



Effects of sealant and self-etching primer on enamel decalcification. Part I: An in-vitro study

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Introduction: The objective of this study was to compare the resistance to enamel demineralization between self-etching primer (SEP) and conventional sealant in vitro. **Methods:** A total of 120 molar sections were randomly assigned to 3 groups: SEP (Transbond Plus, 3M Unitek, Monrovia, Calif), sealant (Light Bond fluoride-releasing sealant, Reliance Orthodontic Products, Itasca, Ill), or control (no enamel treatment). SEP or sealant was applied following the manufacturer's recommendations. The tooth samples were exposed to rotary brushing for 2 minutes. A 2 × 2-mm window of sound enamel was created by using nail varnish. After 48 or 72 hours of acidic challenge with Ten Cate solution (pH 4.46), the samples were sectioned down to a thickness of 200 μm and stained with rhodamine B dye to evaluate lesions, lesion depths, area of lesions, and total fluorescence by using confocal microscopy. Statistical analyses were performed with 1-way analysis of variance (ANOVA) and Tukey-Kramer tests. **Results:** The incidence of lesion was 50% in the sealant group and 100% in both the SEP and the control group. The lesion in the sealant group was present only when the sealant integrity was broken. Lesion depth ($149.9 \pm 20.5 \mu\text{m}$), area ($636 \pm 90 \times 10^2 \mu\text{m}^2$), and total fluorescence ($252 \pm 83 \times 10^4$) in the SEP group were similar to those in the controls. Lesion depth ($107.6 \pm 45 \mu\text{m}$), area ($441 \pm 212 \times 10^2 \mu\text{m}^2$), and fluorescence ($160 \pm 103 \times 10^4$) in the sealant group were significantly less than in the SEP and control groups ($P < 0.05$). **Conclusions:** These results suggest that neither sealant completely protects the teeth against enamel decalcification. The application of sealant provided protection in 50% of the samples, whereas the SEP provided no resistance to enamel demineralization. Protection from acid demineralization depends on the integrity of the sealant. (Am J Orthod Dentofacial Orthop 2009;135:199-205)

Fixed orthodontic appliances make it difficult for young patients to maintain adequate oral hygiene during orthodontic treatment. The tooth surfaces adjacent to bonded attachments are particularly susceptible to decalcification.^{1,2} Several studies found more plaque and white spot lesions around orthodontic appliances.³⁻⁵ Several methods have been suggested to prevent or reduce enamel decalcification during orthodontic treatment, including fluoride in various forms, enamel sealants, oral hygiene regimens, and modified appliances. Conventional bonding of orthodontic brackets involves placement of a sealant after etching enamel with 37% o-phosphoric acid. Previous studies showed that sealants provide caries

protection on etched enamel during orthodontic treatment and increase resin bond strength.⁴⁻⁶ A self-etching primer (SEP) combines etching and priming of enamel into 1 step. This process eliminates the need to rinse and dry the enamel and saves clinicians valuable chair time. Although studies have shown adequate bond strength with SEPs, there is a scarcity of literature evaluating demineralization around orthodontic brackets after the use of SEPs.⁷⁻¹⁰

Confocal microscopy has been extensively used in cell biology. The technique and its application in dentistry have been advocated by Watson¹¹ to visualize the tooth-restoration interface. Other investigators have used confocal microscopy to observe in-vitro secondary caries, structural changes of acid-etched enamel, and inhibition of caries lesions after application of a sealant.^{12,13} The confocal principle is based on the elimination of stray light from the out-of-focus planes by confocal apertures. Images are obtained by scanning the sample with a spot-size laser light source and recording the light reflected from the in-focus plane. In-depth tomographic imaging is enabled by recording a series of consecutive images either in the optical planes, parallel (x and y) or perpendicular (x and z) to the sample surface. This eliminates the need for thin-

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Table I. Group distribution and sample size

Group (n = 20)	Treatment	Demineralization time (h)
I	No treatment	48
II	No treatment	72
III	Sealant	48
IV	Sealant	72
V	SEP	48
VI	SEP	72

section preparation, when valuable information can be lost during the process.

The purpose of this study was to compare the in-vitro resistance of enamel to demineralization after the application of a SEP or a conventional sealant by using confocal microscopy.

MATERIAL AND METHODS

Sixty extracted third molars were used. The selection criteria were teeth with no enamel defects and decalcification. This in-vitro study received approval by the Institutional Review Board at West Virginia University. The extracted teeth were placed in a glass container with deionized water and sterilized in a steam autoclave for 45 minutes at 250°F in liquid cycle. The crowns were then sectioned into buccal and lingual halves and examined under 10 times magnification for enamel defects and decalcification. The sectioned crowns were randomly allocated and divided into 6 groups (n = 20) as shown in Table I.

The teeth in groups I and II received no treatment; the teeth in groups III and IV were air dried for 5 seconds, etched with 37% o-phosphoric acid, rinsed with sterile water, and air dried. A thin coat of Light Bond fluoride-releasing light-cure sealant (Reliance Orthodontic Products, Itasca, Ill) was applied with a Quick Tip micro applicator (Hager, Odessa, Fla) and light cured for 20 seconds with an Ortholux XT curing light (3M Unitek, Monrovia, Calif). The teeth in groups V and VI were air dried for 5 seconds, and a SEP (Transbond Plus, 3M Unitek) was applied to the enamel surfaces. The SEP liquid was rubbed for 3 seconds on the buccal or lingual surface, followed by a gentle burst of dry compressed air and light curing for 20 seconds.

A toothpaste slurry was prepared by stirring 9 g of toothpaste (Colgate Total with 0.24% sodium fluoride, Colgate Palmolive, New York, NY) in 50 mL of deionized water. The slurry was placed on the sample with a 10-cm³ syringe (Becton Dickinson, Franklin Lakes, NJ). An automated toothbrushing apparatus was built with an Ultra Plaque Remover (Braun, Lynnfield, Mass). The toothbrush was positioned so that the brush

head lay passively on the tooth surface. Enough toothpaste slurry was added, and a force of 85 to 100 g was applied. The force was maintained with finger pressure monitored by an electronic scale (model SP5, Sunbeam, Baldwin Park, Calif).

A preliminary study was preformed to determine whether the length of mechanical toothbrushing had any effect on demineralization. The result showed no significant difference in lesion depth, area, or fluorescence at 0, 2, and 6 minutes of mechanical brushing. Therefore, each sample was brushed for 2 minutes to simulate a patient's hygiene maintenance. The Ultra Plaque Remover provides 7600 oscillating movements per minute, and a new brush head was used after 80 minutes of brushing. The toothbrush was recharged after 30 minutes of use to maintain a consistent power supply. After brushing, each sample was rinsed for 30 seconds under running water and then for 10 seconds with deionized water. The samples were stored in deionized water until further use.

Nail varnish was applied to provide a 2 × 2-mm window of exposed enamel in the middle of the sample surface. The samples were arranged around the outer edge of a 120 × 90 mm Pyrex dish (Corning, Corning, NY). To induce demineralization, the teeth were exposed to Ten Cate solution¹⁴ (pH 4.46) for either 48 or 72 hours at room temperature. A 50-mm magnetic stirring rod was placed in the middle of the Pyrex dish, and 400 mL of Ten Cate solution was added. The dish with the samples was then placed on a stirring plate (model 310T, Allied Fisher Scientific Thermix, Hampton, NH) at a speed of approximately 75 rpm. The pH of the solution was checked daily and adjusted if necessary. The samples were removed from the solution after the designated demineralization time (48 or 72 hours), rinsed, and stored in deionized water until further use.

After demineralization, the samples were sectioned serially with an Isomet low-speed saw (Buehler, Lake Bluff, Ill). The blade sectioned perpendicular to the treated surface to yield a 200-μm specimen in the mesiodistal dimension. The sectioned specimens were stored in and stained with 0.1 mmol/L of rhodamine B (Aldrich Chemicals, Milwaukee, Wis) for 24 hours, with no subsequent rinsing. Rhodamine B from the solution is incorporated in the demineralized tooth structure and does not penetrate sound tooth structure or orthodontic resin. The sections were placed on a cover slip. An Axioplan 2 confocal microscope (Carl Zeiss, Oberkochen, Germany) was used. The microscope can emit lasers of 3 wavelengths in addition to capturing an image under transmitted light. By using the nail varnish as a guide, the surface in the window was identified

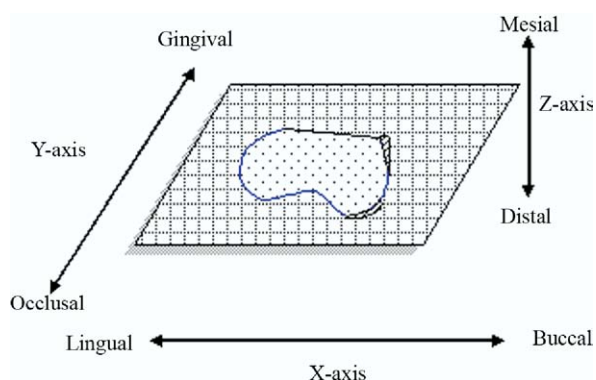


Fig 1. The upper and lower surfaces of a nail-varnish window.

after bringing the specimen into focus (with a 10-times objective [Plan-Neofluar, NA0.30, Carl Zeiss]). The specimens were then illuminated with a helium-neon laser with a 543-nm excitation wavelength. Areas were scanned along the plane parallel to the cut surface of the specimen. The stage of the microscope was adjusted so that the nail varnish appeared on the bottom left of the confocal image screen on the computer monitor. This allowed visualization of the gingival surface of the window created by the nail varnish (Fig 1). In the fluorescent confocal image, rhodamine B dye absorbed in the demineralized tooth structure fluoresces. Since dye does not penetrate sound tooth structure or orthodontic resin, these areas have no fluorescence and appear gray or black. In the transmitted image, since fluorescence was not detected, the area with demineralization appears dark, but the resin and the sound tooth structure were visible. For this study, both fluorescent and transmitted images were recorded. The LSM 510 software (Carl Zeiss) combines the fluorescent and transmitted images to provide a composite image that shows the fluorescent demineralized areas, the sound tooth structure, and the orthodontic resin in 1 image.

The gingival surface of the nail varnish window was scanned in the x-y axis by using the fast x-y function of the LSM 510 program of the confocal microscope and brought into focus. The z-axis settings were adjusted by using the z-stack function of the LSM 510 program of the confocal microscope to provide a 6- μ m slice. A z-axis depth of 66 μ m was achieved, yielding 11 slices of 6 μ m each. Depth beyond 66 μ m yielded poor resolution. Depth of resolution varied by tooth and also by the amounts of demineralization and dye penetration. A similar set of 11 z-stack images of the incisal surface of the nail varnish window was obtained. The sections were stored in deionized water after the

confocal imaging. The images were stored on a CD-ROM disk for use during the measurement part of this study.

All measurements were recorded by using Optimas software (version 6.2, Meyers Instrument, Houston, Tex). Three parameters were measured: area of the fluorescent lesion, average fluorescence, and depth of the lesion. A fourth parameter, total fluorescence, was calculated by multiplying the area of the lesion by average fluorescence. Presence or absence of a lesion was noted for each image. To check intraexaminer reliability of the procedure, measurements were repeated on 12 random samples 3 days later.

The lesion (area with fluorescence) was identified on the composite image and outlined. The software measured the area of the lesion in square micrometers. To calculate the fluorescence in the outlined area, the program assigns each pixel in the area a gray value between 0 and 255. The values are 0 with no fluorescence in the pixel and 255 for maximum fluorescence. The program then adds all the assigned gray values of the pixels and calculates the average gray value for the area of interest. Fluorescence values were obtained from the middle slice, 2 slices above it, and 2 slices below it. This provided fluorescence readings for 5 slices, with each slice 6 μ m apart, for a total of a 30- μ m thickness. The 5 readings were averaged to provide the fluorescence for the sample. Measurements for the depth of the lesion were made at a distance starting 50 μ m from the nail varnish.

A template with parallel lines 100 μ m apart was made on an overhead transparency sheet with a fine-tip marker. This template was placed flat on the computer monitor screen at the outer edge of the lesion where the nail varnish ended. Three measurements on the depth of the lesion were made 100 μ m apart. The measurements were made from the outer surface to the base of the lesion (Fig 2). For broken surfaces, the area was demarcated from a projected outer surface parallel to the base of the lesion. The depth of the lesion was the average value of 3 measurements.

Statistical analysis

Four samples were damaged during sectioning. The final sample size for analysis was 116. Differences in lesion depth, area, average fluorescence, and total fluorescence in and among groups were compared by using analysis of variance (ANOVA). Paired comparisons were performed with the Tukey-Kramer test at $P < 0.05$. An intraclass correlation coefficient of reliability was performed to determine the operator's reliability in measuring the lesion depth and area. Measurements were repeated in 12 samples 3 days apart, and

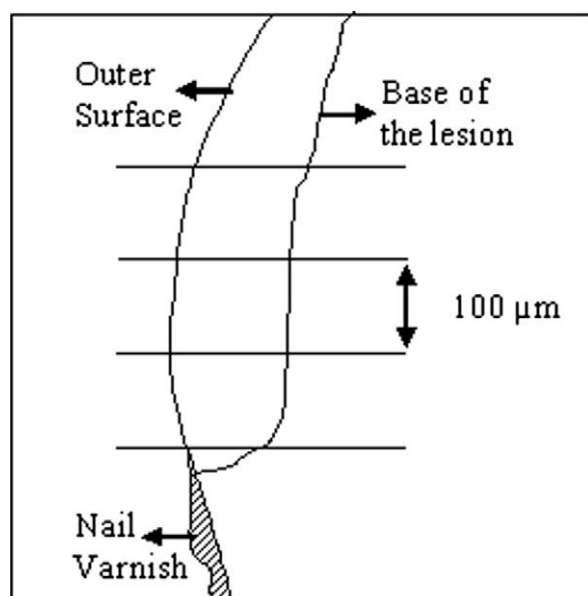


Fig 2. Lesion depth measurements made 100 μm apart.

intraexaminer reliability was recorded in measuring lesion depth and area.

RESULTS

In the control (no surface treatment) and SEP groups, lesions were found in all specimens. In the sealant group, lesions were found in only 19 of 38 (50%) samples. Since no significant differences were found among the 48- and 72-hour demineralization times in either the control, SEP, or sealant groups, the data were combined; this created 3 treatment groups (control, SEP, sealant) for analysis. The apparent lesion depth, area, and total fluorescence were calculated from the values of all samples in the sealant group. Since the incidence of lesions was only 50% in the sealant group, the actual lesion depth, area, and total fluorescence were calculated for the samples that had lesions. Table II shows the mean lesion depth for the collapsed data for the 3 groups.

ANOVA showed significant differences in mean lesion depth among the 3 treatment groups. The Tukey-Kramer test showed significant differences between the sealant and the control groups ($P < 0.05$). Significant differences were also found between the sealant and the SEP groups ($P < 0.05$). No significant differences were found between the control and SEP groups.

The mean lesion area for the collapsed data for the 3 groups is shown in Table III. ANOVA showed significant differences in mean lesion area among the 3 treatment groups. The Tukey-Kramer test found signif-

Table II. Lesion depth for the control, SEP, and sealant groups

Treatment	Lesion depth (μm)			
	Mean	SD	Minimum	Maximum
Control	154.8	23.2	120.7	200.6
SEP	149.9	20.5	74.8	207.4
Sealant [†]	58.7*	58.6	5.5	198.7
Sealant [‡]	107.6*	44.8	28.2	198.7

*Significantly different from the control and SEP groups, $P < 0.05$.

[†]Apparent lesion depth (mean of all samples in the sealant group).

[‡]Actual lesion depth (mean of only samples with lesions in the sealant group).

Table III. Lesion area for the control, SEP, and sealant groups

Treatment	Lesion area $\times 10^2$ (μm^2)			
	Mean	SD	Minimum	Maximum
Control	686	92	497	826
SEP	636	90	321	873
Sealant [†]	245*	248	17	753
Sealant [‡]	441*	212	72	753

*Significantly different from the control and sealant groups, $P < 0.05$.

[†]Apparent lesion area (mean of all samples in the sealant group).

[‡]Actual lesion area (mean of only samples with lesions in the sealant group).

Table IV. Total fluorescence for the control, SEP, sealant groups

Treatment	Total fluorescence $\times 10^4$			
	Mean	SD	Minimum	Maximum
Control	229	81	117	354
SEP	252	83	49	440
Sealant [†]	86*	105	3	394
Sealant [‡]	160*	103	21	394

*Significantly different from the control and SEP groups, $P < 0.05$.

[†]Apparent total fluorescence (mean of all samples in the sealant group).

[‡]Actual total fluorescence (mean of only samples with lesions in the sealant group).

icant differences between the sealant and control groups ($P < 0.05$). Significant differences were also found between the sealant and SEP groups ($P < 0.05$). No significant differences were found between the control and the SEP groups.

Table IV shows the mean total fluorescence for the collapsed data for the 3 groups. ANOVA showed significant differences in mean total fluorescence among the 3 groups. The Tukey-Kramer test found significant differences between the sealant and control

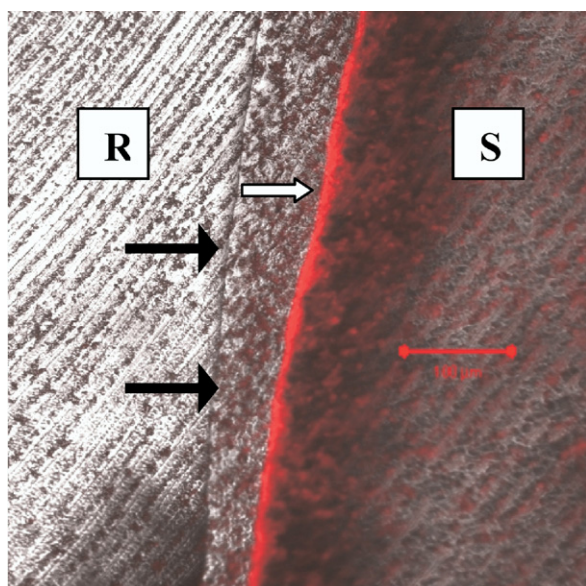


Fig 3. Confocal image of a typical sample from the sealant group with no lesion. *R*, orthodontic resin; *S*, sound tooth structure; *black arrow* points to intact sealant layer; *white arrow* points to intact tooth surface.

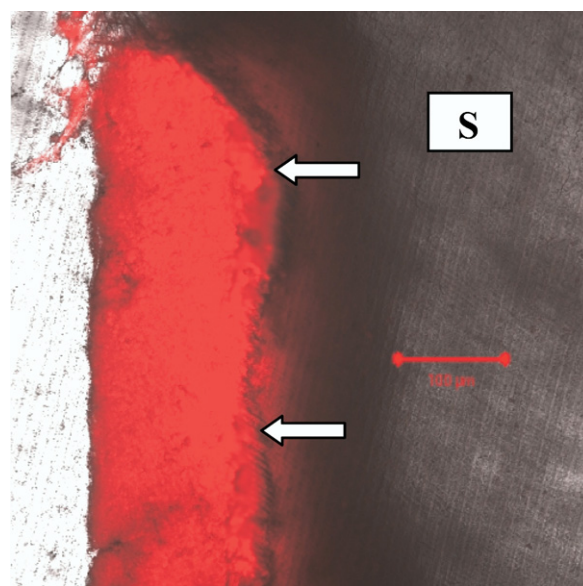


Fig 5. Confocal image of typical sample from the SEP group with rhodamine B dye incorporated in the demineralization areas, producing fluorescence. *Arrow* points to the base of the lesion.

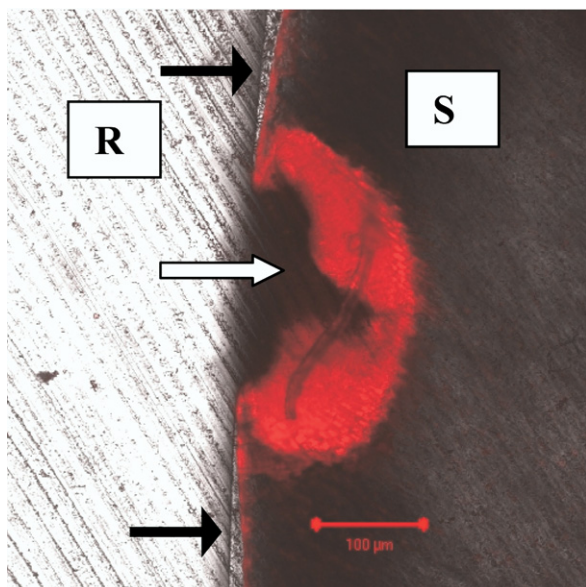


Fig 4. Confocal image of a typical sample from the sealant group with a lesion. *Black arrows* point to intact sealant layer; *white arrow* points to area of lesion with the fluorescence.

groups ($P < 0.05$). Significant differences were also found between the sealant and SEP groups ($P < 0.05$). No significant differences were found between the control and SEP groups.

The intraclass correlation of reliability values, used

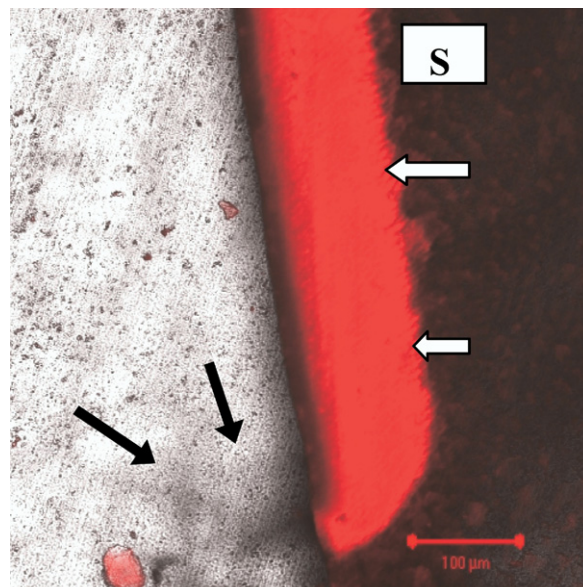


Fig 6. Confocal image of a sample from the control group with rhodamine B dye incorporated in the lesion, producing fluorescence. *White arrow* points to the base of the lesion; *black arrow* points to nail varnish, appearing as darker area on the lower left corner of the image.

to determine intraexaminer error in measuring lesion depth and area, were 99.7% and 97.7%, respectively.

Figure 3 shows a typical confocal image of a sample from the sealant group without lesions. Figure 4

shows the confocal image of a typical sample from the sealant group with a lesion. Figure 5 shows a typical confocal images from the SEP group that is no different from the confocal image of samples from the control group (Fig 6).

DISCUSSION

All samples in the control group showed demineralization of the exposed tooth surfaces. These findings were expected, since enamel with no protection exhibits demineralization when exposed to an acidic challenge, and the findings were consistent with those of other in-vitro studies.^{15,16} Fifty percent of the sealant group specimens had demineralization of the acid-challenged tooth surface. Studies have shown an incidence of 20% to 23% demineralization after the use of sealants.^{4,5,12} Frazier et al¹⁵ reported a 20% incidence of demineralization after application of a sealant. That was much less than the 50% in our study. This could be because, in the study of Frazier et al, 5 ppm of sodium fluoride was added to the demineralization solution, and a filled pit and fissure sealant was used. We used an artificial caries system with no remineralization solution or artificial saliva. In an in-vivo investigation, Banks and Richmond⁴ found a 23% incidence of demineralization in teeth treated with nonviscous light-cured sealant and no significant difference when compared with the control. This different result could be due to the in-vivo design with a modified decalcification index and direct clinical observation. In addition, confocal microscopy of the tooth sections allowed better visualization than direct clinical observation. Ceen and Gwinnett¹⁷ used a chemically cured sealant and found that it failed to prevent white spot lesions. They speculated that it might be caused by the removal of the unpolymerized oxygen-inhibited layer with mouth rinsing and normal function.⁵ Joseph et al¹⁸ found that, for light-cured sealants, although the outer oxygen-inhibited layer might be washed off, the cured layers underneath still protected against demineralization.

In the sealant group, the lesions occurred only when there was a break in the sealant layer's integrity. This agrees with findings in other studies that showed demineralization when there is a break in the sealant layer's integrity or at the periphery of the sealant; the demineralization slowly advances below the sealant layer.^{12,15}

In our study, 100% of the specimens in the SEP group had demineralization of the entire exposed tooth surface. The findings suggest that, similar to the control group, SEP provides insufficient protection against demineralization. SEP combines an etchant and a primer with a pH of 1, and it does not have a filled resin

component that can provide a protective outer layer against demineralization from an acidic challenge or abrasion from toothpaste. Lesion depth in both the control and SEP groups averaged approximately 150 μm and was similar to that found by Frazier et al¹⁵ using polarized light microscopy. In an in-vitro study by Fontana et al,¹⁹ measurements for lesion depth obtained by confocal microscopy correlated with the measurements with polarized light microscopy. In our study, no significant differences were found in lesion area and total fluorescence between the SEP and control groups. In an in-vitro study by Fontana et al,²⁰ measurements for lesion area and total fluorescence obtained by confocal microscopy correlated with the measurements from microradiography.

In the sealant group, no lesions formed when the sealant layer was intact. The apparent mean values for lesion depth, area, and total fluorescence for the group were small and had wide standard deviations. To calculate actual lesion depth, area, and total fluorescence, only the data from the samples with lesions were used. This actual value provides a more accurate representation of the effectiveness of the sealant to resist demineralization of enamel when exposed to an acidic challenge.

Significant differences were found in lesion depth between the SEP and sealant groups ($P < 0.05$). Frazier et al¹⁵ also found that the lesion depths in the sealant group were similar to those in their control group. Significant differences were found in lesion area and total fluorescence between the SEP and sealant groups. In our study, the formation of a lesion was limited to the site of the break in the sealant layer's integrity. In contrast, in the SEP and control groups, lesions were formed over the entire surface no matter what the surface treatment.

A 2-minute brushing was used in the study to simulate short abrasive contacts from toothbrushes and toothpastes during a patient's routine oral hygiene. However, in our preliminary pilot study, no significant differences were found in lesion depth, area, and fluorescence with no (0 minute) and longer (6 minutes) brushing times. Apparently, demineralization occurs independently of mechanical abrasion of the tooth surface.

A SEP makes the clinical procedure a little simpler in a busy office. Fewer steps in the adhesive system also reduce the chance of intraoral contamination. A SEP combines the etching and priming steps. Simultaneous etching and priming allows the primer to penetrate the entire depth of the etch, ensuring good mechanical interlock. As the phosphate group on the methacrylated phosphoric acid ester dissolves the cal-

cium from the hydroxyapatite, instead of being rinsed away, the calcium forms a complex with the phosphate group and is incorporated into the network when the primer polymerizes. The acid etching process is stopped when the complexes are formed. The acid transport also slows as the viscosity of the primer goes up when the solvents are removed with a burst of air and polymerizes when exposed to the curing light. However, this study affirms that even the continuous acidic environment might not have contributed to the increase in lesions; insufficient sealing of the etched surfaces could result in increased demineralization from an acid attack.

CONCLUSIONS

Neither sealant completely protected the teeth against enamel decalcification. The sealant gave protection in 50% of the samples, but SEP provided no resistance to enamel demineralization when exposed to an in-vitro acidic challenge. Protection against acid demineralization depends on the integrity of the sealant layer.

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