CHAPTER II

ABSTRACT

Objective: This in vitro study examined the effects of orthodontic bonding sealants with fluoride-releasing glass filler and fluoridated resin-based orthodontic sealant on caries-like enamel lesion formation.

Materials and Methods: Sound human permanent molar teeth (n=40) were divided into two treatment groups. Group 1: Light Bond, a sodium fluoride-filled sealant; Group 2: Pro Seal, fluoride-releasing glass filled sealant. Sealant material was placed on buccal surfaces according to manufacturer's recommendations. Acid-resistant varnish was applied to each tooth, leaving a rim of exposed sound enamel surrounding the sealants and windows of exposed sound enamel on the lingual surfaces as treatment controls. In vitro caries were created using modified ten Cate solution. Each group underwent synthetic saliva rinsing for 2 weeks; lesion initiation for 2 weeks; synthetic saliva rinsing for 1 week; lesion progression I for 1 week; synthetic saliva rinsing for 1 week; and lesion progression II for one week. At each time period, 3 longitudinal sections were obtained from each tooth. Sections were examined with polarized light microscopy to evaluate lesion depth and enamel/resin interface. Mean (SD) lesion depths were determined and compared (ANOVA, t-test). Results: For all time periods, each of the treatment groups exhibited a significant reduction in mean lesion depth, as compared to the control group (P<.001). Pro Seal exhibited statistically significant reductions in mean lesion depth as compared to Light Bond (P<.01) at all time periods.

Conclusions:

1. Pro Seal and Light Bond enhanced in vitro caries resistance when compared with no treatment controls, as demonstrated by significant reductions in mean lesion depths at each time period (P<.001).

2. There were significant differences between the two treatment groups at each of the three time periods (P<.01). Pro Seal had mean lesion depths that were significantly less than that for Light Bond at each time period.
INTRODUCTION

One of the most detrimental complications of orthodontic treatment is enamel decalcification. Orthodontic bands, brackets, archwires, coils and elastics encourage the collection of food and plaque. Impeccable oral hygiene must be emphasized prior to treatment initiation and throughout the treatment period. However, the reality is that many preadolescent and adolescent orthodontic patients do not possess the skill and self-motivation required for impeccable oral hygiene. In addition to increased plaque retention and substandard oral hygiene, orthodontic patients have been shown to exhibit an increase in the number of both Streptococcus mutans and lactobacilli spp., the primary caries-causing bacterial. This environment leads to a much greater risk for enamel demineralization for the orthodontic population compared to the adolescent population not receiving orthodontic therapy.

Enamel decalcification or demineralization is an early form of dental caries. This initial lesion is formed when bacterial plaque produces acids that cause loss of tooth mineral substance by decreasing the plaque pH to a point where dissolution of calcium and phosphate from the enamel occurs.

Many methods of prevention have been attempted by orthodontists in an effort to eliminate or, at least, reduce the occurrence of enamel demineralization. Previous research has shown that fluoride rinsing protocols are an effective method of reducing enamel demineralization, if compliance is high. However, Geiger found that only 13% of the 206 patients fully complied with a fluoride rinse protocol. Professionally applied fluorides were popularized in an effort to avoid the need for patient compliance. Professionally applied gels, foams and varnish have each been shown to effectively reduce enamel demineralization. The fluoride release from these products is short-lived, requires repeated application during the treatment period, and are not as beneficial in reducing caries and remineralization of white spot lesions. Expenditure of clinician time also has been listed as a deterrent to the use of these products. More recently, fluoride-containing bonding agents have been released in order to address the need for long-term fluoride release, to minimize the clinic time required for professionally applied fluoride, and to decrease the need for multiple topical fluoride applications. Glass ionomers have demonstrated an ability to reduce enamel caries lesion depth by inhibiting demineralization and promoting remineralization. However, the use of glass ionomers as orthodontic bonding
agents has been guarded due to lower bond strengths than composite resins 23. Resin modified glass ionomers attempt to take advantage of some of the caries-preventive characteristics of glass ionomers, such as a sustained fluoride release, especially at low pH, with the ability to take up exogenous fluoride and release fluoride over considerable time periods 24-26. While the fluoride released from these materials is less than that from conventional glass ionomers27, resin-modified glass ionomers have the ability to increase the amount of fluoride released over time with the addition of extrinsic fluoride sources, such as fluoridated toothpastes, home use fluoride rinses and self-applied topical gels28. Resin-modified glass ionomers also possess bond strengths comparable to those for composite resins 11.

This in vitro study examined the effectiveness of a fluoride-releasing orthodontic bonding resin (Light Bond, Reliance Orthodontic Products, Inc., Itasca, IL, 60143) and an orthodontic bonding sealant filled with a fluoride-releasing glass, similar to that found in conventional glass ionomers (Pro Seal, Reliance Orthodontic Products, Inc., Itasca, IL, 60143). The aims of this in vitro study were to determine the efficacy of these orthodontic bonding agents on in vitro enamel caries formation and progression compared with matched controls. In addition, the caries-resistance effects of Light Bond and Pro Seal were compared with each other.

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MATERIALS AND METHODS

Sound human permanent molar teeth (n=40) were selected for this in vitro study. Soft tissue debridement, fluoride-free prophylaxis and stereo-zoom microscopic examination (16x) were carried out for each tooth specimen to ensure that the enamel smooth surfaces were intact and without white spot lesions. The teeth were then divided into two treatment groups; Light Bond and Pro Seal groups. The two groups were bonded with the appropriate adhesive sealants following preparation with a 37% phosphoric acid etchant for 30 seconds, copious air-water spray rinsing, and thorough air-drying. Each sealant was light cured for 20 seconds. Group 1 was bonded with Light Bond, a fluoride-releasing, filled resin sealant (Figure 1, Reliance Orthodontic Products, Inc., Itasca, IL, 60143; n=20 teeth).

Group 2 was bonded with Pro Seal, a light cured, resin sealant filled with a fluoride-releasing glass (Figure 1, Reliance Orthodontic Products, Inc., Itasca, IL, 60143; n=20).

The sealant material was placed on either the buccal or lingual surface of each tooth, leaving the untreated opposing surface to serve as a no treatment, matched internal control. An acid-resistant varnish was applied to the tooth surfaces, leaving a 1 mm rim of exposed sound enamel surrounding the sealant-treated surface and a 2 mm by 5 mm window of exposed sound enamel on the surface opposite the sealant to serve as a no treatment, matched internal control (Figure 1). The varnished teeth underwent synthetic saliva rinsing for a 14 day period (20 mM NaCOs, 3 mM KH2P04, 1 mM Ca at pH 7.0) with daily replenishing of the rinse.

In vitro caries were created in the exposed sound enamel windows using a modified ten Cate solution (2.2 mM calcium, 2.2 mM phosphate, pH 3.90, Figures 2 and 3). Following a 14 day lesion initiation period in the in vitro caries solution, three longitudinal sections per tooth were prepared (Figure 4) using a hard tissue microtome. This resulted in 600 in vitro enamel lesions (240 with each treatment group, 120 with the no treatment matched internal control group). For Group 1, the lesion depths for each of the three sections per tooth specimen were determined for the enamel occlusal to the Light Bond sealant material and the enamel cervical to the Light Bond sealant material. For Group 2, the lesion depths for each of the three sections per tooth specimen were determined for the enamel occlusal to the Pro Seal sealant material and for the enamel cervical to the Pro Seal sealant material.
For the control group, lesion depths for each of the three sections per tooth specimen were determined. This resulted in 240 lesions from enamel surrounding the Pro Seal sealant material, 240 lesions from enamel surrounding the Light Bond sealant material and 120 control lesions being available for lesion initiation evaluation.

The acid-resistant varnish was reapplied to the cut surfaces of the teeth. The teeth were rinsed in synthetic saliva for 7 days with daily replenishment of the rinse, and underwent in vitro caries progression I in the modified ten Cate solution for 7 days. After the lesion progression period, three longitudinal sections per tooth were prepared. This, again, resulted in 240 caries-like enamel lesions with each of the two treatment groups and 120 caries-like enamel lesions with the "no treatment" control being available for lesion progression evaluation.

The acid-resistant varnish was reapplied to the cut surfaces of the teeth. The teeth were again rinsed in synthetic saliva as previously described, and underwent in vitro caries progression II in the modified ten Cate solution for 7 days. After the lesion progression II period, three longitudinal sections per tooth were prepared. This resulted in 240 caries-like enamel lesions with each of the two treatment groups and 120 caries-like enamel lesions with the "no treatment" control being available for lesion progression evaluation.

The longitudinal sections from the lesion initiation, lesion progression I and lesion progression II periods were imbibed with water and examined with polarized light microscopy in a blinded fashion. Images of the lesions were captured and evaluated using computer-interfaced image software (UTHSCSA ImageTool program, developed at the University of Texas Health Science Center at San Antonio, Texas, available from the Internet by anonymous FTP from ftp://maxrad6.uthscsa.edu) for mean lesion depths. In addition, for each treatment specimen at each time period, it was noted if the lesion extended beneath the enamel-resin interface.

Intragroup comparisons were made among the occlusal and cervical lesion depths for each treatment specimen at each time period using analysis of variance (ANOVA) to determine if differences existed between lesions formed at the occlusal site versus those at the cervical site. This analysis was carried out within both treatment groups. No significant differences were found between the occlusal and cervical sites for lesion depth within both treatment groups (P>0.05, ANOVA). Therefore, lesion depths measurements from the occlusal and cervical sites were pooled and considered together within each treatment group.
This allowed for a single mean lesion depth for each time period for each of the treatment groups. In addition, intragroup comparisons were made among the 3 sections prepared from each tooth specimen at each individual time period. Within each of the groups and at each time period, the lesion depth measurements were not statistically different from one section to the next section (P > .05, ANOVA). Furthermore, all matched internal control lesion depth measurements were compared, using ANOVA, for each time period. At each time period, the depths of the matched control lesions for each treatment group were not found to be significantly different (P > .05, ANOVA). This allowed for pooling of all control lesion depths from the 40 tooth specimens at each time period. This statistical evaluation resulted in 60 lesion depth measurements from each treatment group and compared with 60 matched internal control lesion depth measurements for each treatment group (n = 20 teeth per group with 3 measurements per tooth).

Statistical comparisons were made between all lesion depth measurements for the two treatment groups and the controls at each time period using a t-test analysis. The t-test was used because there was no significant difference between the matched internal controls for the Pro Seal and Light Bond tooth specimens, as determined by ANOVA analysis. This allowed for the use of a t-test (alpha level < .05) to compare:

1) Pro Seal vs Matched Internal Control at each time period (n = 20 teeth per group with 3 lesion depth measurements per tooth resulting in 60 lesions depth measurement comparisons per group); and

2) Light Bond and Matched Internal Control at each time period group (n = 20 teeth per group with 3 lesion depth measurements per tooth resulting in 60 lesions depth measurement comparisons per group); and

3) Pro Seal vs. Light Bond at each time period (n = 20 teeth per group with 3 lesion depth measurements per tooth resulting in 60 lesions depth measurement comparisons per group).
RESULTS

Following the lesion initiation period, the mean lesion depths for both the Light Bond and the Pro Seal groups were significantly less than that for the control group (P<01, t-test, Table, Figure 5). Pro Seal sealant resulted in a 43% reduction in lesion depth compared with the no treatment control (P<01). Light Bond sealant resulted in a 38% reduction in lesion depth compared with the no treatment matched internal controls (P<01). In addition, the mean lesion depth for the Pro Seal group was significantly less than that for the Light Bond group (P<01). Pro Seal sealant resulted in a 9% reduction in lesion depth compared with the Light Bond sealant (P<01).

After in vitro caries progression I, lesion depth had increased for all groups (Table, Figure 6). The differences in mean lesion depths that were found after lesion initiation among the groups were maintained following lesion progression I. Lesion depth was reduced by 32% and 23% for the Pro Seal and Light Bond treatment groups, respectively, when compared with no treatment matched internal controls (P<01). Once again, the mean lesion depth for the Pro Seal group was significantly less than that for the Light Bond group (P<01). Pro Seal sealant resulted in an 11% reduction in lesion depth compared with the Light Bond sealant (P<01).

After in vitro caries progression II, lesion depth had again increased for all groups (Table, Figure 7). The differences among the groups in mean lesion depths that were found after lesion initiation and lesion progression I were maintained following lesion progression II. Lesion depth was reduced by 29% and 21% for the Pro Seal and Light Bond treatment groups, respectively, when compared with the no treatment matched internal controls (P<01). In addition, the mean lesion depth for the Pro Seal group was significantly less than that for the Light Bond group (P<01). Pro Seal sealant resulted in a 10% reduction in lesion depth compared with the Light Bond sealant (P<01).

At all time periods, the enamel-resin interfaces with the Pro Seal and Light Bond sealants were examined with polarized light microscopy for extension of the lesions along the interface between the sealant material and the underlying enamel (Figure 8). The interfaces for both Pro Seal and Light Bond groups were intact with all sections examined, and there was no evidence of lesion formation along the enamel-sealant material interfaces for either Pro Seal or Light Bond.
DISCUSSION

Enamel demineralization and white spot lesion formation are well-recognized problems in the orthodontic patient population. The benefits of topical fluoride in diminishing enamel demineralization and encouraging enamel remineralization have also been well-documented and are well accepted as a standard of care. There have been many different forms of topical fluoride that have been created and utilized by the dental profession and in orthodontics over the past several decades. A great deal of research has been dedicated to evaluating methods of fluoride treatment that may result in reduction or elimination of enamel demineralization for the orthodontic patient. Of major concern today in orthodontics is identifying caries-preventive modalities that are not dependent on patient compliance. This is true of fluoride treatments, as well.

One of the most exciting advances in noncompliant fluoride treatment for the orthodontic patient is fluoride-releasing bonding systems. Many products have been studied, but inadequate bond strength, short-term fluoride release, insufficient fluoride release, and degradation and failure of the material have led to disappointing results for many of these products. Glass ionomers are known to be excellent materials for fluoride release and fluoride "rechargeability" and, therefore, provide a long-term fluoride benefit. However, their bond strength is not considered sufficient for orthodontic bonding.

Fluoride-containing composite resins also exhibit insufficient bond strengths and inadequate fluoride release over time. Resin-modified glass ionomers have been created in an effort to maximize the long-term fluoride release and fluoride recharging of glass ionomer, while maintaining bond strengths comparable with composite resins.

In the present study, each of the two sealants (Pro Seal and Light Bond) applied to the teeth led to significantly reduced lesion depths compared with no treatment matched internal controls (P<.01). Each of the sealants tested, Pro Seal and Light Bond, are resin-based materials that contain fluoride. Light Bond uses sodium fluoride as its fluoride-releasing agent. According to the manufacturer, Pro Seal uses a "glass ionomer" filler as its fluoride-releasing agent; however, the filler is most likely a fluoride-releasing glass, similar to that found in a glass ionomer cement. This material should have the ability to take up exogenous fluoride and release fluoride.
With the in vitro caries challenge, there was a statistically significant difference in the caries-inhibitory effect between the two materials, with Pro Seal providing a greater degree of caries resistance for the adjacent enamel. The differences in the mean lesion depths between Pro Seal and Light Bond at lesion initiation, lesion progression I, and lesion progression II, were as follows: 8 urn, 14 urn and 17 pm, respectively. Although the study demonstrated a difference between the two materials, lesions did not form at the enamel/resin interface during any time period in either treatment group. However, in the oral environment, Pro Seal would be exposed to exogenous fluoride that would allow for replenishing its fluoride content and allow for additional fluoride release over a long period of time. This in vitro study did not provide exogenous fluoride for assessment of the recharging effect with Pro Seal or Light Bond materials. In addition, the bonding material was maintained intact during caries formation and progression, and there was no evidence of extension of the lesions along the enamel-sealant interfaces within both treatment groups at any of the time periods. The materials provided protection of the underlying enamel completely.

One might speculate, from previous research findings26'37, that in a clinical setting the "glass ionomer" filled sealant would provide a substantial and prolonged increase in fluoride levels within saliva and dental plaque, due to the ability of the fluoride-releasing glass to recharge with respect to fluoride48'49. This would facilitate remineralization of existing clinically undetectable demineralized and hypomineralized enamel, as well as white spot lesions. It would also promote increased resistance of the adjacent unsealed enamel to a cariogenic attack.

The results of this in vitro study provide evidence to merit a clinical investigation to compare the effects of Pro Seal and Light Bond, with respect to development of decalcification adjacent to bonded orthodontic brackets. This would allow for determining if the difference between the caries-resistance found in this laboratory study would be manifested in a clinical situation. Based on this initial laboratory study, Pro Seal appears to be a promising orthodontic sealant, due to its protection against caries formation. The benefits of such a product would be in those orthodontic patients at moderate to high caries risk, with enamel decalcifications at the time of bonding, and with bulky bonded appliances, such as a bonded palatal expander. The results from the current study require future studies to demonstrate the clinical efficacy of Pro Seal. Areas for future studies include determination of the clinical durability of the product over time, as well as the long-term fluoride release from this product.
CONCLUSIONS

1. Both Pro Seal and Light Bond enhanced in vitro caries resistance when compared with no treatment matched internal controls, as demonstrated by significant reduction in mean lesion depths at each time period (P<.001, t-test).

2. Although both products demonstrated the ability to significantly decrease cariogenic activity when compared to the controls. Pro Seal resulted in a significant reduction in lesion depth compared with Light Bond (P<0.1). The teeth bonded with a sealant using "glass ionomer", or fluoride-releasing glass, as filler. Pro Seal, had a mean lesion depth that was 17 um less than the mean lesion depth for the sodium fluoride-releasing sealant. Light Bond, at the end of lesion progression BE period (146 y.m versus 163 pm).
Table I: Pro Seal and Light Bond Treatment Effects on In Vitro Enamel Caries Initiation and Progression

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Mean Lesion Depth (± SD)</th>
<th>Lesion Reduction(%)</th>
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<tr>
<td>Lesion Initiation Period</td>
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<tr>
<td>Control (n=120)</td>
<td>125±6µm a,b</td>
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<tr>
<td>Pro Seal (n=60)</td>
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<td>38%</td>
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<tr>
<td>Light Bond (n=60)</td>
<td>78±6µm b,c</td>
<td>9 %</td>
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<tr>
<td>Lesion Initiation Period I</td>
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<td></td>
</tr>
<tr>
<td>Control (n=120)</td>
<td>165±8µm d,a</td>
<td>32%</td>
</tr>
<tr>
<td>Pro Seal (n=60)</td>
<td>113±9µm d,c</td>
<td>23%</td>
</tr>
<tr>
<td>Light Bond (n=60)</td>
<td>127±8µm e,f</td>
<td>11 %</td>
</tr>
<tr>
<td>Lesion Initiation Period II</td>
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<tr>
<td>Control (n=120)</td>
<td>207±13µm g,h</td>
<td>29%</td>
</tr>
<tr>
<td>Pro Seal (n=60)</td>
<td>146±12µm g,i</td>
<td>21%</td>
</tr>
<tr>
<td>Light Bond (n=60)</td>
<td>163±10µm h</td>
<td>10 %</td>
</tr>
</tbody>
</table>

a-i: means with same letters are significantly different at P<0.01 (t-test)
REFERENCES


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Figure 1: Prior to lesion initiation, acid-resistant varnish was placed, leaving windows of sound enamel exposed for *in vitro* enamel caries formation A) No Treatment, Internal Control Group; B) Light Bond Group; C) Pro Seal Group.
Figure 2: *In vitro* enamel caries lesion formation with modified ten Cate solution.

Figure 3: Following *in vitro* caries formation, white spot lesions were readily identified within the exposed enamel windows A) Control Group; B) Light Bond Group; C) Pro Seal Group.
Figure 4: The specimens were sectioned using a hard tissue microtome. Longitudinal sections of approximately 125μm in thickness (A) are prepared using diamond impregnated disks. Numerous sections (B) can be obtained from each specimen using this hard tissue microtome, allowing for lesion initiation and lesion progression period comparisons.
Figure 5: Lesion Initiation Period. *In vitro* enamel caries. Compared with the no treatment control (A), lesion depth is markedly reduced for Light Bond (B) and Pro Seal (C) treatment groups. Also note that the lesions do not extend along the enamel-sealant interface (B, C) with either treatment group. The enamel caries lesions terminate at the point where bonding occurs between the enamel and sealant (polarized light microscopy, water imbibition, original magnification x200).
Figure 6: Lesion Progression Period I: *In vitro* enamel caries. Compared with the no treatment control (A), lesion depth is considerably reduced for Light Bond (B) and Pro Seal (C) treatment groups. The enamel caries lesions do not extend along the enamel-sealant interface (B, C) with either treatment group. The enamel caries lesions terminate at the point where bonding occurs between the enamel and sealant (polarized light microscopy, water imbibition, original magnification x200).