ORIGINAL ARTICLE

Prevention of enamel demineralization: An in-vitro study using light-cured filled sealant

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Introduction: Enamel demineralization is an undesirable side effect of orthodontic treatment with fixed appliances. The purpose of this in-vitro study was to evaluate the efficacy of applying a light-cured filled sealant onto the buccal tooth surfaces to prevent demineralization. **Methods:** Fifty extracted human third molars were allocated to 1 of 5 groups: (1) enamel surface untreated (control); (2) surface etched; (3) fluoride varnish applied; (4) enamel etched and coated with a light-cured, unfilled sealant (control sealant); and (5) enamel etched and coated with a light-cured, filled sealant (Pro Seal, Reliance Orthodontic Products, Itasca, Ill). The enamel surface of each specimen was brushed for 15,000 strokes with nonfluoride toothpaste slurry with a piston-action brushing machine under a standardized load. All samples were then cycled for 14 days through a daily procedure of demineralization for 6 hours and remineralization for 17 hours. Then the teeth were sectioned and evaluated quantitatively by cross-sectional microhardness testing. **Results:** Demineralization in the Pro Seal group was significantly less ($P < .05$) than in the other groups. Teeth treated with fluoride varnish exhibited 30% less demineralization than the control teeth, the enamel-etched teeth, and the teeth treated with a light-cured, unfilled sealant ($P < .05$). **Conclusions:** Pro Seal can be considered for use as a preventive method to reduce enamel demineralization adjacent to orthodontic attachments, particularly in patients who exhibit poor compliance with oral hygiene and home fluoride use. (Am J Orthod Dentofacial Orthop 2005;128:592-600)

White-spot formation is an undesirable complication of orthodontic fixed appliance therapy. These lesions are due to demineralization of the enamel by organic acid produced by cariogenic bacteria that readily accumulate around the brackets.[1,2] Previous studies have shown that the rate of decalcification in orthodontic patients was higher than those without orthodontic treatment,[3-5] and teenagers were at higher risk of demineralization than adults.[6] Enamel demineralization can form rapidly because of the high and continuous challenge of plaque when associated with orthodontic appliances. O'Reilly and Featherstone[7] found that demineralization adjacent to orthodontic brackets could develop in just 1 month in orthodontic patients who use a fluoride-containing toothpaste twice daily. Gorton and Featherstone[8] confirmed this observation recently.

Although orthodontists have recognized this negative complication, and most take active steps to minimize it, white-spot formation and development of caries in patients who do not follow aggressive caries-preventive measures during orthodontic treatment still remain problems.[9-14] Because the lesions are unesthetic, unhealthy, and irreversible,[15,16] it is parrticularly discouraging to the specialty whose goal is to improve facial and dental esthetics.

Fluoride plays an important role in the prevention of demineralization during orthodontic treatment. Several fluoride regimens, with varying fluoride concentrations, pH, and delivery systems (varnish, gel, rinse, dentifrice) have been shown to be effective in preventing demineralization.[7,10,17-23] However, the effectiveness of these products is directly related to the patient's compliance. Studies have indicated that full compliance with fluoride regimens is unlikely, and partial or sporadic compliance might result in only limited benefit.[10,17,20]

A method to protect the susceptible area adjacent to bonded attachments, independent of patient compliance, would be extremely beneficial. One approach is to use glass ionomer cement or fluoride-releasing resin to reduce demineralization. Numerous studies have investigated the efficiency of these fluoride-releasing materials on bracket bonding and enamel surface protection,

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but with mixed results.[8,11,13,14,24-29] Many bonding adhesives currently in use release fluoride, but long-term clinical studies have shown that this was often unsuccessful in preventing decalcification near the brackets.[11,13,14,28,29] Another possible way is the application of resin sealant on the enamel surface around and beneath the orthodontic bracket to prevent demineralization. The use of sealant in orthodontics for the prevention of demineralization is not a new idea.[30,31] Placement of sealant after acid etching was thought to provide several benefits: increased bond strength, sealing of etched enamel, and protection against demineralization around the bracket. It was suggested that the sealant could be effective in preventing demineralization during treatment.

Previous studies have proven that most of the chemically cured sealants do not effectively seal smooth enamel surfaces, because of oxygen inhibition of polymerization when the sealant is in contact with the air in a thin layer.[32-34] Only "islands" of cured sealant remain where resin pooling occurs.[32,34] On the other hand, light-cured sealants have been proven to cure completely on smooth enamel surfaces and prevent enamel demineralization effectively in vitro.[34,35] However, subsequent clinical studies did not support the results of the laboratory research.[12,36] The unfilled or lightly filled light-cured sealant could not provide more protection than the chemically cured sealant. Mechanical (tooth brushing) and chemical (acid attack) wear of sealants in vivo could weaken the protection effect, especially for unfilled sealants. Wearing off or breaks in the sealant layer might result in decalcification under the sealant.

A new product, Pro Seal (Reliance Orthodontic Products, Itasca, Ill), claims to protect enamel against demineralization during orthodontic treatment with fixed appliances. This sealant is a highly filled light-cured resin. The manufacturer claims that it stands up to toothbrush abrasion and erosion by oral fluids. If this were true, it would be an important adjunct during orthodontic therapy. Therefore, the purpose of this study was to investigate the mechanical abrasion resistance and the efficacy of this new sealant in preventing decalcification in vitro.

MATERIAL AND METHODS

Tooth preparation and group allocation

Fifty extracted caries-free human third molars were collected and stored in 0.1% thymol solution and then sterilized overnight with gamma irradiation (Cs^{137}) at a dose above 173 kilorad. The roots were removed at the cementoenamel junction with a 15 HC (large) wafering blade on a low-speed saw (Isomet, Buehler, Lake Bluff, Ill). The enamel surfaces were polished with 5-μm aluminum slurry to remove any surface contamination. The teeth were divided randomly into 5 groups of 10.

Group 1: control group, with untreated enamel surface.

Group 2: etched group, enamel surface (whole buccal surface) etched for 30 seconds with 37% phosphoric acid gel, washed under running water for 60 seconds, and air dried thoroughly.

Group 3: fluoride varnish group, with a single thin coat of fluoride varnish (5% sodium fluoride varnish, CavityShield, Omnii Pharmaceuticals, West Palm Beach, Fla) applied to the whole buccal surface of the enamel and allowed to dry for 5 minutes.

Group 4: unfilled sealant group, with enamel surface (whole buccal surface) etched for 30 seconds with 37% phosphoric acid gel, washed under running water for 60 seconds, and air-dried thoroughly; an unfilled, light-cured sealant (Light Bond Sealant, Reliance Orthodontic Products) was applied in a thin, uniform layer on the etched enamel with a brush and then light-cured with a curing light (Optilux VCL401, Demetron Research, Danbury, Conn) at close range for 20 seconds.

Group 5: filled sealant group, with enamel surface (whole buccal surface) etched for 30 seconds with 37% phosphoric acid gel, washed under running water for 60 seconds, and air-dried thoroughly; a filled, light-cured sealant (Pro Seal) was applied in a thin, uniform layer on the etched enamel with a brush and then light-cured with a curing light (Optilux VCL401, Demetron Research) at close range for 20 seconds.

All prepared specimens were stored in 100% humidity for 12 hours at 37°C.

In vitro abrasion and demineralization study

A metal mold with 6 cavities (each 13.8 mm round and 9.5 mm deep) was used to prepare the test cylindrical block of specimens. Each specimen was placed in the middle of the cavity and held by methacrylate, leaving the buccal surface exposed (Fig 1). Specimen blocks were removed from the mold after the mounting resin was cured. An abrasion test was performed by a piston-action brushing machine under a standardized load. This device consisted of 6 heads to hold toothbrushes connected to a camshaft driven by a motor/gearbox system and a control unit. A toothbrush with soft nylon bristles (Oral-B Indicator toothbrush, Oral-B laboratories, Belmont, Calif) was fitted into each head, and the specimen block was mounted in the opposing specimen holder. Care was taken that the filaments in each tuft of the brush were perpendicular to the buccal surface of the enamel. Fifteen thousand strokes were

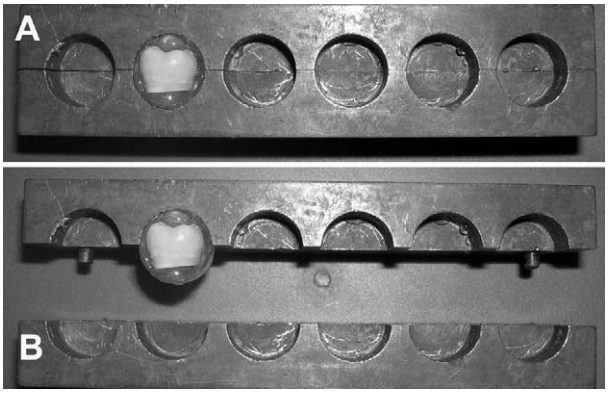

Fig 1. Cylindrical test blocks of specimens for abrasion test. A, Metal mold with 6 cavities. Crown was placed in middle of cavity and held in place with resin. B, Mold was separated after resin was cured, and specimen was removed.

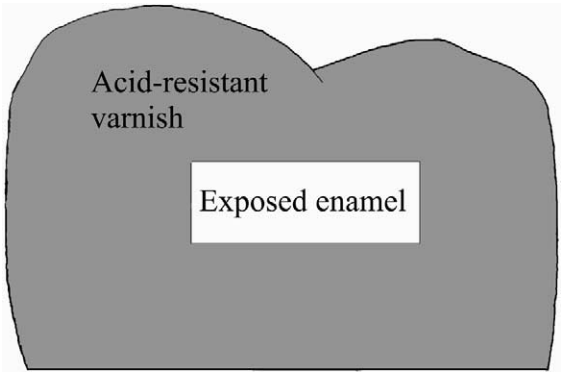

Fig 2. Buccal surface view of specimen painted with the acid-resistant varnish. Window (exposed enamel) was on middle third of surface and was approximately 4.0 × 2.0 mm.

performed on each specimen at a speed of 200 strokes (complete forward and reverse movement) per minute, with a load of 225 g, with 5 mL of nonfluoride toothpaste slurry (weight ratio of toothpaste to deionized water was 1:2, silica as abrasive, Crest toothpaste, Procter & Gamble, Cincinnati, Ohio). After the abrasion test, each sample was rinsed with deionized water, and the resin that held the crown was removed carefully with a cross-cut bur and a low-speed hand piece. The crowns were hemisectioned vertically into buccal and lingual halves with a 15 HC diamond wafering blade on the low-speed saw without contamination on the buccal surface. Only the buccal side of each crown was used in the following pH cycling study. The specimens were rinsed with deionized water and dried with compressed air.

Each crown surface of all groups was painted with acid-resistant varnish, leaving an exposed window of enamel (approximately 4.0 × 2.0 mm) on the middle third of the buccal surface (Fig 2), so that most of the crown was covered by acid-resistant varnish, and only the exposed enamel would be attacked by acid. The daily procedure of pH cycling included a demineralization period of 6 hours and a remineralization period of 17 hours. Each crown was immersed individually in 40 mL of demineralization solution containing 2.0 mmol/L calcium, 2.0 mmol/L phosphate, and 75 mmol/L acetate at pH 4.3 for 6 hours at 37°C. Specimens were then removed from the demineralization solution, rinsed with deionized water, and immersed individually in 20 mL of the remineralization solution at 37°C overnight (17 hours) to simulate the remineralizing stage of the caries process. The remineralization solution consisted of 1.5 mmol/L calcium, 0.9 mmol/L phosphate, 150 mmol/L potassium chloride, and 20 mmol/L cacodylate buffer at pH 7.0. This cycling system was repeated daily for 14 days.

Microhardness profile

After the pH cycling phase, the crowns were thoroughly rinsed with deionized water, sectioned longitudinally through the lesion on a hard-tissue microtome (Series 1000 Deluxe, Scientific Fabrication, Lafayette, Colo), as shown in Figure 3. Half of each specimen was embedded in epoxy resin so that the cut section of the lesion and the underlying normal enamel were exposed, according to methods reported previously.[37,38] After serially polishing the embedded teeth, each lesion was assessed by microhardness profiles across the cut surface with a microhardness tester (Micromet 2100 Series, Buehler) fitted with a Knoop diamond. The first indentation was made 25 μm deep from the outer enamel surface toward the dentin and then at 25-μm steps up to 300 μm from the surface of the tooth. Indentations were made across the sectioned lesion along a line perpendicular to the surface and into the underlying enamel. Two rows of indentations were made across each lesion, 1 at the junction of the gingival third and the middle third of the lesion, and the other at the junction of the occlusal third and the middle third of the lesion (Fig 4).

These indentations were observed under a microscope (Olympus BX50, Olympus Optical Company, Tokyo, Japan) at 500X magnification, and the images were captured by a digital video camera (DVC-1300C, DVC Company, Austin, Tex) (Fig 5). The length of each indentation was measured with Image-Pro Plus 4.5 software (Media Cybernetics, Silver Spring, Md).

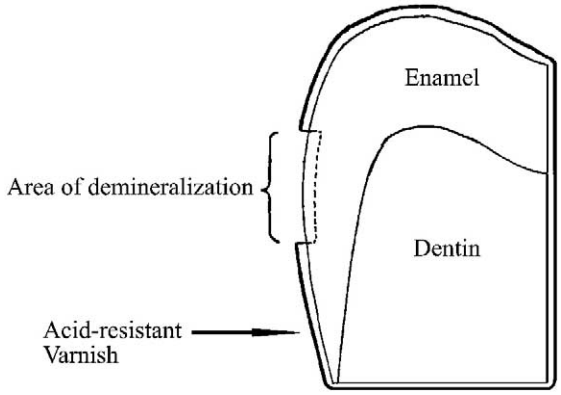

Fig 3. Schematic cross section of crown illustrating placement of window and area of demineralization.

The average length of the 2 indentations at each depth was calculated for each sample. The Knoop hardness number was calculated from each average indentation length and converted to volume percent mineral (VPM) according to the previously established formula.[37] The indentation length is inversely proportional to the mineral content in enamel, enabling microhardness to provide an accurate measure of mineral change.

Underlying enamel at depths of 150 μm or greater was treated as sound. The average VPM of sound enamel is 85%. Therefore, values of VPM for depths between150 and 300 μm from the outer surface were mathematically normalized to an average of 85% mineral, so that each specimen could be directly compared with the others. VPM values for the other depths were then adjusted in proportion. This calculation brings each sample data set to an equivalent normalized underlying enamel value in a similar manner to that used for quantitative microradiography, without eliminating point-to-point variations in each sample. It enables mean VPM values (and standard deviations) to be calculated at each depth for each group of samples. The overall relative mineral loss (ΔZ) for each sample was calculated from the data of mineral content profile by using curve fitting with Simpson's rule to provide integrated mineral loss values for each group in units of volume % × μm (Fig 6).[38]

Statistical methods

The mean ΔZ for each group was calculated, and analysis of variance (ANOVA) was used to determine whether there were any significant differences in mean ΔZ between groups. A post hoc Newman-Keuls multiple comparison test was performed to determine the statistically significant differences ($P < .05$) between groups.

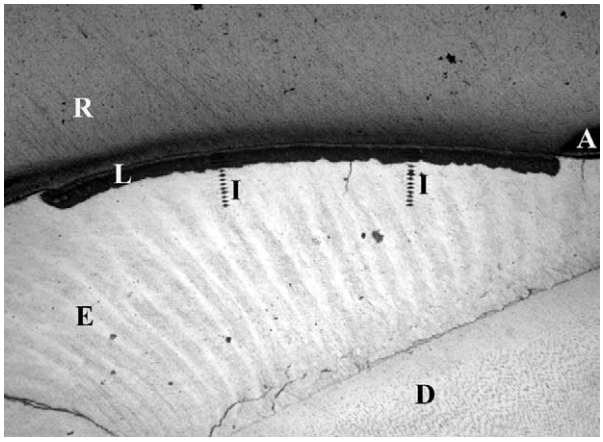

Fig 4. Two rows of indentation (original magnification 50X). *A,* Acid-resistant varnish; *E,* sound enamel; *D,* dentin; *I,* row of indentation; *L,* demineralized lesion; *R,* resin.

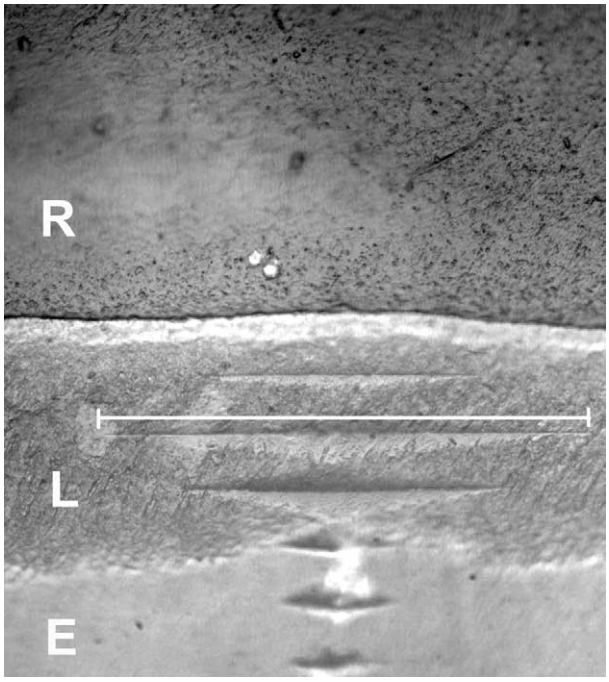

Fig 5. Indentations (original magnification 500X). First was made 25 μm deep from outer enamel surface, second was 25 μm deeper. *White bar* shows length of second indentation. *E*, Sound enamel; *L*, demineralized lesion; *R*, resin.

RESULTS

The mean relative mineral loss (ΔZ), standard deviations, ranges, and minimum and maximum measurements are summarized in Table I. With ANOVA, statistically significant differences ($P < .001$) were

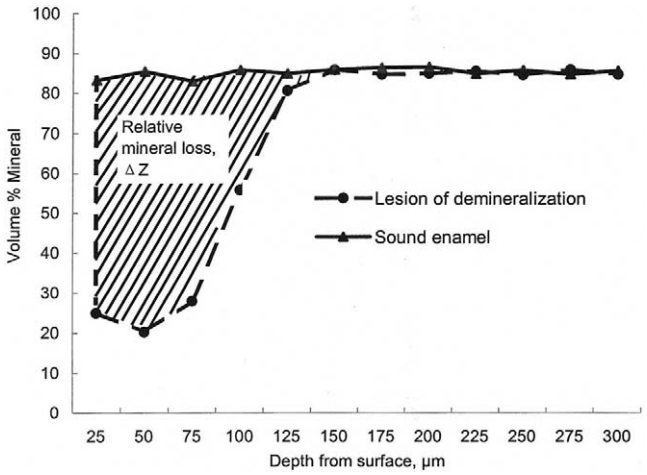

Fig 6. Schematic illustration of relative mineral loss (ΔZ). *Upper continuous line* is microhardness profile of sound enamel (average mineral content is 85% by volume); *dotted line* is microhardness profile of demineralized enamel. Area with oblique lines represents relative mineral loss (ΔZ) in decalcified lesion.

Table I. Mean relative mineral loss (ΔZ) of enamel lesions in different groups

Group	*n*	*Mean ΔZ**	*SD*	*Minimum*	*Maximum*
1 (control)	10	4614	1081	3212	6351
2 (enamel etched)	10	5227	1120	3974	6649
3 (fluoride varnish)	10	3267	1688	739	6299
4 (unfilled sealant)	10	4489	1006	2881	6123
5 (filled sealant)	10	79	293	−253	670

*Unit of mean ΔZ, volume % × μm.

found between the groups (Table II). Group 2 (etched enamel) had the greatest relative mineral loss. Group 5 (filled sealant) had the least amount of demineralization. Group 3 (fluoride varnish) exhibited approximately 30% less demineralization than group 1 (control).

The Newman-Keuls multiple comparison test (Table II) showed that group 5 (filled sealant) had significantly less demineralization ($P < .05$) than the other 4 groups. Meanwhile, group 3 (fluoride varnish) also had significantly less mineral loss in the lesions ($P <. 05$) than the other 3 groups (control, etched enamel, and unfilled sealant), and there were no significant differences between groups 1, 2, and 4 (Table II).

The enamel mineral content profiles for the lesion areas in each group are shown in Figure 7. The mineral content of teeth in the control group at 25 μm from the enamel surface was only 25% and demonstrated approximately 60% mineral loss, compared with the mineral content of sound enamel (85%). It was 20% at 50 μm, increasing to 30% at 75 μm and 55% at 100 μm. Teeth treated with unfilled sealant (group 4) had a similar profile to the control group. The etched teeth (group 2) showed 5% to 10% less mineral content than the control group at the first 4 points. Teeth treated with fluoride varnish (group 3) demonstrated 10% more mineral content than the control group at the first and fourth points, 20% more at 50 and 75 μm, which indicated a partial inhibition of demineralization. Teeth sealed with filled sealant (group 5) had what looked like a normal enamel profile at all points, indicating almost complete inhibition of demineralization.

DISCUSSION

A method that can prevent the demineralization of susceptible areas beneath and adjacent to orthodontic attachments, with its preventive effect independent of patient compliance, would be extremely beneficial for clinical orthodontics. Long-term sealing of enamel with sealant resin before bracket bonding does not require a patient's compliance to prevent or interrupt demineralization related to orthodontic treatment. The duration of protection is influenced by the thickness and abrasion resistance of the sealant. Previous studies have shown that some conventional chemically cured sealants did not polymerize completely in a thin film to cover the etched enamel because of the oxygen inhibition of the curing reaction.[32-34] These sealants cannot be expected to provide protection against demineralization. Light-cured sealants solve the problem of uneven polymerization, and some studies[34,35] in vitro showed that these materials could seal large areas of smooth enamel

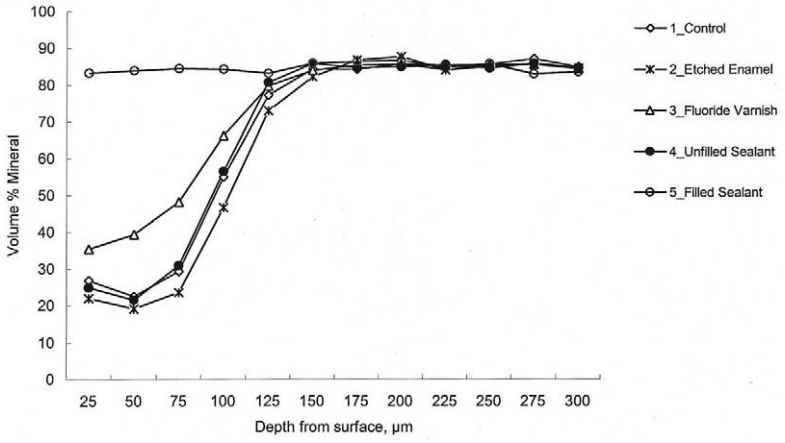

Fig 7. Comparative cross-sectional mineral profiles for different groups.

surfaces effectively and resulted in a significant reduction in enamel demineralization, but most of them failed in clinical research[12,36] and did not show more protective effect than the chemically cured sealant. The reason may be that the products tested in clinical trials were unfilled or lightly filled sealants. These sealants have low abrasion resistance and wear more rapidly than filled sealants.[39] Therefore, several authors have recommended adding filler particles into the sealant to improve performance and increase the likelihood of a thin resin layer being retained throughout treatment.[12,32] The result of our study indicated that Pro Seal, a highly filled sealant, offers adequate resistance against wear during tooth brushing and essentially complete protection against decalcification compared with the unfilled light-cured sealant in vitro.

On the contrary, the unfilled sealant showed nearly no protective effect to the underlying enamel. This resulted in the etched enamel underneath the sealant being exposed to the acid attack and consequently the demineralization lesion formed, which was not different from the lesions in the control group. The result is different from some previous studies, which found that the protection afforded to the enamel did not just rely on retention of the superficial unfilled resin coverage.[40,41] Even after mechanical removal of the sealant, the remaining surface enamel proved to be resistant to carious attack as long as the resin tags were present, which have been shown to extend from 80 to 170 μm into the enamel surface.[42] However, our result is consistent with the reported clinical studies.[12,36] Those researchers found no significant difference between the decalcification rates of the unfilled sealant group or the control group. It can be suggested that, once the covered sealant wears off, the enamel is exposed to acid attack directly, and demineralization could develop when the acid challenge is strong and that it continues, even though some part of the exposed area is sealed by the tags of the remaining sealant.

The teeth that appear to be most prone to demineralization and most in need of protection are the maxillary anterior teeth and the mandibular canines and premolars.[3,5,12,43] The new sealant could be painted on the etched enamel before bracket bonding; perhaps the whole labial or buccal surface should be covered, especially the gingival area of the bracket, which is the most susceptible.[5,12] Furthermore, this sealant could protect patients whose oral hygiene is compromised after placement of fixed appliances, and when the orthodontist believes that caries might develop before the end of treatment. This sealant is transparent when it is cured. It can be removed by fine polishing or a finishing bur. Further research, especially a clinical study, is warranted to confirm that this technique is effective in demineralization protection in the mouth. Meanwhile, the possible irritation of the gingivae by this product and the long-term staining of the residual resin tags should also be investigated in a clinical study.

The use of toothbrushing to simulate mechanical wear in vivo is commonplace in studies assessing wear of dental materials.[44-46] In our study, a piston-action brushing machine with an Oral B toothbrush and nonfluoridated toothpaste were used to simulate abrasion by everyday toothbrushing. The hypothesis was that 5-7 strokes per smooth surface per brushing episode, 3 times a day, would be considered good oral hygiene practice for orthodontic patients. At an average treatment time of 2 years, 15,000 strokes of tooth brushing would be a 2-year equivalent of toothbrush abrasion in vivo.

Table II. Statistical comparison of relative mineral loss of enamel lesions in groups

DF	*F value**	*Probability*	*Mean ΔZ*[†]	*Group*	*Newman-Keuls test*[‡]
4	33.264	<.001	4614	1 (control)	A
			5227	2 (enamel etched)	A
			3267	3 (fluoride varnish)	B
			4489	4 (unfilled sealant)	A
			79	5 (filled sealant)	C

DF, Degree of freedom.
*ANOVA.
[†]Relative mineral loss (volume % × μm).
[‡]Groups with different letters are significantly different ($P < .05$).

To study the cycles of demineralization and remineralization of enamel that occur under dental plaque in the mouth, a laboratory pH cycling model was previously developed to mimic the processes of acid attack (demineralization) and remineralization by saliva in the mouth.[47] Therefore, our well-established laboratory pH cycling model was used in this study. The many cycles in the mouth were successfully simulated with an acid challenge at pH 4.3 for 6 hours daily, followed by an overnight remineralization stage for 17 hours. The cycle was repeated daily for 14 days.

Many fluoride regimens can achieve similar reductions of demineralization in vitro. However, clinical studies found that this inhibition effect relies on excellent compliance by the patient. Stratemann and Shannon[20] found a 32% reduction in decalcification in a group of stannous-fluoride-gel users. Geiger et al[10] reported a 25% reduction in white-spot lesions in their sodium-fluoride-rinse users. In these studies, although much higher reductions of demineralization were seen in patients who complied fully with the fluoride regimen, only a small percentage of patients in the whole sample demonstrated good compliance. Therefore, the variable of patient compliance remains the major limitation of fluoride prevention in orthodontic patients. Professionally applied fluoride varnish can protect against demineralization without patient compliance. Therefore, in this study, the fluoride varnish group was used as a positive control. The specimens in the third group were coated with fluoride varnish and then left for 12 hours in a humid environment before tooth brushing. This method was adopted to simulate the usual instructions issued to patients in whom fluoride varnish was applied: to refrain from tooth brushing until the morning after the application. The relative mineral loss of the demineralization lesions in the fluoride varnish group was 30% less than the control group, but greater than the mineral loss in the filled sealant group. This is similar to the results of previous studies.[21,22] Those authors found that the fluoride varnish slowed the progress of demineralization significantly but did not completely inhibit the enamel lesion from forming. A high bacterial challenge cannot be completely overcome by fluoride alone.

The relative mineral loss of group 2 (etched enamel) was higher than that of group 1 (control), although there was no significant difference. The reason might be that etched enamel has a porous surface and a higher solubility rate than normal enamel.[40] The organic acid would readily penetrate into deep enamel and dissolve the calcium and phosphate ions that would also easily diffuse out of the enamel. Remineralization in vitro brings about a reduction in solubility rate, but the enamel is still not the same as normal enamel.[40] Gangler and Hoyer[48] found that etched enamel could be remineralized in vivo, but the process was always incomplete. The demineralization adjacent to brackets might be partly due to the rough, retentive, and decalcified surface of enamel produced by acid etching and lack of sealant. Therefore, particular care should be taken during the acid etching in the clinic to ensure that only the area where the bracket is to be placed is etched or to use an effective sealant to seal the etched enamel completely.

CONCLUSIONS

The new sealant, Pro Seal, results in a significant reduction of enamel demineralization in vitro, even with a severe acid challenge. Such a light-cured, filled sealant can effectively seal the smooth enamel surface and greatly resist toothbrush abrasion. This method might be useful in orthodontics, but further clinical investigations are warranted, based on the positive results of our study.

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