Effect of fluoride- and nonfluoride-containing resin sealants on mineral loss of incipient artificial carious lesion.

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The purpose of this study was to evaluate the difference in mineral loss of incipient enamel artificial carious lesions that were sealed with fluoride- or nonfluoride-containing resin sealant. Two artificial lesions (2x2 mm2 each) were created on buccal surface of 60 premolars by using Carbopol demineralizing solution. Lesions in the first group (30 teeth) was sealed with Delton® and Delton®Plus, the second group (30 teeth) was sealed with Helioseal® and Helioseal®F. All teeth were immersed in artificial saliva for 7 days and sectioned through the lesions. The cross-sectioned surfaces were polished and then subjected to Vickers hardness measurement at 20 mm from the resin-enamel interface, and every 10 mm inwards across the lesion to the underlying sound enamel. Mineral content was converted from hardness number. Mean mineral loss was calculated and was compared between lesions sealed with fluoride- or nonfluoride-containing sealants. Mean±SD of mineral loss for Delton, Delton Plus, Helioseal and Helioseal-F were 1423±441, 1287±421, 1223±284, and 1165±267 VPM·mm, respectively. Paired t-test showed that the mineral loss of incipient enamel carious lesions sealed with fluoride-containing sealants, Delton Plus and Helioseal-F, were not significantly different from those of nonfluoride-containing sealants, Delton and Helioseal (P>0.05).

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INTRODUCTION

Caries preventive effect of pit and fissure sealants has been extensively documented over the past decades.[1,8,10,20] Key conditions for the success are that the sealant is properly placed, remains intact and is maintained over time. It was shown that when sealants were detached from molars, the caries rate was equal to the unsealed controls.[20] The addition of fluoride will enhance the effectiveness of sealant in the area where the resin is fractured or worn away and to the surrounding tooth structure prone to caries. Fissure sealant that is able to supply fluoride will play an important role not only as passive, but also active cariostatic agent.

Several approaches to develop fluoride-containing resin sealant have shown promise.[3,24,25,29] Caries formation around fluoride-releasing resins was reduced compared to conventional sealants in laboratory studies.[11,12,13] There has been a concern that the incorporation of fluoride into resin sealant may adversely affect other properties. However, there was no significant difference in microleakage[22] and bond strength[18] between fluoride- and nonfluoride containing sealants. More importantly, the retention rate of fluoride-containing sealant was not different from that of the conventional one.[14,15]

The ideal fluoride-containing sealants should release continuous level of fluoride for prolonged period of time.[21] In addition, the fluoride released should be able to incorporate into enamel.[3,29] The pattern of fluoride release usually started with a bursting amount in the first 24 hours, dropped sharply during the following days, then decreased slowly afterwards.[4,7]

Traditionally, tooth surface with questionable active caries is a contraindication for sealant treatment. Sealed decalcified area will no longer remineralize. Remaining bacteria, if any, can be trapped and survive.[2] However, sufficient scientific evidences have indicated sealant as an effective therapeutic treatment for early pit and fissure carious lesions.[5] There was no difference in retention of sealants between sound and carious molar teeth.[10] The possibility of fluoride release from sealants, and being uptake by dental hard tissues, will further support this concept.

The evidence that fluoride released from sealant prevents demineralzation in the adjacent tooth structure is well established.[12,13] The effect of fluoride-containing sealant to facilitate remineralization of underlying enamel has not yet been shown, especially when sealant is applied into an incipient lesion such as a clinically undetected fissure caries. Accordingly, the purpose of this *in vitro* study was to evaluate the difference in mineral loss of incipient enamel artificial carious lesions that were sealed with fluoride- and nonfluoride-containing resin sealants. The amount of mineral loss in the lesion was quantified using microhardness testing.

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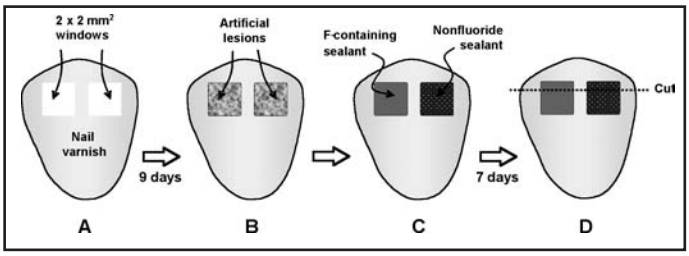


Figure 1. Diagram showing the experimental procedures. A. Two windows (2x2 mm2) on buccal surface of a premolar. B. Artificial lesions formed after 9 days. C. Fluoride- and nonfluoride-containing sealants were applied on the lesions. D. After 7 days, the tooth was sectioned through the sealant-covered lesions for microhardness measurement.

MATERIALS AND METHODS

Preparation of Tooth Specimens and Material Application.

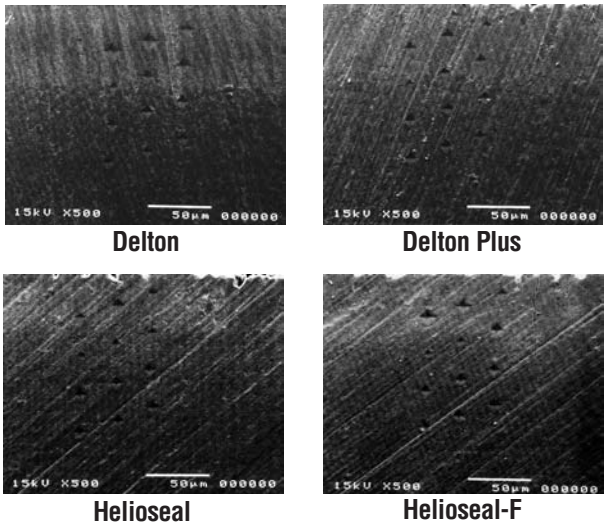
60 caries free extracted permanent premolars were selected. The entire tooth surface was painted with nail varnish except for two windows, approximately 2x2 mm^2, on the buccal surface as shown in Fig 1A. The lower border of the windows was on the line separating the occlusal and middle third of the tooth. The windows were evaluated with a binocular stereomicroscope (SZH 10, Olympus Optical Co., Ltd, Tokyo, Japan) at 40x magnification to ensure that these surfaces were intact and caries-free. To create the artificial lesions, the teeth were immersed separately in 16 ml of Carbopol demineralizing solution at 37_C (Adapted from Oral Health Research Institute, Indianapolis, IN, USA). The demineralized solution consisted of 0.2% polyacrylic acid and hydroxyapatite in 0.1 M lactic acid and adjusted to pH 5.0 with 1.0 M sodium hydroxide.

After 9 days, the teeth were removed from the demineralizing solution, and rinsed with water for 10 seconds. Sixty teeth were randomly assigned to two treatment groups. Operator etched both windows with 35% phosphoric acid gel (Densply, York, PA, USA) for 20 seconds, rinsed thoroughly with an air-water syringe for 10 seconds, and dried with a stream of oil free air. In each tooth, fluoride- and nonfluoride-containing sealants were applied to the windows in random order. Group 1 received Delton® (Densply, USA) and Delton®Plus (Densply, USA). Group 2 received Helioseal® (Vivadent Inc., Tonawanda, NY, USA) and Helioseal®F (Vivadent, USA). Delton®Plus and Helioseal®F are fluoride-containing sealants. The sealants were cured for 20 seconds using a visible-light curing unit (XL 3000, 3M Dental Products, St Paul, MN, USA). After that the teeth were separately submerged in 10cc. of artificial saliva at 37ºC for 7 days. The artificial saliva, pH 7, consisted of 0.11 g/L calcium phosphate, 1.2 g/L potassium chloride, 0.84 g/L sodium chloride, 0.052 g/L magnesium chloride, 42 ml sorbitol, 10 g carboxymethyl cellulose, 0.7602 g/L sodium phosphate, and trace of sodium hydroxide for pH adjustment.

Cross-sectional Microhardness Evaluation.

After 7 days, the teeth were removed from artificial saliva, rinsed with water and sectioned perpendicular to the long axis of the tooth at 0.5 mm below the upper margin of the window (Fig 1D). The specimens were embedded in epoxy resin and the sectioned surfaces were serially polished with a Metaserv 2000 Grinder and Polisher (Buehler, Lake Bluff, USA) using 320, 600, and 1200 grit silicon carbide paper, followed by 0.05 µm alumina suspension on a polishing cloth.

Figure 2. Lesion images (x50) taken from scanning electron microscope showing positions of Vickers indentations. The upper border of each image is sealant-enamel interface. Demineralized area (upper third) appeared lighter than the underlying sound enamel (lower two-third).



The demineralized lesions were investigated by cross-sectional microhardness method. One investigator carried out all measurements without knowing the type of sealant on each specimen. MHN test was performed with a Mitutoyo MVK-G3 (Akashi Corp., Japan) using Vickers indentor with 5-, 10- and 25-gram loads for 15 seconds. The first indentation was done with 5-gram load at 20 µm from the sealant-enamel interface. The next indentation was moved 30 µm laterally and 10 µm towards the underlying sound enamel (Fig 2). Ten-gram load was used for the second to the sixth indentations, and 25-gram load for the seventh to the twelfth indentations. Two areas in each window were tested to calculate an average hardness value for each distance from the sealant-enamel interface.

Calculation of Parameters Used to Quantify the Demineralized Lesion.

Vickers hardness number (VHN) was converted to Knoop hardness number (KHN) (Zwick 3212 Instruction manual, Germany). KHN was then converted to volume percent mineral by using an empirical formula: volume percent mineral $= 4.3 \div KHN + 11.3$.[6] The mineral profile of the demineralized lesion was achieved by plotting volume percent mineral (VPM) as a function of depth from enamel surface. The amount of mineral loss, _Z (VPM·mm) of each mineral profile was integrated from the area between the mineral profile of the lesion and the average volume percent mineral of sound enamel, which was extrapolated from the enamel underneath the lesion.[30]

Statistical Analysis

Means mineral loss (_Z) of the lesions sealed with fluoride-containing and non-fluoride containing resin sealants were compared within each group: Helioseal vs Helioseal-F and Delton vs Delton Plus, using Paired-t-test.

RESULTS:

Figure 2 shows the images taken from Scanning electron micro-

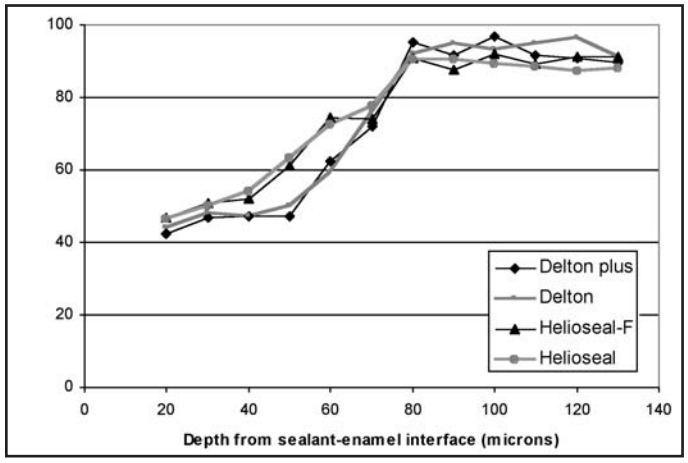


Figure 3. Mineral profiles of artificial carious lesions under fluoride- and nonfluoride-containing resin sealants.

Table 1. Mean mineral loss of lesions under fluoride- and nonfluoride-containing resin sealants. (N=30)

Sealants	Mean ± SD of _Z (VPM·mm)
Delton	1422 ± 441
Delton Plus	1287 ± 420
Helioseal	1222 ± 284
Helioseal-F	1164 ± 266

Vertical lines connect mean _Z that were not significant different (paired t-test, P>0.05)

scope (x500) for illustrating purpose. The upper border of each picture is the sealant-enamel interface, the gray area is the artificial carious lesion, and the dark area under the lesion is sound enamel. These pictures show diamond-shape indentations from Vickers indentor. Figure 3 depicted typical mineral profiles of artificial carious lesion under fluoride- and non-fluoride containing sealants. Mean _Z and standard deviation from each group are listed in Table 1. Paired-t-test revealed no significant difference in _Z between Helioseal and Helioseal-F, and between Delton and Delton Plus (P>0.05).

DISCUSSION:

At present the requirement of pit and fissure sealants has not yet included the protective aspects related to Fluoride. Fluoride-containing sealant is expected to provide localized protection against caries to the surrounding tooth structure and to the underlying enamel in case a portion of sealant fractured or worn away. Studies showed that enamel under fluoride-containing sealant had higher fluoride content[3,29] and the adjacent enamel exhibited substantially reduced demineralization.[12,13] This study hypothesized the effect of fluoride-containing sealant another step further, whether the demineralized lesion sealed with fluoride-containing sealant can reverse.

The mineral content of incipient artificial carious lesions may alter after the lesions have been sealed with fluoride-containing sealant if fluoride can be incorporated into demineralized enamel. The result of this study indicates that the mineral loss in fluoride-containing sealants, Delton plus and Helioseal-F, was not significantly different from the conventional sealants.

The best way to evaluate the effect of fluoride-containing sealants on demineralized lesion is to measure the mineral content before and after treated with sealants, then compare the change in mineral content between the experimental and the control groups. However, we could not evaluate the same lesion twice, because we had to section the specimens for microhardness testing. In order to compare the amount of mineral loss between the two groups, we posted some assumptions. First, the two windows had the same mineral content to begin with, and second, the demineralized process created similar incipient lesions. Purdell-Lewis et al[23] showed that enamel areas at a certain level of occluso-gingival height had the same mineral content. In our study both windows subjected to demineralized solution under the same condition. Pilot study revealed that demineralized lesions of the two windows were similar in terms of hardness profile.

Hardness change is a simple method used as a direct measure of mineral gain or loss as a consequence of demineralization and remineralization.[6] Baseline microhardness values for enamel in this study ranged from 280-350 VHN. These values are similar to many studies, which ranged between 298 and 352 VHN.[19,28] The operator accuracy in the hardness measurement was confirmed statistically by having the a -coefficient of 0.9. We used 5-g load with the first indentation to avoid damaging the sealant-enamel interface. The next indentations in the demineralized region were done with 10-g load, and the last six indentations in the sound enamel region were done with 25-g load. This method gave appropriate size of the indentations for our measurement without any damage and within the limited working area. The variation in loading has been accounted for in the formula used to calculate hardness number: VHN = loadx189000/(indentation length2). Although the different loads can affect the results, it should be noted that the procedures for all samples were carried out the same way and thus were comparable.

Rawls and Zimmerman[24] showed that a fluoride-exchanging resin decreased lesion body and increased thickness of dark zone when applied over artificial carious lesion. This sign of remineralization was observed after the fluoride-releasing resin was in place for 10 days, then the tooth were subjected to the second demineralization for 2 months. We did not observe any statistically significant difference in our study, although the mere mineral loss of both fluoride-containing sealants was lower than the nonfluoride-containing sealants. It should be stressed that these values can highly be experimental variable. Our negative results may result from unsuitable environment for the remineralizing process, or because fluoride release from these sealants is too low to affect any mineral change. As pointed out by Simonsen in his recent review,[27] no clinical benefit of fluoride-containing resin sealant has been documented. The addition of fluoride to resin sealant seems to be a marketing strategy rather than actual clinical advantage.

The method of studying remineralization of incipient carious lesions should provide an environment conducive to the remineralizing process. Calcium and phosphate ions are building blocks for mineral deposit. Exposure of demineralized area to fluoride in the aqueous phase around the tooth enhances the precipitation process.[16] In our study, the incipient lesion was totally covered with sealant, thus the ion exchange had to occur through a solid state reaction. Tanaka *et al*[29] reported more than a thousand ppm fluoride uptake into enamel at 60 mm under a fluoride sealant *in vivo*. Capilouto *et al*[5] showed in vitro fluoride uptake as much as 600 ppm to enamel

50 mm underneath a boron trifluoride resin. In addition, more than 50% of the deposited fluoride was bound to enamel mineral in apatite form. Both studies demonstrated the efficacy of fluoride deposit from sealants without the suitable environment for remineralization. As a matter of fact, demineralized enamel like the incipient lesion that we used in this study should be more susceptible to ion exchange than sound enamel. It is also possible that there was some remineralization, but in a lower scale than our detection limit.

Helioseal F released measurable amount of fluoride.[7] Both *in vitro* and *in vivo* studies showed that the majority of fluoride release from resin materials occurred within the first few days.[4,7,17] The fluoride released should diffuse and absorb promptly to the underlying enamel. Silverstone[26] reported that remineralized process could occur within a week. It is possible that, although fluoride-containing sealants used in this study released certain amount of fluoride which were uptaken by the underlying lesion, fluoridated apatite that formed was not enough to increase the hardness of the lesion. We placed the first hardness measurement at 20 mm from resin-enamel interface to avoid the edge effect, especially from resin tags along the interface. At such location, we did not see any increase in hardness near the interface as shown in the mineral profile in Figure 3. Longer period of contact between fluoride-containing sealant and incipient enamel lesion may provide more fruitful result. On the other hand, without external supply of calcium and phosphate ions as building blocks, remineralization may not occur to the measurable level. Suggestion for further study to show the effect of fluoride-containing sealant on incipient lesion should include acid challenge after the detachment of sealant.

Researchers have concluded that well applied pit and fissure sealant can inhibit the progression of decay especially in white spot lesions.[8,9] Fluoride should enhance the caries preventive efficacy of sealant, presumably by facilitating remineralization of decalcified enamel. Previous studies have shown that fluoride released from sealants was able to produce an inhibitory effect against S. mutans.[17] The antibacterial activity of fluoride together with the sealing ability supports the application of fluoride-containing sealant on incipient carious lesions. Although this study cannot prove the effect of fluoride-containing sealant on increasing mineral content of an underlying lesion, there is no harmful consequence. Sealing incipient carious lesion with fluoride-containing resin sealants may not be the best choice for remineralization, but where the sealant has remained intact, evidence has been provided to show that the caries have not progressed.[1,5]

CONCLUSION

Incipient enamel artificial carious lesions that were sealed with fluoride- non-fluoride-containing resin sealants for 7 days did not show distinct sign of remineralization, as observed by no significant difference in the amount of mineral loss.

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