Fluoride-releasing materials for orthodontic appliances
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CHAPTER 4

Protective Effects of Resin Impregnation on Demineralization of Enamel


Abstract
Purpose: The purpose of this study was to determine if resin penetrated into the enamel etch pattern, in the absence of a polymerized outer surface film, could reduce the degree of demineralization of enamel subjected to a simulated caries challenge, and to evaluate whether the addition of fluoride to the resin would enhance reductions in demineralization.

Materials and Methods: Enamel surfaces of extracted human incisors were acid-etched. One-half of the etched area was treated with an unfilled bonding resin, while the other one-half was left untreated as a control. In another group, this same procedure was followed except the unfilled bonding resin contained fluoride. The applied resin was aggressively air thinned to ensure oxygen inhibition throughout the external surface film thickness. The thinned film was visible light cured and the area was wiped with an ethanol swab to remove the inhibited layer. The specimens were exposed to a buffer solution of pH=4.7 for four days, and were sectioned and examined by polarized light microscopy and microradiography.

Results: In each of the two test groups, the demineralization of the resin-treated side was significantly lower than the control side (p< 0.015). Under the conditions of this study, the experimental fluoride resin did not produce statistically significant reductions in demineralization compared with the non-fluoride resin.

Clinical Significance: Resin impregnated into etched enamel surrounding restorations or orthodontic appliances can reduce the degree of demineralization of that enamel, even when a surface film is not present.
Introduction

Demineralization of enamel adjacent to dental materials is of interest in many areas of dentistry and has been the subject of numerous studies. In such studies, an aspect of design that is sometimes overlooked is the influence that bonding resin may have on the demineralization of the enamel. While it is obvious that a cured resin film on top of an enamel surface will protect it from demineralization, the resin that has penetrated below the surface into the etched microporosities may also reduce demineralization, even when there is no cured resin film on the surface. It is important that this be taken into consideration when a study is designed to compare bonded materials with unbonded materials, or when the study is designed to examine the effect on demineralization by other factors, such as fluoride release from a bonded material. Failure to consider the resin effect could result in erroneous conclusions.

This resin effect can have implications in many areas of dentistry, but for much of this article, the area of orthodontics will be emphasized. A persistent problem in orthodontic treatment of teeth is enamel demineralization surrounding orthodontic appliances, which may result in white spot lesions. White spot lesions can develop within one month after placing appliances, [1,2,3] and can persist for years following removal. [4] The incidence of white spot lesions due to orthodontic appliances has been shown to be variable depending on factors such as topical fluoride treatments and patient oral hygiene. In a clinical study [5] where no topical fluoride was used, 50% of patients and 10.8% of teeth where orthodontic bands or brackets were used showed white spot lesions, compared to a control group, without orthodontic treatment, where 24% of patients and 3.6% of teeth had white spot lesion formation. Another study [4] showed a significant increase in white spot lesions for orthodontically treated patients compared to untreated patients. The mean white spot score for the treated group was a little more than twice the score for the untreated group. These studies would indicate that the problem is severe enough to make preventive measures highly desirable.

One preventive measure that has been investigated is sealing the enamel surrounding an orthodontic appliance with a bonding resin. Several studies have found that chemically cured unfilled resins were not effective in providing
complete surface sealing. [6,7,8] The reason cited for failure of the resin systems used in these studies to seal enamel was oxygen inhibition of polymerization. Since the investigators were only concerned with complete protection from the presence of a cured surface film, they did not evaluate reductions in demineralization due to the treatment. In contrast, studies utilizing either UV light curable [9] or visible light curable resins, [10,11] have shown that these resins can produce a cured film on the enamel surface, and provide complete protection from acidic attack. These light cured resins are less affected by oxygen inhibition and are able to seal the etched enamel.

Two studies, that used UV light-cured resins, showed a degree of protection from demineralization by subsurface resin tags which remained in the etched enamel after removal of the cured outer surface film. [12,13] In these studies, the presence of an outer film prior to its removal ensured better polymerization of these subsurface resin tags. An additional observation showed similar results if the resin was allowed to penetrate the etched enamel for one minute, followed by wiping away the surface resin with a tissue, and then UV light-curing. [12]

In some clinical situations, excess resin materials may be removed prior to polymerization. For example, this could occur around a composite restoration where excess bonding resin or restorative materials are removed from the enamel surrounding the margins either by air thinning or scraping away with an instrument. This would allow inhibition to minimize curing in those areas. In orthodontics, it is common to remove excess materials from around bonded appliances with an instrument, such as a scaler, prior to curing. [14] Again oxygen inhibition would be expected to minimize the ability of the residual resin to polymerize. The purpose of this study was to examine the effect of resin in the subsurface of etched enamel on demineralization when a light cured surface film is not possible due to oxygen inhibition. A secondary objective was to compare this effect for a fluoride-containing bonding resin and a non-fluoride resin.

**Materials and methods**

Ten caries-free human incisors, which were inspected under 3x magnification and found to be free of cracks and defects, were used in this study. The middle 2 mm
of the facial surface of each tooth was isolated between strips of firmly attached vinyl tape for the purpose of attaching a Z-100® resin-based composite divider to separate the tooth into two test areas. This enamel area was acid etched with 35% phosphoric acid gel for 60 seconds and then was rinsed and dried. A thin layer of an unfilled bonding resin, Scotchbond™ Multi-Purpose Dental Adhesive (SBMP®), was applied with a brush to the etched area between the tapes, and was gently air thinned and light cured for 20 seconds with a Visilux™ 2 visible light curing unit. The resin-based composite restorative divider was carefully placed between the tapes and light cured for 20 seconds.

The tapes were removed and the test areas of enamel on either side of the resin-based composite divider were then acid etched with 35% phosphoric acid gel for 60 seconds, rinsed and dried. The teeth were randomly divided into two test groups. In Group 1, bonding resin containing no fluoride (SBMP), was applied to the etched enamel on one side of the resin-based composite divider. No resin was applied to the control area of the tooth on the other side of the resin-based composite divider. The bonding resin layer was immediately air thinned with a strong air stream, such that maximum removal of resin was accomplished. This thinned resin layer was then light cured for 20 seconds, followed by an alcohol wipe over the resin area to remove the oxygen-inhibited layer. This procedure prevented surface film formation due to oxygen inhibition, but still allowed for potential polymerization of resin tags in the subsurface region. In Group 2, a bonding resin consisting of SBMP containing 30% (wt/wt) of an organic fluoride, tetrabutylammonium- tetrafluoroborate (TBATFB), was used as described for Group 1. Each specimen was inspected at 3x magnification to assure that there was no cured resin film on the enamel surface.

The individual specimens were stored in 100% humidity until all were completed. The teeth were coated with an acid resistant nail varnish to within 1 mm of the resin-based composite divider and each group of teeth was then suspended in 500 milliliters of an unstirred acidic buffer solution (2.2 mM Ca²⁺, 2.2 mM PO₄³⁻ and 50 mM acetic acid at pH=4.7 and 22° C) for four 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 resin-based composite divider 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 composite divider were measured to a distance corresponding to 800 µm away from the composite divider, 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.

Following the polarized light micrographic analysis, the tooth sections were used for microradiographic analysis. It was necessary to thin the sections for this analysis, so they were polished to approximately 100 µm thickness on 600 grit silicon carbide papers. Approximately 40% of the sections from Group 1 were lost during this process due to fracture of the demineralized enamel, which is a common problem in preparing such specimens. Microradiographs of the remaining specimens and a 12-step aluminum stepwedge were taken on photographic plates with an X-ray source at 20 kV and 20 mA. The microradiographs were scanned with a microdensitometer to determine optical density as a function of depth, which was converted to digital data and entered into a computer. Each scan included the region from the edge of the resin-based composite divider to 300 µm on either side of the composite divider. This data was compared with that from the aluminum stepwedge and analyzed by computer to provide mineral profiles. These were further analyzed to provide integrated mineral loss (IML) values. Details of the methodology have been published previously [15].

Values for the demineralization of the control and treated sides for both polarized light and microradiography were obtained for each tooth by averaging the sections
from that tooth. These values were then used to obtain mean values for the
demineralization of the control and treatment sides for both groups. Statistical
comparisons of the mean values for the treatment and control side of each group
were determined using a one-tailed t-test for paired measurements [16].
Comparisons between the mean values of the two groups were made using a two-
tailed t-test.

Fluoride release from the experimental bonding resin was measured over a 20
week period. Discs (d=2.2 cm; h=0.12 cm) of the bonding resin were made by
polymerizing the material in a Teflon mold with polyester lined glass plates on
either side. These discs were suspended in polyethylene containers containing 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 TISAB⁴. 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. At the end of the demineralization experiment, the fluoride
concentrations in the demineralizing solutions were determined using the same
method described above.

Scanning electron microscopy was used to visualize the acid resistance of resin-
treated enamel as used in the demineralization experiment. Human incisors were
used with the facial enamel ground to a 600 grit finish. This surface was treated
in a similar manner to the specimens used in testing demineralization. After
completing treatment, one-half of the area was etched for 60 seconds with 35%
phosphoric acid gel and rinsed with water. This procedure was used to simulate
the artificial caries process in a shorter time with a more aggressive acid, and
show the relative acid resistance of the two areas. These specimens were prepared
for conventional SEM observation.

To observe the amount of resin left on the surface after the aggressive air thinning
procedure used in the demineralization experiment, human incisors had the facial
surfaces treated as for the experiment, but instead of light curing in the open
atmosphere, they were cured under vacuum to prevent oxygen inhibition. The
teeth were sectioned perpendicular to the facial surface and the sectioned surface
was etched for 60 seconds with a 50% (v/v) nitric acid solution, rinsed and dried, and then processed for SEM observation.

**Results**

**Polarized Light Evaluation**

The average areas of the lesions, as measured from the edge of the resin-based composite divider to a distance 800 μm from the divider, are shown in Table I. Within each group, the average area of the lesion on the resin-treated side was significantly smaller than the control side (p < 0.015). Figure 1a shows a polarized light micrograph (25x) of a representative enamel lesion present on control side of Group 2 specimens.

### Table I

Mean lesion area and lesion reduction for polarized light experiment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Lesion Area (μm² x 10⁴)</th>
<th>Lesion Reduction</th>
<th>Statistical Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control side</td>
<td>Treated side</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.30 (1.37)</td>
<td>2.42 (2.54)</td>
<td>54%</td>
</tr>
<tr>
<td>2</td>
<td>4.00 (0.39)</td>
<td>2.35 (1.01)</td>
<td>41%</td>
</tr>
</tbody>
</table>

**Figure 1a.**

Polarized light micrograph (25x) of a representative enamel lesion present on control side of Group 2 specimens.

**Figure 1b.**

Polarized light micrograph (25x) of a representative enamel lesion present on the resin-treated side of the same Group 2 specimen as in Fig. 1a.
light micrograph of a representative enamel lesion on the control side of a specimen from Group 2, while Figure 1b is a representative lesion on the resin-treated side of the same specimen. Group 2 showed somewhat smaller lesions on the control side compared to Group 1, but the difference was not statistically significant.

**Microradiography**

The average integrated mineral loss (IML) values for the lesions on the control and resin-treated sides of Groups 1 and 2 are shown in Table II. The microradiographic data shows trends similar to the polarized light experiment. The sample size for Group 1 was reduced by approximately 40% due to shattering during thinning of the sections for the microradiography experiment. For this reason, the Group 1 values shown in Table II are unreliable in terms of actual IML and are probably lower than would have been expected. The mineral loss was smaller for the resin-treated sides of both groups compared to the control sides. The difference for IML mean values between resin-treated and control sides for Group 2 was statistically different at a high level (p<0.001), while for Group 1 the difference was statistically different only at a p<0.05 level, due to the reduced number of specimens.

**Table II**

Mean IML and IML reduction for microradiography experiment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean IML (Vol % μm)</th>
<th>IML Reduction</th>
<th>Statistical Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control side</td>
<td>Treated side</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>1</strong></td>
<td>*1553 (369)</td>
<td>*1010 (689)</td>
<td>35%</td>
</tr>
<tr>
<td>2</td>
<td>1302 (123)</td>
<td>840 (251)</td>
<td>36%</td>
</tr>
</tbody>
</table>

* based on reduced sample size

**Fluoride Release**

The amount of fluoride measured in the demineralizing solution from Group 1, using the non-fluoride containing resin, was 0.009 ppm, while the demineralizing solution used for Group 2, with fluoride-containing resin, measured 0.014 ppm.
Figure 2. Fluoride release of resin which contains 30% organic fluoride into distilled water.

Measurements at these low levels may be somewhat inaccurate, but the ranking of the values is as expected. Figure 2 shows the fluoride released into distilled water over time for the fluoride-containing bonding resin used in Group 2.

**SEM Analysis**

Figure 3a shows an SEM micrograph of an etched enamel surface that was treated with resin, as it remains after the alcohol wipe, while Figure 3b is the same surface after subsequent etching with 35% phosphoric acid. The resin protective effect can be seen compared to a control area as shown in Figure 3c, where the enamel was acid etched, but not coated with resin.

The SEM micrograph in Figure 4 shows a specimen that was light cured under vacuum after aggressive air thinning of the applied resin. Since oxygen inhibition is eliminated, the resin will cure and its morphology can be observed, indicating resin tags of about 10 μm length, and a surface film estimated to be about 4 μm thick.
Figure 3a. Scanning electron micrograph showing the etched enamel surface after application of an aggressively thinned, light cured resin film, which had the oxygen inhibited layer removed. Marker equals 5 µm.

Figure 3b. An SEM of the same enamel surface as shown in Figure 3a, that was subsequently acid etched with phosphoric acid, shows some protection from the acid attack. Marker equals 5 µm.

Figure 3c. Etched enamel with no resin applied (control). Marker equals 5 µm.
Discussion

It was the purpose of this in vitro study to determine if polymerized resin within the etched microporosities of an enamel surface would provide the enamel some protection against subsequent acidic demineralization. It is clear that a fully polymerized film on the outer surface will act as a barrier to acid attack, [9-11] and even when such a cured film is removed from the surface, it has been found that there is protection by the polymerized resin remaining beneath the surface. [12,13] However, air thinning and cleaning procedures such as employed around an orthodontic bracket or a bonded restoration, may reduce the thickness of the resin to an extent that oxygen inhibition can prevent the formation of a polymerized film on the outer surface. In this study, the applied resin was air thinned aggressively immediately after application and prior to light curing, to ensure that the outer surface film would be completely inhibited by oxygen. The thickness of SBMP resin that will be inhibited has been estimated to be about 8 μm, from unpublished work by the authors. Figure 4 shows an SEM micrograph of a specimen that was light cured in vacuum to prevent any oxygen inhibition of polymerization. This micrograph demonstrates that the aggressively air thinned surface film is about 4 μm thick. This small amount of resin thickness will not cure as a surface film, but can provide protection to the underlying resin from oxygen in the atmosphere. Upon light curing, the resin impregnated into the etched microporosities of the enamel may be polymerized and act as a protective seal to reduce demineralization. The photomicrographs of Figure 1 provide a
visual confirmation of this protective effect, as evidenced by the smaller enamel
lesion on the resin-treated side (Figure 1b). Figure 3 also shows the protective
effect of the subsurface resin against acid attack on the enamel, where there is
clear evidence that with phosphoric acid etching of the treated enamel (Fig. 3b),
there is less attack of the enamel compared to untreated enamel (Fig. 3c).

The data shown in Table I indicates that the demineralization of the resin-treated
side was statistically significantly less than the control side (p<0.015). This was
true for both the non-fluoride resin and the fluoride-containing resin. The
percentage reduction in demineralization was substantial, being 54% for Group 1
and 41% for Group 2. There were similar findings for IML values. Table II
shows the results for Groups 1 and 2 indicating statistically significant differences
in IML for the resin- treated sides compared to the control sides. The percentage
reductions in IML for Group 1 and Group 2 were similar at 35% and 36%
respectively.

Because so many sections in Group 1 were lost in thinning them for
microradiography, the mean IML was most likely skewed toward a lower value,
especially for the control side which had the most vulnerable enamel. The lost
sections were those that generally had the largest lesion areas in polarized light
measurements and therefore, would presumably have the greatest demineralization
and highest IML values in the microradiography experiment. Figure 5 shows the
plot of the matched data comparing the polarized light lesion areas to the IML, for
all available sections for both groups. The good correlation (r = 0.82) supports the
presumption that the lost sections, which were those with the largest polarized
light lesion areas, would have increased the mean IML. Despite the loss of
sections, an analysis of the reduced set of data for Group 1 still indicated a
statistically significant reduction (p< 0.05) in IML by resin treatment compared to
the control.

While the data in Tables I and II show that within Groups 1 and 2 there were
significant reductions in demineralization due to the presence of resin
impregnation on the treated enamel compared to the control enamel, there were no
statistically significant differences between Groups 1 and 2 for polarized light
measurements or IML. The polarized light data in Table I, show a reduction in
Figure 5. Correlation between polarized light lesion area and IML obtained by microradiography.

lesion area for the control side of Group 2 compared to Group 1. While this suggests that fluoride may have influenced the demineralization, there was no statistically significant difference between the two groups. Because of the lost specimens from Group 1, the difference in IML between Group 1 and 2, especially for the control side, is not as large as expected and there was no statistical difference found.

Studies [17,18] have shown that fluoride amounts in the 0.024 - 0.06 ppm range in a demineralizing solution can significantly reduce the demineralization of enamel. The measured fluoride content of the buffer solution for Group 2 at the end of the four days was 0.014 ppm, which within the accuracy of the measurement appears to be consistent with what would be expected from the fluoride release profile of Figure 2. This fluoride concentration is lower than cited in the above studies, but with an unstirred solution, as used here, diffusion limitations would cause the fluoride concentration near the tooth surface to be higher than what is finally
measured in the entire solution volume. What was observed was the loss of many sections from Group 1 during the thinning process, particularly because of the shattering of the untreated side. The fact that few sections were lost from Group 2 suggests that the enamel, especially on the untreated control side, was less fragile than in Group 1. There may well have been a fluoride effect that provided this resistance to fracture. This can also be seen through examination of individual mineral loss profiles obtained by microradiography, where on the control sides of Group 2, there was consistent evidence of a pronounced mineral surface zone, whereas for Group 1, even in the sections that did not fracture, there was minimal evidence of a surface zone. For Groups 1 and 2, the mineral profiles showed little surface zone formation on the resin-treated sides. This observation, along with the similar demineralization values on the resin-treated sides of Groups 1 and 2 shown in Table I, indicates the demineralization process on the resin-treated sides, is dominated by the resin impregnation effect, and fluoride did not have much effect under the conditions of this study.

The results of this study show that substantial reduction in demineralization can occur from resin impregnation of etched enamel with no cured resin on the outer surface. This has implications in designing and interpreting results of studies where the factors affecting demineralization are of interest. For example, it is important, when comparing demineralization around a bonded and a non-bonded restoration, to not attribute demineralization differences only to internal cavity sealing differences, but to be aware that the outer enamel surface in the bonded group will be more resistant to demineralization because of resin impregnation, unless measures are taken to prevent this from happening, or the surface is abraded to remove the entire resin impregnated enamel layer.

An orthodontic study [19] where light-cured resins were applied to the etched enamel before placing brackets has some parallels to the present study. Two different materials were used, one having no fluoride, and the other where both the bracket composite and the unfilled bonding resin contained fluoride. Since the unfilled resins were light cured, it is possible that there was a cured outer surface film, but the methodology presented did not give enough detail to know whether there was air thinning or not. Also, the two resin systems used were different and so curing efficiency could have been different. But one can presume that there
could be subsurface resin present in the enamel surrounding the orthodontic brackets. The results of the study indicated that there was no observable demineralization of enamel on the teeth with the fluoride system, but 12.6% of the teeth in the non-fluoride control group, showed observable demineralization. The question then becomes how much of this difference in performance was due to fluoride, and how much may have been due to the resin treatments of the surfaces.

Aside from the implications for design controls in studies, there are also some potential clinical implications from the results found. Whether it be restorative procedures or orthodontic procedures, if etched enamel is bonded with a light-cured resin, there may be a degree of protection conferred to that enamel, even if the surface film is removed. The reductions in demineralization could be about 50%, which is a consequential amount. Enamel wear under normal conditions is estimated to be from 4-6 µm per year [12, 20]. At such a rate, one might expect the beneficial effect of subsurface resin, with tag lengths of approximately 10 µm, to last for one to two years. In orthodontics however, demineralization is most frequently observed adjacent to brackets, where enamel removal through toothbrush abrasion is unlikely to occur, so the effect could be longer. Similarly, interproximal areas near bonded restorations aren’t abraded during toothbrushing, so the resin impregnation effect could be long lasting. Further clinical investigations would be required to validate these potential benefits.

a. 3M Dental Products, St. Paul, MN, USA.

b. Scientific fabrications, Layfayette, CO, USA.

c. Summagraphics Corporation, Seymour, CT, USA.

d. Orion Research Inc., Boston, MA, USA.
References


