Abstract

Purpose: The purpose of this study was to examine the reduction in enamel demineralization provided by fluoride release from a conventional glass-ionomer, a resin-modified glass-ionomer and an experimental fluoride-releasing resin-based composite compared to a conventional resin-based composite control, and to correlate the level of fluoride release with demineralization.

Materials and Methods: Enamel surfaces of extracted human incisors had a 0.4 mm thick layer of the specified test material carefully placed in a band across the mid-facial enamel to simulate a cement layer beneath an orthodontic bracket. The top surface of the test material was covered with nail varnish, leaving only the edges of the material exposed to release fluoride. The teeth were additionally covered with nail varnish to within 1 mm of the test material. Each group of teeth was placed into separate volumes of unstirred demineralizing solution at a pH of 4.7 for four days. The specimens were sectioned and examined by polarized light microscopy. Lesion areas were measured at distances from 100 – 800 μm away from the test material. Fluoride release for the test materials was measured for periods up to five months.

Results: All of the fluoride-releasing materials demonstrated a statistically significant (p<0.05) degree of protection of enamel from demineralization compared to the non-fluoride control material. The degree of protection was greatest near the material, but lesion areas increased with distance in an inverse relationship to the amount of fluoride release. Lesions were displaced from the region near the materials and the mean displacement was directly related to amount of fluoride release. The mean lesion areas for each distance decreased with the logarithm of the cumulative fluoride release.
Clinical Significance: The fluoride releasing materials provided protection against enamel demineralization adjacent to the material. The degree and range of protection was directly related to the amount of fluoride release.

Introduction

The usefulness of fluoride-releasing materials for prevention of enamel demineralization has been demonstrated with various model systems. [1] One particular application where model systems have been used to study the effectiveness of fluoride-releasing materials is orthodontics, where protection of enamel surrounding a bracket or band is of interest, and white-spot lesion formation is a common problem due to the inability to easily remove plaque from around brackets with arch wires in place [2,3,4] (See Figure 1).

In an in vitro study by Valk and Davidson [5], orthodontic brackets were bonded to bovine enamel with either a resin-based composite or a conventional glass-ionomer. After exposure to a demineralizing solution, the lesions formed were displaced from the bracket bonded with the glass-ionomer by about 800 μm, but the resin-based composite resulted in lesion formation up to and undermining the composite material. A similar result was observed by Basdra et al. [6] using two fluoride-releasing resin based materials compared to a conventional composite. In this study, the material with greater fluoride release displaced the lesion by a mean distance of 142 μm, whereas the material with lower fluoride release had a mean displacement of only 20 μm. Kindelan [7] compared two resin-based composites,

Figure 1. Photograph showing white-spot lesions present surrounding orthodontic brackets after their removal.

106
two fluoride-releasing resin-based composites and a glass-ionomer for their ability to reduce demineralization around orthodontic brackets as determined by measuring the mineral content of the demineralizing solutions. The best performing material was the glass-ionomer, but it was not significantly different from one of the fluoride-releasing resin-based composites. The other fluoride-releasing resin-based composite was similar to the two standard resin-based composite materials. Relative amounts of fluoride released from the various materials were not presented. Recently Vorhies et al. [8] compared a resin-based composite, a fluoride-releasing resin system and a resin-modified glass-ionomer. These materials were used to bond brackets to human premolars which were treated by cycling between a synthetic saliva and 60 minutes per day in a demineralizing solution. Lesion formation adjacent to the brackets was examined by polarized light microscopy and the lesions adjacent to the fluoride-releasing materials were found to be smaller than those adjacent to the resin-based composite, but were not significantly different from one another. The fluoride release for the materials was not measured.

In vivo models have also examined the effect of fluoride-releasing materials on reduction of enamel demineralization. Rezk-Lega et al. [9] looked at the enamel positioned in gaps under orthodontic bands cemented with two glass-ionomer cements compared to a conventional cement. Both glass-ionomers showed reductions in measured mineral loss compared to the control, and these relative reductions in mineral loss were 49% and 27% respectively. The fluoride release of the two materials was not presented. In another in vivo study [10], brackets were bonded to premolars, scheduled for extraction, with either a fluoride-releasing resin-based composite or a conventional resin-based composite. The demineralization immediately adjacent to the bracket was examined by microradiography and there was a statistically significant reduction in demineralization when the fluoride-releasing composite was used compared to the control material. It has also been shown by Hallgren et al. [11], that the fluoride concentration in plaque surrounding orthodontic brackets was consistently higher when they were cemented with a glass-ionomer compared to a resin-based composite. These measurements were carried out over a period of six months. The fluoride release of the glass-ionomer was not presented.
While the studies above indicated benefit of fluoride release from some materials, only a few of the studies provided information regarding relative levels of fluoride release. It is, however, clear that there were differences between the effectiveness of materials that was likely related to their levels of fluoride release. The purpose of this in vitro study was to measure the fluoride release characteristics of three types of fluoride-releasing materials, to determine their ability to reduce enamel demineralization compared to a non-fluoride releasing control material, and to examine the relationship between the level of fluoride release and enamel demineralization.

**Materials and methods**

*Preparation of samples* - Twenty caries-free human incisors were chosen for this study, and randomly divided into four test groups having five teeth in each group. The middle 2 mm of the facial enamel of each tooth was isolated between strips of firmly attached vinyl tape for the purpose of attaching a 0.4 mm thick layer of test material in a band across the tooth. The tape also assured that the enamel was protected from alteration during the process of bonding the test materials. Standard bonding procedures were used for each of the materials. The test materials were a conventional glass-ionomer, a resin-modified glass-ionomer, an experimental fluoride-releasing resin-based composite and a common resin-based composite control as shown in Table I. The experimental resin-based composite is a Bis GMA/HEMA resin system which contains an organic fluoride and is filled at a ratio of 19% monomer to 81% filler. [12]

**Table I**

Test materials used in study.

<table>
<thead>
<tr>
<th>Test material</th>
<th>Abbreviation</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>3M Z100™ Restorative</td>
<td>Z100</td>
<td>Hybrid resin-based composite</td>
</tr>
<tr>
<td>Experimental Composite</td>
<td>Exp</td>
<td>Fluoride-releasing resin-based composite</td>
</tr>
<tr>
<td>3M Vitremer™</td>
<td>Vit</td>
<td>Resin-modified glass-ionomer</td>
</tr>
<tr>
<td>Ketac-Fil™</td>
<td>KF</td>
<td>Conventional glass-ionomer</td>
</tr>
</tbody>
</table>

108
**Measurement of demineralization** – Once the test materials were bonded and the protective tapes removed, they were allowed to mature in 100% humidity for about 2 hours. Nail varnish was applied to the teeth to within 1 mm of the test materials and also to the top surface of the test materials, leaving only the 0.4 mm edges exposed over a length of approximately 4 mm. Each group of teeth was then suspended in 500 milliliters of an unstirred acidic buffer solution (2.2 mM Ca\(^{2+}\), 2.2 mM PO\(_4^{3-}\) and 50 mM acetic acid at pH=4.7 and 22°C) for 4 days to induce artificial lesion formation.

All specimens were thoroughly rinsed with distilled water upon removal from the acidic solution and multiple sections were cut perpendicular to the enamel surface, using a Silverstone/Taylor hard tissue microtome. The sections were cut approximately 150 μm thick to minimize damage to the demineralized enamel surface. Only sections with the demineralized enamel intact were kept for analysis. The lesions on both sides of the test material were then examined by polarized light microscopy in a water medium, and photomicrographs were obtained on 35 mm transparency film. The polarized light transparencies were projected and tracings at a magnification of 200x were made from them. Areas of the magnified lesions adjacent to the test material were measured at distances corresponding to 100, 200, 400, 600 and 800 μm away from the test material, utilizing a digitizing board (SummaSketch II Plus). Because of the fragility of demineralized enamel, only several representative sections were individually hand polished to 80-100 μm for high quality photographic recording. The lesion areas measured at 200x were subsequently converted back to areas in square micrometers.

**Displacement measurement** – From the same tracings used for lesion area, the distance from the test material to where the lesion began was measured. Measurements were converted to micrometers.

**Fluoride release** - Fluoride release from the test materials was measured for up to 5 months. Discs (d=2.2 cm; h=0.12 cm) of the fluoride-releasing materials were made by polymerizing the material in a Teflon mold with polyester lined glass plates on either side. These discs were individually suspended in polyethylene containers with 25 mls of deionized water and stored at 37°C until the time of
each measurement. To measure the fluoride concentration in the water, a 10 ml aliquot was removed and added to 10 mls of TISAB®. An Orion Model 96-09 fluoride selective electrode was used to measure the concentration. The discs were returned to their respective containers with fresh deionized water and stored at 37°C for the next period of time. Standard fluoride solutions were diluted with TISAB and a calibration curve was determined for the fluoride electrode. Calibration was reevaluated in the same way prior to each measurement period.

*Statistical analysis* - Values for the demineralization at each test site were obtained by averaging the sections from that site. These values were then used to obtain mean values for the demineralization. Statistical comparisons of the mean values for each group were determined using ANOVA and a Tukey-Kramer post hoc test was used to compare individual means at a 5% level of significance.

**Results**

*Fluoride release*
Cumulative fluoride release for the three test materials is shown in Figure 2. The experimental resin-based composite showed linear fluoride release, unlike the two glass-ionomers which had the typical decreasing rate of release over time. Over longer periods of time, the experimental resin-based composite has fluoride release comparable to glass-ionomers.

*Demineralization*
Mean lesion areas at the five measurement distances for all materials are shown in Table II. All three of the fluoride-releasing materials showed statistically significant reductions in demineralization, at all measured distances, compared to the control (p<0.05). The experimental resin-based composite and Vitremer were not statistically different at any distance. Similarly, Ketac-Fil and Vitremer were statistically equivalent at all distances. The data is shown graphically in Figure 3.

The relationship between lesion area and 4-day cumulative fluoride release is shown in Figure 4 for each test material at each measured distance. For Z100, which does not release fluoride, an arbitrary minimal value of 0.01 μg/cm² was chosen. The relationship between lesion area and the logarithm of fluoride release
Figure 2a. Long-term fluoride release of Ketac-Fil, Vitremer and the experimental composite into distilled water.

Figure 2b. Short-term fluoride release of test materials into distilled water.
### Table II
Mean enamel lesion areas (μm²) measured at various distances from the test materials. Shown with standard deviation ( )

<table>
<thead>
<tr>
<th>Material</th>
<th>area (100μm)</th>
<th>area (200μm)</th>
<th>area (400μm)</th>
<th>area (600μm)</th>
<th>area (800μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z100</td>
<td>2.17 (0.32) a</td>
<td>4.54 (0.57) a</td>
<td>9.24 (1.39) a</td>
<td>14.34 (2.20) a</td>
<td>20.74 (4.24) a</td>
</tr>
<tr>
<td>Exp</td>
<td>0.25 (0.25) b</td>
<td>0.67 (0.59) b</td>
<td>1.78 (1.52) b</td>
<td>3.30 (2.58) b</td>
<td>5.61 (3.77) b</td>
</tr>
<tr>
<td>Vit</td>
<td>0.17 (0.43) b</td>
<td>0.33 (0.69) bc</td>
<td>1.03 (1.73) bc</td>
<td>1.94 (3.22) bc</td>
<td>3.11 (5.18) bc</td>
</tr>
<tr>
<td>KF</td>
<td>0 b</td>
<td>0 c</td>
<td>0 c</td>
<td>0.01 (0.04) c</td>
<td>0.23 (0.74) c</td>
</tr>
</tbody>
</table>

Values with the same letter in each column are not statistically different (p<0.05)
**Figure 3.** Mean enamel lesion areas at five measurement distances for all test materials.

**Figure 4.** The relationship between lesion area and 4-day cumulative fluoride release at all measured distances for the test materials.
is linear, with correlation coefficients \( r^2 \) between 0.99 and 1.0.

**Displacement**

For the fluoride-releasing materials, the demineralization lesions were frequently displaced away from the materials, whereas, with the Z100 control, the lesions tended to undermine the material. Typical lesions are shown for Z100, the experimental resin-based composite and Ketac-Fil in Figure 5, where the displacement and undermining can be noted. In particular, the displaced lesion for Ketac-Fil (Figure 5c) is just apparent at the edge of the photomicrograph. The mean displacement for Z100 was \(-29.6 \pm 8.0 \, \mu m\) and for the experimental resin-based composite was \(339.5 \pm 207.4 \, \mu m\). Definitive displacement values for the other two materials could not be determined because for some specimens, there was no lesion within the unvarnished area.

**Discussion**

There is sufficient evidence from a variety of studies to support the belief that fluoride released from materials inhibits the demineralization of enamel adjacent to those materials. [1, 5-10] What has generally been lacking is information on the relationship between fluoride release characteristics of the materials studied and their relative effect on enamel demineralization. Such a relationship was examined with an in vitro study of enamel demineralization where the demineralizing solutions had different fluoride concentrations, [13] and a linear-log relationship was found between demineralization and fluoride concentration. However, little has been done to examine such relationships where the fluoride source is a restorative material. Dijkman et al. [14] related fluoride release into water with in situ enamel demineralization for several fluoride-releasing resin-based composites and also derived a logarithmic relationship between demineralization and cumulative fluoride release. It was the purpose of the authors of the present study to use an in vitro model to examine the relationship between fluoride release and enamel demineralization adjacent to three different fluoride-releasing materials compared to a resin-based composite control.

The three test materials had different fluoride release profiles, both in amount and rate of release, but the long term cumulative fluoride release of all of them fall at
Figure 5a. Polarized light micrograph (25x) of a representative enamel lesion present adjacent to Z100.

Figure 5b. Polarized light micrograph (25x) of a representative enamel lesion present adjacent to Experimental Resin-based Composite.

Figure 5c. Polarized light micrograph (25x) of a representative enamel lesion present adjacent to Ketac-Fil.
the high end for fluoride releasing materials (Figure 2). The experimental resin-based composite displays a linear fluoride release after several days. Such a fluoride release profile has been previously described for composites filled with fluoride containing glasses. [14,15] It has been suggested that the relationship describing the fluoride release for such a material is: [15]

$$[F] = \frac{[F]_1 \cdot t}{t^{1/2} + t} + \alpha t$$

and the fit to this relationship for this material yields values for the parameters of $[F]_1 = 14$, $t^{1/2} = 3$ and $\alpha = 1.9$.

Ketac-Fil and Vitremer show typical glass-ionomer fluoride release profiles. A relationship that describes the fluoride release for glass-ionomers is: [15]

$$[F] = \frac{[F]_1 \cdot t}{t^{1/2} + t} + \beta t^{1/2}$$

For Ketac-Fil, $[F]_1 = 65$, $t^{1/2} = 1.2$ and $\beta = 30$, while for Vitremer, $[F]_1 = 18$, $t^{1/2} = 1.2$ and $\beta = 14$.

The current study focused on the short-term response of fluoride, so the curves of Figure 2b show the pertinent time range in more detail. Future studies will examine the relationship of long-term fluoride release to demineralization.

The data of Table 2 and plot of Figure 3 show a substantial reduction in demineralization for the fluoride releasing materials compared to the control. In the case of Ketac-Fil, there is nearly total inhibition of demineralization out to the maximum range of measurement of 800 µm. The unstirred solution of this study creates an environment where the fluoride diffuses outward from the test materials creating a decreasing concentration gradient of fluoride as you move away from the material. The concentration will also be dependent upon the relative release rate of fluoride from the material. At any given distance, the local fluoride concentration will be reflected by the degree of enamel demineralization. This can be seen in the curves for the lesion areas at each of the measured distances (Figure 3). The gradient effect can also be observed in the photograph for the experimental resin-based composite in Figure 5b.

Hallgren [11] has measured an increase in plaque fluoride concentration around
orthodontic brackets bonded with glass-ionomer materials compared to resin-based composite over a period of six months. The model of the current study is intended to simulate such diffusion of fluoride into the saliva and plaque on a tooth surface, and although it is simplistic, it is useful for examining relative effectiveness of materials. The range to which fluoride can diffuse with effective concentration is limited, and at some distance from the material, the lesion formation will be similar to the resin-based composite.

Although there are some statistical differences in mean lesion areas between materials, the standard deviations are relatively large, so that statistical differences were not demonstrated at the greater distances where they might be expected; however, all of the fluoride-releasing materials were significantly different from the Z100 control. A likely reason for the high variability is because human incisors used for this study had intact enamel surfaces that were not altered in any way prior to their use, leaving them with variable surface fluoride levels, and variable amounts of prismatic and aprismatic enamel. This is indicated in the raw data where some specimens had no demineralization at any distance, while others had substantial demineralization. For example, for Vitremer, 40% of the specimens showed no lesion formation out to 800 μm, while the other 60% of the specimens showed lesion formation at 100 - 200μm from the material. It is expected that if the surface enamel had been ground away, the variability might have been decreased, and other statistical differences may have been demonstrated between materials. This variability likely occurs clinically as well, and for this reason, the decision was made to leave the enamel surface intact.

As discussed above, lesion formation was displaced in some cases by greater than 800 μm. Measurements of the mean displacement were made for the experimental resin-based composite and Z100, and they were 339.5 ± 207.4 μm and –29.6 ± 8.0 μm respectively, the latter number indicating undermining of the control composite material. Definitive numbers could not be obtained for Vitremer and Ketac-Fil because no lesion formation was found within the measurable distance for 40% of the Vitremer specimens, and 90% of the Ketac-Fil specimens. However, estimates can be made which suggest the mean displacement for Vitremer would be > 500 μm, and for Ketac-Fil it would be > 800 μm. Others have also found lesion displacement from various materials using in vitro model
systems. In a study by Valk and Davidson [5], the mean displacement on ground bovine enamel was 818 μm for a glass-ionomer. Basdra et al. [6] measured lesions produced on unaltered human premolars in an acidic gel system after 4 weeks (pH=4.8), and found a mean lesion displacement of 142 μm for a fluoride containing resin material which had a cumulative fluoride release of approximately 40 μg/cm² after 1 month. The other material used in their study had a displacement of only 20 μm and its cumulative fluoride release after 1 month was about 10 μg/cm². This fluoride release measurement is confirmed by Chadwick and Gordon. [16] Even though the displacements measured by Basdra et al. [6] were in a gel system, the results are reasonably consistent with the results of the current study, which used an aqueous demineralization system.

To examine dose response relationships, the lesion areas for each material were plotted against the logarithm of the 4-day cumulative fluoride release as suggested by Dijkman et al [14]. This was done for each of the five distances away from the material. For Z100, an arbitrary fluoride release was assigned at a low level of 0.01. Since there is no zero value for a logarithmic scale, a finite value needs to be assigned, and while Z100 has no measurable fluoride release, there will still be a low level of fluoride in the demineralizing solution near the enamel due to release from the enamel. Figure 4 shows the data plotted as described above. The data fit in all cases had correlation coefficients which ranged from $r^2 = 0.99$ to 1.0. This relationship would probably break down at distances much larger than 800 μm where effective levels of fluoride no longer were obtained.

The results of this study indicate that fluoride, released from materials, can exert an inhibiting effect on demineralization of enamel adjacent to the material. The range and degree of protection is directly related to the level of fluoride release, and the lesion area was logarithmically related to fluoride release for distances up to 800 μm away from the material.

For prevention of white spot lesions around orthodontic brackets, it is desirable to be able to inhibit demineralization to a range of at least 1.0 mm and preferably further (Figure 1). In the present model, at 0.8 mm, the reduction in demineralization was 73% for the experimental resin-based composite, 85% for Vitremer and 99% for Ketac-Fil. At least in this model, the range and degree of
protection afforded is promising, but it is also clear that relatively high levels of fluoride release are required. An examination of the long-term relationship between fluoride release and demineralization in model studies is needed. Moreover, in vivo studies are also needed to determine the relevance of these in vitro model results.

a. 3M Dental Products, St. Paul, MN, USA.
b. ESPE, Seefeld, Germany
c. Scientific Fabrications, Lafayette, CO, USA.
d. Summagraphics Corporation, Seymour, CT, USA.
e. Orion Research Inc., Boston, MA, USA.
References


