Clinical Trial of an Antimicrobial Pit and Fissure Sealant

Short title: Antimicrobial Pit and Fissure Sealant

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Abstract

Purpose: Pits/fissure sealant containing an organo-selenium was evaluated for clinical retention, plaque and caries formation. **Methods:** 120 subjects, age 7-20 years old, at either moderate or high caries risk status, received SeLECT Defense sealant (SD) on one tooth and UltraSeal XT Plus (UXT) on the corresponding tooth on opposite side. Sealants were evaluated quarterly for 12 months for retention, plaque and caries formation around sealant. Lost sealants were re-applied but tooth was considered as failure in future analysis. Differences in evaluated factors between UXT and SD at each assessment period were analyzed statistically by McNemar's test. Logistic regression was used to test any association between evaluated factors and other variables. **Results:** Retention was significantly ($p=0.0004$) higher in SD (96%) than UXT (81%) after 12 months. No significant difference in retention between the two sealants at 3, 6 or 9 months. SD exhibited 100% prevention of plaque growth around sealant, while plaque growth was observed around 7% and 12% of UXT at 9^{th} and 12^{th} months respectively. Subjects with plaque around UXT have no plaque around their SD. No caries formation around either sealant. **Conclusions:** SD completely prevented plaque growth around sealant while UXT offered only limited protection against plaque growth.

Keywords: Sealants, Caries prevention, SeLECT™ Defense sealant

INTRODUCTION

Majority of the increment in dental caries among school-aged children has been detected on pit and fissure surfaces of first and second molars[1]. Sealing is a recommended procedure to prevent caries of the occlusal surfaces of permanent molars[2]. Pit and fissure sealants have been used successfully for many years to prevent caries by eliminating plaque stagnation areas, thus preventing plaque accumulation that promotes decay in occlusal pits and fissures. Sealants may be indicated for children and adults who for a variety of reasons, may be at moderate or high risk of developing dental caries; have incipient caries (limited to enamel of pits and fissures); have sufficiently erupted permanent teeth with existing pits and fissures that are anatomically susceptible to caries.

Two main types of pit and fissure sealant materials are available, resin-based and glass ionomer sealants. The most commonly used being the visible light-activated fluoride containing sealants. The effectiveness of resin-based sealants, which depends on the longevity of sealant coverage (i.e. clinical retention), has been demonstrated in many studies[7,8,9]. Whether the fluoride release from sealants has any additional beneficial effects in caries prevention is still questionable[10]. However, fluoride-releasing sealants may strengthen the tooth surface to increase its resistance to caries development as well as remineralize an underlying enamel caries lesion. Apart from lack of retention, a major cause of sealant failure or caries formation in the presence of sealant is plaque formation around the edge of improperly contoured sealant and underneath a leaking sealant.

Thus, the development of a sealant with antibacterial action should be an advance in caries prevention. Element34 Technology Incorporated has developed an antibacterial pit and fissure sealant, SeLECT Defense™ Pit and Fissure Sealant (Element34 Technology Inc., Lubbock, TX),

which has received 510(k) clearance (#K09059). The present clinical study assessed the effectiveness of SeLECT Defense™ Pit and Fissure Sealant (SD) in children and adolescents by evaluating (a) clinical retention of the sealant, (b) caries formation around the sealants, (c) plaque formation on and/or around the sealant (evidence of bacterial growth), and (d) the safety of the sealant. The effectiveness of SD was compared with that of a commonly used commercially available resin-based fluoride-releasing sealant, UltraSeal XT Plus™ (Unltradent Inc., South Jordan, UT, USA).

MATERIALS and METHODS

Study Design

This was a randomized double-blind split-mouth study involving paired tooth surfaces. The study recruited 120 children and adolescents of age 7-20 years old, of mixed gender, and from different ethnic origins, varied socioeconomic status, at either moderate or high caries risk status. Subjects were selected such that there were mixed of tooth surface conditions, sound or incipient caries limited to enamel of pits and fissures. Every subject received SeLECT Defense sealant (SD) on occlusal pits and fissures of molars or premolars on either left or right side of the dentition and UltraSeal XT Plus (UXT) on the corresponding tooth on the opposite side. The side to receive SD sealant was randomly selected by computer output. Pits and fissures of sealed paired teeth shared similar health status either sound or with incipient caries limited to enamel. Sealants were evaluated every 3 months for 12 months for clinical retention (total retention, partial or total loss), plaque and/or caries formation around and/or underneath the sealant, and presence of any side effect. Lost sealants were re-applied but tooth was considered as failure in the future analysis.

Subject Population

This study was approved (Approval #: HSC20100380H) by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. The study recruited 120 children and adolescents of age 7-20 years old, of mixed gender, and from different ethnic origins, varied socioeconomic status, at either moderate or high caries risk status. The caries status was assessed using American Dental Association (ADA) caries risk assessment tools and determined based the recommendation of the ADA Council on Scientific Affairs[11]. Majority of the subjects (approximately 95%) were recruited from two elementary schools in San Antonio, where the sealants were placed in a mobile dental facility, while the rest subjects were recruited via a flyer and where treated at the Clinical Research Facility of the University of Texas Health Science Center dental school. The sample size was calculated based on the main objective of the study, which was to compare SD sealant versus the control sealant (UltraSeal XT Plus) in a split-mouth, paired-sample design at each follow-up interval. Comparisons involving binary outcomes measured on a categorical scale (i.e., caries formation, retention/mobility, side effects) were tested using McNemar's procedure for paired percents. Assuming a 5% caries incidence in the SD sealed teeth and a 17% caries incidence in the UltraSeal XT sealed teeth and a 1% joint caries incidence (caries in both teeth), then for a sample size of n = 100, McNemar's test will have power equal to 0.80 under a two-sided alternative hypothesis and alpha = .05. Comparisons involving outcomes measured on a continuous scale (i.e., plaque formation) were tested using Student's t-test for paired samples. In this case, a sample size of n = 100 will provide power equal to .77 to detect an effect size d = 0.3 under a two-sided alternative hypothesis with alpha =

.05, and a correlation between paired teeth equal to 0.4. To make provision for a potential 20% dropout over 12 months of study monitor, 20 subjects were added to the total subjects recruited.

The approval of the Institutional Review Board of University of Texas Health Science Center at San Antonio, who ensured that all proper procedures to protect human subjects were followed, was obtained. Subjects were recruited based on the following inclusion and exclusion criteria. Besides, age and caries risk status, subjects were included into the study only if they (or their surrogate if a child) was able to read and sign a written informed consent form that explains the study, have at least one existing occlussal pit or fissure in at least one fully erupted paired permanent tooth surfaces (e.g., left and right lower first molars) that were anatomically susceptible to caries and were in similar health status, sound or with incipient caries limited to enamel of pits and fissures. Subject must also agree to give a full medical and drug history, visit every three months for assessment, and have telephone contact for scheduling appointment and monitoring adverse effects. Subjects below the age of seven or above 20 years as well as those with restoration or sealant coverage in the pit and fissure system were excluded from the study.

Study Protocol

Following confirmation of eligibility and obtaining consent, sealant was placed on each subject by one experienced dentist licensed in the state of Texas, and who has been involved in school sealant programs for elementary schools in San Antonio and Laredo, all in Texas. Subjects received instructions on good oral health behavior and were individually shown how to clean their teeth by trained oral health educators prior to the start of the treatment. Every subject received SD on one side of the dentition (upper or lower quadrant) and UltraSeal XT Plus on the corresponding tooth on the opposite side i.e. if SD was placed on the left first molar, then the right lower first molar received UXT. This is to eliminate the possible influence, on sealant

retention, of the habit of using one side of the mouth more than the other for food mastication, exhibited by some individuals. The side to receive SD sealant was randomly selected by computer output. The sealed occlusal pits and fissures of the paired teeth shared similar health status, both were either sound or have incipient caries limited to enamel. Only pits and fissures in occlusal surfaces of molars and premolars deemed to be anatomically susceptible to caries, or to have incipient caries limited to enamel of pits and fissures, were sealed.

Sealant Procedures

Following selection and identification of the teeth to be sealed, sealants were placed in accordance with the recommended clinical procedures as described by Govoni,[12] and in accordance with the sealant manufacturer's instruction. No fissurotomy was performed on incipient caries on pits and fissures prior to sealant placement.

Clinical Evaluation

The same dentist that placed the sealant performed all the evaluations. The sealant was evaluated every 3 months for a total of 12 months for (1) clinical retention, (2) mobility of the sealant (evidence of ingress of saliva underneath the sealant), (3) plaque formation on and around sealant, (4) caries formation around the sealant, and (5) presence of any side effect on oral soft tissues.

Retention and mobility evaluation: All sealed surfaces were examined and recorded at the baseline (immediately after sealant placement). These surfaces were then evaluated at 3, 6, 9 and 12 months visit intervals. Sealant retention was evaluated using both visual and tactile techniques i.e. in addition to visual presence or absence of sealant; an explorer was used to determine mobility and marginal integrity of sealant. The following sealant retention criteria were used: 1 = totally present and immobile; 2= partially lost and immobile; 3 = partially lost and mobile; 4=

totally lost or totally present and mobile. At any assessment visit, sealants that scored 2, 3 or 4 are replaced; however, the affected tooth was considered as failure in the subsequent clinical assessment.

Plaque formation: This assessment was performed using the Quantitative Light-Induced Fluorescence (QLF Pro™, Inspektor Research, Inc., Amsterdam, The Netherland) in red fluorescence mode as described by Heinrich-Weltzien et al.[13] QLF Pro™ utilizes the principle of red fluorescence emission by some bacteria/plaque to detect and monitor the presence of bacterial plaque and hence, bacterial activities on tooth surfaces[13-16].

Caries formation: Surfaces with incipient caries in the pits and fissures prior to sealant placement were noted at the baseline (immediately after sealant placement). The development of new caries lesions in sealed pits and fissures were diagnosed as (1) incipient or (2) cavitated caries, following the current clinical criteria for caries diagnosis using the conventional visual-tactile method. No radiograph was employed.

Adverse/Side effect evaluation: Before the placement of the sealant, the subject's oral mucosa was examined for any soft tissue lesion. Following placement of the sealant, each subject was contacted through telephone calls by the Study Coordinator on a monthly basis to inquire of any side or adverse effect that may relate to the sealant. Side effects were also monitored on every evaluation visit.

Statistical Analysis:

For statistical evaluation of retention, scores 2, 3 and 4 were classified as failed sealant, while score 1 was classified as successfully retained. McNemar's test, a test of paired proportions, was employed to evaluate statistical differences in retention between the paired UltraSeal XT Plus and SD sealants at each period of assessment. For statistical evaluation of caries outcome on

sealed surfaces, effectiveness was defined as the difference between the numbers of tooth pairs where the control (UltraSeal XT Plus) tooth developed caries and the number of tooth pairs where the test (SD sealant) tooth developed caries, expressed as a proportion of the number of tooth pairs where the control tooth developed caries during the period of the study. Similarly, the effectiveness of the sealant in preventing plaque formation (bacterial growth) was calculated as above. Logistic regression test was used to evaluate the association between sealant retention or plaque formation and other variables ((gender, age, molar or premolar, upper or lower jaw and left or right side).

RESULTS

A total of 144 fissure sealants were placed on 120 subjects (46 males, 74 females) at baseline. 70 pairs of sealants were placed with SD on left and UXT on right while 74 pairs had SD on right and UXT on left. Six subjects, with 13 pairs of sealants, withdrew from the study at the last quarter due to either change of school or relocation from San Antonio.

The overall result of the study is shown in Table. After 12 months of application, 96% of the SeLECT Defense and 81% of the UltraSeal XT Plus sealants were completely retained (Score 1), and this difference in retention proportion was statistically significant ($p=0.0004$). There was no statistically significant difference in retention between the two sealants at 3, 6 or 9 months evaluations. With SeLECT Defense sealant, 3.0% were partially lost (score 2 and 3), while 1.0% were completely lost (score 4). With UltraSeal XT Plus, 5% were partially lost, while 14% were completely lost. There were no plaque growth around the SD applied in those subjects in whom 7% and 12% of the UXT had plaque growth around them after 9 and 12 months respectively (Table). These subjects with plaque growth around the UXT were among those at high caries risk due poor oral hygiene. Logistic regression test showed no association between any tested

variable (gender, molar or premolar, upper or lower jaw and left or right side) and sealant retention or plaque formation. There was no adverse/side effect reported throughout the study.

Condition assessed	3 months		6 months		9 months		12 months	
	SeLECT Defense sealant	UltraSeal XT Plus	SeLECT Defense sealant	UltraSeal XT Plus	SeLECT Defense sealant	UltraSeal XT Plus	SeLECT Defense sealant	UltraSeal XT Plus
Retention	100%	100%	98.6%	87.5%	96.5%	84.7%	96.2%*	80.9%*
Mobility	0	0	0	0	0	0	0	0
Caries formation	0	0	0	0	0	0	0	0
Plaque growth	0	0	0	0	0	7%	0	12%
Adverse Effect	0	0	0	0	0	0	0	0
Side-effect	0	0	0	0	0	0	0	0

Table: Summary of the study results. *Difference is statistically significant (p=0.0004).

DISCUSSION

Although the effectiveness of a resin-based pit and fissure sealant as a caries preventive device depends on the longevity of sealant coverage (i.e. clinical retention), caries can develop in the presence of sealant due to plaque formation either around the edge of improperly contoured sealant or underneath a shrunk sealant due to ingress of saliva/bacteria under the sealant. With regards to this, the present study investigated the ability of a resin-based pits and fissure sealant containing an organo-selenium to exhibit antimicrobial action, preventing the growth of dental plaque around the sealant, while still maintaining an adequate clinical retention for a considerable period of time. In this study the antimicrobial SeLECT Defense™ sealant (SD) was compared with a commercially available UltraSeal XT Plus™ sealant (UXT) for prevention of plaque and caries formation around the sealants, clinical retention, and safety. The result of the

study demonstrated that the SeLECT Defense™ sealant inhibited the growth of dental plaque in subjects where plaque growth was observed around UltraSeal XT Plus™ sealant applied to corresponding teeth on opposite side of the dentition. This observation is attributed to the organo-selenium molecule component of the SD, which has been shown to serve as a catalytic generator of superoxide radicals ($O_2^{\bullet-}$) from the oxidation of thiols,[17] an abundant element in human saliva. The superoxide radical is known to be toxic to different bacteria such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Escherichia coli in vitro*.[18-22] A recent in vitro study also demonstrated the inhibition of bacterial (*S. mutans* and *S. salivarius*) attachment and biofilm formation on the surface of as well as underneath an organo-selenium dental sealants.[23] Thus, it is also envisioned that, in event of sealant with microleakage with bacteria ingress underneath the sealant, the selenium-containing SD sealant would not trap bacteria that could live to form dental caries under the sealant.

It is pertinent to mention that effort was made to ensure that the subjects selected for the present study have the potential to develop dental plaque and caries lesions, by recruiting only children and adolescents at either moderate or high caries risk. The minimum qualification for participation was presence of at least one factor that may increase caries risk.[11] It was interesting to observe that the subjects that developed plaque around their UXT sealants were those at high caries risk due to poor oral hygiene, despite the fact that all subjects received instructions on good oral health behavior and were individually shown how to clean their teeth by trained oral health educators prior to the start of the treatment.

In this study, the presence of bacterial plaque was detected as red/orange fluorescence along the edges of the sealant using the QLF Pro™. This device uses the principle of green and red

fluorescence imaging for detecting caries and bacterial activity on teeth. It has been demonstrated that several strains of bacteria that are linked to caries and gingivitis produce metabolites that emit red fluorescence, especially in matured plaque (> 1 day), when illuminated with blue light emitted by QLF Pro™.[13, 14.] This principle of red fluorescence is utilized by QLF Pro™ to detect and monitor the presence of bacterial plaque and hence, bacterial activities on tooth surfaces[13-16].

In the present study a significantly higher proportion of the SeLECT Defense™ sealant was retained than the UltraSeal XT Plus™ sealant after 12 months of application. The SD were retained in subjects were UXT failed and verse versa. Thus retention or failure of either sealant did not follow any particular pattern, and logistic regression showed that it does not depend on gender, type of tooth (molar or premolar), or position in dentition (upper or lower jaw and left or right side). Some people have the habit of using one side of the mouth more than the other for food mastication. So to eliminate the possible influence of this factor on sealant retention, the positioning of the sealants were altered based on computer output randomization number.

Adverse and side effects were monitored throughout the study, and none was reported nor clinically observed in the present study. We believed that this has to do with the fact that selenium does not leach out of the sealant material due to the covalent attachment of selenium to the polymer of the sealant. This property of non-leaching of selenium from the sealant, enables the sealant to retain its antibacterial effect overtime, and this is an advantage of the organo-selenium dental sealants over fluoride-containing sealants, in which the gradual release of fluoride compromises the antibacterial effect of the fluoride containing sealants.[24-26] Fluoride-containing sealants would require the recharge of the sealant with more fluoride salt to enhance their antimicrobial activity.

CONCLUSIONS

The results of the present study demonstrated that SeLECT Defense Pit and Fissure Sealant completely prevented plaque growth around sealant with better clinical retention than UltraSeal XT Plus sealant, which offered only limited protection against plaque growth.

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Disclosures

The authors have no conflicts of interest to report.

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