations (10wt% and 30wt%) of a commercially available MBAG (S53P4, Vivoxid Ltd, Turku, Finland) to RMGIC (Fuji II LC, GC Corporation, Tokyo, Japan).[66] They measured concentration changes in calcium, phosphorous, and fluoride over time in neutral SBF and evaluated the glass surfaces using SEM. Relative to the control group with no added MBAG, calcium concentrations in SBF increased over time in the experimental group with 30wt% MBAG. SBF calcium concentrations in the 10wt% MBAG group ultimately decreased in SBF over time. Phosphorous decreased over time in the 30wt% MBAG group and remained relatively unchanged in the 10wt% MBAG group. The experimental groups released more fluoride over time than the control group, with the 30wt% MBAG group releasing the highest concentrations. The RMGIC containing 30wt% of MBAG showed the most reactivity with SBF. Although the surface changes did not represent clear calcium phosphate precipitation layers, a Ca-P-like precipitate was observed after 336 hours, which would explain the decrease in phosphorous over time. Phosphorous was present in lower concentrations than calcium, explaining the excess release of calcium into solution.

RMGIC containing MBAG has also been shown to inhibit growth of cariogenic bacteria such as Streptococcus mutans *in vitro*.[67] Despite this added benefit, the formation of BAG-induced apatite crystals surrounding bracket margins could be

detrimental, as it may create an irregular surface for increased bacterial accumulation. Although studies using MBAG have indicated surface apatite formation, sol-gel BAG in combination with RMGIC may not yield the same results. Cerruti *et al.* showed that surface area influences the rate of ion release and CaP surface re-precipitation in MBAG.[68] Smaller particles yielded faster ion release and thinner Ca-P precipitation layers. The smaller and more uniform particles in the sol-gel derived bioactive glass could allow for a thin surface layer of Ca-P without inducing apatite or crystal formation.

PROPOSED RESEARCH

Research has yet to discuss an orthodontic composite resin containing sol-gel derived BAG. The results from the aforementioned studies[66-68] as well as Crowe *et al.*, 2008 (unpublished) indicate BAGs potential as a filler in resin composites, which provides bioavailable calcium and phosphate ions when incorporated into both GICs and RMGICs. In theory, a sol-gel BAG used as a filler in an orthodontic composite resin that releases bioavailable calcium and phosphate ions at the enamel/plaque/bracket interface would provide a bonding agent capable of preventing WSLs while offering mechanical properties superior to those of GICs and RMGICs.

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APPENDIX 1

FUTURE RESEARCH

Results from this study are promising but continued efforts to improve the properties of BAG-Bond and to evaluate the viability of its clinical applications are needed. Hence, the opportunities for future research with these BAG-Bond materials are great. The following is a list of future research opportunities:

1) The bond strength of the BAG-Bonds could be evaluated over time. As bioactivity continues with time, the integrity of the BAG as the filler may be changing. What are the implications of this on bond strength?

2) The precipitation of apatite onto the BAG-Bond disks used in this study could be analyzed by scanning electron microscopy, transmission electron microscopy, infrared spec, or xray diffraction. This would confirm whether or not the calcium and phosphate ions are nucleating onto the surface of the BAG-Bonds.

3) Multiple *in vitro* experiments with varying concentrations, types and particle sizes of BAG and monomer ratios are needed to determine how sol-gel BAG can best be used as a filler material for an orthodontic composite resin. Then a test of the mechanical properties and ion release of these compositions would be indicated.

4) Ultimately, *in vivo* experiments could be designed to test the hypotheses that the incorporation of BAG into the resin will a) not increase the number of bond failures and b) decrease the occurrence and size of white spot lesions surrounding fixed appliances during orthodontic treatment.

5) Perform the same experiment but take sample measurements at longer time points to see what happens to the ion concentrations as time progresses.

6) Culture cariogenic bacteria (Streptococcus mutans and lactobacilli) and expose BAG-Bond to the environment to determine if BAG-Bonds have an antibacterial effect.

7) Test the rechargeability of the BAG-Bonds by cycling them in fresh SBF at specific time intervals and examine the change in ion concentrations in the different SBFs.

8) If bond strength is an issue, try adding a silane-coupling agent to the BAG/monomer mixture to see if bond strength increases.