Scientific Article

Protective Effect of Pit and Fissure Sealants on Demineralization of Adjacent Enamel

A. Alsaffar, DDS, MS[1] • D. Tantbirojn, DDS, MS, PhD[2] • A. Versluis, PhD[3] • S. Beiraghi, DDS, MSD, MS[4]

Abstract: ***Purpose:*** *This study's purpose was to evaluate the in vitro effect of sealants in protecting adjacent enamel from acid demineralization.* ***Methods:*** *Occlusal fissures of extracted molars (N=10) were sealed with: conventional nonfluoride (DO; Delton Opaque) resin-based sealant (RBS); fluoride-containing RBS (US; UltraSeal XT plus, and CP; Clinpro); amorphous calcium phosphate-containing RBS (BW; Bosworth Aegis); or glass ionomer sealant (FT; Fuji Triage). The specimens were immersed in lactic acid gel for 20 days to create demineralized lesions on the occlusal enamel. Cross-sectional microhardness was measured at the lesion 0.5 mm from the sealant margin. Mineral loss (ΔZ, volume % mineral x μm) was calculated from the microhardness values and subjected to analysis of variance and student-Newman-Keuls tests.* ***Results:*** *Mineral loss values (mean ±SD) were: 1,975±806, 1,802±512, 1,004±421, 1,275±375, and 88±124 for DO, US, CP, BW, and FT, respectively; ΔZ for DO and US was significantly higher, and ΔZ for FT was significantly lower than that for CP and BW (P=.05).* ***Conclusions:*** *Resin-based sealants containing fluoride or amorphous calcium phosphate may provide some protective effect on demineralization of adjacent enamel vs conventional nonfluoride sealant. Glass ionomer sealant was the most effective in protecting adjacent enamel from acid demineralization.* *(Pediatr Dent 2011;33:491-5) Received March 12, 2010 / Last Revision May 12, 2010 / Accepted May 12, 2010*

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Pits and fissures of the tooth are the most susceptible areas for dental caries due to the morphology that is favorable for plaque accumulation. Pit and fissure sealant, when properly applied, acts as a physical barrier to protect the pits and fissures from the biofilm and their acid-byproduct.[1] Partial or complete loss of sealants reduces their effectiveness in caries prevention.[2] Although the relative risk of caries development in teeth with partial or complete loss of sealants was not found to be higher than those that never received sealants,[3] partially retained sealants with compromised margins could conceivably provide a niche for bacteria and their nutrients.

Teeth with a partially retained sealant had more than triple the chance to develop caries vs teeth with a missing sealant.[4] In addition, a concern about inadvertent sealing over caries has been reported as one of the reasons limiting the use of sealants.[5] Therefore, sealants with anticariogenic properties are considered desirable, especially in populations with high caries risk and where sealant retention cannot be assured. Currently, sealants with such potential are commercially available, either in the form of glass-ionomer materials or resin-based products containing fluoride or calcium phosphate. The question whether better protection is gained by particular types of fissure sealants remains to be answered.

Calcium, phosphate, and fluoride in the surrounding aqueous phase are essential to maintain the integrity of the tooth surface against caries development.[6] Accordingly, one prospect to increase the potential for caries prevention can be achieved through the release of these ionic substances in addition to the physical protection of pit and fissure sealants. Glass ionomer materials are known for their ability to release fluoride.[7] The clinical evidence of glass ionomer sealants (**GISs**) to prevent caries formation is inconclusive, however, due to the low retention rate.[8] Resin-based sealants (**RBSs**), with their preferred retentive property, can be a source of slow-release fluoride.[9]

Fluoride-containing sealants have been a subject of interest over the years.[10-12] Interestingly, a clinical study could not clearly show whether the caries preventive effect of a fluoride-containing sealant was the result of sufficient retention or the fluoride release.[13] Recently, a RBS containing amorphous calcium phosphate has been introduced. Resin-based composites with amorphous calcium phosphate have been shown to release calcium and phosphate ions, which remineralized a caries-like lesion in enamel.[14] The abilities of these different types of sealants to prevent the development of caries as an added benefit to the obliterating property of conventional sealants have, however, not yet been compared.

In the current study, acid demineralization of enamel adjacent to the sealant margin was used as a surrogate for the initial stage of caries formation, simulating a lesion developed in enamel surrounding a partially lost sealant or area with high caries risk. The severity of demineralization was determined using a cross-sectional microhardness method.[15,16] Additional caries protection provided by various types of sealants, namely RBSs containing fluoride or amorphous calcium phosphate and GIS, were compared to a conventional RBS.

[1]*Dr. Alsaffar is a pediatric dentist, and a part-time instructor, Faculty of Dentistry, Kuwait University, Kuwait City, Kuwait;* [2]*Dr. Tantbirojn is an associate professor, Department of Restorative Dentistry, and* [3]*Dr. Versluis is a professor, Department of Bioscience Research, both at the College of Dentistry, University of Tennessee Health Science Center, Memphis, Tenn; and* [4]*Dr. Beiraghi is a professor and director, Division of Pediatric Dentistry, Department of Developmental and Surgical Sciences at the School of Dentistry, University of Minnesota, Minneapolis, Minn.*

Correspond with Dr. Tantbirojn at dverslui@uthsc.edu

The purpose of this in vitro study was to evaluate the effect of various types of pit and fissure sealants in protecting adjacent enamel from acid demineralization. The null hypothesis was that there was no difference in mineral loss at the demineralized enamel lesion 0.5 mm from the margin of tested sealants.

Methods

This in vitro study using extracted human teeth was approved in the exemption category by the Institutional Review Board (study no. 0706E11161) of the University of Minnesota, Minneapolis, Minn. Fifty human mandibular molars extracted for periodontal reasons or extracted third molars that were free of macroscopic defects, caries, hypocalcifications, and fluorosis were selected for this study. The teeth were cleaned with pumice slurry and randomly divided into 5 groups (N=10). The central groove of the occlusal surfaces was sealed with one of the following dental sealants: conventional RBS (**DO**; Delton Opaque, Dentsply, York, Pa); fluoride-containing RBS (**US**; UltraSeal XT Plus, Ultradent Products Inc, South Jordan, Utah, and **CP**; Clinpro, 3M ESPE, St. Paul, Minn); amorphous calcium phosphate-containing RBS (**BW**; Bosworth Aegis, Bosworth Company, Skokie, Ill); and GIS (**FT**; Fuji Triage, GC America, Alsip, Ill). The sealant information and application methods are summarized in Table 1.

After sealant placement, all surfaces of the teeth except the occlusal surface were sealed with 2 layers of acid resistant nail polish. The teeth were separately immersed in 20 ml acid gel at 37°C to create a demineralized lesion on the occlusal enamel. The acid gel, modified from the study by Arends et al.,[17] consisted of 6% by weight hydroxyethylcellulose in a 0.1 mol/l lactic acid solution adjusted to pH 5.1 with 1.0 mol/l NaOH.

The specimens were removed from the acid gel after 20 days, sectioned buccolingually with a slow speed diamond disc under water coolant (Buehler, Lake Bluff, Ill), and embedded in Orthodontic Resin (LD Caulk, Milford, Del). The sectioned surfaces were serially polished with an Ecomet 3 Grinder-Polisher (Buehler) using 400- and 600-grit silicon carbide paper, followed by 1.0 µm and 0.05 µm alumina suspensions.

A cross-sectional microhardness technique[15,18] was used to evaluate the demineralized enamel lesion at 0.5 mm from the sealant margin. Two sites per tooth were randomly chosen for the measurement. A series of Knoop indentations was performed with a Micromet 5104 Microhardness tester (Buehler) at a 25-g load for 10 seconds. The indentations were made at 25 µm increments starting from the outer enamel surface and moving inwards until reaching the underlying sound enamel, determined by 3 consecutive measurements of approximately 300 to 350 Knoop hardness number (**KHN**). The orientation of the microhardness measurement is shown in Figure 1.

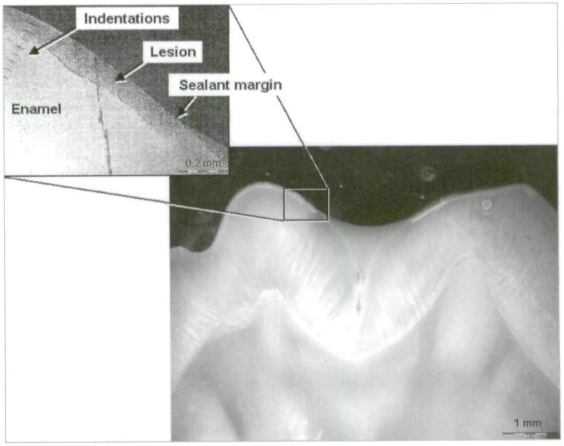

Figure 1. Cross-sectioned specimen of extracted human molar with sealant covering the fissure and demineralized lesion on the cusp slope. The shown inset is an image from the microhardness tester showing the demineralized lesion as a dark area adjacent to the sealant margin. A series of Knoop indentations were made perpendicular to the tooth surface in 25-µm increments across the lesion into underlying sound enamel.

The KHNs were converted to volume percent mineral using an empirical formula: volume % mineral = $4.3\sqrt{KHN} + 11.3$.[16] The 300 to 350 KHN determined as sound enamel is, thus, equivalent to 85 to 92 volume % mineral. The mineral profile was achieved by plotting volume percent mineral as a function of depth from the outer enamel surface (Figure 2). The amount of mineral loss (ΔZ; volume % mineral x µm) of each mineral profile was calculated by integrating the area between the mineral profile and the average volume % mineral extrapolated from the underlying sound enamel. Consequently, the ΔZ represents the severity of demineralization adjacent to each sealant. The differences in ΔZs among the tested sealants were analyzed statistically using 1-way analysis of variance (ANOVA) followed by student-Newman-Keuls posthoc test (SuperANOVA, Abacus Concepts Inc, Berkeley, Calif).

Table 1. SEALANTS USED AND APPLICATION METHODS IN THE STUDY

Sealant	Description	Manufacturer	Lot	Application technique
Delton Opaque (DO)	Conventional resin-based sealant	Dentsply, York, Pa	090626	Enamel etched with phosphoric acid gel for 45 s, washed, dried, sealant applied, light cured* for 40 s
UltraSeal XT plus (US)	Fluoride-containing resin-based sealant	Ultradent Products Inc, South Jordan, Utah	725	Enamel etched with phosphoric acid gel for 45 s, washed, dried, sealant applied, light cured* for 40 s
Clinpro (CP)	Fluoride-containing resin-based sealant	3M ESPE, St. Paul, Minn	6HC	Enamel etched with phosphoric acid gel for 45 s, washed, dried, sealant applied, light cured* for 40 s
Bosworth Aegis Pit & Fissure Sealant (BW)	Amorphous calcium phosphate-containing resin-based sealant	Bosworth Company, Skokie, Ill	0610-481	Enamel etched with phosphoric acid gel for 45 s, washed, dried, sealant applied, light cured* for 40 s
GC Fuji Triage capsule (FT)	Glass ionomer sealant	GC Corp, Tokyo, Japan	0305101	Mixed† for 10 s, sealant applied; setting time=6 min

* Spectrum QHL 75 Curing Light (Dentsply Caulk). † Rotomix (3M ESPE).

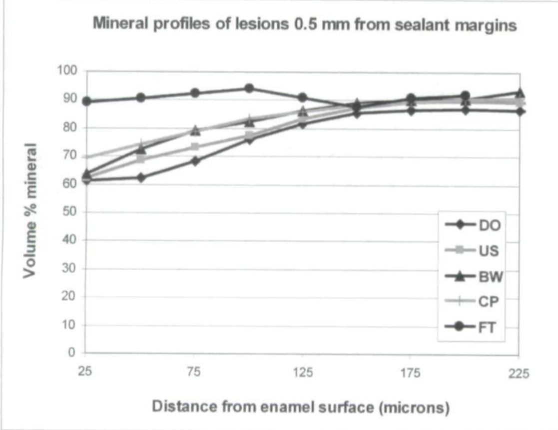

Figure 2. Mineral profiles of the demineralized lesions 0.5 mm from the sealant margins. Each profile is an average of 10 teeth.

Results

The mineral profiles [ie, mineral content (volume % mineral) plotted as a function of depth from the outer enamel surface] of lesions located 0.5 mm from the sealant margin are shown in Figure 2. Each mineral profile is averaged from 10 teeth. The mineral profiles of the RBSs (DO, US, CP, BW) show softening at the surface and a gradual increase in mineral content when approaching sound enamel at approximately 150 µm. For FT, the mineral profile did not show any substantial drop in mineral content.

Mean mineral loss (ΔZ) values, standard deviations, ranges, and statistical results are shown in Table 2. The amount of ΔZ represents the extent (severity) of demineralization. There was a significant difference in ΔZs of the adjacent enamel lesions among the tested sealants (1-way ANOVA followed by student-Newman-Keuls test, $P<.05$). The lesions adjacent to the nonfluoride and one of the fluoride-containing RBSs (DO and US) had significantly more extensive demineralization (higher ΔZs) than the other groups. The lesions adjacent to FT had significantly lower ΔZ than the other groups. There were no significant differences between ΔZs of CP and BW.

Discussion

In this study, the amount of ΔZ from demineralization was compared in enamel adjacent to various types of sealants. Each tooth demineralizes differently, depending on composition such as fluoride or carbonate content. The differences between teeth may have contributed to the relatively high standard deviations. The calculation of ΔZ assumes homogeneous enamel hardness within each tooth (ie, hardness of enamel under the lesion is the same as hardness of surface enamel prior to demineralization). Using this assumption, the mineral content of the underlying sound enamel was extrapolated to calculate ΔZ. If the enamel is protected from demineralization, ΔZ will be 0. We observed that enamel under the sealants did not show signs of demineralization. Mean (±SD) mineral content calculated from microhardness, measured at 25, 50, 75, and 100 µm from the sealant/enamel interface was 86.8 (1.7) volume % (N=200). The mean (±SD) ΔZ of enamel under sealants was -0.6 (11.3) volume % mineral x µm (N=50). Mean ΔZs of the enamel lesions adjacent to the sealants ranged from 88 to 1975 volume % mineral x µm (Table 2), indicating various degrees of demineralization.

This study's results rejected the null hypothesis, since various degrees of protection from acid demineralization on enamel adjacent to tested sealants were demonstrated. GIS clearly showed a superior protective effect. The relatively low ΔZ of enamel adjacent to the GIS indicated a minimal demineralization of the lesion. One fluoride-containing RBS and the amorphous phosphate RBS showed a small but significant protective effect in this in vitro setting. Interestingly, the other fluoride-containing RBS did not have a significant protective effect in comparison to the conventional nonfluoride control. It should be emphasized that this protective effect on adjacent enamel is not the main rationale for sealant application. It can, however, provide added benefit to the obliterating property of sealants in high caries risk cases and/or when compromised sealing ability raises a concern about caries development.

Our finding that GIS is superior to RBS in inhibiting lesion formation agrees with other in vitro studies.[10-12] The clinical merit of using GIS, however, has been a subject of debate. Systematic reviews comparing resin-based and GISs either reported inconclusive results[19] or found no evidence that one was superior to the other in caries prevention.[20] Despite a better retention rate of RBSs, their caries preventive effect is equivalent to GIS.[8] These clinical findings may be explained by observations from laboratory studies that GISs are very effective in preventing demineralization. This suggests that the caries preventive effect is either derived from a well-retained RBS or from the ability to inhibit lesion formation of GIS. Even after macroscopic loss of GIS, the pit and fissure areas still have some resistance to demineralization.[21]

A clinical study where more than 80% of resin-based and GISs were lost after 5 years in service showed that the caries preventive effect in the re-exposed pit and fissure areas was 3 to 4 times better with GIS than RBS.[22] Likewise, glass ionomer cement was shown to provide more protection against dental caries than RBS in recently erupted first molars of high caries risk children.[23] A report by the American Dental Association Council on Scientific Affairs recommended that sealants should be monitored and reapplied as needed to maximize effectiveness.[2] This recommendation would particularly benefit GISs, since the superior fluoride-releasing ability of newly applied glass ionomer will ensure a continuous protective effect on the surrounding enamel, as demonstrated in this study.

The present study showed that some degree of caries protection of the adjacent enamel can be achieved by incorporating fluoride or calcium phosphate into RBS. Integrating the chemical effect of fluoride with

Table 2. MINERAL LOSS (ΔZ; VOLUME % MINERAL X µM) AT 0.5 MM FROM THE SEALANT MARGIN*

ΔZ	Delton Opaque (DO)	UltraSeal XT plus (US)	Clinpro (CP)	Bosworth Aegis (BW)	GC Fuji Triage (FT)
Mean (±SD)	1,975±806[a]	1,802±512[a]	1,004±421[b]	1,275±375[b]	88±124[c]
Range	1,025-3,649	1,208-2,973	540-2,069	884-1,575	-18-365

* Same superscript letter denotes mean ΔZs that were not significantly different (1-way analysis of variance followed by student-Newman-Keuls test; $P<.05$).

the physical effect of resin-based pit and fissure sealants has been attempted for several decades.[24-26] It may be argued that a well-retained sealant already obliterates the highly susceptible areas; thus additional caries protection on the adjacent enamel is not necessary. The additional protective effect, however, may benefit patients with high caries activity or when the sealing quality is impaired. Sealants that are still retained on the tooth may have marginal gaps that are not readily detectable, which favors biofilm accumulation and subsequent caries formation. A clinical study showed that teeth with partially retained sealant had triple the chance of developing caries vs those with missing sealant.[4]

Calcium and phosphate ions in the microenvironment around tooth surfaces are part of the protective factors in the caries process.[6,27] Calcium phosphates in various forms have gained much attention lately and are added in preventive products.[28,29] For resin-based dental materials, Bosworth Aegis pit and fissure sealant is currently the only commercial product containing calcium phosphate in the form of amorphous calcium phosphate. Resin-based composite with amorphous calcium phosphate released sufficient calcium and phosphate ions to promote remineralization of artificial enamel lesions.[14] The results of this current study indicate that Bosworth Aegis reduced demineralization of enamel similar to one of the fluoride-containing RBSs tested, but not as substantially as GIS.

The ability to prevent demineralization of RBSs containing fluoride was also investigated in this study. Several commercially available fluoridated RBSs released measurable amounts of fluoride.[9,30,31] Although fluoride release resulted in the reduction of caries formation,[10,32] a sealant with a significant amount of fluoride release in one study did not prevent mineral loss in another study.[9,12] We found that the demineralized lesions with 1 of the 2 fluoride-containing RBSs (US) were not significantly different from the nonfluoride control. It is possible that the amount of fluoride release was not sufficient, since Ultraseal XT released minimal amounts of fluoride vs other fluoridated sealants.[9]

In summary, the results of this in vitro study indicated that GIS was the most effective, whereas RBSs containing fluoride or amorphous calcium phosphate could have some effect in protecting adjacent enamel from acid demineralization. Considering the superior retentive properties of RBS in caries prevention, the protective effect against demineralization of adjacent enamel is an additional benefit. When sealing is likely to be compromised and caries development is a concern, glass ionomer is a sensible alternative to RBS.

Conclusions

Based on this study's results, the following conclusions can be made:

1. Glass ionomer sealant is effective at protecting adjacent enamel from acid demineralization.
2. Resin-based sealants containing fluoride or amorphous calcium phosphate may provide some protective effect on demineralization of adjacent enamel vs conventional nonfluoride sealants.

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