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The Effects of Bioactive Glass on Bonding to Dentin

by

Diana N. Zeiger

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

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in the

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of the

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Dedicated to Susan and Harvey Zeiger: Thank you for *everything*...

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## **The Effects of Bioactive Glass on Bonding to Dentin**

*Diana N. Zeiger*

### **ABSTRACT**

Since the introduction of adhesive dentistry in the 1950s, leakage has been a major problem affecting the dentin bonding process. The micromechanical nature of the bond leaves it vulnerable to hydrolytic and enzymatic degradation, reducing the strength and lifetime expectancy of the restoration. Bioactive glasses are known to promote the formation of apatite in aqueous environments that contain calcium and phosphate (e.g. saliva); it is hypothesized here that their presence at the bonded interface will improve the quality of the resin-dentin bond through self-sealing caused by the formation of apatite, in the presence of leakage. Determination of the relative success of incorporation of bioactive glass into the dentin bonding process requires assessment of the ability of the glass to reduce leakage, as well as its effect upon the strength of the bond. Leakage was measured by both a silver nitrate method and a methylene blue method. The ability of microparticles of commercially available bioactive glass to infiltrate etched dentin was tested, and hybrid layer formation and leakage were assessed by SEM and EDX. A novel bioactive glass containing fluoride and magnesium was made, and the same tests were applied to teeth that were vacuum-deposited with microparticles of this glass powder. The effects on bond strength of the presence of bioactive glass at the bonded interface were tested by the single plane lap shear test, and the failure modes investigated by SEM. Finally, bioactive glass powder was incorporated into a commercially available adhesive, and the effects on leakage and bond strength were investigated. Both the commercially available glass and the novel glass were found to reduce leakage with no negative effects on bond strength, either when vacuum-deposited into etched dentin or when incorporated into adhesive.

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## **Chapter 1**

### **Introduction**

Of the millions of dental restorations that are placed annually, many utilize the process of bonding a resin to dentin. While the use of polymeric materials in restorative dentistry has many advantages, including aesthetics and ease of placement<sup>1</sup>, there are, nevertheless, problems still associated with their use.

Issues with bonding to enamel are largely resolved, thanks to the introduction of acid etching prior to the application of resin,<sup>2</sup> a method developed by Buonocore in 1955. In a 1976 review, Kidd discusses the need for a material that will form a chemical bond, and thus a perfect seal, to dentin.<sup>3</sup> Over thirty years later, we are still in search of that material. Effectively bonding resin to dentin remains troublesome for a number of reasons. The first is the character of the bond itself—it is micromechanical in nature, and there is little chemical bonding present due to the hydrophilic nature of dental tissue in contrast with the largely hydrophobic nature of most of the polymeric resins. The bond of the polymer to the dentin is created by the permeation of dentin and the dentin tubules by the bonding adhesive, but permeation of the dentin is limited.<sup>4</sup> A second problem is leakage of the bond. Attempts to increase the hydrophilicity of the adhesive monomer, and thus the ability of adhesive resin to permeate dentin, also increase the vulnerability of the bonded dentin to hydrolytic attack<sup>5</sup> and breakdown of the bond. Additionally, enzymes from either saliva or oral tissues can further break down the bond.<sup>6</sup> The result is either direct failure of the bond, or development of carious lesions that can, in turn, erode the tooth. The intrinsic properties of the restorative material also can cause leakage—upon curing, the resin-based materials shrink, thus creating gaps and inducing stress at the material interface.<sup>7</sup>

In the years since the introduction of resin bonding, many developments have occurred to improve what began as a three-step system: etch the dentin to remove surface contaminants<sup>2</sup> (e.g. the smear layer created when preparing a cavity surface with a bur); priming of the surface; and application of the adhesive resin, followed by curing.

Etchants are generally phosphoric, maleic, nitric, or citric acid in gels or liquid form, and are used to remove the smear layer and lightly demineralize dentin. Similar to etchants are conditioners, whose action can be considered analogous, but may additionally clean or modify the dentin so as to increase its adhesive ability.<sup>4</sup> The purpose of the primer is to, in effect, bridge the difference in hydrophilicity that exists between the dentin and resin. Primers contain molecules with hydrophilic groups that are capable of infiltrating and adhering to dentin, as well as hydrophobic groups that adhere to the resin. Monomers contained in primers include hydroxyethyl trimellitate anhydride (4-META) and biphenyl dimethacrylate (BPDM).<sup>8</sup> Adhesives can be unfilled or filled (less frequent), and are a mixture of hydrophobic molecules that polymerize upon curing. Commonly used monomers include 2-hydroxyethylmethacrylate (HEMA), bisphenyl-A glycidyl methacrylate (Bis-GMA), urethane dimethacrylate (UDMA), and triethylene glycol dimethacrylate (TEGDMA); HEMA is hydrophilic, while the rest of the monomers listed are hydrophobic.<sup>4</sup>

Several “generations”<sup>8</sup> of bonding agents have followed, with the dual aims of increasing ease of use and decreasing technique sensitivity. Systems have been developed that combine two of the three steps, or even all three steps into one bottle.<sup>7</sup> Thus there are self-etching primer systems, etch-and rinse adhesive systems, and self-etching adhesive systems.

The efficacy of each of these methods has been tested by various means, both *in vitro* and *in vivo*. The most common of the laboratory tests are assessments of bond strength by microtensile bond strength ( $\mu$ TBS) and shear bond strength tests. Laboratory studies of bond strength indicate that the three-step method of etching, priming, and bonding creates the strongest and most durable bond; that there is no significant difference between the two-step methods; and that one-bottle systems are the least effective of all.<sup>7</sup>

Clinical studies have also been used to assess the different restorative methods. Commonly, a Class V non-cariou lesion is prepared with the restorative method to be tested. Ideally, a negative control restoration is prepared in the same subject. After a prescribed period of time, the teeth are evaluated by multiple examiners and effects on the restorative material are measured.<sup>7,9,10</sup> Such studies have demonstrated various levels of effectiveness for each of these methods, with only the self-etch adhesive systems approaching the “gold standard” of the three-step method for bonding effectiveness.<sup>11</sup> Specific criteria are used to evaluate the success of the bonding: these include color, marginal adaptation, and loss of retention.<sup>12</sup>

The goal of resin bonding systems is to create a high quality and durable bond. This is likely to include a hybrid layer in which the collagen fibril network that remains after etching is fully permeated by the bonding material, with no area of unsupported fibrils in between the dentin and adhesive.<sup>13</sup> The depth and durability of the hybrid layer are indicative of the quality of the bond formed; a well-formed hybrid layer suggests a long-lived bond. The hybrid layer, as described by Nakabayashi *et al* in 1982, is demineralized dentin that has been infiltrated by monomers which are later polymerized.

This interface appears to diffuse mechanical stress, thereby reducing the possibility of failure of the restoration.<sup>13</sup>

However, as previously mentioned, the nature of the resin bond is primarily not chemical, although some restorative materials do form chemical bonds with dentin. Glass ionomer cements were introduced by Wilson and Kent<sup>14</sup> and bond to teeth both micromechanically (as the cement diffuses through the collagen network, which is exposed by treatment with polyalkenoic acid) and chemically (interaction of carboxyl groups of the acid with calcium ions attached to the collagen fibrils).<sup>15</sup> While the chemical bonding is a positive aspect of glass ionomers, in addition to the fact that the glass itself may be used as a fluoride reservoir,<sup>16</sup> nevertheless, glass ionomers are far from ideal as restorative materials. Firstly, there are aesthetic disadvantages;<sup>17</sup> secondly, material properties of these cements are not conducive to a durable bond.<sup>7</sup> In order to address the latter of these issues, metals have been added to the cement to reinforce them;<sup>18</sup> however, this practice is diminishing in use.<sup>19</sup> More recently, resin-modified glass ionomers have been developed; however, even these materials require many improvements before they can be considered to be a superior restorative material, as they have been found to leach cytotoxic compounds.<sup>20, 21</sup>

Bioactive glasses have also been used in ceramic cements for bone bonding,<sup>22</sup> but these particular materials have yet to be used in dental applications. However, these glasses have been used as filler in composites.<sup>23</sup> This may not be an ideal application for bioactive glasses, as their dissolution may create voids in the resin in which they are embedded, allowing for bacterial infiltration. Additionally, bioactive glass particles can

be used in dentifrices, for the purpose of reducing tooth sensitivity when mineral is precipitated and occludes dentin tubules.

There is a need for restorative materials that bond strongly and durably to dentin; equally, there is a need for these materials to be aesthetically pleasing. Resin-based restoratives meet the latter qualification, but there is much room for improvement in the case of the former. Perhaps incorporating additional materials into the resin-bonding procedure can produce this improvement—specifically, incorporating bioactive glasses. Because of their ability to promote the formation of apatite in aqueous environments that contain calcium and phosphate (e.g. saliva), their presence at the bonded interface will improve the quality of the resin-dentin bond through self-sealing in the presence of leakage caused by the formation of apatite.

According to Hench, bioactive glasses elicit a response at the glass/tissue interface such that a bond is formed.<sup>24-26</sup> They possess unique compositional traits, such as low silica content, high sodium and calcium content, and a high ratio of CaO to P<sub>2</sub>O<sub>5</sub>.<sup>24</sup> Because of these characteristics, a distinct series of chemical reactions occurs when these glasses are brought into contact with tissue, or any aqueous environment that contains calcium and phosphate, and carbonated hydroxyapatite (HCA) is formed.<sup>24</sup> As the glass is exposed to this environment, alkali ions are almost immediately leached out, leading to the formation of SiOH bonds at the surface of the glass. These SiOH bonds condense and a silica-rich layer is formed on the glass surface. Ca<sup>2+</sup>, PO<sub>4</sub><sup>3-</sup>, and HCO<sub>3</sub><sup>2-</sup> ions then adsorb to this layer in the form of amorphous, substituted calcium phosphate that later crystallizes to HCA. Following these reactions, which normally occur within the first two hours, a sequence of biological reactions transpires, beginning with the adsorption of

cellular growth factors to the layer of apatite, through the attachment of stem cells which later differentiate and secrete extracellular matrix, that eventually leads to bone growth within a period of days to weeks.<sup>24</sup> The resultant bond is mechanically strong and is believed to be chemical in nature; force is required to separate the tissue from the glass.<sup>27</sup> The exact character of the bonds is not known, though it has been suggested that the forming mineral bonds to specific amino acids within the collagen that is the main structural component of bone.<sup>25, 26</sup> In addition to bone, bioactive glasses have been shown to react favorably with dentin, creating a mechanical bond.<sup>28</sup> forming carbonated apatite similar to tooth mineral,<sup>29, 30</sup> and displaying antibacterial properties that would be beneficial to the prevention of secondary caries.<sup>31</sup>

The chemical composition of bioactive glasses has significance. Sodium is included because of the oxide's solubility in an aqueous environment, its ability to aid in maintenance of physiological ionic balance and pH, and ease of use in glass production; while silica maintains the glass structure.<sup>26</sup> Calcium and phosphate are necessary because they are the principal constituents of the mineral in mineralized tissues. The proportions of these have been varied with differing results.<sup>26</sup> Other components may be added for their beneficial effects either on the manufacture of the glass or on the behavior of the glass once implanted.<sup>32</sup> Boron,<sup>32</sup> aluminum,<sup>32</sup> magnesium,<sup>32, 33</sup> iron,<sup>34</sup> titanium,<sup>34</sup> fluorine,<sup>32, 34-37</sup> and silver<sup>38, 39</sup> have all been added to bioactive glass. Boron-containing bioactive glasses are reported to enhance resorption.<sup>34</sup> Glasses that will be used to coat metal-alloy implants may be doped with those metals<sup>34</sup> (such as iron, aluminum, or titanium) to increase their compatibility (for example, the coefficient of thermal expansion) and reduce the likelihood of delamination. Silver has been added to bioactive



glass for its antibacterial properties.<sup>38, 39</sup> The addition of fluorine to bioactive glass can be rationalized for the following reasons: firstly, that the rate of fluorapatite formation at the glass surface can be enhanced;<sup>36</sup> and secondly, that the fluorapatite formed will be less vulnerable to acid attack,<sup>40</sup> both of which are desirable in bioactive glass to be utilized in dental applications. Magnesium can also be a beneficial addition to bioactive glass, as it has been shown to slow down the rate of apatite precipitation, thus leading to more controlled mineralization.<sup>29</sup>

Determination of the relative success of incorporation of bioactive glass into the dentin bonding process will require assessment of the ability of the glass to reduce leakage, as well as its effect upon the strength of the bond. In order to evaluate these effects, a combination of methods will be applied. Leakage will be measured by both the silver nitrate method and the methylene blue method. Bond strength will be quantified using the single plane lap shear method. In Chapter 2, the ability of microparticles of commercially available bioactive glass to infiltrate etched dentin is tested, and hybrid layer formation and leakage are assessed by SEM and EDX. Chapter 3 applies the same tests to a novel bioactive glass containing fluoride and magnesium. The effects on bond strength of the presence of bioactive glass at the bonded interface are tested by the single plane lap shear test in Chapter 5, with the failure modes investigated by SEM. In Chapter 6, bioactive glass powder is incorporated into a commercially available adhesive; the effects on leakage and bond strength are investigated.

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## **Chapter 2**

### **Bioactive glass reduces microleakage in resin-bonded dentin**

## **ABSTRACT**

A major disadvantage of resin-dentin bonding is the tendency of the restorations to leak, due to incomplete infiltration of the demineralized collagen network with bonding resin, hydrolytic degradation of the resin, or attack of exposed collagen by native enzymes.

Objective: To test the hypothesis that the presence of bioactive glass, which is known to precipitate apatite when placed in aqueous environments that contain calcium and phosphate, will reduce leakage of resin-bonded dentin. Methods: The occlusal dentin of human third molars was ground and etched, and a slurry of bioactive glass powder in ethanol was vacuum-deposited into the etched surface. The samples were then bonded with commercially available adhesive and composite. Samples were stored in simulated body fluid (SBF) for one week, two weeks, or tested without storing in SBF. Leakage was evaluated in one of two ways: soaking in silver nitrate followed by scanning electron microscopy and energy dispersive x-ray analysis (SEM/EDX); or soaking in methylene blue followed by ordinal ranking by two independent operators and analysis of the results with the non-parametric Mann-Whitney test. Results: SEM/EDX analysis and x-ray mapping showed reduced leakage of samples treated with bioactive glass when compared to negative controls. Statistical analysis of the ranked samples showed a significant difference (Mann-Whitney  $U = 39.0$ ,  $p = 0.0005$ ) between glass-treated samples and negative controls. Significance: Reduced leakage of resin-bonded dentin in the presence of bioactive glass may increase the lifetime expectancy of these restorations.

## **INTRODUCTION**

The resin-dentin bonding process consists of three steps: etching with an acid to partially demineralize the dentin matrix and remove the so-called smear layer that has been shown to be a poor substrate for bonding; priming with a monomer that can penetrate the collagen-rich network that remains after the etching procedure; and application of a bonding agent that usually is cured and bonds to a hydrophobic resin composite. The resulting stratum of polymer-infiltrated collagen is called the hybrid layer.<sup>1</sup> Since the introduction of adhesive dentistry, methods have been developed that combine these steps: self-etching primers, etch-and-rinse-adhesives, and “one-bottle” systems that combine all three steps. Good infiltration of the demineralized collagen generally leads to a good hybrid layer and a stable bond, yet the thickness of the layer does not necessarily affect the bond’s quality.<sup>2,3</sup> The bond between the resin and the dentin is primarily micromechanical, similar to the concept proposed by Buonocore<sup>4</sup> that resin penetrates microporosities in enamel that has been acid-etched, resulting in the micromechanical bond.<sup>5</sup> Therefore, it is crucial to permeate the collagen network with resin and to cure it *in situ* so that the fluids of the oral environment cannot disrupt the bond. Over time, the resins may absorb water, leading to degradation,<sup>6-9</sup> or fluids within the oral environment may carry with them chemical compounds or enzymes that can break down the polymer.

An additional factor that can contribute to leakage of bonded dentin is unprotected collagen fibrils at the base of the restoration. The etchant may demineralize the dentin to a depth greater than that penetrated by the adhesive—this leaves the collagen network

unshielded by mineral or polymer and exposed to oral fluids. Matrix metalloproteinases or MMPs (for example, collagenases and gelatinases) present in these fluids<sup>10, 11</sup> can then break down the network and lead to failure of the restoration. Recent work has also shown that shown that MMPs present in the dentin matrix can be activated by self-etching primers<sup>12, 13</sup> or “etch-and-rinse” bonding systems.<sup>14</sup>

Many modifications and improvements have been made to resin bonding products in the years since their inception. However, despite such improvements, leakage of restorations remains a significant issue. One possible modification that may serve to attenuate the leakage problem is the incorporation of microparticulate materials into the bonding process; materials which may create a chemical bond in addition to the micromechanical one, or potentially precipitate mineral into any open space that remains after polymerization of the adhesive.

One such material is bioactive glass, the best-studied and -characterized of which is Bioglass<sup>®</sup>, first described by Hench in 1971.<sup>15</sup> Bioactive glasses, including Bioglass<sup>®</sup> (formula 45S5), form apatite in aqueous environments that contain calcium and phosphate, and bond to bone<sup>15</sup> and soft tissue<sup>16</sup> without toxicological consequences. Bioglass<sup>®</sup> 45S5 is currently utilized in bone-repair applications and under the trade name Perioglas<sup>®</sup>, particulate Bioglass<sup>®</sup> is also used to repair bony defects resulting from periodontal disease.<sup>17</sup> Additionally, particulate Bioglass<sup>®</sup> has been used in pulp-capping procedures and to reduce tooth sensitivity. However, in these applications, the particle sizes can be as large as 300  $\mu\text{m}$ .<sup>18</sup>

Such large particles would be inappropriate for incorporation into the resin bonding process since the dentin tubule diameters are on the order of 1  $\mu\text{m}$ .<sup>19</sup> The spaces



in the three-dimensional mesh of the collagen network exposed through demineralization are smaller still. The kinetics of dissolution of small ( $\sim 5 \mu\text{m}$ ) particles of Bioglass<sup>®</sup> 45S5 have been studied,<sup>20</sup> and the material retains its ability to precipitate apatite even at that small size. Additionally, very recent studies of nanoparticulate bioactive glass have demonstrated the ability to remineralize dentin that has been chemically demineralized.<sup>21</sup>

The hypothesis of this study is that bioactive microparticulate material, specifically Bioglass,<sup>®</sup> may be effectively added to the dentin-resin bonding procedure to ultimately reduce leakage of bonded restorations.

## **MATERIALS AND METHODS**

### *Preparation of tooth samples*

The occlusal enamel of six human third molars collected according to a protocol approved by the UCSF Committee on Human Research was removed using a belt sander with 240-grit silicon carbide paper, following which the roots were removed using a slow-speed saw (Isomet, Buehler Ltd., Lake Bluff, IL) with water coolant to produce tooth discs  $\sim 5$  mm thick. The exposed dentin was polished with 320-grit silicon carbide paper. The discs were mounted on open-ended tubes with hot glue, and then attached to a vacuum trap (Figure 1). The occlusal dentin was etched for 15 seconds with Scotchbond gel etchant (3M/ESPE, St. Paul, MN) and rinsed for a further 15 seconds with deionized water. A slurry of 20% or 40% (w/v) bioactive glass of the formula 45S5 (SEM-COM, Toledo, OH) in ethanol was applied to the top of the sample. The average particle size of the ground bioactive glass powder was  $\sim 1 \mu\text{m}$ , and it was prepared by planetary ball milling of glass chips. The glass slurry was prepared with ethanol, rather than with

water, so as not to affect the surface chemistry of the glass prior to its contact with the tooth. Other samples were prepared using a slurry of 40% Al<sub>2</sub>O<sub>3</sub> powder (particle size 1µm; Buehler, Lake Bluff, IL) in water, to test the ability of particles of that size to penetrate etched dentin. Vacuum was applied at 530 mm Hg for one minute; the sample surface was kept moist by re-applying the slurry every few seconds. After removal from the vacuum, any excess solid was gently rinsed away with deionized water. Negative controls were vacuum-aspirated with water alone. The sample surface was then bonded with Single Bond (3M/ESPE, St. Paul, MN) adhesive and light-cured per manufacturer's instructions. The samples were cryofractured and mounted on aluminum stubs, or prepared for leakage studies, for which two coats of Filtek Z-250 Universal Restorative Composite (3M/ESPE, St. Paul, MN) were applied and cured per manufacturer's instructions.

#### *Silver nitrate leakage study*

Silver nitrate is commonly used to determine the extent of leakage of dental materials—silver ions in solution are able to penetrate into areas where leakage has occurred. After exposure to a photographic developer, the ions precipitate as an easily detectable solid.<sup>22</sup> After the bonded samples were soaked in simulated body fluid<sup>23</sup> for a specified length of time (no storage, t=1 week, t =2 weeks), three serial slabs were cut from the central region of each tooth with a slow-speed saw. Each slab was coated with nail polish to within 1 mm of the bonded interface and soaked in ~ 2 ml of 50% (w/v) silver nitrate for two hours without exposure to light. The samples were then transferred to ~2 ml of developing solution for four hours under fluorescent lights, after which they

were thoroughly rinsed with deionized water. The nail polish was removed with acetone, and the samples were finished through 1200 grit SiC, then mounted on aluminum stubs for SEM analysis.

#### *Scanning electron microscopy and energy-dispersive x-ray analysis (SEM/EDX)*

SEM (Topcon ISI ABT SX-40A, Milpitas, CA) visualization of the samples was executed in one of two ways: either in secondary electron mode for topographical information or in backscattered mode, using a charge-free anticontamination system (CFAS) and energy dispersive x-ray analysis for chemical information (Thermo-Noran Sigma2, Middleton, WI). Samples viewed in secondary electron mode (15keV) were first sputter-coated with a 200 nm-thick layer of gold/palladium under argon atmosphere. Samples designated for EDX were not coated with gold/palladium. Their chemical composition was mapped (dwell time, 5600 $\mu$ s; 128 x 128 pixels) with regard to the following elements: silicon, sodium, calcium, and phosphorus for cryofractured Bioglass-impregnated samples; and silicon, sodium, calcium, phosphorus, and silver for silver nitrate leakage studies.

#### *Methylene blue leakage study*

Dye penetration studies are also commonly used to assess leakage of dental restorations, as these techniques produce results that are easy to visualize and do not require complex chemical reactions or the use of radioactive isotopes<sup>22</sup>. Methylene blue is frequently used for this purpose.<sup>22</sup> Prepared and bonded teeth were soaked in simulated body fluid<sup>23</sup> for ~16h, then thermocycled in water baths for 500 cycles in the

following pattern: 5 seconds at 5°C, 20 seconds at 37°C, 5 seconds at 55°C, and 20 seconds at 37°C. After thermocycling, the samples were coated with nail polish on the tooth surface to within ~1 mm of the bonded interface and inverted in individual wells of a multi-well plate so that they were about two-thirds submerged in a 2% (w/v) methylene blue solution for 14 hours. They were then removed from the methylene blue and rinsed with tap water for 30 minutes. The samples were allowed to air-dry, and then the nail polish and superficial methylene blue were removed by polishing. Longitudinal slices ~1 mm thick were cut in an occlusal to apical direction from the center of each tooth, using a slow-speed saw and xylene as a coolant. The slices were allowed to air-dry and mounted on glass microscope slides, then examined under a light microscope by two independent scorers and ranked. The ranking methodology was determined as follows: teeth were visually divided in half, and two scorers graded each half. Thus, each tooth received four scores. Teeth with no dye penetration were given a score of zero, while penetration into the outer third was scored as 1, the middle third was scored as 2, and the inner third was scored as 3. The non-parametric Mann-Whitney Rank Sum Test was used to analyze the results.

## **RESULTS**

SEM images of the cryofractured samples show that an apparently normal hybrid layer formed in the dentin that had been vacuum-infiltrated with Bioglass<sup>®</sup> (see Figure 2). The adhesive was able to permeate etched dentin and enter the tubules to form resin tags, thus sealing the microparticulate matter into the dentin. The vacuum aspiration process

did not seem to have a deleterious effect on the dentin tubules; they did not appear to be narrowed, collapsed, or altered in any manner.

Although neither glass nor  $\text{Al}_2\text{O}_3$  particles were easily detected in the SEM images, EDX maps (Figure 3) showed that particles were embedded in the dentin to a depth of 5-10  $\mu\text{m}$  below the surface. The maps further suggested that the materials are present in both the adhesive and the dentin itself.

Silver nitrate leakage studies indicated that the presence of bioactive glass appeared to reduce leakage in comparison to negative controls as seen by a reduction of the intensity in the Ag x-ray map (Figure 4). Bioactive glass-treated samples that were studied prior to soaking in simulated body fluid exhibited leakage similar to that of negative controls; however, after storage in SBF for one or two weeks, Ag was not detected and thus leakage was apparently reduced in the glass-treated samples.

In the case of the methylene blue leakage studies, a statistically significant difference between glass-treated teeth and negative controls (Mann-Whitney  $U = 39.0$ ,  $p = 0.0005$ ) was found. Although 12 teeth were prepared, 1 sample was physically lost; of the others, rankings were discarded if the interface was obscured by either residual enamel or by overhanging composite. In all, there were eighteen separate rankings for six glass-treated teeth, and fourteen separate rankings for five negative controls. The rankings from both scorers were pooled in order to evaluate the difference between treatment groups: there was no significant difference between scorers ( $p=0.85$ ); there was, however, a significant difference between sides among the groups, which was more pronounced for the negative controls ( $p=0.084$ , glass-treated teeth;  $p=0.012$ , negative controls).

## **DISCUSSION**

Current dentin bonding procedures involve formation of a hybrid layer between the adhesive layer and the dentin<sup>1</sup>. The micromechanical nature of the bond requires, therefore, that the dentin be thoroughly permeated with adhesive to produce a good quality bond.<sup>1, 3, 24</sup> The experiments performed in this chapter were designed to test the hypothesis that bioactive glass powder can be incorporated into resin-bonded dentin for the purpose of reducing leakage, without deleterious effects on the hybrid layer itself. The introduction of an additional material into the bonding process has the potential to disrupt the formation of a proper hybrid layer. This potential depends on a number of factors, including but not limited to: how well the dentin is etched; the size of the particles of the material that is introduced; and the properties of the material itself. This last is especially important, and one property that stands out as critical is the hydrophilicity of the material in question; that is, the affinity for both the dentin (hydrophilic) and the adhesive (somewhat hydrophobic). It is necessary for the bonding agent to be able to wet the material in such a manner that it remains within the dentin and the adhesive upon curing. Evidence suggests that bioactive glasses<sup>25</sup> are wettable, though less hydrophilic than pure apatite,<sup>26, 27</sup> with variations in the level of hydrophilicity caused by surface impurities or irregularities, and should be able to maintain contact with the dentin and adhesive. From the SEM micrographs and the EDX maps, it appears that Bioglass<sup>®</sup> does not penetrate the etched dentin to a uniform depth; this is likely explained by the range of particle sizes of the powder. The Bioglass<sup>®</sup> used in these experiments was ground by planetary ball milling to an *average* particle size of 1

μm, likely to have a broad size distribution, implying that some particles may be substantially smaller. The smaller particles are more likely to be pulled into and deposited in the dentin to a greater depth during vacuum aspiration. Figures 2(a) and 3(a) illustrate that the presence of the glass particles does not interfere with the formation of the hybrid layer. Additional studies, such as with transmission electron microscopy, are needed to further confirm the structure of the glass-containing hybrid layer.

The reduction of leakage that is apparent in the SEM micrographs and EDX maps of glass-treated samples could be due to a number of different factors: firstly, the glass particles are simply occluding any gaps that are possible means for water ingress; secondly, that the glass particles facilitate a better bond between the adhesive and the dentin owing to some intrinsic property that complements both; or thirdly, that the contact with the aqueous environment via leakage has led to apatite precipitation, which has sealed the resin and dentin together. While the first and second possibilities cannot be entirely ruled out, it is the last which seems most likely, as previous studies have shown that calcium phosphate deposits form quickly on the surface of particles of bioactive glass powders, appearing as soon as 1 hour after immersion of 45S5 powder in SBF;<sup>20</sup> these deposits exist as both crystalline apatite and amorphous calcium phosphate. A recent study<sup>21</sup> has shown that bioactive glass is capable of remineralizing dentin, and smaller glass particles are substantially more effective at doing so. Mere occlusion of the tubules is not a likely explanation for the reduction of leakage, for two reasons: firstly, fluid from the tubules is not the sole cause of leakage—fluid trapped within the hybrid layer contributes substantially to leakage;<sup>6,9,28</sup> and secondly, leakage appeared in areas of the interface that were not necessarily tubule-dense.

The use of dyes for the evaluation of leakage is a long-standing and widely used technique.<sup>29</sup> Due to the problems of interpretation of results<sup>22</sup> and the frequently subjective nature of ranking tests<sup>29</sup> that are associated with dyes and tracers, two separate processes (silver nitrate and methylene blue) were used in the anticipation that the findings from one would substantiate those from the other. The results of the methylene blue leakage studies confirm the findings generated from the silver nitrate leakage studies. While this method may be considered to be somewhat subjective, the use of two independent scorers serves to better regulate the findings. Additionally, the two scorers' individual sets of rankings appear to be in good agreement with one another, strongly indicating a good level of standardization, as demonstrated by the statistically insignificant difference between the scorers. The non-parametric Mann Whitney Rank Sum Test was chosen, as it does not make assumptions about the normality of the data distribution, therefore it was not necessary to nest the data with regard to operators. Additionally, this test is appropriate for use with samples that have been ordinally ranked. Since the samples were bonded, thermocycled, and treated with methylene blue before they were cut, they cannot be considered as truly independent; however, leakage on one side does not guarantee leakage on the other, as was evident from the statistical analysis. The characteristic assumptions made by the Mann Whitney Rank Sum Test allow for the data to be pooled, rather than nested.

## **CONCLUSION**

Microparticles of bioactive glass may be effectively incorporated into the resin-dentin bonding process, allowing the adhesive to penetrate the exposed collagen network and



form an apparently normal hybrid layer upon polymerization. Once incorporated into the bonded dentin, the glass particles appear to reduce leakage when samples are soaked in simulated body fluid; the likely cause of this is precipitation of apatite by the glass.

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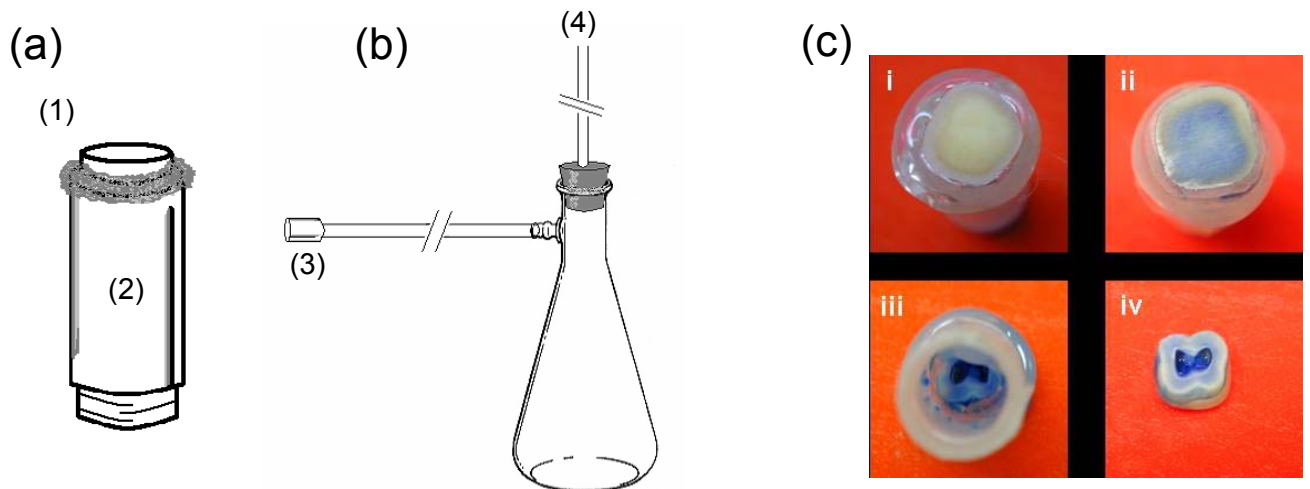
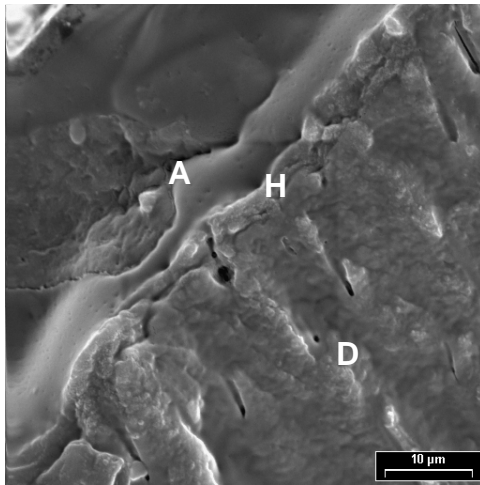


Figure 1. Vacuum-infiltration device: (a) enlargement of sample. A 5-mm-thick tooth disk (1) is secured to a cryofuge tube (2) with hot glue (end of tube was sliced off) so that an airtight seal is formed. A slurry is applied to the top of the exposed occlusal dentin. Figure (b) illustrates the vacuum trap: the sample screws into a cryofuge tube cap (3) that has been cemented to a silicone tube that is attached to an Erlenmeyer flask; the flask is attached to a vacuum at (4). An experiment using a methylene blue solution shown in (c)i-iv, shows that liquid applied to the top of the sample is pulled all the way through the dentin only. Figure 1(c)(i) shows a sample mounted in preparation for vacuum aspiration; 1(c)(ii) shows the same sample after vacuum aspiration for one minute with methylene blue. Figure 1(c)(iii) and (iv) show the underside of the same sample, with (iii) still mounted, and (iv) removed from the apparatus.

**(a)**



**(b)**

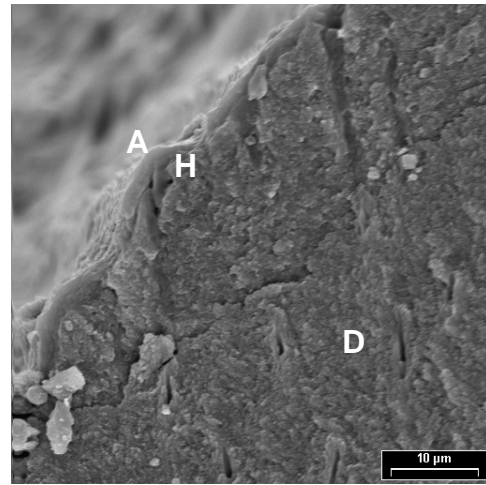


Figure 2. SEM micrographs of cryofractured teeth at 2000x: (a) vacuum-impregnated with Bioglass<sup>®</sup>, (b) negative control. An apparently normal hybrid layer has formed in the Bioglass<sup>®</sup>-treated teeth. A-adhesive; H-hybrid layer; D-dentin.

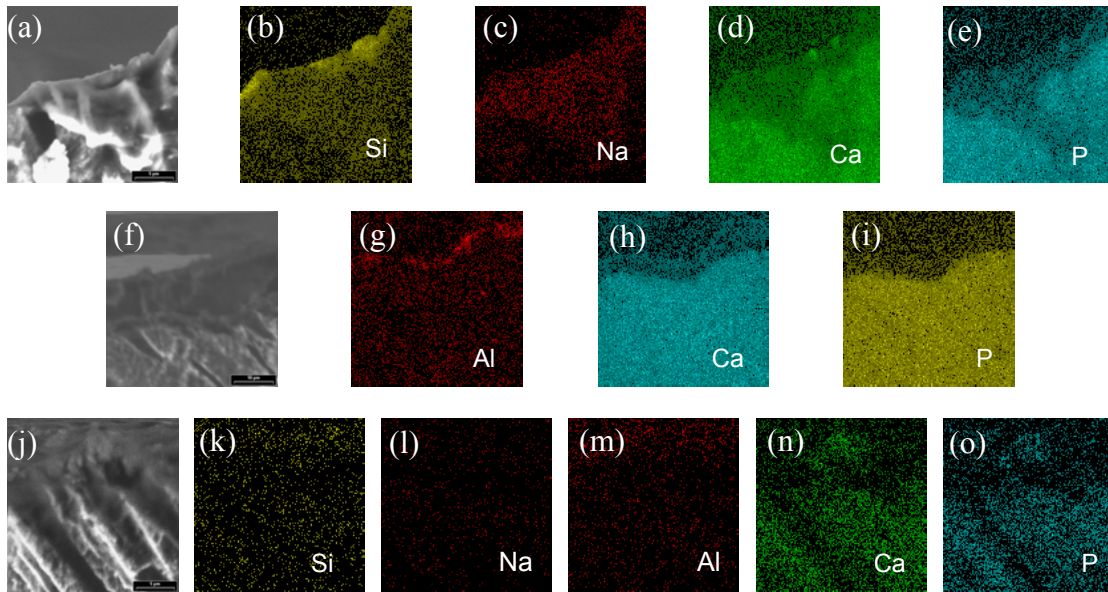


Figure 3. SEM micrographs and EDX maps of cryofractured, vacuum-impregnated teeth: (a-e) Bioglass<sup>®</sup>-treated; (f-i) Al<sub>2</sub>O<sub>3</sub>-treated; (j-o) negative control. Silicon and sodium are evident in the adhesive and hybrid layers of the Bioglass<sup>®</sup>-treated teeth (b, c), as is aluminum in the Al<sub>2</sub>O<sub>3</sub>-treated teeth (g), yet are not seen in the negative control (k, l, m). Calcium (d, h, n) and phosphate (e, i, o) are also shown.

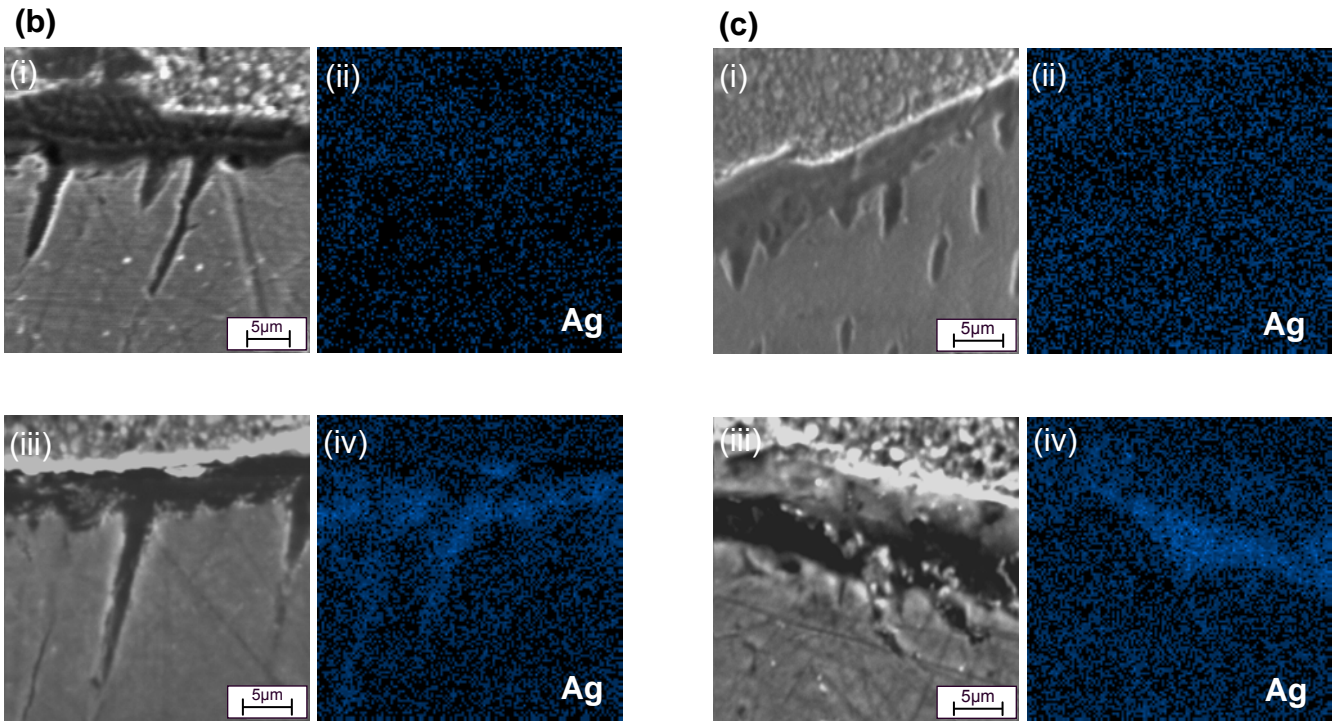
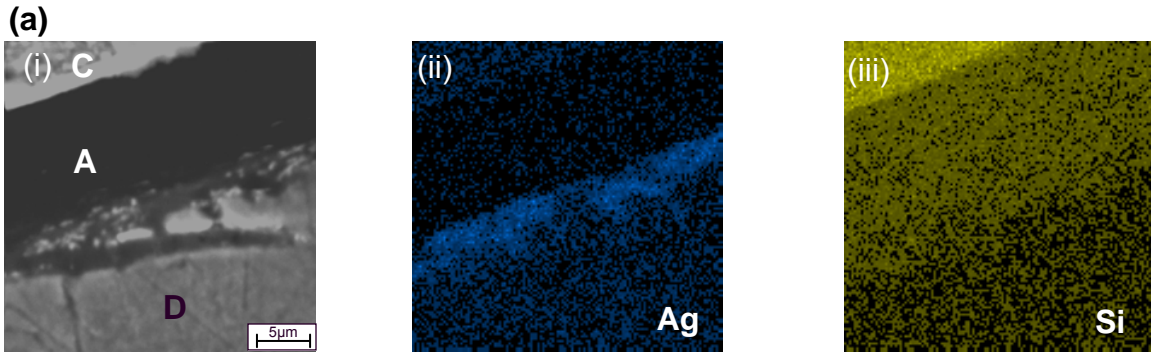


Figure 4. SEM micrographs and EDX maps of teeth that have been bonded and soaked in simulated body fluid (SBF) for 0 days (A i-iii), one week (B i-iv), and two weeks (C i-iv). Glass-treated samples that have not been exposed to simulated body fluid exhibit leakage between the adhesive and dentin, as represented in areas of highly concentrated blue pixels (A ii). Silicon (yellow pixels) is present in high concentrations in the composite, in reduced concentrations in the adhesive, and in further reduced concentrations in the dentin. Glass-treated samples (B i, ii; C i, ii) show little to no leakage, whereas negative controls (B iii, iv; C iii, iv) exhibit extensive leakage. (**C** = composite, **A** = adhesive, **D** = dentin)

### **Chapter 3**

**A novel, fluoride- and magnesium-containing bioactive glass reduces microleakage  
in resin-bonded dentin**



## **ABSTRACT**

Microleakage is a problem that has afflicted adhesive dentistry since its introduction more than five decades ago. This occurs for a number of reasons, including incomplete infiltration of the demineralized collagen network with bonding resin, hydrolytic degradation of the resin, or attack of exposed collagen by native enzymes. Previous work has shown that bioactive glass, which is known to precipitate apatite when placed in aqueous environments that contain calcium and phosphate, can reduce leakage in resin-bonded dentin. This chapter tests the hypothesis that a novel bioactive glass that contains magnesium and fluoride can produce similar or improved effects. The occlusal dentin of human third molars was ground and etched, and a slurry of bioactive glass powder in ethanol was vacuum-deposited into the etched surface. The samples were then bonded with commercially available adhesive and composite. Samples were stored in simulated body fluid (SBF) for one week, two weeks, or tested without storing in SBF. Leakage was evaluated in one of two ways: soaking in silver nitrate followed by scanning electron microscopy and energy dispersive x-ray analysis (SEM/EDX); or soaking in methylene blue followed by ordinal ranking by two independent operators and analysis of the results with the non-parametric Kruskal-Wallis and Mann-Whitney tests. SEM/EDX analysis and x-ray mapping showed reduced leakage of samples treated with bioactive glass when compared to negative controls. Statistical analysis of the ranked samples did not show a significant difference (Kruskal-Wallis  $H=1.50$ ,  $p=0.471$ ) between any of the glass-treated samples and negative controls, though a trend of reduced leakage was suggested. This reduced leakage in the presence of bioactive glass may increase the lifetime expectancy of these restorations.

## **INTRODUCTION**

Buonocore's introduction of the acid-etching technique for resin bonding to dentin<sup>1</sup> initiated the rapid growth of the field of adhesive dentistry. While resin-based restorations are superior to amalgam restorations in terms of aesthetic appeal, a major problem with these materials remains unsolved: microleakage. Microleakage, which can be defined as the movement of fluids that may or may not carry ions, enzymes, or bacteria, into a zone between a restoration and the host tooth,<sup>2</sup> is the major issue of concern.

The adhesives that are currently used do not form chemical bonds to the collagen network that is exposed by etching; instead, the bond is based on the ability of the adhesive monomer to penetrate the collagen before it is polymerized *in situ*, and is thus micromechanical in nature. This creates the so-called "hybrid layer," first described by Nakabayashi in 1982.<sup>3</sup> Penetration of the adhesive into the collagen is limited,<sup>4</sup> however, and voids around the unprotected collagen fibrils can permit entry of oral fluids that carry enzymes capable of breaking the bond.<sup>5</sup>

Increasing the hydrophilicity of the adhesive monomers enhances their ability to penetrate the collagen layer, but also increases their vulnerability to hydrolytic attack<sup>6</sup> and breakdown. When the adhesive and supporting layer have been weakened, oral fluids carrying potentially destructive bacteria or enzymes may enter the area,<sup>2</sup> leading to failure of the bond and possible loss of the restoration.

Over the years, many improvements have been made to the original three-step method (etch, prime, bond) of applying adhesive restorations. Several "generations"<sup>7</sup> of bonding agents have been developed in the last few decades, but the

advancements have mainly increased ease of use and reduced technique sensitivity. Systems have been developed that combine two of the three steps, or even all three steps into one bottle<sup>8</sup>. Thus there are self-etching primer systems, etch and rinse adhesive systems, and self-etching adhesive systems. To date, however, little progress has been made with regard to reducing the propensity of the adhesive towards microleakage, though the achievement of improved bond strengths has significantly reduced gross gaps from occurring.

There is a need for restorative materials that bond strongly and durably to dentin; equally, there is a need for these materials to be aesthetically pleasing. It was hoped that glass ionomer cements, introduced by Kent and Wilson,<sup>9</sup> would meet both of these needs. While glass ionomer cements do bond both micromechanically and chemically to the collagen network in dentin,<sup>10</sup> the aesthetics of glass ionomer cements are inferior to those of resin based composites.<sup>11</sup> In addition, the mechanical properties of glass ionomer cements are not likely to produce durable bonds.<sup>8</sup> Resin-based restoratives do possess positive aesthetic qualities, but there is much room for improvement where strength and durability are concerned. Perhaps the addition of auxiliary materials into the resin-bonding procedure can produce the desired result—specifically, the addition of bioactive glasses. Because of their ability to promote the formation of apatite in aqueous environments that contain calcium and phosphate (e.g. saliva), their presence at the bonded interface may improve the quality of the resin-dentin bond through self-sealing that results from the presence of leakage which leads to the formation of apatite.

According to Hench, the developer of the original bioactive glasses, these materials elicit a response at the glass/tissue interface such that a bond is formed.<sup>12-14</sup>

They possess unique compositional traits, such as low silica content, high sodium and calcium content, and a high ratio of CaO to P<sub>2</sub>O<sub>5</sub>.<sup>12</sup> Because of these characteristics, a distinct series of chemical reactions occurs when these glasses are brought into contact with tissue, or any aqueous environment that contains calcium and phosphate, and carbonated hydroxyapatite (HCA) is formed.<sup>12</sup> As the glass is exposed to such an environment, alkali ions are rapidly leached out, which leads to the formation of SiOH bonds at the surface of the glass. These SiOH bonds condense and a silica-rich layer is formed on the glass surface. Ca<sup>2+</sup>, PO<sub>4</sub><sup>3-</sup>, and HCO<sub>3</sub><sup>2-</sup> ions then adsorb to this layer in the form of amorphous, substituted calcium phosphate that later crystallizes to HCA. These reactions normally occur within the first two hours; afterwards, a sequence of biological reactions transpires in bone, which would not occur in the tooth upon inclusion of bioactive glass; however, it is expected that the precipitated apatite would bond to the exposed collagen as occurs in bone. Studies have shown that such bonds to bone are mechanically strong and believed to be chemical in nature; force is required to separate the tissue from the glass.<sup>15</sup> The exact character of the bonds is not known, though it has been suggested that the forming mineral bonds to specific amino acids within the collagen that is the main structural component of bone.<sup>13,14</sup> In addition to bone, bioactive glasses have been shown to react favorably with dentin, creating a mechanical bond,<sup>16</sup> forming carbonated apatite similar to tooth mineral<sup>17,18</sup> and displaying antibacterial properties that would be beneficial to the prevention of secondary caries.<sup>19</sup>

The chemical composition of bioactive glasses is significant. Sodium is included because of its solubility in an aqueous environment, its ability to aid in maintenance of physiological ionic balance and pH, and ease of use in glass production; while silica

maintains the glass structure.<sup>14</sup> Calcium and phosphate, as the principal constituents of biominerals, are required. The proportions of these have been varied with differing results.<sup>14</sup> Other components may be added for their beneficial effects either on the manufacture of the glass or on the behavior of the glass once implanted.<sup>20</sup> Magnesium<sup>20</sup>,<sup>21</sup> and fluorine<sup>20, 22-25</sup> have been added to bioactive glass, among other elements, and could provide special benefits when included in applications for restorative dentistry. Magnesium can be a beneficial addition to bioactive glass, as it has been shown to slow down the rate of apatite precipitation, thus leading to more controlled mineralization.<sup>17</sup> The addition of fluorine to bioactive glass can be rationalized for the following reasons: it increases the formation of fluorapatite at the glass surface,<sup>23</sup> and that the fluorapatite formed will be less vulnerable to acid attack,<sup>26</sup> both of which are desirable in bioactive glass to be utilized in dental applications. Additionally, fluoride is important in caries prevention and remineralization—anti-caries slow-release materials are very beneficial, since they reduce the likelihood of recurrent caries as well as promote remineralization. The bonded interface is the most susceptible location for caries to start, hence the presence of fluoride in this area would be very helpful in the prevention of secondary caries.

In this chapter, the hypothesis is that the presence of a novel bioactive glass that contains magnesium and fluorine will reduce leakage in resin-bonded dentin, in a manner similar or even superior to the manner in which 45S5 reduced leakage in chapter 2.

## **MATERIALS AND METHODS**

### *Glass Preparation*

Bioactive glass of the formulation 45S5 was donated by SEM-COM, Inc. (Toledo, OH) in chip form. These chips were ground to an average particle size of  $\sim 1 \mu\text{m}$  by planetary ball milling.

Based on modification of another bioactive glass prepared by our group, a novel glass incorporating fluoride (“F glass”) was prepared<sup>27</sup> using the following compounds, with weight percentages in parentheses:  $\text{SiO}_2$  (44),  $\text{Na}_2\text{O}$  (23),  $\text{CaO}$  (10),  $\text{MgO}$  (4.5),  $\text{P}_2\text{O}_5$  (6), and  $\text{CaF}$  (12.5). The reagents were suspended in ethanol and mixed using a high-speed stirrer. This mixture was dried at  $80^\circ\text{C}$  for 12 h, then fired in air at  $1400^\circ\text{C}$  for 4 h in a Pt crucible. The liquid was cast into a graphite mold, obtaining plates of ( $\sim 50 \times 50 \times 5 \text{ mm}$ ) that were annealed at  $500^\circ\text{C}$  for 6 h.<sup>28</sup> The resulting glass was ground to an average particle size of  $\sim 1 \mu\text{m}$  as before.

### *Glass analysis: X-ray diffraction and scanning electron microscopy (SEM)*

Slabs of F glass were cut from the prepared glass bars using a diamond blade and ethanol coolant, then polished through 1200 grit in an ascending series of silicon carbide grits to final dimensions of  $\sim 25\text{mm} \times \sim 24\text{mm} \times \sim 2\text{mm}$ , also with ethanol coolant. The glass slabs were soaked in simulated body fluid for 6 weeks. X-ray diffraction analysis was performed on a Siemens D5000 diffractometer with Cu radiation, 40 kv, 30 ma and diffracted beam monochromator; step scan  $0.04^\circ 2\theta$  at 2 sec per step. The scan ranges were usually  $24\text{--}55^\circ 2\theta$ ; although  $5\text{--}24^\circ 2\theta$  was also examined. The resulting patterns were compared with Joint Committee on Powder Diffraction Standards (JCPDS) files for

hydroxyapatite (9-432) and brushite (9-0077). SEM (Field Emission Variable Pressure SEM Model S-4300SE/N, Hitachi Hi-Technologies, London, UK) was performed at an accelerating voltage of 15kV in order to visualize the samples.

### *Vacuum Infiltration and Bonding*

The occlusal enamel of human third molars extracted as part of dental treatment and following a protocol approved by the UCSF Committee on Human Research (N=6 for each treatment group, including negative controls) was removed using a belt sander with 240-grit silicon carbide paper, following which the roots were removed using a slow-speed saw (Isomet, Buehler Ltd., Lake Bluff, IL) with water coolant to produce tooth discs ~ 5 mm thick. The exposed dentin was polished with 320-grit silicon carbide paper. The resulting discs were mounted on open-ended tubes with hot glue, and then attached to a vacuum trap. The exposed occlusal dentin was etched for 15 seconds with Scotchbond gel etchant (3M/ESPE, St. Paul, MN) and rinsed for a further 15 seconds with deionized water. A slurry of 10% (w/v) bioactive glass of the formula 45S5 (SEM-COM, Toledo, OH) or 10% F glass in ethanol, was applied to the top of each sample. The glass slurry was prepared with ethanol, rather than with water, so as not to affect the surface chemistry of the glass prior to its contact with the tooth. Vacuum was applied at 530 mm Hg for one minute; the sample surface was kept moist by re-applying the slurry every few seconds or as needed. After removal from the vacuum, any excess solid was gently rinsed away with deionized water. Negative controls were vacuum-aspirated with water alone. Samples were removed from their mountings and the etched surface was then bonded with Single Bond (3M/ESPE, St. Paul, MN) adhesive and light-cured per

manufacturer's instructions. Two coats (each ~1 mm thick) of Filtek Z-250 Universal Restorative Composite (3M/ESPE, St. Paul, MN) were applied and light-cured per manufacturer's instructions. Samples were stored in simulated body fluid<sup>29</sup> at 37°C for one or two weeks prior to either cryofracturing or preparation for the silver nitrate leakage study. Additionally, one set of samples (N=6 for F glass, 45S5, and negative control groups) was prepared for the silver nitrate leakage study immediately after bonding .

#### *Silver nitrate leakage study*

Silver nitrate is commonly used to determine the extent of leakage of dental materials—silver ions in solution are able to penetrate into areas where leakage has occurred.<sup>30</sup> After exposure to a photographic developer, the ions precipitate as an easily detectable solid. After soaking the vacuum-infiltrated, bonded samples in simulated body fluid for a specified length of time (1 week, 2 weeks), or immediately after vacuum-infiltrating and bonding the teeth (to investigate leakage behavior without benefit of SBF exposure), three serial slabs were cut from the central region of each tooth (N=4 for each treatment group; 2 from each group were cryofractured and mounted for SEM analysis) with a slow-speed saw. Each slab was coated with nail polish to within 1 mm of the bonded interface and soaked in ~2 ml of 50% (w/v) silver nitrate for two hours without exposure to light. The samples were then transferred to ~2 ml of developing solution for four hours under fluorescent lights, after which they were thoroughly rinsed with deionized water. The nail polish was removed by grinding with 240 grit silicon carbide



paper, the samples were finished through 1200 grit SiC, and then mounted on aluminum stubs for SEM analysis.

*Scanning electron microscopy and energy-dispersive x-ray analysis (SEM/EDX)*

SEM (Topcon ISI ABT SX-40A, Milpitas, CA) visualization of the samples was executed in backscattered mode, using a charge-free anticontamination system (CFAS) and energy dispersive x-ray analysis for chemical information (Thermo-Noran Sigma2, Middleton, WI). Their chemical composition was mapped (dwell time, 5600  $\mu$ s; 256 x 256 pixels) with regard to the following elements: calcium, magnesium, phosphorus, silicon, silver, and sodium.

*Methylene blue leakage study*

An additional method of leakage assessment was deemed necessary to corroborate the results from the silver nitrate studies, therefore it was decided to use a dye penetration method. Dye penetration studies are also commonly used to assess leakage of dental restorations, as these techniques produce results that are easy to visualize and do not require complex chemical reactions or the use of radioactive isotopes<sup>31</sup>. Methylene blue is frequently used for this purpose. Samples (N=6 for each treatment group, including negative controls) were soaked in simulated body fluid<sup>29</sup> overnight, then thermocycled in water baths for 500 cycles in the following pattern: 5 seconds at 5°C, 20 seconds at 37°C, 5 seconds at 55°C, and 20 seconds at 37°C. After thermocycling, the samples were coated with nail polish on the tooth surface to within ~1 mm of the bonded interface and inverted in individual wells of a multi-well plate so that they were about two-thirds

submerged in a 2% (w/v) methylene blue solution for 14 hours. They were then removed from the methylene blue and rinsed with tap water for 30 minutes. The samples were allowed to air-dry, and then the nail polish and superficial methylene blue was removed by polishing. Longitudinal slices ~1 mm thick were cut in an occlusal to apical direction from the center of each tooth, using a slow-speed saw and xylene as a coolant. The slices were allowed to air-dry and mounted on glass microscope slides, then examined under a light microscope by two independent scorers and ranked. The ranking methodology was determined as follows: teeth were visually divided in half, and each scorer ranked each half. Thus, each tooth received four scores. Teeth with no dye penetration were given a score of zero, while penetration into the outer third was scored as 1, the middle third was scored as 2, and the inner third was scored as 3. If the interface was obscured by composite (“flash”), that side was not included for analysis. The non-parametric Kruskal-Wallis Test was used to analyze the results where three groups were compared; the non-parametric Mann Whitney Rank Sum Test was used when only two variables were compared.

## **RESULTS**

After storage in SBF for 6 weeks, x-ray diffraction analysis (Figure 1A) of the glass slabs that were produced in our lab showed peaks consistent with apatite, thus demonstrating its ability to form apatite when placed in an aqueous environment that contains calcium and phosphate. The 002 peak is larger than in the JCPDS file, indicative of preferred orientation, which is further suggested by the apparent uniform orientation of the crystals as seen in the SEM analysis (Figure 1B) of the samples.

Crystal formation that is consistent with apatite is evident; this is the fundamental characteristic that defines a bioactive glass.

Leakage was evident to a similar degree in all samples (Figure 2) on which silver nitrate leakage assessment was performed immediately after bonding (no soaking in SBF at all). Results from this study show lower Ag content and thus a reduction in leakage in the samples that were treated with either the F glass or the 45S5, after soaking in SBF, when compared to the negative controls (Figure 3). Cryofractured samples illustrate the formation of an apparently normal hybrid layer in the glass-treated teeth (Figure 4).

The methylene blue leakage test following thermocycling (see Table 1 for a summary of results) did not suggest a statistically significant difference between treatments (Kruskal-Wallis  $H=1.50$ ,  $p=0.471$ ). When analyzed with the Mann-Whitney Rank Sum Test, appropriate for comparison of only two variables, there was no significant difference between operators for any of the three groups (negative control,  $p=0.52$ ; F glass,  $p=0.97$ ; 45S5,  $p=0.98$ ); nor was there a significant difference between sides for any of the three groups (negative control,  $p=0.70$ ; F glass,  $p=0.24$ ; 45S5,  $p=0.34$ ). These non-parametric tests were appropriate given the ordinal ranking design of the experiment, and the lack of assumption of a normally distributed data set. However, a trend was suggested by the data in that there were more sides with “0” scores in each of the two glass-treated groups (F glass, 4 scores of 0; 45S5, 10 scores of 0) than in the negative controls (3 scores of 0).

## **DISCUSSION**

In a previous study, it was demonstrated that an apparently normal hybrid layer forms when powdered bioactive glass of the formula 45S5 is vacuum-deposited into etched dentin prior to application of adhesive and bonding (Zeiger, Chapter 2). In Figure 4, it can be seen that the same is true for the novel F glass, formulated in our laboratory. The presence of bioactive glass does not appear to prevent the adhesive from permeating the etched dentin.

The silver nitrate study clearly shows that the presence of the bioactive glasses is able to reduce leakage when the samples are soaked in simulated body fluid. The glass-treated samples of both kinds leak in a similar manner to the negative controls prior to soaking in SBF: this suggests that the glass is reacting in the aqueous calcium- and phosphate-containing environment and in proximity to dentin<sup>16, 18</sup>. If leakage were being reduced simply because the glass particles were obstructing the tubules, preventing fluid ingress, then the glass-treated samples would not leak even when not soaked in SBF.

It appears, then, that the mechanism by which the leakage is reduced is the precipitation of calcium phosphate mineral, in the form of apatite<sup>12</sup>. X-ray diffraction and SEM analysis of samples of the F glass show that crystals that are consistent with apatite morphology do form on the surface after the glass is soaked in SBF. The preferred orientation in the apatite 002 peak appears large, and therefore oriented to diffract. This is corroborated by the SEM image, which shows crystals of a homogeneous orientation that is consistent with growth along the Z axis. Vacuum deposition of the powdered glass into etched dentin places it in contact with the collagen network that has been exposed in the etching process. When the treated dentin is then exposed to the SBF, in simulation of

the oral fluids that would be present *in vivo*, the sequence of reactions that leads to the formation of apatite is initiated.

Although the methylene blue study did not demonstrate a statistically significant difference between glass-treated samples and negative controls, nor was there a significant difference between the two types of glass (Kruskal-Wallis  $H=0.43$ ,  $p=0.51$ ), a trend of glass-induced leakage reduction was suggested by the results. More zero scores were obtained for the glass-treated samples than for the negative controls, which implies that at least in some cases, the bioactive glass may be able to arrest leakage completely. It should be noted that the non-parametric Kruskal-Wallis and Mann Whitney Rank Sum Tests were chosen as they do not make assumptions about the normality of the data distribution. It was unnecessary, therefore, to nest the data with regard to operators. Additionally, these are appropriate tests to use with samples that have been ordinally ranked. Since the samples were bonded, thermocycled, and treated with methylene blue before they were cut, they cannot be considered as truly independent; however, leakage on one side does not guarantee leakage on the other, as was evident from the statistical analysis, which showed differing amounts of leakage on either side of many of the teeth. The characteristic assumptions made by both the Kruskal-Wallis Test and the Mann Whitney Rank Sum Test allow for the data to be pooled, rather than nested.

## **CONCLUSION**

The presence of bioactive glass at the interface of resin-bonded dentin appears to reduce leakage when bonded teeth are stored in an aqueous calcium- and phosphate-containing

environment. This is likely due to the precipitation of apatite by the glass, a known chemical reaction that is initiated in such environments. Two different formulations of bioactive glass, the commercially available Bioglass® formula 45S5, and a novel glass prepared in our lab, produced similar results, demonstrating that an apparently normal hybrid layer forms in their presence. Further studies are required to determine other effects of bioactive glass powder at the bonded interface, including effects on bond strength or induction of chemical reaction with exposed collagen. .

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**Table 1. Summary of methylene blue leakage results**

Operator 1								
Negative Control			F glass			45S5		
Sample	L	R	Sample	L	R	Sample	L	R
A	1	1	G	1	N/A	N	0	1
B	1	1	H	0	1	P	0	3
C	1	1	J	0	1	Q	1	0
D	3	3	K	1	1	R	1	0
E	1	0	L	3	N/A	S	3	3
F	2	2	M	1	2	T	0	1

Operator 2								
Negative Control			F glass			45S5		
Sample	L	R	Sample	L	R	Sample	L	R
A	1	1	G	1	N/A	N	0	1
B	1	0	H	0	1	P	0	3
C	1	1	J	0	1	Q	1	0
D	1	3	K	1	1	R	1	0
E	1	0	L	3	N/A	S	3	3
F	2	2	M	1	2	T	0	1

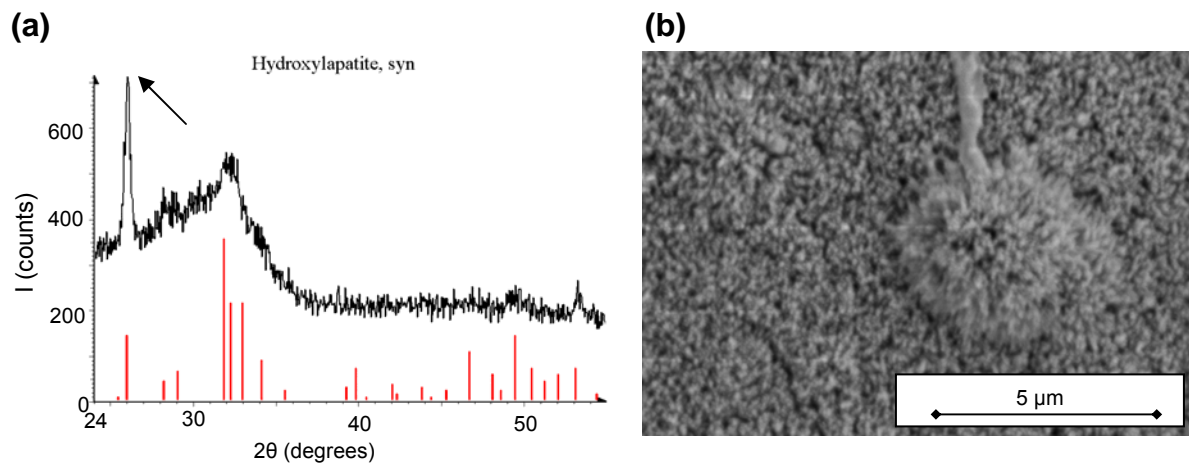


Figure 1. Analysis of an F glass slab that had been soaked in simulated body fluid for 6 weeks . Figure 1(a) shows an x-ray diffraction pattern, with preferred orientation of the 002 peak evident (indicated by arrow); the red lines correspond to the JCPDS hydroxyapatite pattern. Figure 1(b) is an SEM image of the same sample at 10,000x, showing growing crystals of what appears to be apatite.

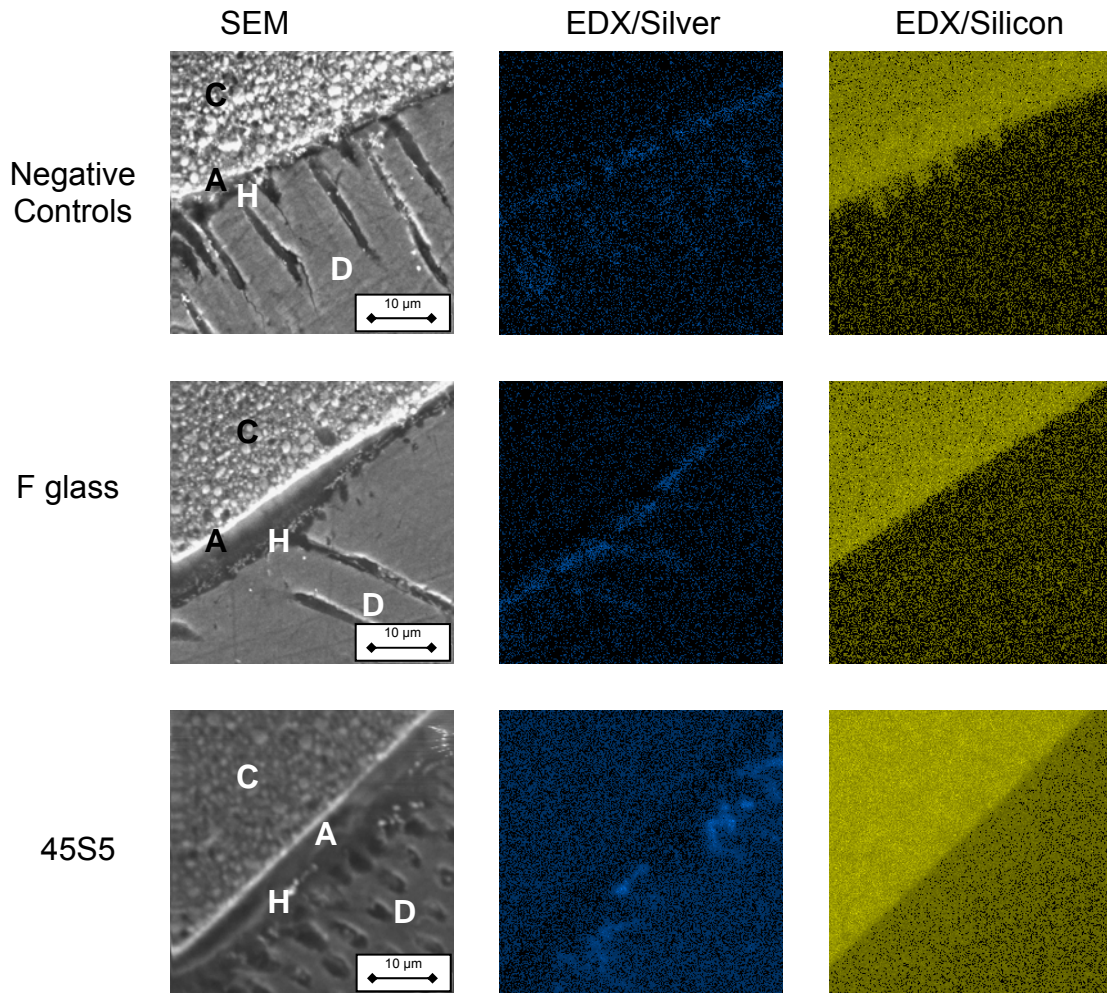


Figure 2. Leakage of samples prior to soaking in simulated body fluid. Etched samples were treated with glass powder and bonded, or bonded without glass, then immediately subjected to soaking in a silver nitrate solution. All samples display a similar amount of leakage, as shown by a high concentration of dots in the silver x-ray maps. The map for silicon, a major component of the composite, is shown for comparison of intensity. Leakage is primarily in the hybrid layer. C=composite; A=adhesive; H=hybrid layer; D=dentin

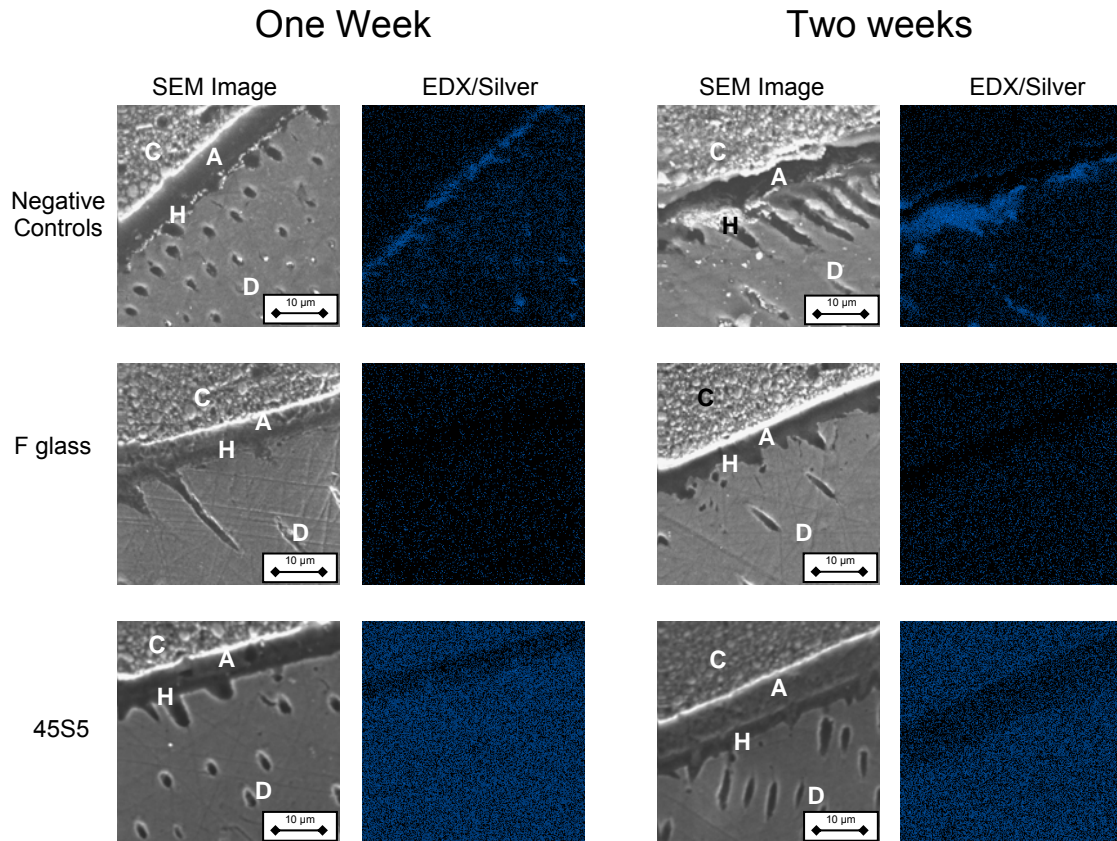


Figure 3. Leakage after soaking in simulated body fluid. Glass-treated samples do not demonstrate the leakage that is shown by the negative controls after soaking in SBF for a period of one or two weeks. Variations of background pixel brightness occur because intensity of signal is depicted as relative, rather than absolute; 45S5 images were collected at a longer dwell time, producing greater levels of background noise than in other maps. Additionally, the dark zone in the Ag map for 45S5 corresponding to the bonded layer is likely due to the topographic differences in the image, this area is slightly depressed and therefore x-rays cannot escape as easily. C=composite; A=adhesive; H=hybrid layer; D=dentin

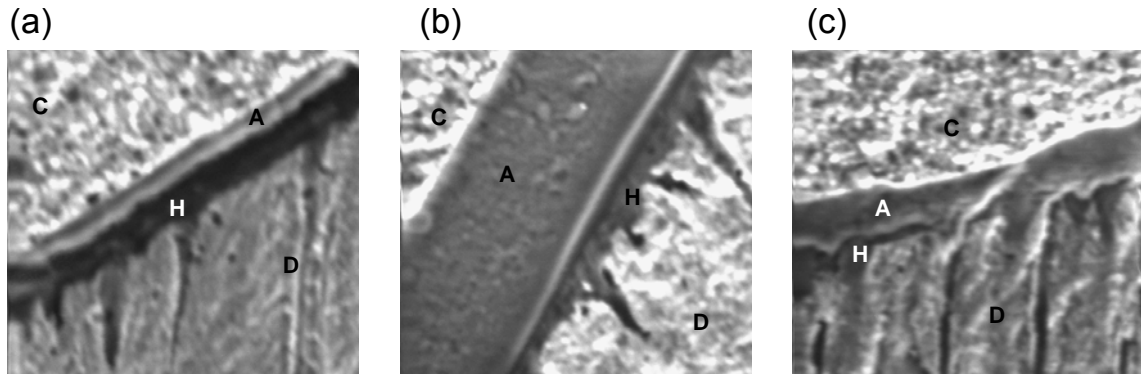


Figure 4. Apparently normal hybrid layers form in the presence of bioactive glass powders. Cryofractured samples reveal that a hybrid layer is able to form in teeth that have been vacuum-deposited with bioactive glass. Neither the glass nor the vacuum-depositing process appear to have a negative impact on the dentin. (A) represents a negative control tooth that was soaked in SBF for one week; (B) is a tooth that was treated with F glass and soaked in SBF for one week; (C) is a tooth that was treated with 45S5 and soaked in SBF for two weeks. C=composite; A=adhesive; H=hybrid layer; D=dentin

**Chapter 4**  
**The effects of two types of bioactive glass on the strength of bonded dentin**

## **ABSTRACT**

Many attempts have been made to increase durability and reduce technique sensitivity when bonding to dentin. While the latter goal has been achieved to some degree, much work remains in order to fulfill the former. Varying the formula of the adhesive has had some positive effects; however, the results have not been entirely satisfactory. Including additives in the adhesive, notably glass, has shown potential. Adding bioactive glasses to the dentin bonding process is an especially promising prospect, as they are known to form apatite in aqueous environments that contain calcium and phosphate, and have been shown to reduce leakage in bonded dentin. The aim of this study is to test the hypothesis that bioactive glasses that have been shown to reduce leakage in bonded dentin do not cause deleterious effects on bond strength. Materials and methods: The occlusal enamel of human third molars was removed by grinding, then etched. Powdered bioactive glass, either of the commercially available formula 45S5, or a novel fluoride- and magnesium-containing formula ("F glass") created in our laboratory, was vacuum-deposited in the etched dentin. The dentin was then bonded and composite was applied within a Single-Plane Lap Shear Test (SPLST) apparatus. Samples were stored in either water (~16 h, 8 days, or 65 days) or simulated body fluid (SBF; 15 days). After storage, samples were tested to failure using the SPLST. Statistical analysis was performed using one-way ANOVA at a significance level of  $p < 0.05$ ; fracture surfaces were examined by scanning electron microscopy. Results: no significant difference was observed between the F glass-treated samples and the 45S5-treated samples at any time period, in any medium (water: ~16h  $p = 0.10$ , 8 days  $p = 0.57$ , 65 days  $p = 0.35$ ; SBF  $p = 0.14$ ). Failure of all types (adhesive, cohesive in dentin,

and cohesive in composite) was observed for all types of samples, but cohesive failure was particularly pronounced in the F glass-treated samples, suggesting the strength of the interface may exceed that of bulk dentin or composite. Conclusion: Bioactive glasses may be incorporated into the dentin bonding process without detrimental effects on bond strength.

## **INTRODUCTION**

In the more than five decades since the era of modern adhesive dentistry was ushered in by Buonocore,<sup>1</sup> numerous attempts have been made to improve the strength and longevity of the bond to dentin, as well as the ease of use and technique-sensitivity of the materials. Variations on the adhesive formula have been made to increase its ability to bond to dentin;<sup>2</sup> fillers have been added to reduce adhesive shrinkage upon curing;<sup>3, 4</sup> and the canonical three steps of etch-prime-bond have been reduced to two steps (combining either etching and priming or priming and bonding) or even one step.<sup>5, 6</sup> For many years, the “gold standard” for bond strengths remained those values that result from the traditional three-step method in the case of bonding to dentin;<sup>6, 7</sup> more recently, however, self-etch and total-etch systems have produced results close or even superior to these.<sup>8</sup>

In resin bonding, retention of a restoration is dependent upon micromechanical bonding—liquid adhesive monomer permeates the collagen network left behind after dentin is acid etched and is polymerized *in situ*, forming the so-called “hybrid layer,” as first described by Nakabayashi in 1982.<sup>9</sup> Glass ionomer cements are also used for adhesive restorations, and were introduced in 1969 by Kent and Wilson;<sup>10</sup> these rely upon an acid-base setting mechanism and a chemical bond with the tooth. While glass ionomer



cements can offer durable, long-wearing seals when used in appropriate situations, and are not as prone to leakage as are resin-bonded restorations, there are still a number of drawbacks to their use. Firstly, glass ionomer cements lack the aesthetic benefits of resin—while they can attain a match to tooth color, the translucency that is characteristic of natural tooth structure has been difficult to achieve with these materials. Secondly, they offer low wear resistance when placed on chewing surfaces.<sup>11</sup> Thirdly, glass ionomers are brittle and tend to fracture relatively easily.<sup>11</sup>

More recently, glasses and resins have been combined in restorative materials. In the early 1990s, resin-modified glass ionomers were introduced.<sup>7</sup> This modification has resolved some of the problems inherent with glass ionomer cements: these newer materials have an increased working time and shorter setting time; additionally, their aesthetics are improved with regard to translucency.<sup>11</sup> Another manner in which resin and glass have been combined is as filled adhesives, in which micro- or nanoparticles of a silicate glass are added to the adhesive monomer. This addition reduces adhesive shrinkage (a major cause of leakage and failure of restorations) without a negative effect on bond strength.<sup>3</sup> The glasses in glass ionomer cements release fluoride, but to our knowledge bioactive glass has yet to be incorporated into commercial dental adhesive.

The best-studied and -characterized bioactive glass is the commercially available Bioglass<sup>®</sup>, first described by Hench in 1971.<sup>12</sup> Bioactive glasses, including Bioglass<sup>®</sup> (formula 45S5), form apatite in aqueous environments that contain calcium and phosphate, and bond to bone<sup>12</sup> and soft tissue<sup>13</sup> without toxicological consequences. Bioglass<sup>®</sup> 45S5 is currently utilized in bone-repair applications and under the trade name

Perioglas<sup>®</sup>, particulate Bioglass<sup>®</sup> is also used to repair bony defects resulting from periodontal disease.<sup>14</sup> Bioglass<sup>®</sup> has been used in some dental therapies—it has been utilized for pulp-capping procedures and to reduce tooth sensitivity. However, in these applications, the particle sizes can be as large as 300  $\mu\text{m}$ ,<sup>15</sup> and such large particles would be inappropriate for incorporation into the resin bonding process since the dentin tubule diameters are on the order of 1  $\mu\text{m}$ .<sup>16</sup> Other bioactive glasses have been formulated besides Bioglass<sup>®</sup>;<sup>17-23</sup> these materials also have yet to be utilized in dental bonding applications.

There are a number of ways to test bond strength, but the two most commonly used are the microtensile test and shear test. There is currently no standard test to assess bond strength. Finite element analysis has shown that sample shape<sup>24</sup> and loading geometry are crucial to determining the strength of a bond.<sup>25</sup> While the modes of failure appear different upon analysis with scanning electron microscopy,<sup>26</sup> shear testing produces many failures in the adhesive,<sup>27</sup> and therefore may offer valuable insights into bond strength.

Previous studies [Zeiger et al; Chapters 2 and 3] have shown that the incorporation of bioactive glass into the adhesive process reduces leakage of bonded dentin. The hypothesis tested in this chapter is that bioactive glass can be added to the dentin bonding process without deleterious effects on the shear bond strength.

## **MATERIALS AND METHODS**

### *Glass Preparation*

Bioactive glass of the formulation 45S5 was donated by SEM-COM, Inc. (Toledo, OH) in chip form. These chips were ground to an average particle size of  $\sim 1 \mu\text{m}$  by planetary ball milling.

Based on modification of another bioactive glass prepared by our group, a novel glass incorporating fluoride (“F glass”) was prepared<sup>20</sup> using the following compounds, with weight percentages in parentheses:  $\text{SiO}_2$  (44),  $\text{Na}_2\text{O}$  (23),  $\text{CaO}$  (10),  $\text{MgO}$  (4.5),  $\text{P}_2\text{O}_5$  (6), and  $\text{CaF}$  (12.5). The compounds were mixed in ethanol with a high-speed stirrer, dried at  $80^\circ\text{C}$  for 12 hours, and then fired in air in a Pt crucible for 4 hours at  $1400\text{-}1500^\circ\text{C}$ . The melt was cast into a graphite mold; the  $\sim 50 \times 50 \times 5$  mm plates obtained were annealed at  $500^\circ\text{C}$  for 6 hours. The resulting glass was ground to an average particle size of  $\sim 1 \mu\text{m}$  as described before (Chapter 3).

### *Vacuum Infiltration and Bonding*

The occlusal enamel of human third molars (N=54) extracted as part of dental treatment and according to a protocol approved by the UCSF Committee on Human Research, was removed using a belt sander with 240-grit silicon carbide paper, following which the roots were removed using a slow-speed saw (Isomet, Buehler Ltd., Lake Bluff, IL) with water coolant to produce tooth discs  $\sim 5$  mm thick. The exposed dentin was finished with 320-grit silicon carbide paper, which has been shown to result in a similar surface to clinical dental instruments.<sup>8</sup> The resulting discs were mounted on open-ended tubes with hot glue, and then attached to a vacuum trap. A strip of Mylar with a circular

(3.2 mm diameter) window was affixed to the dentin surface. The exposed occlusal dentin was etched for 15 seconds with Scotchbond gel etchant (3M/ESPE, St. Paul, MN) and rinsed for a further 15 seconds with deionized water. A slurry of 10% (w/v) bioactive glass of the formula 45S5 (N=18; SEM-COM, Toledo, OH) or 10% F glass in ethanol (N=18), was applied to the top of each sample. The glass slurry was prepared with ethanol, rather than with water, so there was no effect on the surface chemistry of the glass prior to its contact with the tooth. Vacuum was applied at 530 mm Hg for one minute; the sample surface was kept moist by re-applying the slurry every few seconds. After removal from the vacuum, any excess solid was gently rinsed away with deionized water. Negative controls (N=6) were vacuum-aspirated with water alone. We recognize that this is not a clinically applicable technique, but wanted to determine if the presence of a layer of bioactive glass would have measurable effects on adhesion. Samples were removed from their mountings and placed into shear plates for the Single Plane Lap Shear Test.<sup>28</sup> The sample surface was then bonded with Single Bond (3M/ESPE, St. Paul, MN) adhesive and light-cured per manufacturer's instructions. The remainder of the shear test apparatus was assembled, following which two coats of Filtek Z-250 Universal Restorative Composite (3M/ESPE, St. Paul, MN) were applied and light-cured per manufacturer's instructions. Samples were stored in deionized water at 37°C for ~16 h, for 8 days, or for 65 days.

Additionally 9 samples (3 negative controls, 3 F glass-treated, and 3 45S5-treated) were prepared as before, but were stored in simulated body fluid<sup>29</sup> for 15 days. This time period was chosen as the pertinent chemical reactions between glass and SBF would have occurred to completion by that point.

### *Bond Strength Test*

About 1 hour prior to testing, samples were removed from the storage medium. Excess water was removed from around the tooth, and dental stone (Tuff Rock, Talladium, Inc., Valencia, CA) was poured to anchor the sample in the device. Once the stone hardened, samples were tested to failure on a universal mechanical testing machine (Instron Model 1122, Canton, MA) at a crosshead speed of 5 mm/min. Means and standard deviations were calculated and statistical analysis was performed using one-way ANOVA at a significance level of  $p < 0.05$ .

### *Scanning electron microscopy (SEM)*

After mechanical testing, samples were sectioned through the area of failure using a slow-speed saw (Isomet, Buehler, Ltd., Lake Bluff, IL) in the direction that force was applied. The cut surface was polished through ascending grits of silicon carbide paper (through 1200 grit), then with 6  $\mu\text{m}$ , 3  $\mu\text{m}$ , 1  $\mu\text{m}$  and 0.25  $\mu\text{m}$  diamond paste (Metadi Monocrystalline Diamond Suspension, Buehler, Ltd., Lake Bluff, IL). Samples were then mounted on aluminum stubs and sputter-coated with a 200 nm thick layer of gold/palladium in preparation for scanning electron microscopy. SEM (Field Emission Variable Pressure SEM Model S-4300SE/N, Hitachi Hi-Technologies, London, UK) was performed at an accelerating voltage of 20kV.

## **RESULTS**

Bond strength tests showed no significant difference between F glass-treated teeth, 45S5-treated teeth, and negative controls at all time periods for samples that had been stored in water (Table 1). While the general trend showed a decrease in bond strength over time, none of the losses was statistically significant: for the negative controls,  $p=0.27$ ; for the F glass-treated samples,  $p=0.22$ ; and for the 45S5-treated teeth,  $p=0.35$ . Similarly, there was no significant difference between groups that had been stored in simulated body fluid for 15 days (Table 2). The very large standard deviations indicate that much further investigation is necessary into the effects of storage in SBF on bond strength. By the end of the storage period, the SBF had changed color, from clear to blue; this effect was determined to be due to pigment leaching from the sample labels and probably did not affect the outcome of the bond strength tests.

SEM observation revealed that samples of all types failed in a variety of modes across all time periods, in both types of storage medium. In each category, adhesive failure was observed, as well as cohesive failure of both dentin and composite (See Figures 1 and 2). However, it did appear that cohesive failure, either of dentin or of composite, appeared to be much more pronounced in samples that had been treated with F glass than either negative controls or 45S5-treated samples, for both types of storage medium (See Figures 1(a), (b), and (c); and Figures 2(a) and (b)).

## **DISCUSSION**

A previous study has shown that the addition of microparticles of glass to adhesive does not produce negative effects on bond strength to dentin<sup>3</sup>. Although the

mode of incorporation is different in this study, the results here also show no significant difference between glass-treated samples and negative controls. Interestingly, however, two trends appear in the studies over time: firstly, the standard deviations appear consistently smaller for the F glass-treated samples than for either the negative controls or the 45S5-treated samples; and secondly, the general reduction in strength that occurs for all samples in water appears to be less for the F glass-treated samples. This behavior is apparent for samples stored in water only, and the reduced performance of F glass treated samples stored in SBF requires further investigation.

The smaller standard deviations suggest that the addition of F glass to bonding to dentin may reduce the technique sensitivity of the dentin bonding process. The exact mechanism of this is not certain, but is potentially due to the formation of apatite in the bonded area upon exposure to the aqueous environment. This also provides a potential explanation for the apparently reduced loss of bond strength over time for the F glass samples stored in water. The fact that these effects appear to be enhanced in the F glass samples in comparison to the 45S5 samples could be explained by the presence of magnesium, which has been shown to slow the rate of apatite precipitation, leading to controlled (and possibly superior) mineralization;<sup>30</sup> and fluoride, whose presence promotes the formation of fluorapatite, a form of mineral that can be stronger and more resistant to acid attack<sup>31</sup> than carbonated apatite.

SEM analysis of the failures shows that while adhesive failure, and cohesive failure of dentin and composite occur for all categories of samples, the cohesive failures are predominant in F glass-treated samples that have been stored in either water (Figure 1) or simulated body fluid<sup>29</sup> (Figure 2), occurring to some extent in all F glass-treated

samples over approximately 60% of the interface. Such results imply that the strength of the adhesive bond may be greater than that of the bulk dentin or composite. Again, this could be due to the precipitation of apatite at the bonded interface. Previous studies have found that bioactive glass forms bonds between dentin<sup>32</sup> and soft tissue<sup>13</sup> such that substantial mechanical force is required for separation; both studies concluded that the collagen in the respective tissues was chemically bonding to the glass, as was suggested by Hench.<sup>33</sup>

## **CONCLUSION**

Bioactive glasses were incorporated into the dentin bonding process without deleterious effects on the strength of the bonds formed. A novel glass that contains both fluoride and magnesium shows promise of improving durability of bonds and reducing technique sensitivity when deposited on dentin during bonding, although further investigation is needed.

## **ACKNOWLEDGEMENTS**

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**Table 1.** Shear bond strengths of samples stored in water

	~ 16 h		8 Days		65 Days	
	<i>N</i>	Mean±SD (MPa)	<i>N</i>	Mean±SD (MPa)	<i>N</i>	Mean±SD (MPa)
Negative Control	6	31.4±11.1	6	38.6±6.6	6	29.2±11.7
F Glass	6	41.9±3.6	6	36.2±6.0	6	38.6±6.4
4SS5	6	37.6±7.3	6	40.8±9.0	6	31.7±14.2
		p=0.10		p=0.57		p=0.35

**Table 2.** Shear bond strengths of samples stored in simulated body fluid

	<i>N</i>	Mean±SD (MPa)
Negative control	6	35.2±5.2
F glass	6	23.6±12.1
45S5	6	33.4±11.6

p=0.14

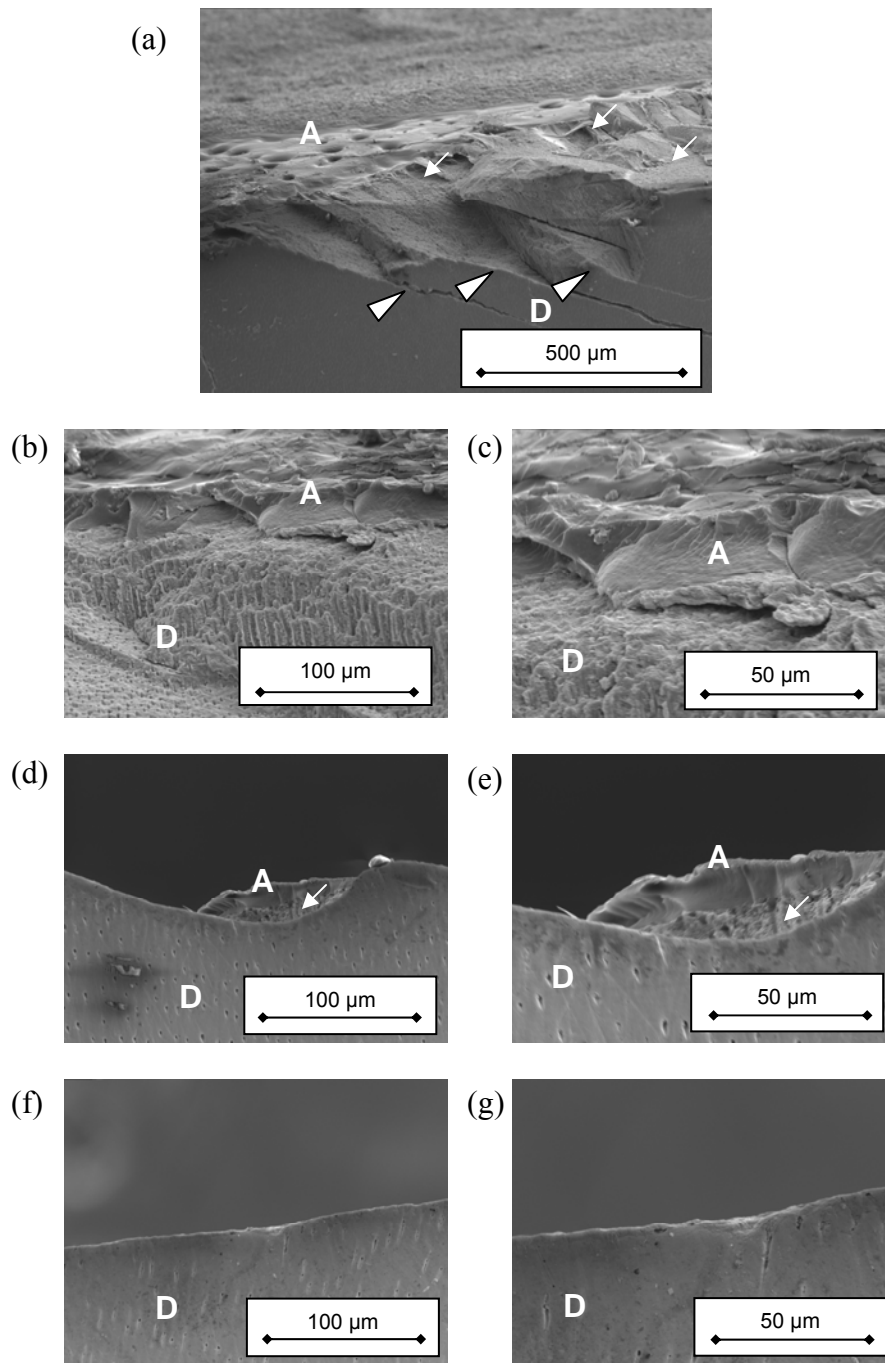


Figure 1. SEM images of teeth that have been bonded, stored in water for 65 days, and tested to failure using the single plane lap shear test. Figure 1(a), at 100 $\times$  magnification, illustrates the dramatic nature of failure in a tooth that has been treated with F glass—note the large sections of dentin that have been pulled away (white arrows), and deep cracks radiating from the area (white triangles). Figures 1(b) and 1(c) are close-ups of the same tooth at 500 $\times$  and 1000 $\times$ , respectively. Figures 1(d) and 1(e) show a tooth that has been treated with 45S5 glass; cohesive failure of the dentin is evident here as well (white arrows). In the negative control, shown in figures 1(f) and 1(g), adhesive failure of a more modest nature is apparent. Figures 1(d) and 1(f) are at a magnification of 500 $\times$ , while figures 1(e) and 1(g) are at a magnification of 1000 $\times$ .

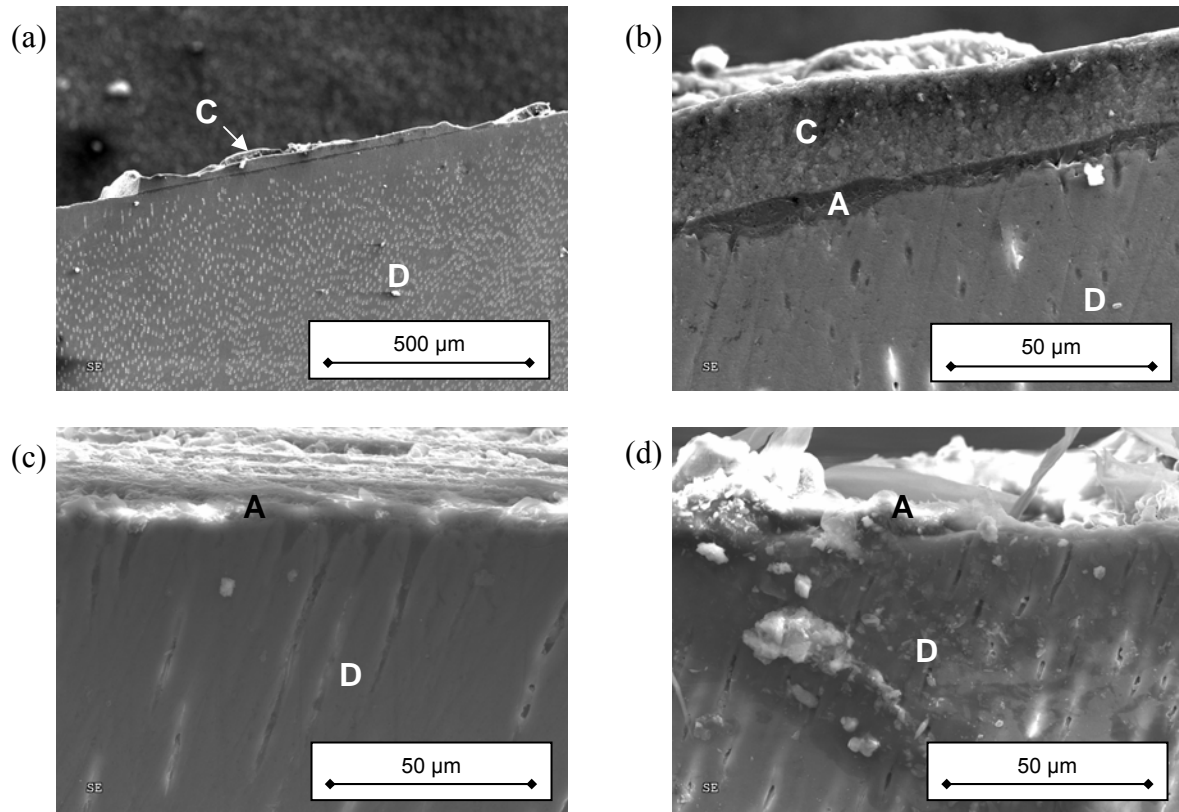


Figure 2. SEM images of teeth that have been bonded, soaked in simulated body fluid for 15 days, and tested to failure in the single plane lap shear test. Figures 2(a) and 2(b), at 100 $\times$  and 1000 $\times$ , respectively, show the pronounced cohesive failure of an F glass-treated tooth, with large chunks of composite remaining firmly attached to the dentin. In contrast, both Figures 2(c) and 2(d), both at 1000 $\times$ , show adhesive failures in a 45S5-treated tooth and a negative control, respectively.

## **Chapter 5**

**Addition of a novel fluoride-and magnesium- releasing glass to adhesives reduces leakage and increases bond strength**

## **ABSTRACT**

During the last several decades, many significant strides have been made in the field of adhesive dentistry, with improvements in the areas of aesthetics, ease of use, and technique sensitivity, among others. However, one major problem has yet to be resolved: the tendency of these materials to leak. Previous studies have suggested that powdered bioactive glass, both of the well-characterized 45S5 formulation and of a novel, fluoride- and magnesium-releasing formulation (“F glass”), reduce leakage when vacuum-deposited into etched dentin. The objective of this study is to test the hypothesis that powdered bioactive glass will reduce leakage when incorporated into adhesive that is used for bonding dentin without sacrificing bond strength. Materials and Methods: To determine bond strength, the dentin of human third molars (N=10 for each group) was exposed by grinding and etched. Powdered bioactive glass, average particle size ~1µm, was mixed with either One Step or part A of All Bond 3 adhesives at a weight percentage of either 0.5% or 1%. Adhesives were applied and light cured per manufacturer’s instructions, as was composite. Shear bond strength testing was carried out per the Ultradent method (RD-022). For leakage studies, the dentin of human third molars was again exposed by grinding; etching, bonding, and composite were conducted as before. Serial slabs were cut through the restoration in an occlusal to apical direction. Slabs were covered to within 1mm of the bonded interface with nail polish, soaked in ~2mL 50% (w/w) silver nitrate and developer, then rinsed. The side to be examined was finished through 1200 grit SiC, then the samples were mounted and examined with scanning electron microscopy/energy dispersive x-ray analysis. Results: SEM/EDX analysis indicated reduced leakage in all cases except for adhesives containing a load of 0.5%



45S5. The bond strength of samples that were bonded with One Step was not significantly reduced when compared to negative controls ( $F=2.57$ ,  $p=0.051$ ). Bond strength for samples bonded with All Bond 3 was increased ( $F=2.79$ ,  $p=0.037$ ) for the samples that were glass-treated. For One Step, the bond strength for 45S5-treated samples was significantly reduced compared to negative controls ( $F=4.55$ ,  $p=0.020$ ); F glass-treated samples did not yield a significant reduction ( $F=0.63$ ,  $p=0.542$ ). , while significantly higher bond strength was seen for F glass-treated teeth that were bonded with All Bond 3 in comparison to negative controls ( $F=3.76$ ,  $p=0.036$ ), but this was not the case for 45S5-treated teeth ( $F=0.42$ ,  $p=0.663$ ). Additionally, the standard deviation for glass-treated samples was reduced compared to the negative controls in all but one case. Conclusion: When mixed into adhesive, the novel F glass reduced leakage and increased bond strength. A commercially available bioactive glass failed to produce similar beneficial effects at the concentrations studied. .

## **INTRODUCTION**

Adhesive dentistry began to evolve towards its currently practiced form in the mid-1950s, with Buonocore's introduction of acid etching.<sup>1</sup> Resin-based restorations are superior to amalgam restorations in terms of aesthetic appeal, yet they are far from ideal. Leakage is a major problem with these materials that remains unresolved. Microleakage, which can be defined as the movement of fluids that may or may not carry ions, enzymes, or bacteria, into a zone between a restoration and the host tooth,<sup>2</sup> is the major issue of concern, as it can lead to secondary caries and failure of the restoration.

The adhesives that are currently used form micromechanical bonds within the collagen network that is exposed by etching, and the quality (strength and longevity) of

the bond is based on the ability of the adhesive monomer to penetrate the collagen before it is polymerized *in situ*. The so-called “hybrid layer,” first described by Nakabayashi in 1982,<sup>3</sup> is created as the monomer permeates the collagen network. There is limited penetration of the adhesive into the collagen,<sup>4</sup> however, and voids around the unprotected collagen fibrils can permit entry of oral fluids that carry enzymes capable of compromising the interface.<sup>5</sup>

The ability of the adhesive monomers to penetrate the collagen layer may be enhanced by augmenting their hydrophilicity. This modification has the unfortunate side effect of increasing the vulnerability of the cured polymer to hydrolytic attack<sup>6</sup> and breakdown. The adhesive and supporting layer can be weakened simply by the presence of the oral fluids, then these same fluids may enter the tissue underneath the restoration, carrying with them potentially destructive bacteria or enzymes.<sup>2</sup> This in turn may lead to failure of the bond and loss of the restoration.

Several “generations”<sup>7</sup> of bonding agents have been developed in the last few decades. The advancements that have been made during this time have mainly focused on increasing ease of use and reducing technique sensitivity. Many developments have been made in order to reduce the number of steps from the canonical three (of etch, prime, and bond), and bonding systems have been developed that combine two of these three steps, or even all three steps into one bottle.<sup>8</sup> Thus there are self-etching primer systems, etch-and-rinse adhesive systems, and self-etching adhesive systems. To date, however, little progress has been made in reducing the tendency to leak.

Restorative materials must form strong and durable bonds to teeth, while at the same time presenting an aesthetically appealing appearance. It was hoped that glass

ionomer cements, introduced by Wilson and Kent,<sup>9</sup> would fulfill both of these criteria. The bonds formed by glass ionomer cements are both micromechanical and chemical in nature,<sup>10</sup> but these cements lack<sup>11</sup> the necessary aesthetic appeal that can be achieved with resin-based restoratives. In addition, the mechanical properties of glass ionomer cements are not likely to produce durable restorations<sup>8</sup>—they tend to be quite brittle. While resin-based restoratives are able to successfully mimic the appearance of natural teeth, there is much room for improvement when it comes to the robustness of the mechanical properties of the bonds. The addition of auxiliary materials, such as bioactive glasses, into the resin-bonding procedure may be able to produce the desired result. Because of their ability to promote the formation of apatite in aqueous environments that contain calcium and phosphate (e.g. saliva), the presence of bioactive glasses at the bonded interface will improve the quality of the resin-dentin bond through self-sealing caused by the formation of apatite in the presence of leakage (Chapters 2 and 3).

Bioactive glasses possess unique compositional traits, such as low silica content, high sodium and calcium content, and a high ratio of CaO to P<sub>2</sub>O<sub>5</sub><sup>12</sup> and according to Hench, the developer of the original bioactive glasses, these materials elicit a response at the glass/tissue interface such that a bond is formed.<sup>12-14</sup> A distinct, though as yet not completely understood, series of chemical reactions occurs when bioactive glasses are brought into contact with tissue, or any aqueous environment that contains calcium and phosphate, and the formation of carbonated hydroxyapatite (HCA) is the ultimate result.<sup>12</sup> The following key reactions normally occur within the first two hours of exposure to such environments: alkali ions are rapidly leached out, and SiOH bonds then quickly form at the surface of the glass. These SiOH bonds condense and a silica-rich layer is formed on

the glass surface.  $\text{Ca}^{2+}$ ,  $\text{PO}_4^{3-}$ , and  $\text{HCO}_3^{2-}$  ions then adsorb to this layer in the form of amorphous, substituted calcium phosphate that later crystallizes to HCA. It is expected that the apatite that is precipitated when the glasses are included in the bonding procedure would bond to the exposed collagen, since it has been suggested that the forming mineral bonds to specific amino acids within the collagen of bone.<sup>13, 14</sup> Bioactive glasses have been shown to react favorably with dentin as well as bone: mechanical bonds have been created,<sup>15</sup> carbonated apatite similar to tooth mineral has been formed,<sup>16, 17</sup> and antibacterial properties beneficial to the prevention of secondary caries have been demonstrated.<sup>18</sup>

The chemical composition of bioactive glasses is significant. Silica maintains the glass structure, and sodium is soluble in an aqueous environment, aids in maintenance of physiological ionic balance and pH, and facilitates the production of glass.<sup>14</sup> Calcium and phosphate are required, as they are the principal constituents of apatite mineral. The proportions of all these constituents have been varied with differing results.<sup>14</sup> Other components may be added for their beneficial effects either on the manufacture of the glass or on the behavior of the glass.<sup>19</sup> Specifically, magnesium<sup>19, 20</sup> and fluorine<sup>19, 21-24</sup> are two elements whose addition to bioactive glass has proved beneficial, and could improve the resin-dentin bond. Magnesium can slow the rate of apatite precipitation, thus leading to more controlled mineralization.<sup>16</sup> The addition of fluorine to bioactive glass can enhance the rate of fluorapatite formation at the glass surface.<sup>22</sup> In addition, the fluorapatite formed will be less vulnerable to acid attack than carbonated apatite.<sup>25</sup>

Adding powdered glass to adhesive resins has produced varying results.<sup>26-28</sup> Filling the adhesive with glass is expected to reduce polymerization shrinkage,<sup>28</sup> which is

likely to improve marginal integrity and reduce leakage. To date, the glasses used in these applications have been conventional silica glass or fluoride-releasing, but the use of bioactive glass as a filler in adhesive has not been attempted to our knowledge.

Previously, two types of bioactive glass have been shown to reduce leakage without detrimental effects on the bond strength (Zeiger *et. al.*, Chapters 2, 3, and 4). However, the method of application of the glass—vacuum deposition—is not possible in the clinic. Hence, the objective of this study is to test the hypothesis that bioactive glass can produce the same effects when it is integrated into the adhesive in manner that is practicable in the clinic.

In this chapter, the hypothesis that the addition of bioactive glasses to adhesive resin will reduce leakage in bonded dentin, without producing negative effects on bond strength, will be tested.

## **MATERIALS AND METHODS**

### *Glass Preparation*

Based on modification of another bioactive glass prepared by our group, a novel glass incorporating fluoride (“F glass”) was prepared<sup>29</sup> using the following compounds, with weight percentages in parentheses: SiO<sub>2</sub> (44), Na<sub>2</sub>O (23), CaO (10), MgO (4.5), P<sub>2</sub>O<sub>5</sub> (6), and CaF (12.5). Bioactive glass of the formulation 45S5 was donated by SEM-COM, Inc. (Toledo, OH) in chip form. All glass was ground to an average particle size of ~1 μm by planetary ball milling. The ability of the F glass to precipitate apatite in an aqueous calcium- and phosphate- containing environment was confirmed by x-ray diffraction and scanning electron microscopy (Zeiger *et. al.*, Chapter 2).

### *Shear Bond Testing*

Either 1% or 0.5% (See Table 1) wt/wt of the bioactive glass was combined with One Step or Part A of All Bond 3 (Bisco, Inc., Schaumburg, IL). Water-free adhesives were chosen to avoid immediate setting upon mixing as well as alteration of the surface chemistry of the two types of glass. Shear bond testing was performed using the Ultradent method as follows: human teeth were set in PMMA, then ground on an orthodontic grinder to expose the dentin and to provide a flat surface to allow for very close contact of the Ultradent mold (cylindrical, with a contact area of 0.044 cm<sup>2</sup>, filled to a height of approximately 2 mm) to the surface of the tooth. It must be ascertained that excess flash of the composite does not occur when uncured composite is packed into the mold, and the surface area of the bonded composite is consistent. The dentin was exposed by grinding, and the teeth were finished in a randomized figure-eight motion for 30 s on moist 320 grit sandpaper, rinsed, and placed into warm water. The dentin surface was then etched with UniEtch (Bisco, Inc., Schaumburg, IL) for 15 s. Teeth were then rinsed, and 2 coats of adhesive (One Step; or All Bond 3 Primer B combined in a 1:1 ratio with part B, part C, or part D) were applied on moist dentin (moisture was controlled by using a foam pellet), air thinned, and light cured for 10 s. Aelite composite (Bisco, Inc., Schaumburg, IL) was used to fabricate the post. Controls were prepared using the same procedure, only without inclusion of any glass in the adhesive. After fabrication, all specimens were stored for 2 hours at 37° C, after which they were tested until failure at a crosshead speed of 1 mm/min (Instron 4466 C1879). Results were analyzed using One-Way Analysis of Variance (ANOVA).

*Silver Nitrate Leakage Study and Scanning Electron Microscopy/Energy Dispersive X-ray Analysis (SEM/EDX)*

Silver nitrate is commonly used to determine the extent of leakage of dental materials,<sup>30</sup> as silver ions in solution are able to penetrate into areas where leakage has occurred; the locations in which these ions are precipitated may then be determined by SEM/EDX using x-ray mapping. For this section of the study, teeth were cut in half in an occlusal to apical orientation. The occlusal dentin of each half was exposed by grinding, and the teeth were finished in a randomized figure-eight motion for 30s on moist 320 grit sandpaper and rinsed; the etching and adhesive application procedures were the same as stated previously. Light Core Blue composite was applied and cured in 3-4 increments with a cure of 40 s/increment. Controls were prepared using the same procedure, only without inclusion of any glass in the adhesive. Teeth were stored in water at room temperature for two weeks. Three serial slabs were cut from the central region of each half-tooth with a slow-speed saw (Isomet, Buehler Ltd., Lake Bluff, IL). Each slab was coated with nail polish to within 1 mm of the bonded interface and soaked in ~2 mL 50% (w/v) silver nitrate for two hours without exposure to light. The samples were then transferred to ~2 mL developing solution for four hours under fluorescent lights, after which they were thoroughly rinsed with deionized water. The nail polish was removed by grinding with 240 grit SiC paper, the samples were finished through 1200 grit SiC, and then mounted on aluminum stubs for SEM analysis.

### *Scanning electron microscopy and energy-dispersive x-ray analysis (SEM/EDX)*

SEM (Topcon ISI ABT SX-40A, Milpitas, CA) visualization of the samples prepared for SEM /EDX analysis was executed in backscattered mode at an accelerating voltage of 15 keV, using a charge-free anticontamination system (CFAS) and energy dispersive x-ray analysis for chemical information (Thermo-Noran Sigma2, Middleton, WI). Their chemical composition was mapped (dwell time, 5600 $\mu$ s; 256 x 256 pixels) with regard to the following elements: calcium, magnesium, phosphorus, silicon, silver, and sodium.

## **RESULTS**

The results of the bond strength tests are summarized in Table 2. There was a not statistically significant reduction in bond strength when all glass-treated samples that were bonded with One Step were compared with the negative controls ( $F=2.57$ ,  $p=0.051$ ). There was a significant difference among samples bonded with All Bond 3 ( $F=2.79$ ,  $p=0.037$ ); where the bond strength was increased for the samples that were glass-treated. When separated by glass type, additional differences became evident: For One Step, the bond strength for 45S5-treated samples was significantly reduced compared to negative controls ( $F=4.55$ ,  $p=0.020$ ); this was not the case for samples treated with F glass ( $F=0.63$ ,  $p=0.542$ ). Samples that were treated with 45S5 and bonded with All Bond 3 were not significantly different from negative controls ( $F=0.42$ ,  $p=0.663$ ), while those treated with F glass and bonded with All Bond 3 exhibited significantly higher bond strength than negative controls ( $F=3.76$ ,  $p=0.036$ ). In all cases but one, the standard deviation for glass-treated samples was reduced compared to the negative controls.



The appearance of the samples to which the glass was added differed from the controls. The bonded surface appeared grainy to varying degrees; the shine that is typical after curing was somewhat reduced; and the F glass-treated teeth that were bonded with All Bond 3 were slightly sticky after curing.

SEM/EDX analysis revealed formation of an apparently normal hybrid layer for all glass-treated samples. Leakage was evident in negative controls, as shown by areas of high silver concentration (Figure 1). Leakage appeared to be reduced in all samples to which F glass had been added, and did not appear dependent upon filler load or adhesive type (Figure 2) for F glass samples; however, samples that had been treated with 45S5 appeared to require a filler load of at least 1 wt% to demonstrate reduced leakage (Figure 3).

## **DISCUSSION**

It was previously reported that bioactive glass of Hench's formula 45S5 (Chapter 1), and F glass (Chapter 2) can reduce leakage in bonded dentin without deleterious effects on bond strength (Chapter 3). However, in each of those studies, the powdered glass was vacuum-deposited into etched dentin, and the adhesive and composite were applied separately from the glass. In this chapter, glass powder was incorporated directly into the adhesives. Although the amount of glass vacuum-deposited into the dentin was greater than the amount of glass in the adhesives (10-20 wt% glass in ethanol vs. 0.5 wt%-1 wt% glass in adhesive), similar effects were observed. Leakage was apparently reduced for samples treated with 1 wt% 45S5 in One Step and All Bond 3; and 0.5 wt% and 1 wt% F glass in One Step and All Bond 3. The addition of 0.5 wt% 45S5 to both adhesives did not appear to have this effect; it would seem that this amount of the

material is not sufficient. However, the amount of glass that may be added to the adhesive was limited: larger amounts of powder tended to settle out of suspension. To a degree, this may be remedied by agitating the mixture, but this effect is only temporary. Future work should investigate the maximum amount of glass powder (and optimum particle size) that may be effectively added to adhesive, stay in suspension, and produce the desired results.

The effects on bond strength can be broken down by both adhesive type and glass type. The 45S5-treated samples exhibited significantly ( $p=0.020$ ) reduced bond strength for One Step, an acetone-based adhesive; and no significant difference in bond strength for All Bond 3, an ethanol-based adhesive. The F glass-treated samples, on the other hand, exhibited no significant difference in bond strength for One Step; but the bond strength for F glass-treated samples bonded with All Bond 3 was significantly increased. These results suggest that the addition of F glass is beneficial in a way that 45S5 is not, and that the bioactive glasses may interact more positively with the ethanol based adhesive. Chemical interactions involving the solvent may affect the behavior of the glass, with ethanol producing more favorable results in combination with the bioactive glasses. It is also possible that a load of 1% may be the lower effective limit for leakage reduction when 45S5 is incorporated into adhesive. Further understanding of the chemistry of various adhesives including solvents, is needed, along with their interactions with bioactive glasses, as is investigation into the effects of loading levels of glass powder. This is suggested not only by the analysis of the bond strength differences, but also by the observations of the different appearances of the powder-containing adhesives after curing, such as the apparent graininess and stickiness of the cured surface. The

presence of the glass may have led to incomplete curing of the adhesive or possibly incomplete evaporation of the solvent, hence the sticky surface.

It is known that the addition of a glass to a resin can reduce polymerization shrinkage, yet it has been shown that a polymer with added glass had no beneficial effects upon leakage or bond strength.<sup>28</sup> However, a resin that included fluoride-releasing glass reduced leakage and increased marginal integrity of restorations while providing good adhesion.<sup>27</sup> In this study, it was found that while a commercially available bioactive glass did not significantly reduce leakage at lower concentration or increase bond strength, a novel fluoride-releasing bioactive glass did have beneficial effects on both properties. However, to further improve these properties additional investigation is needed to determine the optimal particle size and loading level of the glass powder that is added to the adhesive. Additionally, the results suggest that while commercial bioactive glass may have some potential advantages when added to adhesive, it is possible that the presence of fluoride (or magnesium) augments the benefits. Additional studies to optimize the bioactive glass composition are also warranted, as are determination of the appropriate monomer and solvent to provide for optimal chemical interactions.

It was also noted that in all cases but one (0.5% F glass, All Bond 3, for reasons which require further investigation), the standard deviation of the bond strength values was reduced when glass was added to the adhesive resin. It may be inferred from these results that the addition of bioactive glass could reduce technique sensitivity of bonding to dentin, thus producing more consistent results among operators. This was an unexpected but nevertheless encouraging consequence of the addition of bioactive glasses.

## **CONCLUSION**

Bioactive glasses of two different compositions were added to two different adhesive bonding systems. The most favorable results were obtained with the addition of a novel fluoride- and magnesium-containing glass to an ethanol-based resin bonding system, with a significant increase in bond strength and substantially reduced leakage when compared to negative controls. Addition of this novel glass to an acetone-based adhesive reduced leakage, but did not increase bond strength. Lower concentrations of a commercially available bioactive glass in acetone- and ethanol-based adhesives did not produce beneficial effects. Future studies should endeavor to determine the optimum amount of glass that may be successfully added to the resin in order to produce maximum benefits, while remaining suspended in the liquid monomer. Additional investigation should also be conducted to establish the most favorable particle sizes and size distributions that result in minimizing leakage and maximizing bond strength. Further investigation of compositional variations in the bioactive glass are also needed to improve these properties and to identify mechanism associated with the reduced leakage and increases in bond strength observed here. One of the possible mechanisms is that apatite is precipitated that reduces leakage; the mineral may also bond to exposed collagen fibrils, thereby increasing bond strength.

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**Table 1.** Materials used

Material	Name*	Lot Numbers
Etchant	UniEtch	0500001425
Adhesive	One Step	543-139-A; 543-138A; 543-142-A; 543-142-B
Adhesive (primer)	All Bond 3 Primer B	0700001681
Adhesive	All Bond 3	part B 543-139-B; part C 543-139-C, 543-142-C ; part D 543-142-D
Composite	Aelite All-Purpose Body Composite	0600010578
Composite	Light Core Blue	lot 0600010807

\* All products listed from Bisco, Inc., Schaumburg, IL

**Table 2.** Bond strengths (MPa)

Filler Type	Wt %	N	One Step	N	All Bond 3
45S5	1	10	30.4± 3.6*	10	33.9±5.9†
	0.5	10	33.4±3.2*	10	36.2±5.1†
F glass	1	10	34.6±5.2	10	34.9±5.4†
	0.5	10	35.0±4.4	10	42.2±8.3†
None	N/A	10	37.3±7.6	10	35.4±6.0

\* - indicates significantly reduced bond strength in comparison with negative controls

† - indicates significantly increased bond strength in comparison with negative controls



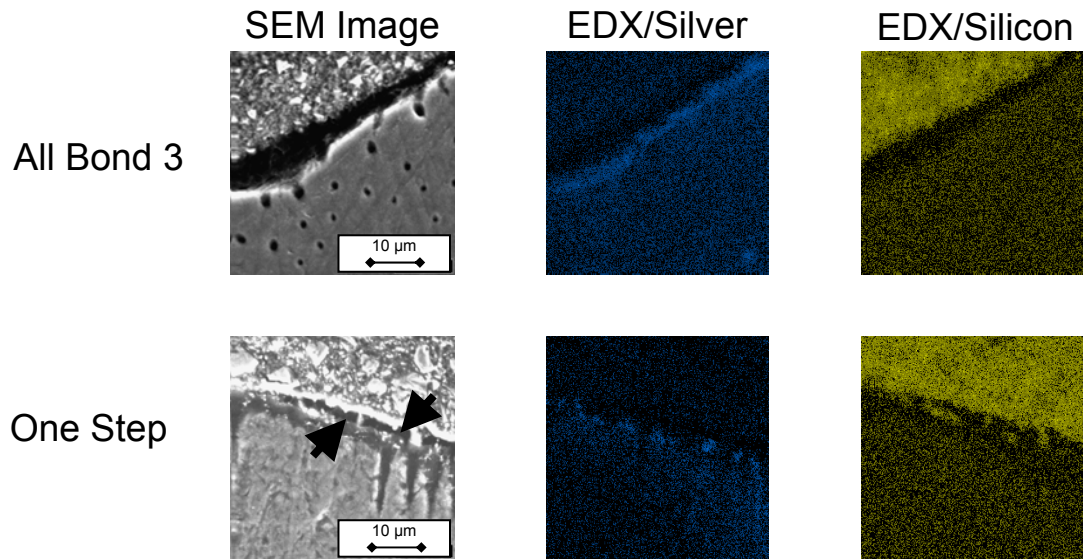


Figure 1. Scanning electron microscopy and energy dispersive x-ray analysis of negative controls. The top row shows images of dentin bonded with All Bond 3, the bottom row shows dentin bonded with One Step. X-ray mapping reveals extensive leakage for both bonding systems, represented as concentrated areas of dots in the silver map. SEM images of One Step samples show debonding of the adhesive and composite, as shown with arrows. X-ray maps of silicon are included for spatial reference. C=composite; A=adhesive; D=dentin.

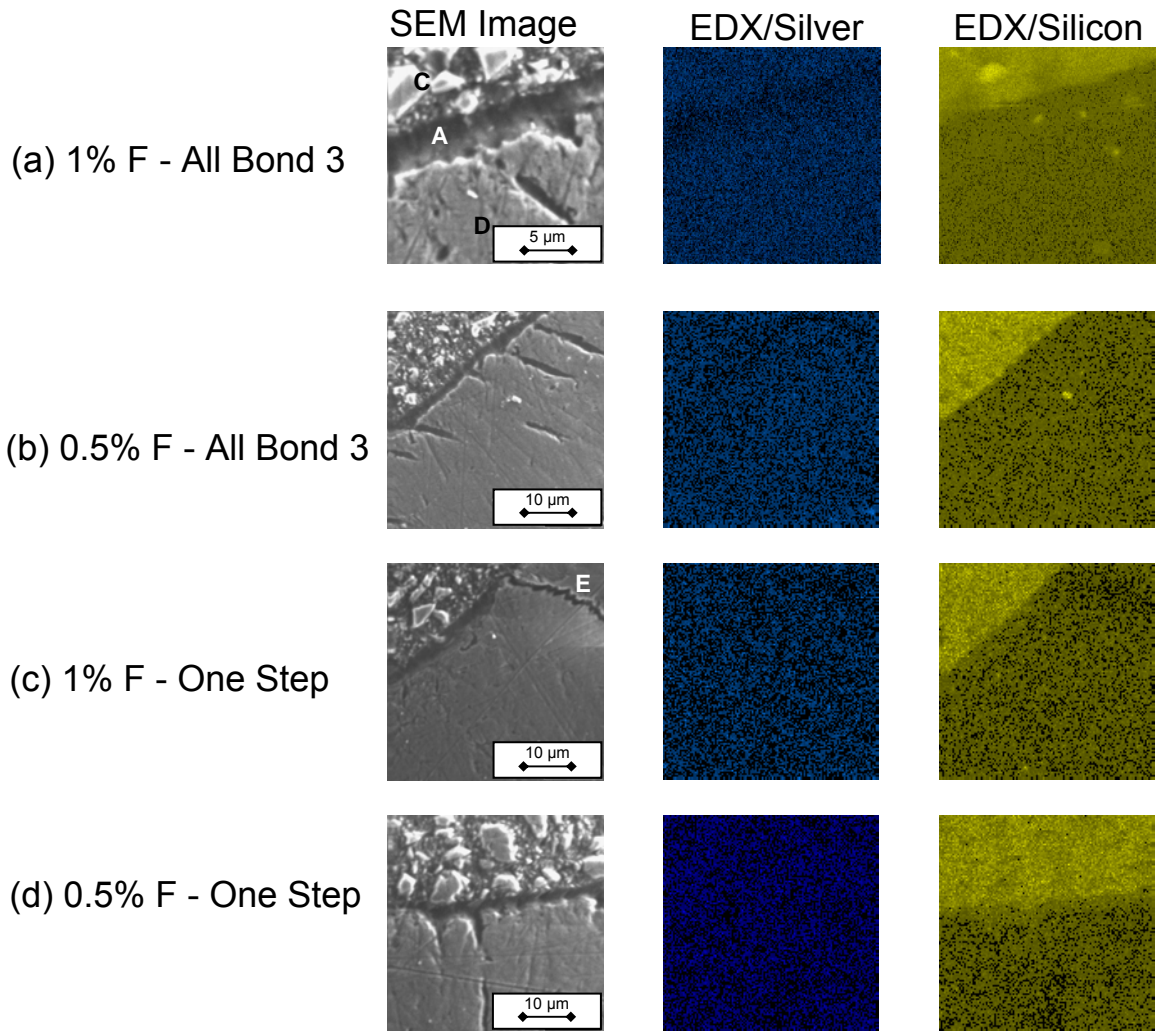


Figure 2. Scanning electron microscopy and energy dispersive x-ray analysis of dentin bonded with adhesive containing F glass. (a) images of dentin bonded with All Bond 3 and 1 wt% F glass; (b) dentin bonded with All Bond 3 and 0.5 wt% F glass; (c) dentin bonded with One Step and 1 wt% F glass; and (d) dentin bonded with One Step, and 0.5 wt% F glass. X-ray mapping does not show leakage for either bonding system when the novel glass is included. The dark zone in the Ag map at the top corresponding to the bonded layer is likely due to the topographic differences in the image, this area is slightly depressed and therefore x-rays cannot escape as easily. Debonding of adhesive and composite that was evident for negative controls is not present. X-ray maps of silicon are included for spatial reference. C=composite; A=adhesive; D=dentin; E=enamel

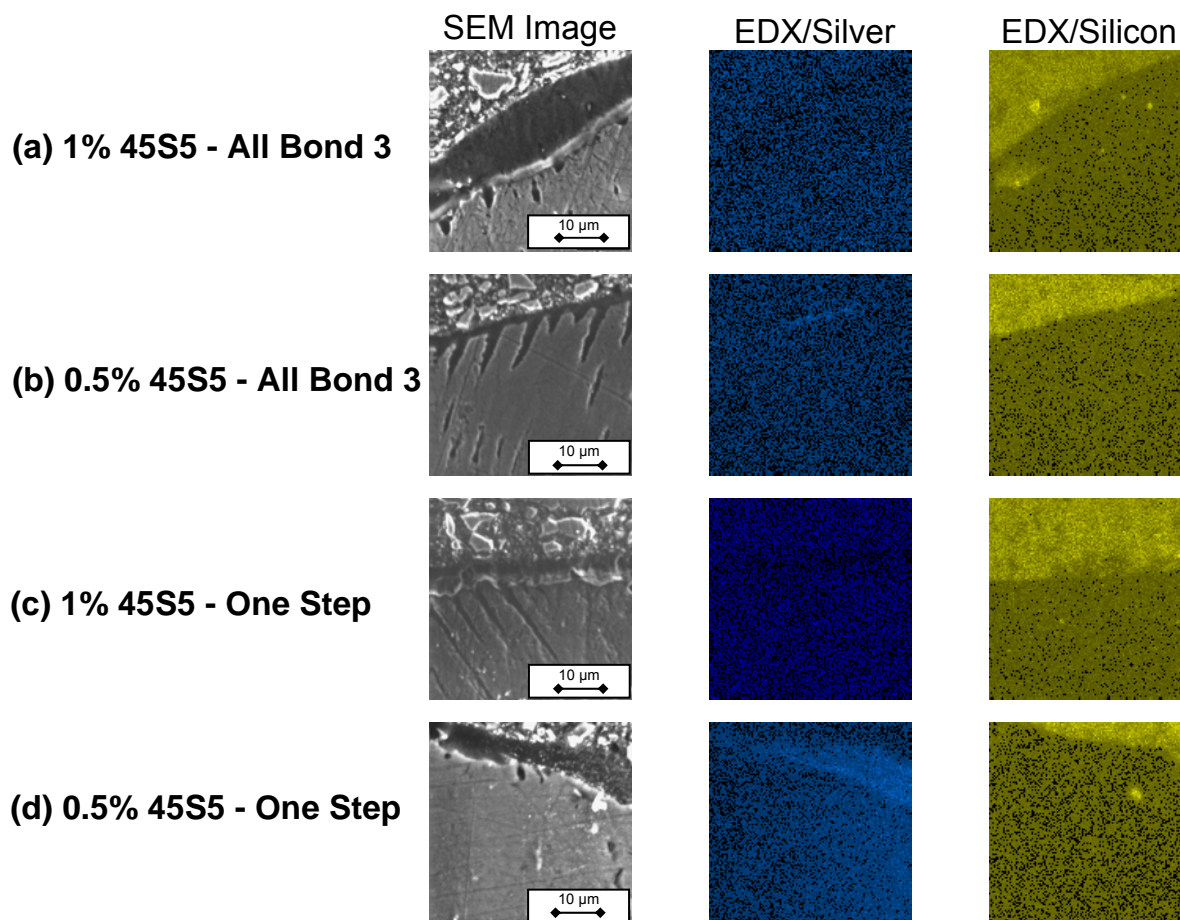


Figure 3. Scanning electron microscopy and energy dispersive x-ray analysis of dentin bonded with adhesive containing powdered 45S5. (A) dentin bonded with All Bond 3 and 1 wt% 45S5; (B) dentin bonded with All Bond 3 and 0.5 wt% 45S5; (C) dentin bonded with One Step and 1% 45S5; and (D) dentin bonded with One Step and 0.5 wt% 45S5. X-ray mapping does not show leakage for either bonding system when 45S5 is included at the higher concentration, but leakage is apparent when only 0.5 wt% of that glass is used (B, D). Debonding of adhesive and composite that was evident for negative controls is not apparent even in the presence of leakage. X-ray maps of silicon are included for spatial reference. C=composite; A=adhesive; D=dentin.

## **Chapter 6**

### **Conclusions**

The introduction of resin bonding to restorative dentistry was a great advancement, allowing clinicians to give patients aesthetically pleasing restorations relatively quickly and easily. However, the process was, and is, not without its faults, and chief among those faults is the frequent occurrence of leakage that can lead to failure of the bond and loss of the restoration. Twenty-one years after Buonocore's introduction of acid-etching,<sup>1</sup> Kidd discussed the need for a leak-resistant material;<sup>2</sup> thirty-one years after that, such a material has yet to be realized.

The premise of the resin-based adhesive restorative process is that the adhesive monomer will penetrate the collagen network exposed by acid-etching the dentin and be cured *in situ* to form the so-called hybrid layer.<sup>3</sup> Composite is then applied over the adhesive and cured. While the adhesive and composite are able to form chemical bonds, the bond created by the polymerized resin and the collagen is micromechanical in nature. Such a situation provides ample opportunity for leakage to occur beneath the restoration: oral fluids may penetrate any areas that are inadequately infiltrated with resin, carrying with them acids and enzymes that can attack either the exposed collagen<sup>4</sup> or cause hydrolytic degradation to the polymer;<sup>5</sup> host matrix metalloproteinases may also attack the exposed collagen;<sup>6</sup> if there are openings of sufficient size, it is even possible for bacteria to infiltrate and cause secondary carious lesions beneath the restoration.<sup>2</sup> Additionally, it has been suggested that self-etching primers may continue to etch even after the adhesive is placed and cured, thus removing additional, unintended, mineral and leaving collagen unsupported by either resin or apatite.<sup>7</sup>

Although the decades since the introduction of resin-based restorative dentistry have brought many changes and improvements to both the process and the materials,<sup>8</sup> the

problem of leakage has yet to be resolved. Improvements have been made in the areas of aesthetic appeal, ease of use, and reduction of technique sensitivity,<sup>9</sup> bond strength and setting contraction gap formation, but few advances have been made with regard to the reduction of leakage.

In the introductory chapter of this thesis, it was proposed that including bioactive glass into the resin-bonding process might reduce leakage. This hypothesis was based on the established fact that when bioactive glass is in an aqueous environment that contains calcium and phosphate, apatite will be precipitated via a distinct series of chemical reactions.<sup>10-14</sup> Furthermore, it is known that bioactive glass forms mechanically strong bonds to tissues, likely through a chemical bond to collagen,<sup>12, 15, 16</sup> which is abundantly present in dentin. Given these properties, bioactive glass appears to be a good candidate material to use when attempting to attenuate leakage.

The first goal was to establish if bioactive glass, in powder form, could even be included in the resin-to-dentin bonding process. In Chapter 2, a method was established whereby bioactive glass powder of the well-characterized 45S5 formulation and of an average particle size of ~1  $\mu\text{m}$  could be vacuum-deposited into etched dentin to a depth of ~5  $\mu\text{m}$ . When a commercially available adhesive was applied over the deposited glass and light-cured, it was able to form an apparently normal hybrid layer. The bonded samples were then soaked in simulated body fluid, and SEM/EDX analysis revealed reduced leakage. Samples were also aged by thermocycling in water, and again reduced leakage was found.

Following this promising start, it was decided to determine if a novel glass (“F glass”) formula could also reduce leakage in resin-bonded dentin. This glass contained,

in addition to the traditional bioactive glass components (silica, sodium, calcium, and phosphate), magnesium and fluoride. Magnesium has been shown to slow the deposition of calcium phosphate, which is believed to lead to better-controlled (and better quality) mineralization; while the presence of fluoride causes the precipitation of fluorapatite, which is much less vulnerable to acid attack than is carbonated apatite. Again, it was shown that an apparently normal hybrid layer was able to form in the presence of the vacuum-deposited glass powder, and SEM/EDX analysis showed that leakage did indeed appear to be reduced after soaking in simulated body fluid. However, thermocycling and methylene blue leakage studies were less definitive than in the previous chapter. Nevertheless, a trend was suggested that the presence of both bioactive glasses may be capable of reducing leakage.

It was then important to determine whether or not the presence of the glass would have a deleterious effect on the strength of the bonds formed. The study in chapter 4 looked at the shear bond strength of samples treated with both types of bioactive glass and found that there was no difference between experimental samples and negative controls. Analysis of the failure modes displayed a pronounced tendency for the glass-treated samples to fail cohesively in the dentin or in the composite, while the negative controls tended to adhesive failure. Other trends appeared in this study: the standard deviation of the bond strengths of the F glass-treated samples was reduced, suggesting that the presence of the glass could reduce technique sensitivity when bonding; and also the degree of loss of bond strength that occurred in all samples over time was least for the F glass-treated samples, suggesting that the glass may help to maintain bond strength (and therefore restoration durability) over time.

Until this point, the glass had only been added to the resin-bonding process via the vacuum-deposition method described in chapter 2. It remained to be seen whether the glass could be added to an adhesive, and if the subsequent bonds would be successful. In chapter 5, small amounts of both types of glass powder were added to commercially available adhesives, and teeth were bonded for the purpose of carrying out both bond strength tests and leakage studies. The most favorable results were obtained with the addition of F glass to an ethanol-based resin bonding system: bond strength was significantly increased, while leakage was substantially reduced when compared to negative controls. Addition of F glass to an acetone-based adhesive was less successful, as leakage was reduced, although bond strength was unchanged. Addition of 45S5 to an acetone-based adhesive significantly reduced bond strength, although at a concentration of 1% (w/w), leakage was reduced. When 45S5 was added to an ethanol-based adhesive, bond strength was unaffected, and again, leakage was reduced at a concentration of 1% (w/w). Lower doses of 45S5 to both adhesives did not produce beneficial effects in any case. This series of experiments demonstrated that it should be possible to incorporate bioactive glass into the bonding process in a clinically relevant manner.

While the first steps toward improving dental adhesives with regard to leakage have been made, much work remains to be done. Continuing studies should be performed in order to optimize the glass composition, particle size, amount included in the adhesive, and appropriate solvent for the adhesive. Additionally, it would be important to learn the durability of these bonds over a greater time span; bond strengths and leakage should be examined after storage of the samples for several months, or even years. It would also be useful to determine the behavior of restorations containing the



glasses under conditions such as pH cycling and cyclic loading. Finally, in vivo studies in animal models and humans should be conducted.

Bioactive glass shows a great deal of promise as a possible addition to the resin-dentin bonding process. It appears to reduce leakage, one of the major causes of restoration failure, while producing no negative effects on bond strength. In fact, under the right set of circumstances, bond strength may even be increased in the presence of a bioactive glass. It is also possible that the addition of bioactive glass powder may help to reduce the technique sensitivity of dentin bonding. Finally, these glasses may add to the lifetime durability of a restoration, as it appears that glass-treated bonds show a smaller loss of bond strength over time.

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