Determinants of enamel decalcification during simulated orthodontic treatment

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ABSTRACT

Objective: To determine the relative effects that phosphoric acid etching, metal brackets, composite resin adhesives, and filled resin sealants have on enamel decalcification.

Materials and Methods: One hundred and fifty teeth were randomly divided into six groups to test the effects of acid etching, brackets, and a composite resin adhesive. The right side of each tooth was sealed. The teeth were submitted to a simulated carious challenge by Streptococcus mutans in a nutritive media for 42 days. Scanning electron photomicrographs (SEMs) were used to qualitatively evaluate the tooth surfaces. DIAGNOdent laser fluorescence was used to quantify the decalcification changes that occurred.

Results: SEMs showed normal enamel on the control teeth, etching patterns on the acid-etched teeth, surface degradation on the teeth that had resin, and sealant tags on the sealed side. The untreated control group showed significantly less decalcification ($P < .05$) than did all groups except the no etch + bracket group. The no etch + bracket group showed significantly less decalcification than did the etch + bracket, no etch + resin, and the etch + resin groups. Acid-etched teeth demonstrated significantly more decalcification (34%) than did those that were not etched. The teeth with resin showed significantly more decalcification (24%) than did those without resin. There was no significant difference between teeth that did and did not have brackets. The unsealed side of the teeth showed small amounts of—but significantly more—decalcification (5%) compared to the sealed side.

Conclusions: Acid etching and composite resin adhesives increase enamel decalcification in vitro, while brackets do not. Filled resin sealants protect the teeth from decalcification. (Angle Orthod. 2011;81:836–842.)

KEY WORDS: Dental enamel decalcification; White Spot Lesions (WSL); In vitro experiment; Acid etching; Composite resins; Filled sealants; Dental brackets

INTRODUCTION

Enamel decalcification/white spot lesions (WSL) are common problems that occur in 2% to 96% of orthodontic patients.1,2 WSL develop in areas associated with orthodontic appliances, such as locations for bands, brackets, arch wires, and wire or elastomeric ligatures. Moreover, most orthodontic patients are adolescents with poor oral hygiene practices, which increases the likelihood of plaque accumulation; this in turn leads to demineralization of intact enamel.3

Acid etching prior to enamel bonding is a possible causative factor in the decalcification associated with orthodontic treatment. Etching demineralizes the enamel surface at depths ranging from 5 μm to 25 μm.6,7 Acid-etched enamel can remineralize, but the amount of time required for this to occur is variable, and the extent of recovery is incomplete.8–10 Importantly, the acid-etched surface allows the less mineralized underlying enamel to be exposed to a potentially acidic microenvironment.11 Etched enamel exposed to cariogenic solutions has been repeatedly shown to be more severely affected than is unetched enamel.
Enamel decalcification may also be caused by the adhesive resin used to bond brackets to enamel. The polymeric structure of resins hosts a variety of microorganisms. Increases in bacterial accumulation have been reported in composite sites; bacteria not only adhere to, but also consume and colonize within, the composite resin. There are no published studies evaluating the effects that resin adhesives have on enamel demineralization.

Demineralization might also be expected to be associated with the orthodontic appliance used. An increase in bacterial accumulation following orthodontic appliance placement has been observed. Appliances provide more areas for plaque and bacteria retention and make plaque removal more difficult, but it remains unclear what role the actual appliance plays in decalcification.

The aim of this in vitro study was to compare how acid etching, composite resin, and placement of orthodontic brackets influence decalcification of enamel surfaces. The relative effects of these three components have not been previously studied. A secondary aim was to evaluate whether a new filled resin sealant prevents WSL.

MATERIALS AND METHODS

A total of 150 teeth with intact buccal enamel surfaces were obtained from various private practices in Louisiana and from clinics at the Baylor College of Dentistry in Dallas, Tex. The project was approved by the Baylor College of Dentistry. The teeth were randomly divided into six groups, with nine incisors, five canines, 10 premolars, and one molar per group (25 teeth each). The right side of each tooth incising-gingivally was acid etched with 37% phosphoric acid gel (Reliance, Itasca, Ill) for 20 seconds. The teeth in group A (control) were untreated and served as controls (Table 1). Group B (etch only) teeth were acid etched on the entire buccal surface; group C (etch+bracket) teeth were acid etched on the entire buccal surface, and a twin bracket was bonded on the mid-surface of the tooth using Transbond XT primer and resin (3M Unitek, Monrovia, Calif). Group D (no etch-bracket) teeth had the entire buccal surface covered with a thin coat of sticky wax, except for a small window the size of a twin bracket. This ensured that acid etching was restricted to the exposed window, onto which a twin bracket was bonded. The wax was then removed with a dental scaler. Group E (etch+resin) teeth were treated identically to group D teeth, except that the exposed window was created to resemble the size of a small, round pad of resin (Transbond XT 3M Unitek). Group F (etch+resin) teeth were acid etched on the entire buccal surface, and a small, round pad of resin (Transbond XT 3M Unitek) was placed on the mid-surface of the tooth. After preparation, all teeth were stored in distilled water. Everything used in the experiment was sterilized with ethylene oxide gas for 12 hours.

A growth media was formulated using trypticase soy broth without dextrose (Becton Dickinson and Company, Sparks, Md). Sucrose (Sigma Aldrich, Milwaukee, Wis) and 2.5 g/L mucin from porcine stomach, Type III (Sigma Aldrich), were added to the media. Between days 1 and 4, a 1% sucrose was used; 0.2% sucrose was used for days 5–9, and 0.15% sucrose was used for days 10–42. The sucrose concentration was high initially in order to enhance initial plaque accumulation and was later reduced to a level that maintained the pH of the solution within a range of 5.5–6.0.

Procedures

An overnight culture of Streptococcus mutans UA159 in chemically defined medium containing glucose as the carbon source was used as the starter culture for the broth incubation of the teeth. One hundred microliters of the starter culture was added to the trypticase soy broth growth media covering the specimens. All manipulations of the containers and teeth were conducted within a standard tissue culture hood. The containers were sealed and the teeth were incubated for 48 hours at 37°C. After 48 hours, the media was exchanged for fresh sterile media. The media was not re-inoculated as a result of the viable biofilm on the
teeth. The procedure was repeated every Monday, Wednesday, and Friday. Following the media change, the pH of the old media was checked to ensure it was in the 5.5–6.0 range and that it was not contaminated.

The experiment was run for a total of 35 days for four of the batches (as a result of media contamination) and for 42 days for the remaining 20 batches. The media was changed a total of 17 times over the 42-day period. Following experimentation, the teeth were manually debrided of plaque accumulation and the brackets were removed. A 30-fluted carbide bur was used to remove any resin and sealant that remained on the teeth.25

Figure 1. Scanning electron photomicrographs (SEMs) taken at 1000× magnification.
Analysis

Prior to tooth preparation (T0), the buccal surface of each tooth was scanned using the DIAGNOdent (KaVo, Biberach, Germany). A maximum reading of fluorescence was obtained after scanning the entire buccal surface. A preliminary evaluation of 24 healthy teeth with intact enamel surfaces showed no statistically significant differences in maximum DIAGNOdent scores between the right and left sides. Following experimentation (T1), maximum DIAGNOdent scores were taken of the right and the left sides of each tooth. A series of scanning electron photomicrograph (SEM) images were obtained for teeth from each group displaying typical amounts of change from T0 to T1.

Statistical Analysis

SPSS version 15.0 (SPSS Inc, Chicago, Ill) was used to analyze (using a significance level of .05) the changes in the DIAGNOdent scores that occurred between T0 and T1. Because some of the changes were significantly skewed, a Wilcoxon signed rank test was used to compare the right and left sides of all teeth (N = 143). A Kruskal-Wallis test was used to determine if differences existed between all groups, followed by a Mann-Whitney test to compare differences between pairs of groups.

RESULTS

The reliability of the DIAGNOdent readings was evaluated based on 70 replicate measures. The intraclass correlation coefficient was 0.962; paired comparisons showed no statistically significant systematic difference.

The SEM evaluations demonstrated minimal residual sealant filler tags on the sealed side of teeth (Figure 1). The unsealed side of the control group demonstrated a typical homogeneous enamel pattern. The unsealed side of the etch only teeth displayed a honeycomb etch pattern, while the no etch resin teeth showed an etching pattern with an irregular topography. The maximum DIAGNOdent readings on the sealed side were significantly (P = .008) smaller than on the unsealed side (Figure 2).

The control group exhibited significantly lower DIAGNOdent scores than the etch only (P ≤ .013), etch+bracket (P < .001), no etch+resin (P < .001), and etch+resin (P < .001) groups on both the sealed and unsealed sides (Figure 3). The no etch+bracket group demonstrated significantly lower scores than the etch+bracket (P ≤ .005), no etch+resin (P ≤ .005), and the etch+resin (P ≤ .004) groups on both the sealed and unsealed sides. The unsealed side of the no etch+bracket group showed significantly lower DIAGNOdent scores (P = .005) than the etch only group.

The etched teeth had significantly higher DIAGNOdent scores (P ≤ .001) than those that were not etched for both the sealed and nonsealed sides (Table 2). There was no significant difference between teeth with and without brackets (Table 3). The teeth with resin showed significantly higher DIAGNOdent scores (P ≤ .017) than the teeth without resin (Table 4).

DISCUSSION

The SEMs indicated that the sealant protected the underlying enamel surface because none of the groups displayed an irregular or etched enamel appearance at the end of the experimental period. This could explain why the sealed side demonstrated significantly less decalcification than the unsealed side. Decreases in decalcification following the placement of highly filled resin sealants adjacent to orthodontic brackets have recently been shown.26,27 Although less decalcified than on the unsealed side, there was decalcification on the sealed side, as verified in the SEMs. The increased fluorescence could have been due to resin tags on the enamel surface. Elevated DIAGNOdent readings have been shown to occur adjacent to composite resins; micromechanical interlocking of the dental sealant and enamel rods projects 25 μm to 50 μm into the etched surface.5,30 It is also possible that some demineralization of the sealed enamel surface actually occurred. Both sides demonstrated very similar differences between the groups, indicating that the sealant provided less than 100% protection from the acidic challenge. The sealant
used in the present study was 38% filled (by weight), and it has been shown\textsuperscript{31} that filled resin sealants ranging between 42% and 78% undergo surface degradation after submersion into acidic solutions. While some sealant remained on the teeth throughout experimentation, it is possible that the sealant surface was changed as a result of the acidic environment. Even though filler particles allow for a more resistant sealant surface,\textsuperscript{32} prolonged exposure to an acidic solution may promote the breakdown of the sealant components. Previous research\textsuperscript{26} showing that filled resin sealants prevent decalcification was based on a sealant with a higher concentration (58%) of filler particles. However, the amount of filler content alone does not determine the wear resistance of sealants; wear resistance properties decrease with increased

Table 2. Changes from Preexperiment to Postexperiment in Maximum DIAGNOdent Fluorescence of Etched and Non-Etched Buccal Enamel Surfaces of the Teeth

<table>
<thead>
<tr>
<th>Etch (N = 72)</th>
<th>No Etch (N = 71)</th>
<th>Difference</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left side</td>
<td>4.39</td>
<td>3.04</td>
<td>2.00</td>
</tr>
<tr>
<td>Right side</td>
<td>4.44</td>
<td>3.04</td>
<td>2.00</td>
</tr>
</tbody>
</table>

* prob < .05

Table 3. Changes from Preexperiment to Postexperiment in Maximum DIAGNOdent Fluorescence of Bracketed and Nonbracketed Buccal Enamel Surfaces of the Teeth

<table>
<thead>
<tr>
<th>Bracket (N = 47)</th>
<th>No Bracket (N = 96)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left side</td>
<td>3.73</td>
<td>3.72</td>
</tr>
<tr>
<td>Right side</td>
<td>3.26</td>
<td>3.83</td>
</tr>
</tbody>
</table>
filler content after the saturation point has been attained.\textsuperscript{33}

The etched teeth demonstrated significantly greater decalcification (34%) than did those that were not etched. Acid etching demineralizes the enamel surface, exposes the enamel rods and prisms, and removes the protective acquired pellicle.\textsuperscript{3,5} Etching demineralizes teeth to a depth of approximately 5–25 μm; the exposed enamel rods provide a passageway for the bacteria and cariogenic by-products. Acid etching increases the surface area and free energy of the enamel surface,\textsuperscript{34} producing greater “wetability” of the enamel and, therefore, greater penetration of solutions.\textsuperscript{6} The deeper enamel surface is much softer than the surface enamel because it contains a lower concentration of elements such as fluoride, lead, and zinc, which makes the deeper enamel structures more acid soluble.\textsuperscript{11} Studies\textsuperscript{8,9,11} have demonstrated that partial rehardening or remineralization occurs after acid etching, but the original level of mineralization cannot be restored. Even with partial enamel remineralization or surface hardening, the unsightly visual appearance of a clinical WSL will remain.\textsuperscript{35,36}

The DIAGNOdent proved to be an effective tool for evaluating decalcification. It has previously been shown\textsuperscript{37} that the DIAGNOdent has a high specificity for lesions in the outer half of the enamel. Aljehani et al.,\textsuperscript{28} reported a correlation of 0.76 between the DIAGNOdent scores and incipient enamel lesion depths adjacent to orthodontic brackets. Recently, Benham et al.\textsuperscript{26} showed that the DIAGNOdent was better able than visual assessment to detect demineralization on teeth that formed WSL. Since Bamzahim et al.\textsuperscript{38} showed no significant difference in DIAGNOdent readings before and after acid etching with 37% phosphoric acid, it is reasonable to assume that the significant differences produced experimentally in the present study pertain to lesions that extend beyond the outer 5–25 μm. The results also demonstrate that the DIAGNOdent scores are highly reliable.

Albeit smaller than the effects of etching, the resin pad produced 24% more decalcification than expected. Because \textit{S} \textit{mutans} has an affinity to the polymeric structure of composite resins,\textsuperscript{17} bacterial adherence to resin increases at the bracket bases\textsuperscript{15,17} and on the surface of composite restorations.\textsuperscript{17} Effort should be made to develop alternative materials and methods of bracket fixation that do not include acid etching.

The addition of a bracket to the teeth did not produce decalcification in the in vitro setting. This was unexpected, because increases in plaque accumulation\textsuperscript{39} and WSL\textsuperscript{3} formation have been reported following the placement of orthodontic bonded brackets. Metal brackets may have a weaker adherence to \textit{S} \textit{mutans} than do plastic or ceramic brackets.\textsuperscript{40} It is also possible that adherence of plaque around brackets in vivo may be different than that observed in the in vitro setting.

Nothing is more disconcerting than removing a patient’s braces and finding visible WSL. The present study clearly shows that bonding techniques increase the risk of developing WSL. Because WSL are irreversible and remain on the surface of teeth, it is essential for the orthodontist to be proactive in oral hygiene control during treatment and in monitoring all patients closely. Early removal of appliances may be necessary to prevent formation or progression of WSL. The application of highly filled sealants is presently the only effective way to reduce WSL. Given the medico-legal implications, orthodontists should ensure that patients are informed about the possible occurrence of WSL and that proper consent forms have been executed prior to initiating treatment.

CONCLUSIONS

- There was significantly less decalcification (5%) of teeth that were sealed.
- Acid-etched teeth undergo significantly more decalcification (34%) than do teeth that are not etched.
- There is significantly more enamel decalcification (24%) when bonding resin is placed than when it is not.
- The bracket alone does not predispose the enamel surface to decalcification in vitro.

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REFERENCES


