Scientific Article

Microleakage of Self-etching Sealant on Noncontaminated and Saliva-contaminated Enamel

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Abstract: ***Purpose:*** *The purpose of this study was to compare the microleakage of a self-etching sealant with a traditional phosphoric acid-etched sealant under noncontaminated and saliva-contaminated conditions.* ***Methods:*** *Fifty-two sound extracted human molars were randomly divided into 4 groups (N=13). Teeth in Groups 1 and 2 were cleaned with pumice, etched with phosphoric acid, rinsed, coated with a drying agent, placed with sealants (UltraSeal XT Plus), and light cured. Teeth in Groups 3 and 4 were cleaned with a proprietary flour pumice and rinsed prior to being sealed with a self-etching sealant (Enamel Loc). Teeth in Groups 2 and 4 were contaminated with saliva and thoroughly air-dried prior to the sealant placement. All teeth were subjected to a thermocycling process, stained with silver nitrate, and sectioned, and images of the sealant on the occlusal surface were recorded. Microleakage distance was measured in millimeters and subjected to a 2-way analysis of variance.* ***Results:*** *Significantly larger microleakage distances were found for the self-etching sealant vs the traditional sealant (P<.001). Saliva contamination did not significantly affect the microleakage distance (P<.17).* ***Conclusions:*** *Under the conditions used in this in vitro study, the self-etching sealant, regardless of contamination condition, had extensive microleakage distances vs. little microleakage in the traditional phosphoric acid-etched sealant. (Pediatr Dent 2011;33:479-83) Received July 9, 2010 | Accepted August 16, 2010*

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Sealing pits and fissures of posterior teeth with dental sealant is a recommended procedure to protect these caries-susceptible areas.[1,2] Sealant is a physical barrier to prevent accumulation of biofilm and a chemical barrier to bacterial acid-byproducts.[3] For a sealant to convey such benefits, it must remain intact and be maintained on the tooth surface over time.[4] Sealants with compromised margins could provide a niche for bacteria and increase the chance for caries development.

The first successful application of resin to the pits and fissures of teeth as a possible caries prevention was reported in 1967,[5] utilizing a technique developed by Dr. Michael Buonocore in 1955 to prepare enamel with a weak acid.[6] Since then, phosphoric acid etching has become standard practice to achieve enamel adhesion in resin-based sealants as well as other resin-based restorative materials.

Using the so-called total-etch technique, enamel is etched, rinsed, and thoroughly dried before the placement of a resin-based sealant. If the etched enamel is contaminated with saliva, a film of surface coating can form,[7] which subsequently affects the sealant's bonding efficacy.[8] When working with children, this contamination process can happen very quickly and easily. Therefore, a sealant procedure that is brief in length and has few associated stimuli is desirable.

A self-etching primer using acidic monomer to condition and prime dentin in a single step was introduced as part of a dentin adhesive system.[9] In addition to the main advantage of eliminating the technique-sensitive rinsing step to remove phosphoric acid from tooth structures, self-etching primers shorten the duration of adhesive procedures. The effectiveness of the self-etching primer on enamel, however, is less predictable than the standard phosphoric acid-etched primer, especially on intact enamel.[10] The most simplified dental adhesive, an "all-in-one" system using 1 solution to etch, prime, and bond, is promising, since the bond strength and marginal integrity are reliable in both enamel and dentin.[11] Laboratory and clinical studies reported a 1-year bond strength and a 2-year clinical success of an all-in-one self-etching adhesive in bonding sealant to enamel to be equivalent to the standard phosphoric acid-etched sealant, while the simplified method shortened treatment time almost in half.[12,13] Due to its acidic nature, self-etching primers were less influenced by saliva contamination compared to the conventional phosphoric acid-etched procedure.[14-16]

Recently, a new sealant product, Enamel Loc (Premier Dental, Plymouth Meeting, Pa), was introduced as a self-etching material, which eliminates the acid etching step prior to sealant placement. This simplified procedure could have a significant impact on the application of sealants if proven to provide good seal and retention.

Therefore, the purpose of this in vitro study was to evaluate the sealing ability of the self-etching sealant Enamel Loc in comparison with a traditional phosphoric acid-etching sealant under ideal and saliva-contamination conditions. Microleakage at the sealant-enamel interface after a thermocycling process, simulating oral temperature fluctuations, was used to assess the materials' sealing ability.

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Table 1. FOUR STUDY GROUPS IN A 2x2 DESIGN

Sealants	Non-contaminated	Saliva-contaminated
Phosphoric acid-etched/sealant	Group 1	Group 2
Self-etching sealant	Group 3	Group 4

Methods

This in vitro study, using extracted human teeth and saliva, was exempt from review under federal guidelines 45 CFR Part 46.101(b) category no. 4 by the Institution Review Board of the University of Minnesota, Minneapolis, Minn. (study no. 0801E24515). The extracted molars were collected over a period of 8 weeks at surgical centers around Minneapolis and stored in a 0.2% sodium azide solution before being randomly assigned, using a randomization program (Excel Randomization Function, Microsoft Corp, Redmond, Wash), into 1 of 4 groups, as shown in Table 1 (N=13).

Teeth in Groups 1 and 2 were cleaned with pumice and a disposable prophy brush (Acclean Disposable, Henry Schein, Melville, NY) and rinsed with copious amounts of water for 15 seconds. Thirty-four percent phosphoric acid gel (Ultradent Etch Gel, Ultradent, South Jordan, Utah) was applied to the occlusal surface for 15 seconds and rinsed for 15 seconds. A drying agent (PrimaDry, Ultradent) was scrubbed into the surface and air dried until a chalky white enamel appeared. A resin-based sealant (UltraSeal XT Plus, Ultradent) was applied on the prepared enamel of teeth in Group 1 and light cured for 40 seconds (Spectrum Curing Light, Dentsply Caulk, Milford, Del). The light tip was rested on the cusps of each molar to standardize the light-curing process. Group 2 teeth were contaminated with saliva and air dried thoroughly prior to sealant placement. The saliva was obtained from 1 volunteer subject at the time of the experiment by collecting whole saliva from the floor of the mouth with a pipette. The subject did not eat, drink, or brush the teeth for 6 hours prior to the saliva collection.

Teeth in Groups 3 and 4 were cleaned with a disposable prophy-brush (Acclean Disposable) using the proprietary flour pumice from the Enamel Loc kit and rinsed for 15 seconds. The Enamel Loc sealant was placed on the occlusal surfaces of Group 3's teeth and left undisturbed for 15 seconds prior to being light cured for 40 seconds (Spectrum Curing Light). Group 4 teeth were subjected to the same steps as Group 3, but were contaminated with saliva and air dried prior to the Enamel Loc sealant placement.

Table 2. MEAN (±STANDARD DEVIATION) MICROLEAKAGE DISTANCE (MM) OF EACH STUDY GROUP*

Sealant	Noncontaminated microleakage distance Mean±(SD)	Saliva-contaminated microleakage distance Mean±(SD)
UltraSeal XT Plus, phosphoric acid-etched sealant	0.07±0.07a	0.18±0.12a
Enamel Loc, self-etching sealant	1.25±0.18b	1.29±0.29b

* Same letter denotes mean values that were not significantly different (2-way analysis of variance, pairwise comparison; $P<.03$).

All sealed teeth were subjected to a thermocycling process[17] consisting of 500 cycles, alternating between hot water (55°C) and cold water (5°C) with a 30 seconds immersion time in each of the water baths (Thermo Immersion Bath, Neslab, Newington, NH). The teeth were immediately immersed in 50% silver nitrate ($AgNO_3$) solution (Sigma-Aldrich Co, St. Louis, Mo) in the dark for 2 hours. Excess $AgNO_3$ solution was rinsed off with tap water. The teeth were placed in a developing solution under fluorescent light for 8 hours.

Each tooth was sectioned into halves in a buccolingual direction using a low-speed diamond wheel sectioning machine (model no. 650, South Bay Technology, Inc, San Clemente, Calif). The image of sealant on the occlusal surface was captured at 90x magnification under a stereomicroscope (model no. SMZ-2T, Nikon, Tokyo, Japan) with attached CCD camera (Spot Insight QE, Diagnostic Instruments Inc, Sterling Heights, Mich) and stored in a digital format (Figure 1A). The extent of microleakage, seen as a dark line of silver nitrate penetration at the sealant/enamel interface, was measured in millimeters using Image-Pro Plus software 4.5 (Media Cybernetics, Inc, Silver Spring, Md), as shown in Figure 1B. Microleakage distances were recorded for the buccal and lingual inclines on each sample, providing 4 measurements per tooth. These 4 measurements

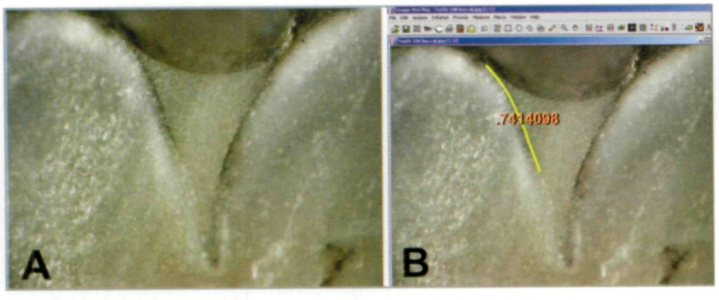

Figure 1. (A) Microleakage at the buccal and lingual slopes of the occlusal sealant. (B) Microleakage distance (yellow line) in millimeters (red font) measured along the enamel-sealant interface using imaging software.

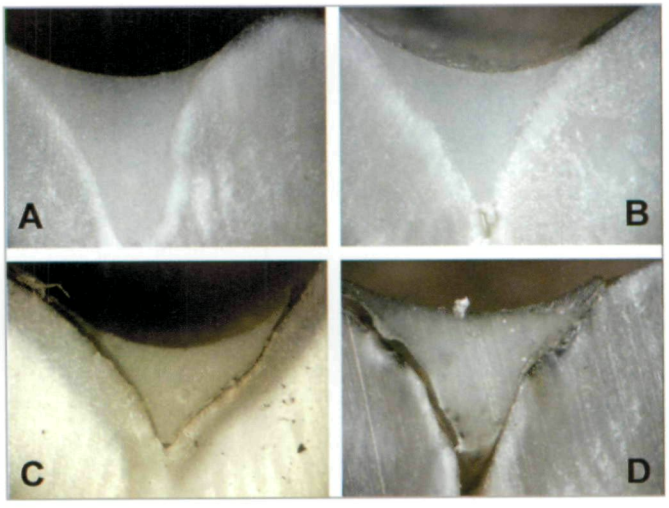

Figure 2. Representative examples of each study group. (A) Group 1: Phosphoric acid-etched sealant without saliva contamination. (B) Group 2: Phosphoric acid-etched sealant with saliva contamination. (C) Group 3: Self-etching sealant without saliva contamination. (D) Group 4: Self-etching sealant with saliva contamination.

were averaged into a single number for each tooth. The measurements were taken by 2 independent evaluators, who were blinded to the group of each tooth. The final microleakage distance was determined as the average of the 2 evaluators. If the microleakage measurements of both evaluators differed more than 10%, the image was reviewed with both operators present to determine a consensus distance.

Results

Microleakage mean distance and standard deviation are shown in Table 2. The results of the Two-way analysis of variance indicated that:

1. Sealant type significantly affected the microleakage distance ($P<.001$).
2. Saliva contamination did not significantly affect the microleakage distance ($P<.174$).
3. The interaction between sealant type and saliva contamination was not statistically significant ($P>.51$).

This lack of interaction means that the microleakage distance due to sealant types did not depend on the saliva contamination; and the microleakage distance due to saliva contamination did not depend on the sealant type. Pairwise comparison ($P>.05/<.03$) also indicated no statistical difference between noncontaminated and saliva contaminated conditions within the same type of sealants, as denoted by the same letters in Table 2.

Images representative of each study group are shown in Figure 2. Groups 1 and 2, the phosphoric acid-etched sealant either with or without saliva contamination, had slight microleakage at the sealant margins. Groups 3 and 4, the self-etching sealant either with or without saliva contamination, had microleakage along the entire interface and debonded from the cuspal inclines. Evidence that sealant did not penetrate into the deepest part of the fissure can be observed in Figure 2D and, although less clearly, in Figure 2B and 2C.

Discussion

Good sealing properties and retention are vital for the success of pit and fissure sealant applications. Microleakage at the sealant margin can lead to bacterial accumulation and increases the chance for caries development. This in vitro study used microleakage as a measure for sealing ability, allowing comparison between the self-etching sealant and the traditional phosphoric acid-etched sealant with or without saliva contamination. The results clearly indicate that the self-etching sealant did not provide a good seal after the thermocycling process that simulated temperature changes encountered in an oral environment; the microleakage distance in the traditional phosphoric acid-etched sealant, however, was minimal. Our results agree with the only other known peer-reviewed study on Enamel Loc self-etching sealant, which found dye penetration in 96% of the self-etching sealant vs. 68% of the traditional acid-etching sealant that had no dye penetration.[18]

Eight abstracts about Enamel Loc self-etching sealant have been presented at recent general or regional International Association for Dental Research meetings. All of these abstracts (1 sealant penetration, 3 microleakage, and 4 bond strength studies) demonstrated significantly lower performance of the self-etching sealant vs. traditional phosphoric acid-etched sealants.[19-26] Although those results were obtained from in vitro studies and have not been published in a full-length peer-reviewed format, all agree with and confirm our findings. Although the ability of in vitro studies to predict clinical performance may be debatable, they are valuable for screening materials. In this case, based on the disappointing performance during the in vitro evaluations, the self-etching sealant should not have been eligible for clinical trial.

At an opinion-posting website,[27] 11 clinicians reported they had used the self-etching sealant Enamel Loc with their patients and found high failure rates after 6 months to 1 year, with some mentioning failure rates of 99% or higher. Only 1 posting was pleased with the results. Interestingly, initial comments had been positive for the simplicity of the self-etching concept, especially when being used in young patients. But the subsequent experience was that most patients had lost the sealants at recall visits. Enamel Loc was available to clinicians until it was taken off the market in 2008 due to a temperature issue during shipping.

In addition to simplifying bonding procedures, the concept of a self-etching primer is fundamentally beneficial for dentin adhesion, since it eliminates the risk of collagen collapse from overdrying dentin after phosphoric acid etching and rinsing.[28] On the other hand, adhesion of self-etching systems to enamel has, thus far, not been reliable. Etching patterns on intact enamel ranged from absent to moderate compared to deep interprismatic etching patterns obtainable with phosphoric acid.[10] Microtensile bond strengths of self-etching adhesives to intact enamel were lower than those of total-etch adhesives.[10] The aggressiveness of self-etching primers, reflected in their low pH values, is one of the contributing factors. An all-in-one self-etching adhesive with low pH provided good bonding between sealant and enamel.[12,13,29] It may be surprising that Enamel Loc did not perform well, despite containing 2 acidic monomers, methacrylated phosphoric acid esters and 4-methacryloxyethyltrimellitic acid.[30] Pashley and Tay reported, however, that bonding of self-etching primers to unground enamel depended on etching efficacy as well as strength of other resin components.[31] Another factor that may affect the performance of dental sealants is their ability to penetrate into pit and fissure areas, although correlation between microleakage and penetrability has been disproved.[32,33] It is possible that the poor performance of Enamel Loc was due in part to its relatively high viscosity, which did not enhance flow, as was observed by the operator in this study and is evident in Figure 2D.

Clinicians working with children know that younger patients are able to better tolerate procedures that are brief in length and have few stimuli associated with them. During the sealant application, young children may become agitated and move or a reflexive swallow may contaminate the tooth surface with saliva. Saliva contamination resulted in the formation of surface coatings that could not be removed by a water wash.[7] Studies showed that self-etching adhesives were less influenced by saliva contamination, perhaps because the acidic primers could infiltrate the surface in the presence of bacteria and associated pellicles.[14-16,31] Therefore, the concept of self-etching sealants is attractive if this benefit can be proven.

In the present study, we tested the traditional and self-etching sealants with and without saliva contamination, but did not find an advantage of the self-etching sealant under the contaminated condition. Our results also showed that saliva contamination did not significantly affect the microleakage in either

type of sealant, although there was a nonsignificant trend of lower microleakage in the noncontaminated groups (Table 2). The finding that saliva had no significant effect on microleakage is contrary to previous studies,[7,8,34] which could have resulted from our efforts to thoroughly dry the contaminated teeth prior to sealant placement. This method was chosen in the present study since clinicians may at times feel they can still seal a tooth if it is dried off after being contaminated. We believe that it remains advisable to avoid saliva contamination, since our result may have been specific for the conditions used in this study and the saliva of the single subject. It should also be noted that a drying agent was applied in the traditional sealant group after acid etching and rinsing, which may have enhanced the sealant adhesion.

In summary, the concept of self-etching is attractive because of the simplicity of sealant placement. The tested self-etching sealant, however, failed to provide good sealing under in vitro conditions that simulated temperature fluctuations in the oral cavity. Despite their limitations, in vitro studies are indispensible for screening new products before clinical testing. It is the responsibility of dental manufacturers as well as clinicians to ensure that products have adequate scientific support before being used in patients.

Conclusions

Under the conditions used in this in vitro thermocycling study, we conclude:

1. The tested self-etching sealant did not have good sealing ability, as shown by significantly higher microleakage compared to a traditional phosphoric acid-etched sealant.
2. Microleakage was not significantly different between noncontaminated and saliva-contaminated surfaces that were air dried thoroughly prior to sealant placement.

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Abstract of the Scientific Literature

General anesthesia informed consent: what do parents remember?

The purpose of this study was to evaluate the practice of "informed consent," supporting the individual's right to self determination. The study received IRB approval from the University of Michigan including a waiver of documentation of consent. Included in the study group were 263 parents of children aged newborn to 18-years-old, who received simple elective procedures under general anesthesia. In the hospital setting, consent for general anesthesia is gained as part of the overall surgery preparation process. There were standard written forms and documents to be completed by surgical and anesthesia staff who interviewed the parents and securing a signed consent. After the children were in surgery the parents were interviewed again to discover their memory of specific categories of information related to them by the anesthesia personnel. Trained research assistants recorded their answers verbatim. The transcribed results were graded by two assessors who were uninformed of those providing information and compared to the children's preoperative care plans. Knowledge of the following categories was graded using scores of 0=no recall, 1=partial recall, and 2=total recall: the anesthesia plan, risks and benefits, post operative pain care, and side effects of the pain care. How parents recalled their own level of understanding was graded on a 1-10 scale with 10=total understanding. Parents were also asked to fill out a questionnaire to discover what they desired to know about the anesthesia process prior to the surgery date. The major areas asked about were 1) what pre-surgical information did they seek and where did they look for the answers, 2) how well did the anesthesia interviewer relate to them, 3) in what format did they want to receive future information about the anesthesia procedures, and 4) what was their family demographics and social histories? Statistical analyses were applied and a significance of $P<0.05$ was accepted unless corrected. One major finding was that the parents' self perceptions of recall and understanding of the anesthesia consent information was less than that observed by the assessors. Parents scored poorly on recalling the anesthesia risks and benefits and post operative pain care. Their recall was significantly better for consent information reviewed within one week to the day of surgery by the person providing the anesthesia care (CRNA) versus the surgical personnel. Also, the higher the parents' education level and the more satisfied they were, the more they recalled.

Comments: *In pediatric dentistry getting informed consent is an important aspect of our treatment protocol for clinical and general anesthesia procedures. Parents may not fully understand or recall what we present as treatment options. We should be attentive to know the parents, full comprehension of our recommended treatment; likewise we should be well aware of the parents' expectations of our treatment plans. Misunderstandings can easily occur; clear oral communication is pertinent for adequate informed consent.* ***JGJ***

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