Effect of fluoride varnish, sealants and caries infiltration in reducing the progression of proximal incipient caries in primary teeth

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Abstract

Objective: The reduction or arrest of proximal white spot lesions in primary teeth due to fluoride varnish, sealants, and resin infiltrants has been reported in previous studies. However, none of these studies reported which treatment gives the best results. Therefore, the aim of this study was to find the most effective treatment approach by comparing white spot lesion progression after treatment with fluoride varnish, sealants, and infiltrants.

Materials and Methods: Artificial proximal white spot lesions were created on 60 extracted primary teeth. Specimens were sectioned perpendicularly across the lesion in two halves. Fluoride varnish (Vanish 5% Sodium Fluoride White), sealant (Helioseal Clear), and resin infiltration (ICON) were applied to both halves. One of the halves was subjected to a demineralizing solution for five days. The other half was used as a baseline. Lesion progression was measured by comparing the mineral loss of the paired halves at eight depth points located within lesion area. This was done using the Cross-Sectional Knoop Microhardness Test.

Results: Differences in caries progression between the four groups (A: no treatment, B: fluoride varnish, C: sealants, and D: resin infiltrants) were statistically significant at three depth points 125µm (P= .005), 175µm (P = .001), and 200µm (P = .003). At a depth of 175µm, caries progressed more significantly in the fluoride varnish group (7.8%) compared to the sealant (-0.5 %) (P < .001) and the infiltrant groups (-1.7%) (P < .001). No significant difference was found

between the sealant and infiltrant groups (P = .94), or between the fluoride varnish and the control groups (P = .118).

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

1- Observing proximal incipient caries or applying fluoride varnish did not reduce caries progression.

2- Sealants (Helioseal) and resin infiltrants (ICON) were capable of significantly reducing the progression of white spot lesions in proximal surfaces when compared with the application of fluoride varnish. The use of sealants and infiltrants seem to be promising novel methods for reducing and inhibiting proximal caries progression in primary teeth.

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Introduction:

Prevalence of proximal caries

Although many preventive dental procedures have played an important role in reducing the prevalence of caries in recent decades, caries on proximal tooth surfaces remains difficult to control, and continues to be a problem in many developed countries. [1] In primary teeth, proximal surfaces are the most susceptible surfaces to caries, followed by occlusal surfaces. [2] In Denmark, cross sectional studies have shown that 25% of 7 years old children have one or more restorations on the proximal surfaces of primary molars, which increases at 9 years of age to 52%. In other countries, the numbers of restorations or extractions due to proximal caries in primary molars are similar or higher. [3] According to the observations mentioned above, it can be concluded that proximal caries in primary teeth is an important health issue worldwide.

Dental caries

Dental caries is a multifactorial disease that is affected by the interaction of various factors such as diet composition and frequency, bacterial dental plaque, the host's tooth surface, and saliva composition. [4] It is a dynamic process involving alternating demineralization and remineralization cycles of the tooth structure within the mouth after the intake of dietary fermentable carbohydrates. [5] In the presence of fermentable carbohydrates and sugars, the microorganisms within the adherent dental plaque biofilm to enamel surface produce organic acids. These acids drop the plaque's neutral pH to an acidic state, and demineralization starts. This results in dissolution of the calcium and phosphate from the enamel hydroxyl appetite crystals. Micro porosities will then be produced within the remaining calcified tooth structure.

This demineralization continues until equilibrium is reached between the oral environment and the enamel. After that, the lesion can be arrested or reversed where the damaged and demineralized enamel structure is reconstructed with the ions released (remineralization). The lesion may also be irreversible, and cavitation occurs after repeated and continuous demineralization episodes. [6]

White spot lesion

The beginning of a proximal carious lesion, also called a subsurface or white spot lesion, starts as an incipient caries that is confined to the enamel layer. White spot lesion is an early non-cavitated enamel lesion that is 10 to 50 times more porous than sound enamel. The incidence of such lesions on proximal surfaces is high. [7] Autio-Gold and Tomar, (2005) reported that the prevalence of proximal white lesions in 5 years old children was 81%. [8]

A white spot lesion is composed of 4 zones histologically: (1) surface zone, (2) body of lesion (3) dark zone (4) translucent zone. The "Surface zone" is considered the most crucial layer in maintaining the integrity of the tooth, since it acts as a barrier to bacterial invasion and has a low pore volume (1-5%). It also helps in the remineralization process and prevents cavitation of the tooth. The second zone, the body of lesion, composes the largest layer of incipient caries, and has a greater pore volume of 5-25%. [9] Featherstone, et al. (2000) explained why the surface zone can be more intact and has less structural damage than the body of lesion zone beneath it. After an acid attack, the dissolved calcium and phosphate ions may re-precipitate onto the surface while escaping the enamel. Also, when the pH returns to neutral, remineralization occurs due to precipitation of the calcium, fluoride and phosphate from the saturated saliva onto

the surface, repairing the dissolved crystallites. [10] The dark zone, with a pore volume of 2%-4%, is found in 95% of the carious lesions examined. The translucent zone, found in only 50% of cases, is the advancing front and deepest area of the lesion, with a pore volume of only 1%. [9]

Porosities of the subsurface explain why white spot lesions have an opaque, chalky white appearance when the surface is thoroughly dry, which disappears when it becomes wet again. [11] Detecting these lesions proximally are difficult and they can be easily missed. Sufficient light, magnifying lenses and good quality digital bitewing radiographs can aid in their diagnoses, in conjunction with other recent diagnostic tools, such as quantitative light [12]induced fluorescence and fiberoptic transillumination. [13]

Treatment of proximal white spot lesions

Today, there are two common treatment approaches for proximal white spot lesions. One includes non-invasive techniques such as oral hygiene instruction, fluoridation, and dietary control, which stop and prevent further initiation of caries. The other is the invasive, restorative approach, which irreversibly removes damaged tooth structure and replaces it to prevent further progression of caries.

1. Noninvasive treatment

Currently, preventive treatment techniques include dietary control, oral hygiene education and local fluoridation. These are the key factors in preventing and/or arresting incipient caries in children with low to moderate caries risk. [7] Non-cavitated lesions were proven to progress further to cavitation if no prevention treatment was applied. [14]

Silverstone, et al (1973) showed that in patients with good oral hygiene practice and regular fluoride exposure, the porous enamel can remineralize and become more resistant to further dissolution than sound enamel. [7] However, the success of these non-invasive preventive measures relies on patient compliance, which is difficult to obtain and maintain. [15]

A 6-year follow up study by Martignon, et al. (2010) showed that 57% of proximal white lesions progressed to deeper radiolucency's or restorations. The authors suggest that this was due to poor patient compliance with dental flossing, which plays an important role in controlling proximal caries. [16] Alternative preventive regimens are needed to arrest this progression, especially with patients who have low exposure to fluoride compounds, and are poorly compliant. [17] Furthermore, once there is enamel surface discontinuity it becomes more difficult to clean, plaque inevitably accumulates, and normal remineralization by salivary mineral components is inhibited. [18]

2. Invasive treatment

Treating high caries risk children having proximal incipient caries with invasive methods (restoration) results in the removal of a considerable amount of sound tissue to reach the carious lesion. Even using minimally invasive preparations, sound tooth structure is compromised. [19] The original anatomy and strength of the tooth structure is altered using cavity preparations that lead to limited tooth structure integrity with the tooth starting a cycle known as the "death spiral of restorations". [20] Therefore, other intervention methods are needed to delay the first restorative treatment for as long as possible.

It is often difficult to make a decision whether to watch and wait, or to restore non-cavitated proximal incipient lesions. There is always a risk of under- or overtreatment with both invasive and noninvasive approaches. [19, 21, 22] Caries sealing, infiltrating resins and remineralizing techniques appear to be the intermediate treatments currently available to fill the gap between prevention and restorative treatments for proximal white spot lesions. [1]

A review reported that three retrospective studies and one clinical study done on primary molars revealed that 73% to 81% of incipient lesions remain in enamel after 12 months with fluoride application. [23] Another study also reported that it took approximately 18 months for caries to progress through primary enamel in high caries risk children with no fluoride therapy. In low caries risk children, caries progression took approximately 40 months in primary enamel receiving regular topical fluoride therapy. [24] With the possibility of slow progression of caries and recent early caries detection devices such as quantitative light induced fluorescence [12] and fiberoptic transillumination, [24, 25] [13] dental professionals will have a better opportunity to intervene and apply intermediate treatment options, such as fluoride varnish, sealants and resin infiltration.

Literature Review

A. Fluoride Varnish

In the past three decades, fluoride has been successfully used for caries prevention. It has been applied in the form of dentifrices, gels and solutions that have been shown to be

effective, but still require patient compliance. Fluoride varnish was developed in the late 1960s and early '70s to lengthen the time of fluoride contact with teeth, since the fluoride release, reactions and uptake strongly depend on the duration of contact. [26] Fluoride varnish has a prolonged fluoride slow release feature, by increasing its retention on the tooth surface until it has been removed by brushing or chewing after 24 hours. This extends the effectiveness of the fluoride for a longer duration, and more efficiently than other topical fluoride agents. [27] In addition, fluoride varnish is well tolerated by the patients, safe, cost effective, and easily and quickly applied by dental professionals. [28] In 2009, Longbottom, suggested that high concentration fluoride vehicles be used as the first treatment approach to incipient caries. [29] The cariostatic effect of professionally applied fluoride on incipient caries has been reported to arrest the progression of active caries and enhance enamel remineralization in both permanent and deciduous dentitions [30-33].

The dissolution of fluoride varnish in a moist environment exposes the carious lesion to saliva saturated with fluoride ions. The primary anti-caries activity of fluoride occurs topically when fluoride ions, together with the calcium and phosphate ions contained in the saliva, precipitate to form a calcium fluoride layer on the tooth surface. This layer acts as a fluoride pH reservoir that releases fluoride ions into the saliva during a cariogenic challenge. [34] Under acidic conditions the calcium fluoride layer releases fluoride and calcium ions. Some of the fluoride ions settle on the tooth structure and replace the hydroxyl ions of the hydroxyapatite, yielding fluorapetite crystals that are more acid stable than hydroxyapatite. Other ions remain in the saliva, preventing the

dissolution of precipitated fluoride. [35] Furthermore, fluoride reduces the formation of cariogenic lactic acid in bacterial plaque, by impairing glucose uptake and glycolysis of Mutants Streptococci. [36]

A number of studies have reported the significant effect of fluoride varnish when applied on proximal incipient carious lesions. [37-40] Trairatvorakul, et al. (2008) compared the efficiency of fluoride varnish and sealants in reducing incipient caries areas of proximal caries in-vitro. Their results revealed that fluoride varnish significantly reduced proximal caries lesion size by 34.85%, compared to 15.27% using sealants. This study also reported that fluoride releasing materials had a greater effect in inhibiting demineralization and enhancing remineralization than materials that cannot release fluoride. [37] Another three years randomized clinical trial showed that the percentage of proximal lesions that did not progress into dentin were 55% in the control group and 84% in the fluoride treatment group receiving two fluoride applications in a year. He also reported that applying fluoride varnish significantly reduced enamel caries progression in medium and high caries risk areas. [41] However, in 1998 Mejare, et al. suggested that the use of fluoride varnish may reduce but not completely prevent the progression of proximal cavitation. [18]

B. Sealants

It has been proven that sealants prevent caries effectively when applied to sound occlusal pits and fissures, by preventing the maturation and accumulation of plaque biofilm. [42] Recently, there has been growing evidence that sealants can also be used therapeutically

in reducing and/or arresting active carious lesions. In several long term studies, the successful application of this mode of treatment to occlusal surfaces has been shown to arrest caries up to 10 years, provided that the sealants remained intact. [43, 44] Griffin, et al. (2008) concluded that sealing pits and fissures with non-cavitated lesions reduced lesion progression by 71.3%. This provided evidence that supported the application of sealants for managing and arresting occlusal caries in permanent teeth. [45] The concept of inhibiting caries progression by the use of sealants is sealing the cariogenic acids pathways, and Isolating the bacteria within the lesion from the substrate they need to survive. This concept of inhibiting caries progression through the use of sealants has been transferred from occlusal to proximal white spot lesions, in an attempt to close the gap between the invasive and noninvasive treatment approaches. In vitro studies [15, 37, 46] and in vivo studies [40, 47, 48] revealed that sealants significantly reduced and/or arrested proximal and smooth carious lesion progression. These studies also suggested the potential of considering sealants as a noninvasive treatment for incipient proximal surfaces. In spite of the previous study results, Gomez, et al. (2005) reported that sealants were applied after temporary tooth separation using orthodontic elastic separators. Eighty eight percent of the fluoride varnish group and 93% of the sealed group showed no caries progression. Results showed that there was no statistical significant difference between two treatment approaches. [40] The 7% progression of sealed lesions was explained by the decrease in sealant effectiveness over time after a single application. [49]

Martignon, et al. (2006) compared the efficacy of sealing proximal active caries versus flossing instructions. Teeth were separated temporarily using orthodontic elastic bands

for two days, then the bands were removed, leaving a space of 0.5mm for sealant application. The authors concluded that sealants were more effective in inhibiting caries progression compared to instructing patients to floss, with caries progression of 44% in the sealant group and 84% in the control group. [47] A similar conclusion was reached by Martington, et al. in 2010, finding that most children had no pain or anxiety during treatment. After one year of follow up, the sealant group showed lesion progression of 27.4% versus 50.7% in the control group. After 2.5 years, caries progression was 46.4% vs. 71.4% respectively. The evidence of lesion progression after 2.5 years could be attributed to not using a rubber dam for proper isolation, or to the poor characteristics of the sealant type used. [48]

The findings of Martignon, et al. (2006, 2010) in two studies suggest that the previous findings can be attributed to two causes: 1- Sealing the surface of non-cavitated enamel lesions; 2- The deep penetration into the demineralized enamel pores, creating a barrier that separates organisms within the lesion from the intraoral substrate required for their survival. [50]

Jensen and Handelman, (1980) reported that sealing caries decreased the viable bacterial count by 99.9% during the first year, and that 75% of the cultivable organisms were reduced by the acid etching procedure itself. [51] Another study by Oong, et al. (2008) also reported many studies that examined the bacteria levels under sealed caries. They showed that viable bacteria levels under sealed caries did not increase significantly, and were reduced by about 47% as measured by total counts of bacteria. [52]

An in vitro study by Mueller, et al. (2006) revealed that complete sealant penetration into the lesion's body pores was not necessary to significantly reduce caries progression, since partially infiltrated sealants had the same effect. [17, 53] However, after long periods of demineralization, partially infiltrated lesions may progress; therefore, complete infiltration would give better results. [17]

C. Resin Infiltrants

The concept of resin penetration into carious porous enamel had been explained by Davila and Robinson et al prior to the 1990's. [54, 55] Since then, several laboratory studies have investigated the inhibition of artificial caries progression by the penetration of different dental adhesives and fissure sealants. [46, 56-61] They discovered, however, that adhesives and sealants penetrated superficially into natural enamel caries, and did not have an optimal penetration due to their properties, and techniques that prevented them from fully penetrating porous enamel. [62]

Robinson, et al. (1976) was the first to report that low viscosity resorcinol-formaldehyde resin effectively infiltrated and reduced the pore volume of enamel caries when applied to carious lesions. However, this material was not accepted clinically due to its toxicity. [63]

A review of the effects of infiltrants and sealants by Kantovitz, et al. (2010) confirmed that the surface layer of non-cavitated carious lesions significantly hampers the resin capillary infiltration process. [64] Therefore, it is important to remove or perforate the surface layer and make the lesion body more accessible for resin infiltration by acid

etching. [62] Gray and Shellis, (2002) reported that etching artificial caries with 36% phosphoric acid for 5 seconds increased resin infiltration depth significantly. [59] However, Davila, et al. (1975) reported that resin would penetrate natural non-cavitated enamel lesions only when conditioned and even with acid etching, the penetration was less than artificial caries. [54] In another study supporting the previous results, in which natural incipient caries was conditioned with 37% phosphoric acid gel for 2 minutes, superficial infiltration depths were reported with a mean depth of 25μm, due to the differences in the structure of the surface layer between both natural and artificial caries. [62] Natural caries have an irregular, 10-25μm thicker and 7-20% higher mineral content surface layer than artificially created caries, due to the alternating remineralization and demineralization cycles in the oral cavity. [7, 65] In addition, natural caries pores are contaminated with organic substances from the saliva that also hamper resin penetration. [62]

In 2007, Meyer-Lueckel, et al. found that etching natural incipient caries with 15% hydrochloric acid for 120 seconds significantly eroded and reduced the irregular and high mineral surface layer, [66] and also improved resin penetration depth, with a mean of 58μm. [62] In primary and permanent teeth, [66] this method of etching makes the pores more accessible for resin infiltration, compared to 37% phosphoric acid. However, with this etching regimen and the complete removal of surface layer in some samples, resin penetration was still considered shallow. [62]

Recently, there have been extensive studies that utilize micro-invasive materials which

control incipient proximal lesions. [67] In the United States, a new improved infiltrant resin, ICON (DMG America, Englewood, NJ), has been introduced. It is an unfilled light cured resin that is composed of 99% Triethylene Glycol Dimethacrylate (TEGDMA) and Champherquinone. The TEGDMA lowers the viscosity of this material and increases its penetration coefficient properties (>100cm/s). [67, 68] This property helps its rapid diffusion and deep penetration into the enamel pores by capillary action, up to 800µm deep. [67] Furthermore, Meyer-Lueckel, et al. (2011) found that lesion depths of 400µm to 500µm could be almost completely penetrated (414µm) after three minutes application of ICON/DMG on natural caries. [69] By occluding enamel pores, the resin infiltrant prevents the transmission of nutrients and organic acids into the lesion and reduces caries progression. [70, 71] It has been proven that depth of penetration is correlated with the ability of the material to hamper lesion progression. [71] In addition, Paris, et al. (2007) reported that completely infiltrated lesions were shown to reduce lesion progression more efficiently than superficially infiltrated lesions. [68] The combination of both low viscosity resin and etching with hydrochloric acid technique for 120 seconds has allowed for the infiltration technique to be used successfully, by almost completely occluding natural enamel caries. [72]

ICON infiltrant application requires no anesthesia or cavity preparation, so it does not change the anatomic shape of the tooth, and thus preserves healthy, hard tissue. Furthermore, infiltration strengthens the fragile enamel mechanically, and prevents cavitation and breakdown of the enamel surface. [61] In a procedure that requires one simple visit, interproximal space is created using a wedge, and the material is applied

using a specific applicator tip, which avoids the need of temporary tooth separation (requiring two appointments). [67] A rubber dam is used to avoid moisture contamination, because this infitrant is sensitive to moisture. The dam also helps to protect the adjacent teeth and surrounding soft tissues from the caustic nature of the 15% HCL used for etching. The resin does not contain any radiographic markers, so an identification card is needed to record infiltrated lesions, since they cannot be differentiated from untreated lesions clinically or radiographically. [73] To evaluate the efficacy of the ICON treated lesions, lesion progression has to be evaluated in follow up visits. [67, 72]

The efficacy of resin infiltration by ICON has been researched in several published articles. One in situ study by Paris et al (2010) [15], two in vivo studies by Paris et al (2010) and Ekstrand (2010) [3, 73], and one in vitro study by Meyer-Lueckel et al (2011) [69]. A clinical trial conducted by Paris, et al. (2010) revealed that 7% of the proximal lesions progressed with infiltrants, and 37% progressed with preventive treatment (diet, flossing and fluoridation). Paris et al (2010) concluded that infiltration is an effective approach in reducing caries progression proximally. [73]

Also, Ekstrand, et al. (2010) reported that treating proximal white spot lesions on deciduous molars with resin infiltration in conjunction with fluoride varnish was 35.7% more efficient in controlling proximal lesions than using fluoride varnish alone. Therefore, they concluded that patients who have greater caries risk and/or poor compliance may benefit from use of resin infiltrants as an alternate or additional approach for prevention. [3]

Another in situ study by Paris, et al. (2010) inserted bovine enamel specimens into intraoral appliances worn by 11 volunteers for 100 days. Results showed that caries progression of ICON treated and sealant treated enamel lesions were significantly reduced compared to untreated enamel lesions. [68] Although the difference between the infiltrated and sealed groups did not reach a significant level (*P*=.071), there was more loss of minerals in the infiltrated group (2,559 vol% x μm) than in the sealed group (1,496 vol% x μm). [15] This could be explained by the inhomogeneous cleft-like structure caused by material shrinkage during light curing, resulting in insufficient degradation resistance and leakage. [68]

Another study reported that a second application can occlude the spaces generated by the first application, and reduce the accessible pore volume. The results also showed that 60% of the lesion pore volume was occluded in the first application followed by a 25 - 35% increase in occlusion of pores after the second application, which provided more protection against further acidic challenges. [58]

Clinical significance of this study

Observing proximal incipient caries is not an adequate option, since there are various intermediate treatment recommendations that have been reported in the literature. [37] These intermediate treatment recommendations showed better and significant reduction of caries progression. It is critical to prevent caries progression at an early stage, since there is a high rate of cavitation even at the early stages of caries progression; and once

there is a cavitation, reversal of the lesion is impossible. [74] It is not clear which treatment approach gives the best results in primary teeth. Understanding the differences between fluoride varnish, sealants and caries infiltration in reducing lesion progression may help dental professionals to make appropriate decisions. Such decisions are especially important when treating primary teeth, which have a higher susceptibility and quicker progression of caries into dentin than permanent teeth. [75] The majority of findings in studies that compared the efficacy of previously mentioned intermediate treatment options relate to permanent and bovine teeth. No studies were done comparing all three together in primary teeth.

Aim of the study

The aim of this study is to find the most effective treatment approach that reduces or arrests proximal white spot lesion progression in primary teeth. This will be done by creating artificial white spot lesions proximally, then comparing lesion progression after treatment with fluoride Varnish (Vanish 5% Sodium Fluoride White), Sealant (Helioseal clear), and resin infiltration (ICON). Caries progression will be evaluated by comparing the enamel mineral profile (vol % mineral) of each treatment group before and after submitting it to cariogenic challenge at 8 depth points within the lesion. The Cross Sectional Microhardness test will be used to measure the length of indentations, which correlates to enamel mineral profiles.

Study Hypothesis

The most effective treatment in reducing proximal white spot lesion progression in primary teeth is sealant (Helioseal Clear), followed by resin infiltration (ICON) and fluoride varnish (Vanish 5% Sodium Fluoride White).

Materials and Methods

Tooth Selection and Preparation

An in-vitro lab study was conducted by selecting 60 therapeutically-extracted or exfoliated primary molars. A sample size was performed using nQuery (Version 7.0) based on the literature review findings of a similar study.[15] Assuming an effect size of Δ^2= 5.26, a sample size of 15 per group is adequate to achieve a Type I error rate of 5% and a power over 99%. There was no identification between biological teeth samples and patients. All teeth were visually inspected for caries and cracks. Only intact and caries-free teeth on mesial or distal surfaces were included in this study. Teeth with hypo-mineralization or hypoplasia were excluded. Teeth were cleaned with distilled water and debrided of soft tissue with a periodontal scaler. Prior to the experiment, the samples were stored in a 0.2% sodium azide solution at an ambient temperature. Carious parts were sectioned using a wheel cutting disk (Isomet 1000, Buehler, Germany). After this, they were polished with non-fluoridated pumice and water, and then embedded up to the cervical line in a block of self-curing acrylic resin (Tehnoviz 4000, cold polymerized resin, Norderstedt, KG). Exfoliated teeth without a sufficient base were embedded using plastic posts to support the crowns.

Formation of Artificial Caries Lesions

An acid-resistant nail varnish (ESSIE, Westfield, NJ) was applied to cover all the enamel except for one window on the mesial or distal enamel surface located at the center of the middle third. The window was rectangular in shape, and measured approximately 1 x 5 mm. Sample teeth were

then immersed in 10 ml of a demineralizing solution (2.2mM $CaCl_2$, 2.2mM KH_2PO_4 and 0.05M $C_2H_2O_2$) each for five days at a room temperature of 37°C. The pH 4.4 was monitored daily and adjusted if needed with a NAOH buffer solution.[76] This was done to create artificial non-cavitated enamel lesions; approximately 150-200μm in depth. Finally, after removing them from the acidic solution, teeth were rinsed with distilled water and dried in absorbent paper.

Teeth Sectioning

All specimens were sectioned perpendicular to the surface. They were sectioned mesio-distally across the lesion into two halves using a sectioning Isomet 1000 (Buehler GmbH, Dusseldorf, Germany) (Figure 1). The cut surfaces were covered with acid resistant nail varnish (ESSIE, Westfield, NJ).

Treatment Application

Samples were divided randomly by computer software (www.random.org) into four groups of 15 teeth each, and assigned into the following categories (Figure 1):

Group A: No treatment applied.

Group B: Fluoride varnish (Vanish 5% Sodium Fluoride White Varnish, 3M ESPE, St. Paul, MN).

Group C: Sealant (Helioseal Clear, Ivoclar Vivadent, Catharines, ON).

Group D: Resin infiltrant (ICON-infiltrant, DMG America, Englewood, NJ).

Group A: No Treatment:

Group B: Fluoride Varnish:

Fluoride varnish was applied on both halves of each sample according to the following manufacturer's instructions: a 5% sodium fluoride white varnish was applied evenly in a thin layer over the exposed area and stored in a neutral artificial saliva pH 7.7 for 24 hours. Then, the varnish was removed by brushing it with a small, soft bristle brush (Colgate, Chicago, IL) for 10 seconds.

Group C: Resin Sealants:

Resin sealants were applied on both halves of each sample according to the following manufacturer's instructions: a 37% phosphoric acid solution (Ivoclar Vivadent, Schaan, Liechtenstein) was applied for 15 seconds and rinsed thoroughly with water. After that, the sample was dried with oil-free air. The etched enamel had to have a matte white appearance. A thin layer of Helioseal Clear was applied directly with a brush and dispersed over the exposed enamel surface. The surplus was removed using a dental floss.[17] After 15 seconds, the surface was cured with a standard light curing unit (Demintron, Kerr, Germany) for 20 seconds.

Group D: Resin Infiltrants

ICON was applied to both halves of each sample according to the following manufacturer's instructions: a 15% hydrochloric gel (ICON-Etch, DMG America, Englewood, NJ) was applied for two minutes with a smooth surface tip attached to the syringe. The samples were rinsed with water for 30 seconds, and then dried with oil-free air for 30 seconds. The lesions were dehydrated with 99% ethanol (ICON-Dry syringe, DMG America, Englewood, NJ) applied by a cannula for 30 seconds and air dried. Using a smooth surface tip, the resin infiltrants were applied onto the test area and were allowed to penetrate the lesion pores by capillary action for three minutes. The

resin surplus was removed from the surface using a dental floss, and then cured for 40 seconds. The light curing unit output was 450nm, and the light intensity was 800mW/cm^2 (Demintron, Kerr, Germany). Subsequently, a second layer of infiltrants were applied for one minute, and then light cured for 40 seconds.

All specimens following treatment protocols were stored in neutral artificial saliva with a pH of 7.7 (mmoles/L): $CaCl_2$ (0.077g), $MgCl_2 \cdot 6H_2O$ (0.049g), KH_2PO_4 (0.544g), KC_l (2.23g), NaN_3 (0.019g), and HEPES buffer (4.76g), at 37^{o}C for 24 hours.[77] Then, all groups were thermocycled for 3500 cycles at 8^{o}C and 55^{o}C, with 15 seconds of dwell time. This represents approximately one year in the oral cavity (Figure 1).[78]

Cariogenic Challenge

One of the halves was randomly selected as a baseline half and the other half was the after caries progression half [71](Figure 1).

1. Baseline Half (BH): The baseline half was used to measure the hardness of the artificial caries lesion previously created.

2. After Caries Progression Half (ACPH): The cut surfaces were covered with a double layer of acid-resistant nail varnish to avoid leakage.[79] Then, for caries to progress, the specimens were subjected to a cariogenic challenge by immersing them in a 10 ml/tooth of demineralizing solution (2.2mM $CaCl_2$, 2.2mM KH_2PO_4 and 0.05M $C_2H_2O_2$) each, for five days at room temperature 37^{o}C (Figure 1).[71, 76]

Cross-Sectional Knoop Microhardness Test

The primary outcome is caries progression measured as mineral loss (Δ vol % mineral). It was calculated for the four groups (A, B, C, D) and compared at eight depth points located within the lesion area (25 μm, 50 μm, 75 μm, 100 μm, 125 μm, 150 μm, 175 μm, and 200 μm). This was done by using Cross-Sectional Knoop Microhardness test.[80]

All specimens were embedded in acrylic resin so that the cut section was exposed for polishing. Polishing was done with a wheel machine (Ecomet Buehler,Lake Bluff, Illinois) up to 600μm grid silicon carbide paper under streaming water. Finally, a paper disk impregnated with diamond paste containing diamond grains of 6μm, followed by 3μm, and then 1μm (Buehler) were used to prepare test samples.[31]

The Cross-Sectional Knoop Microhardness Test was performed using a rhombic-based pyramidal diamond indenter. It produced an elongated diamond-shaped indentation (Figure 2). Indentations were created by subjecting the Knoop diamond tip (Instron Wilson/Shore Instruments, Norwood, MA) to a 25-gf load for five seconds longitudinally across the polished lesion surfaces. The hardness profile of each carious lesion was evaluated in two separate rows of eight indentations each (16 indentations total) located at the lesion area (Figure 3). One of the rows was 100μm above the central region of the lesion and the other row was 100μm below. The indentations were made at intervals of 25μm in depth from the outer enamel up to 200μm (25 μm, 50 μm, 75 μm, 100 μm, 125 μm, 150 μm, 175 μm, and 200 μm). Then, the effect of the four groups (A, B, C, D) on caries progression was compared at these eight different depth points.

The Knoop Hardness Number (KHN) units were calculated by measuring the longest diagonal of the diamond-shaped indentation with an imaging software (Wilson Wolper Measurment system, Instron, MA) (Figure 4).The Knoop hardness number is a function of the test force divided by the projected area of the indent. The diagonal is used in the following formula to calculate the Knoop Hardness.

$$HK = \frac{\text{load(kgf)}}{\text{impression area(mm}^2\text{)}} = \frac{P}{C_p L^2}$$

Where: L = length of indentation along its long axis

Cp = correction factor related to the shape of the indenter, ideally 0.070279

P = load

The KHN of the 16 indentations in each lesion were obtained. Each depth (25 μm, 50 μm, 75 μm, 100 μm, 125 μm, 150 μm, 175 μm, and 200 μm) had two KHN units that were averaged, one on the top row and the other on the second row.[80] Then, the enamel mineral content for both halves was obtained by converting the averaged KHN values into Volume Percentage Mineral according to Featherstone et al. (1983) using the equation:[81]

Volume Percentage Mineral= 4.3 KHN1/2 +11.3

To calculate caries progression measured as mineral loss value in each sample, the volume percentage mineral of the Baseline Half was subtracted from its paired After Caries Progression Half (ACPH):

Caries progression (Δ vol % mineral) = BH vol % min – ACPH vol % min

Finally, caries progression values for each group (A, B, C, D) were averaged at each depth point (25 μm, 50 μm, 75 μm, 100 μm, 125 μm, 150 μm, 175 μm, and 200 μm). This was done to compare the group's effect on white spot lesion progression at these eight depth points.

Statistical Analysis

Statistical analysis was performed using SPSS software (IBM SPSS Statistics, Version 20, Armonk, NY). Data were collected and checked for normality using the Kolmogorov-Smirnov-Test. Data exhibited substantial non-normality; therefore statistical analyses were conducted by non-parametric statistical tests. Differences in caries progression between the four groups (A, B, C and D) at 8 depth points were analyzed using the nonparametric Kruskal Wallis test with Bonferroni correction for multiple testing. Eight Kruskal Wallis tests were performed for depth points of (25µm, 50µm, 75µm, 100µm, 125µm, 150µm, 175µm, and 200µm). The level of significance was set at $0.05 / 8 \approx 0.006$.

To determine more specifically where significant differences exists between the groups, six post-hoc Mann Whitney U tests with Bonferroni correction were done. The level of significance was set at $0.05 /6 \approx 0.008$.

Descriptive statistics were compiled from the results of the study. Medians, interquartile ranges, means, standard deviations (SD), minima and maxima were recorded showing caries progression of the four groups at the eight depth points. Line graphs and boxplot figures were reported.

Results

Sixty primary molars were included in this study. They were randomly divided into four groups with 15 specimens each. Two samples from the control group and three samples from the infiltrant group were damaged during preparation and could not be analyzed. Caries progression of the four groups, measured as mineral loss (Δ vol%mineral), at the eight depth points are reported as medians and interquartile ranges (Table II).

Kruskal-Wallis tests with Bonferroni correction were done at each depth point 25µm, 50µm, 75µm, 100µm, 125µm, 150µm, 175µm, and 200µm.(Table III). The tests revealed that differences in caries progression between the four groups were statistically significant at three depth points only (Table III). The level of significance was predetermined at $0.05 / 8 \approx 0.006$. Differences between the groups are indicated at depths 125µm ($P = .005$), 175µm ($P = .001$) and 200µm ($P = .003$). Accordingly, the null hypothesis was rejected.

Caries progression measurements of the four groups at three depth points of 125µm, 175µm and 200µm are demonstrated in side-by-side box plots (Figures 10-12).
Post-hoc Mann Whitney U tests with Bonferroni correction (with a level of significance set at $0.05 / 6 \approx 0.008$) at depth 125µm indicated that the difference observed between the fluoride varnish and the sealant groups was statistically significant ($P = .007$). Caries progressed by 16.2% in the fluoride varnish group and 4.8 % in the sealant group. In addition, fluoride varnish had a significantly higher caries progression of 16.2%

compared to the infiltrant group (-0.9%) (P= .004). (Figure 10)

At depth 175µm, caries progressed more significantly in the fluoride varnish group (7.8%) compared to the sealant group (-0.5 %) (P< .001). Furthermore, fluoride varnish showed a significant higher caries progression of 7.8% compared to the infiltrant group (-1.7%) (P< .001) (Figure 11)

At depth 200µm, the sealant group had a significantly lesser caries progression (-2.5%) compared to both the control group (6.3%) (P= .002) and the fluoride varnish group (4.3%) (P= .001). (Figure 12)

Finally, no significant differences in caries progression were found between sealant and infiltrant groups, and between fluoride varnish and control groups, at all three depths (125µm, 175µm and 200µm). (Figure 10, 11 and 12)

Although caries progression of the control group was greater than the infiltrant group, this finding was not significant with the cutoff of 0.008.

Discussion

This is the first study conducted that compares the commonly suggested treatment options for proximal artificial white spot lesions in primary teeth. The purpose of this in-vitro study was to compare the effects of fluoride varnish, sealants and infiltrants on the caries progression of artificially-induced white spot lesions at eight depth points (25µm, 50µm, 75µm, 100µm, 125µm, 150µm, 175µm, and 200µm). These three products were selected because they represent commonly used materials that have had positive effects in smooth surface inhibition of white spot lesions. The preventative approach of inhibiting proximal caries progression is an essential goal of progressive dentistry. It is critical to prevent caries progression at an early stage since there is a high rate of cavitation even in the early stages of caries progression. Once there is a cavitation, reversal of the lesion is impossible.[74] For this reason, observing incipient caries or postponing treatment is not an optimal option. In this study, both sealant and resin infiltrant treatment approaches were found to significantly reduce the caries progression of artificial white spot lesions in primary teeth more effectively than the fluoride varnish treatment and no treatment (control) groups at depth points of (125µm, 175µm and 200µm). Furthermore, there was no significant difference in efficiency between the sealant and infiltrant groups, or between the fluoride varnish and control groups. If the level of significance had been set at 0.05, differences between the groups would have been shown at depth 150µm (P= .011) in addition to the three depth points mentioned previously. The Bonferroni correction for multiple testing was used to achieve a Type 1 error rate of 5%.

In this study, 60 primary extracted teeth were collected and proximal non-cavitated artificial white spot lesions were created. Studies have shown that natural caries have an irregular and thicker surface, due to the demineralization and remineralization cycles of the oral cavity.[54] On the other hand, artificial caries have relatively smooth and intact surfaces, due to a continuous acid attack.[59,61] Although the use of artificial caries limits the external validity of the present study, it is difficult to collect 60 primary teeth with proximal non-cavitated natural white spot lesions. In addition, artificial caries are easier to create in high numbers, and produce a more standardized and uniform lesion structure and depth, resulting in a more fair comparison between the treatment groups than is obtained using natural caries.[68] Future studies are needed to compare the effects of fluoride varnish, sealants, and infiltrants on natural white spot lesions.

According to the results of this study, caries progression was significantly less in the sealant group than in the control group. In fact, the sealant group showed an almost complete inhibition of caries progression. This finding can also be supported by two in-vivo studies by Gomez et al. (2005) and Martignon et al. (2010).[40, 48] Gomez et al. (2005) reported that 92%-93% of sealed non-cavitated proximal lesions showed no caries progression after a two year of follow up.[40] Also, Martignon et al. (2010) reported that 27.4% of the lesions in the sealant group and 50.7% of the control group showed caries progression after one year in primary teeth. After 2.5 years, 46.6% of the lesions in the sealant group and 71.4% of the control group showed caries progression. They also reported that most of the participants showed no pain or anxiety during treatment.[48]

The material chosen to represent the sealant group in our study is Helioseal Clear, because many studies reported on its effectiveness in a review of the effects of sealants on non-cavitated enamel

lesions.[82] The result that sealants significantly inhibit caries progression was also reported in this review. Meyer-Lueckel et al. (2005) and Mueller et al. (2006) showed that Helioseal inhibited further demineralization by almost completely penetrating the lesion body in vitro.[17, 83] Paris et al (2006) also reported that Helioseal had significantly deeper penetration compared to other adhesives, and that, since it contains no solvents, it formed a homogenous resin layer within the body lesion, which efficiently inhibited lesion progression in-vitro.[46]

The results of this study also show that leaving the sealant's covering coat on the tooth surface is not necessary. In studies that reported the effectiveness of sealants in caries inhibition of subsurface enamel lesion progression, some reported that the lesions were sealed leaving a coat of resin on top, and others didn't comment explicitly on their application procedure.[58-60] It was not clear if this inhibiting effect resulted from penetrating and occluding the pores within the lesion's body, or if the covering coat on the surface protected the underlying enamel from further dissolution. In this study, the sealant surplus was removed with dental floss to provide a smooth proximal surface after light curing. This was done in accordance by reports from Mueller et al. (2006) and Paris et al. (2006) which stated that in non-cavitated enamel lesions, the penetration and occlusion of demineralized enamel pores with sealants was sufficient to inhibit lesion progression, without the need of a covering resin coat.[17, 46] Due to the anatomical difficulties in diagnosing any marginal imperfection proximally, it is better to remove any excessive resin in this area.[46] It was suggested that sealant coats should be wiped away with dental floss before light curing to avoid resin margins and edges that could accumulate plaque biofilm and induce new caries formation and periodontal inflammation at a later time.[17, 46] In addition, omitting the sealant coat makes the sealant application more applicable to the narrow proximal space in one visit,

without the need for creating space proximally in a two appointments as mentioned in other studies.[47] Instead, a wedge can be used to slightly separate the teeth, or an applicator tip with an ultrathin film perforated on one side can be used to ensure the flow of sealant on the lesion.

Lesions treated with infiltrants almost completely inhibited caries progression. This was in agreement with many studies mentioned previously.[3, 15, 69, 73] It can be explained that infiltrants (ICON) are 99% TEGDMA based resins that contain no solvent, which allows them to deeply penetrate enamel pores, forming a homogenous occlusion layer that prevents nutrients and organic acids from going through the enamel pores into the lesion, resulting in the progression of caries.[84] In addition to its composition, the method of application also increases its effectiveness by removing the surface layer, which hampers its deep penetration, by conditioning the enamel with 15% hydrochloric acid for 120 seconds. Both deep penetration and homogeneity are important in forming a leak-proof seal to inhibit caries progression.[68, 71]

The present study results showed that both the sealant and infiltrant groups showed no significant differences in reduction of caries progression. They had the same effect of reducing caries progression at all depth points. This study confirms an in-situ study done by Paris and Meyer-Lueckel (2010), showing that there were no significant differences between the effects of sealant and infiltrated treatments on caries progression under cariogenic conditions in-situ.[15]

Fluoride varnish was significantly less effective compared with sealants. This can be explained by the different mechanism and longevity of action. Fluoride varnish forms a calcium fluoride layer on the tooth surface, which acts as a fluoride reservoir. Released fluoride ions inhibit the caries

progression and remineralize incipient carious lesions by the formation of the harder fluorapetite crystals and the fluoride's cariostatic effect on bacteria. Fluoride varnish is applied once, and then removed by brushing after 24 hours, according to the manufacturer's instructions, and therefore, it has a short term effect. This effect takes place superficially, and the subsurface layer remains porous, which may create a risk of caries progression in active lesions.[38] On the other hand, sealants have a longer inhibiting effect by deeply penetrating and occluding the demineralized enamel pores of the lesion body, and they prevent the diffusion of acids from the bacterial biofilm through the pores which dissolve enamel at the advanced front of the lesion.[82]

The results revealed that the group treated with infiltrants had significantly reduced caries progression compared to the group treated with fluoride varnish. This is supported by another in-vivo study by Ekstrand et al. (2010), which reported that treating proximal white spot lesions on deciduous molars with resin infiltration in conjunction with fluoride varnish was 35.7% more efficient in controlling proximal lesions than using fluoride varnish alone. They also concluded that patients who have high caries risk and/or poor compliance may benefit from resin infiltrants as an alternate or additional approach for prevention.[3]

Although there were no significant differences between the fluoride varnish group and the control group, we cannot conclude that fluoride does not reduce caries progression. Many studies in the literature support the benefits of fluoride in caries progression inhibition.[30-33] We suggest that, in this study, a possible reason fluoride showed no effect may be that it was applied as a single dose. Other studies have proven that fluoride is most effective when it is applied in low doses over a long period of time, and that single high doses of fluoride are not effective.[85]

Because it is a major research area in dentistry, many techniques have been used to measure the demineralization and remineralization process of dental hard tissue. There are qualitative methods that measure the depth of the caries lesion, such as polarized light microscopy and scanning electron microscopy. Quantitative methods, such as microradiography, the cross-sectional microhardness test, and polarization sensitive optical coherence tomography are more appropriate, as they measure the loss and gain of minerals. Microradiography is the gold standard in measuring mineral loss and lesion depth, but it requires significant amounts of expensive equipment that are not readily available. It also calls for the preparation of thin sections, which is a crucial point in the present study because the mineral loss after five days of demineralization resulted in fragile lesions that may have fractured during sectioning. However, previous studies have demonstrated that the microhardness technique can be used for obtaining quantitative analysis of mineral content profiles within demineralized and remineralized enamel and dentin tissue.[80] It is used with brittle surfaces, requires simple parts, and does not require preparation of thin sections compared to transversal microradiography. There is a linear relationship between microhardness indentation's penetration change and local calcium content loss on partially softened enamel surfaces.[81] Purdell-Lewis et al. (1976) used the same technique and found it very reliable when used with enamel samples.[86] Featherstone et al (1983) reported that there is a significant correlation (r=0.91) between the length of the microhardness indentation and the percent of mineral content in subsurface enamel lesions.[81] Several studies have used the cross sectional microhardness test in measuring demineralization and mineral loss. The two most common types of microhardness tests are Vicker's and Knoop's. The intervals of the indentations have to be wide enough to avoid the indentation overlap effect, so Knoop's measurement was used instead of Vicker's. Indentations produced using Knoop's hardness had an elongated diamond-shaped that

can have more indentations in a very small area compared to Vicker's wider, squared indentations.

Results did not show significant differences between the four groups at the superficial depth points (25μm, 50μm, 75μm, and 100μm). A Cross Sectional Microhardness Test was used on partially softened enamel surfaces.[81] In this study, enamel became so soft and fragile at these depth points that were closer to the demineralizing solution, and the Microhardness Test couldn't accurately measure their hardness. Furthermore, negative caries progression values were reported in Table II. This could be explained by after exposure of the ACPH to the high cariogenic challenge, the enamel in the superficial depth points became very fragile and could have been abraded during the polishing process. This increased the ACPH's hardness and produced negative values after subtracting it from the BH. Another explanation is that caries are three-dimensional by nature, whereas microhardness is a two-dimensional quantitative method for measuring mineral loss. In an attempt to minimize the effects of this difference, the method of taking an average of multiple Knoop hardness number values obtained from two rows with eight indentations each, was followed.

With the limitations of the Microhardness Test in quantitatively measuring demineralization, the use of the transversal microradiography method is suggested, if available. An alternative for measuring demineralization and caries progression is employing the Confocal Laser Scanning Microscopy in conjunction with the Cross Sectional Microhardness Test method.

Teeth used in this study were collected from patients of different ages and fluoride supplementation. Tooth variability is one of the limitations of this study, as shown by the

relatively high interquartile range and spread of Caries progression data within the treatment groups. This affected the results, which showed no significant difference between the infiltrated group and the control group, although the infiltrants almost completely prevented caries progression. It can be suggested that a higher sample would have lowered the interquartile ranges and may have resulted in significant differences between the infiltrant and control groups. Using the post-hoc Mann-Whitney U tests without Bonferroni correction, infiltrants would have shown a significant caries progression reduction compared with the control group at depths 125μm (P= .03) and 200μm (P = .04). The Bonferroni correction for multiple testing was used to achieve a Type 1 error rate of 5%.

To minimize the variability within each tooth in response to acidic solution, caries progression measurements were obtained by sectioning the tooth into halves, then subtracting the baseline half enamel mineral content from the mineral content of its paired after caries progression half.

1. For future studies it is recommended that fluoride varnish should be applied more than once in order to prolong its time of action and to increase its effect in reducing caries inhibition.
2. Because there are differences between the environments of in-vitro and in-vivo studies, the external validity of this study is limited. Comparing the effects of these treatment approaches in the oral cavity would help confirm the effectiveness of both sealants and infiltrants over fluoride varnish in inhibiting caries progression if similar results are found.
3. Trairatvorakul et al. (2008) reported that the application of fluoride-releasing materials had a greater impact on the inhibition of demineralization, and therefore promoted

remineralization more than the materials that did not release fluoride.[37] Future studies are needed to compare the effects of sealants with and without fluoride release on incipient caries.

Conclusion

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

1- Observing proximal incipient caries or applying fluoride varnish did not reduce caries progression.

2- Sealants (Helioseal) and resin infiltrants (ICON) were capable of significantly reducing the progression of white spot lesions in proximal surfaces when compared with the application of fluoride varnish. The use of sealants and infiltrants seem to be promising novel methods for reducing and inhibiting proximal caries progression in primary teeth.

Table I. Product, Material Type, Composition, and Manufacturer of the Materials in the Present Study.

Product	Material Type	Composition	Manufacturer
Vanish 5% Sodium Fluoride White Varnish	Alcohol based solutions of modified rosins	Pentaerythritol Glycerol ester of Colophony resin, n-Hexane, Ethyl alcohol, Sodium Fluoride, Flavor enhancer, Thickener, Food grade flavor, Modified Tricalcium Phosphate	3M ESPE dental products, St. Paul, MN.
Helioseal Clear	Resin based light-curing sealant; unfilled.	Bis-GMA (30-60%) and triethylene glycol dimethacrylate (15-40%)(> 99 wt%), stabilizers and catalysts (<1 wt%)	Ivoclar Vivadent AG, FL-9494 Schaan/Liechtenstein
ICON	Low viscosity and light cured resin (infiltrant); unfilled.	Triethylene-Glycol-Dimethocrylate-based resin (99%), Camphoroquinone, Additives.	DMG-America, Englewood, NJ

Table II. Comparison of Lesion Progression at 8 Depth Points.

Group	N (teeth)	Caries progression (vol % mineral)							
		25µm	50µm	75µm	100µm	125µm	150µm	175µm	200µm
Control	13	-2.7 (18.6)	-5.6 (12.1)	-11.3 (27.0)	1.8 (35.9)	19.2 (44.4)	8.8 (21.7)	3.1 (15.8)	6.3 (11.2)
Fluoride varnish	15	-8.0 (15.2)	-6.5 (18.0)	5.6 (25.1)	15.1 (33.2)	16.2 (30.9)	7.5 (35.6)	7.8 (26.3)	4.3 (10.0)
Sealant	15	-4.8 (18.1)	1.0 (17.5)	-6.1 (23.9)	2.3 (30.1)	4.8 (15.6)	2.3 (13.3)	-0.5 (9.0)	-2.5 (5.7)
Infiltrant	12	1.5 (12.9)	1.2 (4.8)	-2.3 (19.8)	-3.7 (25.3)	-0.9 (7.1)	1.9 (14.9)	-1.7 (6.7)	-0.8 (9.7)

Data shown as medians of lesion progression with interquartile in parentheses.

Table III. P-Values of Kruskal Wallis Tests at 8 Depth Points.

	Depth from the outer surface into enamel							
	25µm	50µm	75µm	100µm	125µm	150µm	175µm	200µm
P-values of Kruskal Wallis test	0.200	0.319	0.147	0.167	0.005*	0.011	0.001*	0.003*

***Statistically significant p < 0.006**

Figure 1. Represents a Brief Description of the Method.

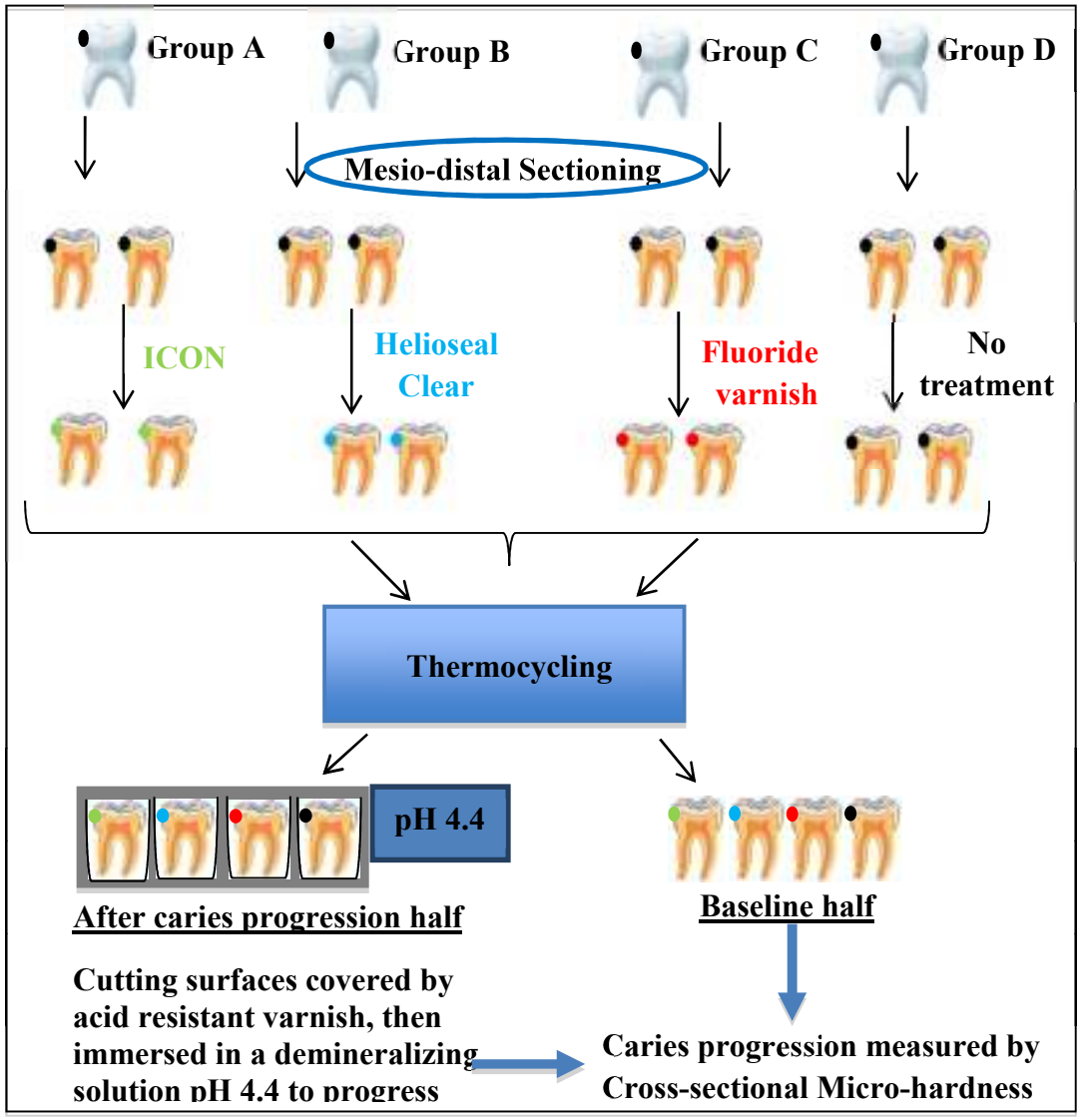


Figure 2. Knoop Hardness Indentation (Courtesy by Google)

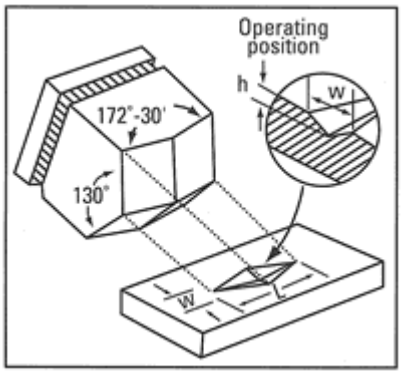


Figure 3. Diagrammatic Representation of Cross-Sectional Microhardness Test Measurements of two Parallel Rows of with Indentations each. The Depths of the Indentations are 25, 50, 75, 100, 125, 150, 175 and 200 µm from the Lesion Surface toward the Pulp (25µm apart).

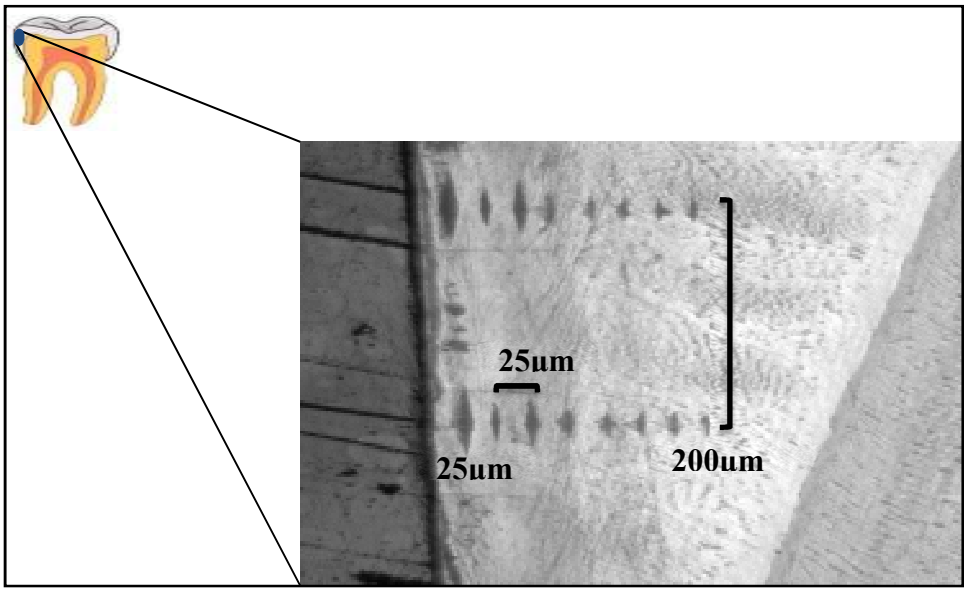


Figure 4. Software Measuring Knoop Hardness Number.

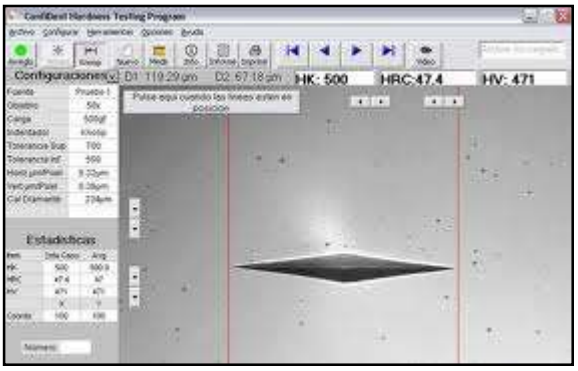


Figure 5. Comparison of the Volume % Mineral between Baseline Half and After Caries Progression Half in the Control Group.

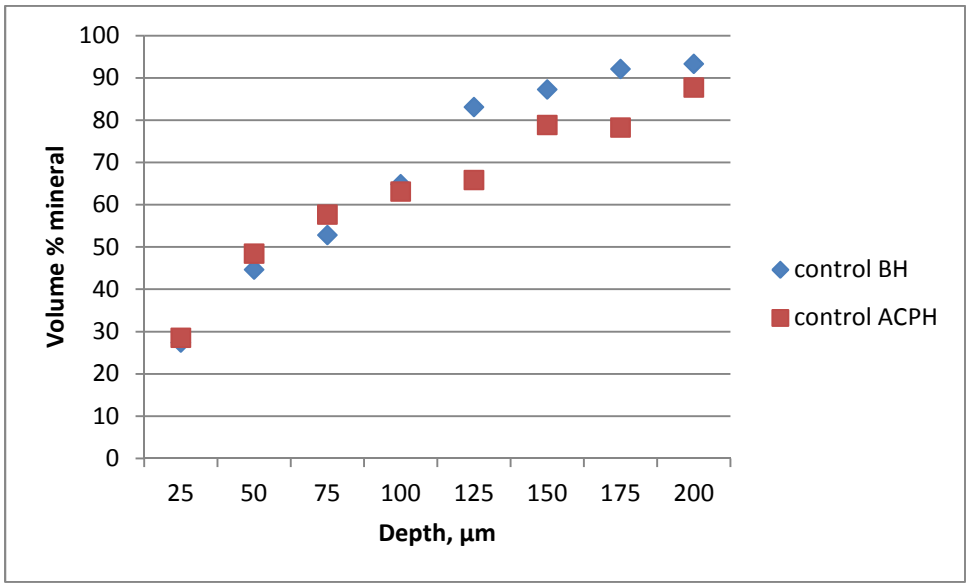


Figure 6. Comparison of the Volume % Mineral between Baseline Half and the After Caries Progression Half in the Fluoride Varnish Group.

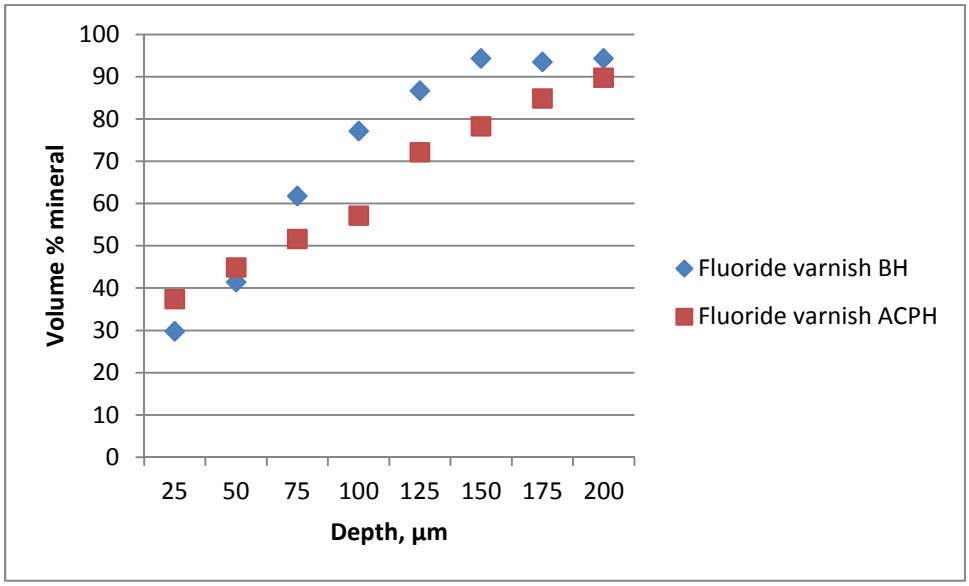


Figure 7. Comparison of the Volume % Mineral between baseline Half and the After Caries Progression Half in the Sealant Group.

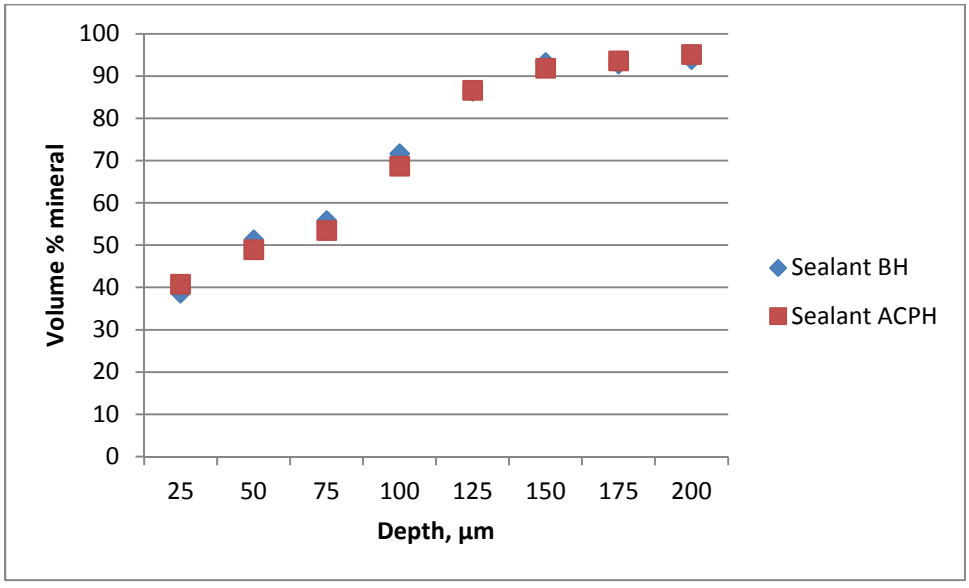


Figure 8. Comparison of the Volume % Mineral between Baseline Half and the After Caries Progression Half in the Infiltrant Group.

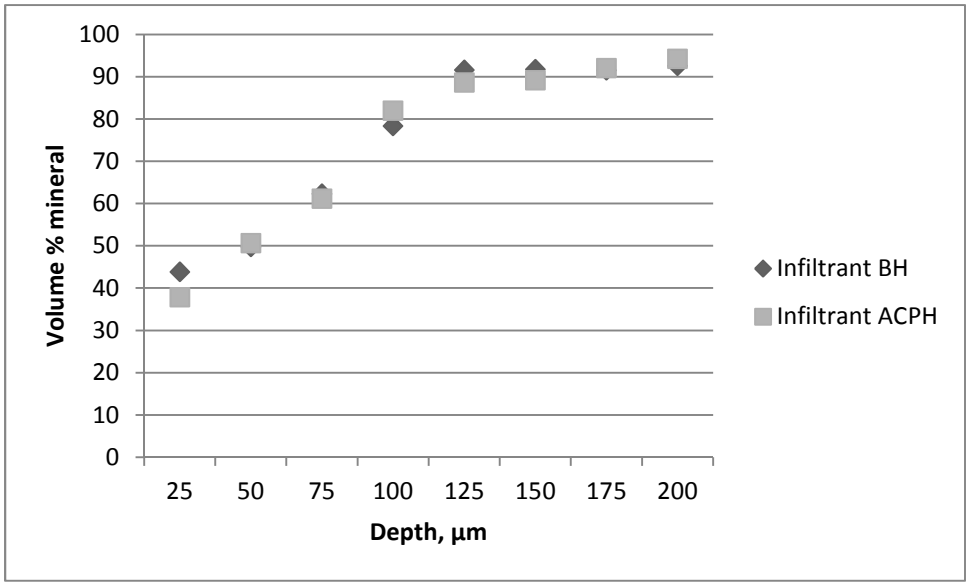


Figure 9. Median Lesion Progression Comparison between the Four Groups at Eight Depth Points.

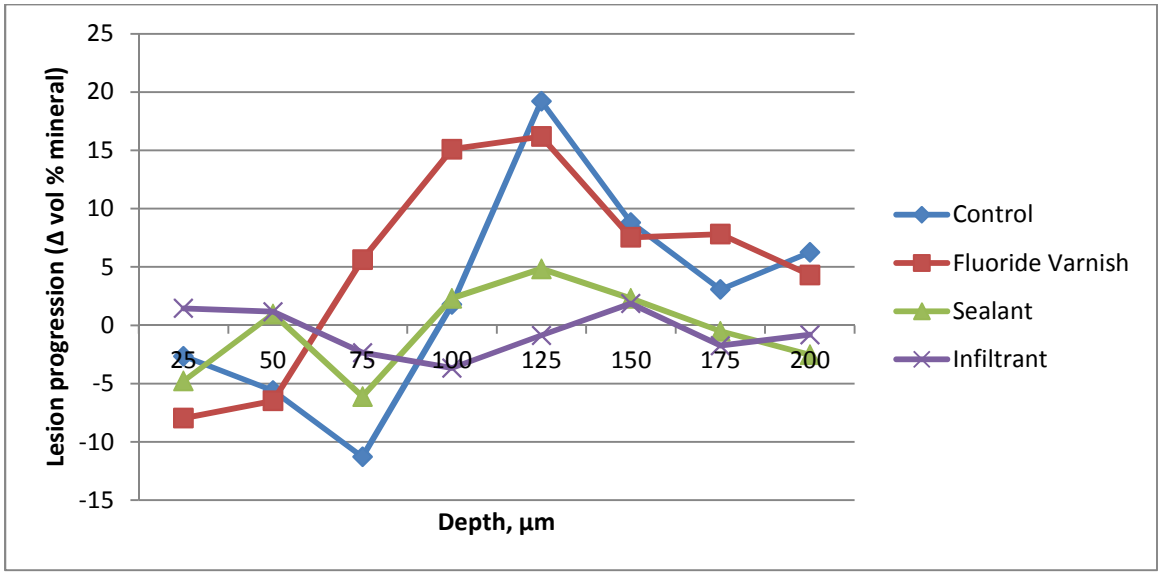
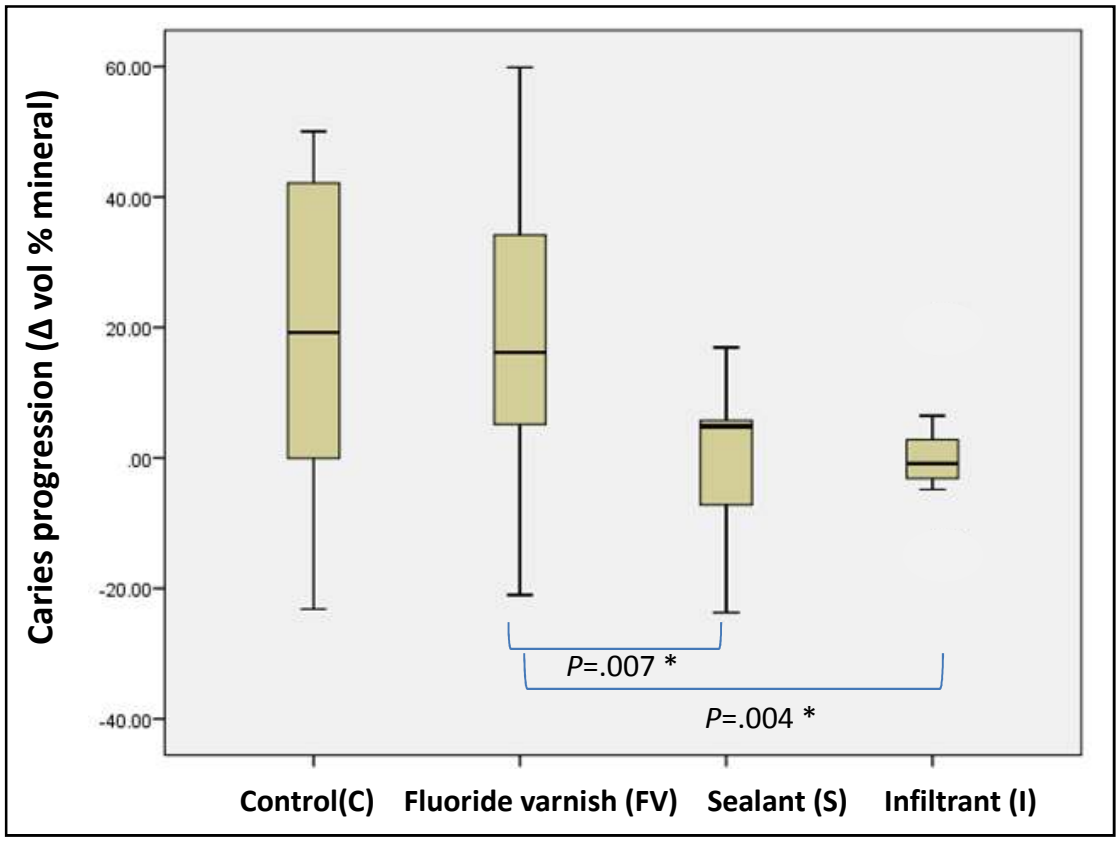


Figure 10. Box Plot Representing Lesion Progression Data of the Four Groups at Depth Point 125µm.



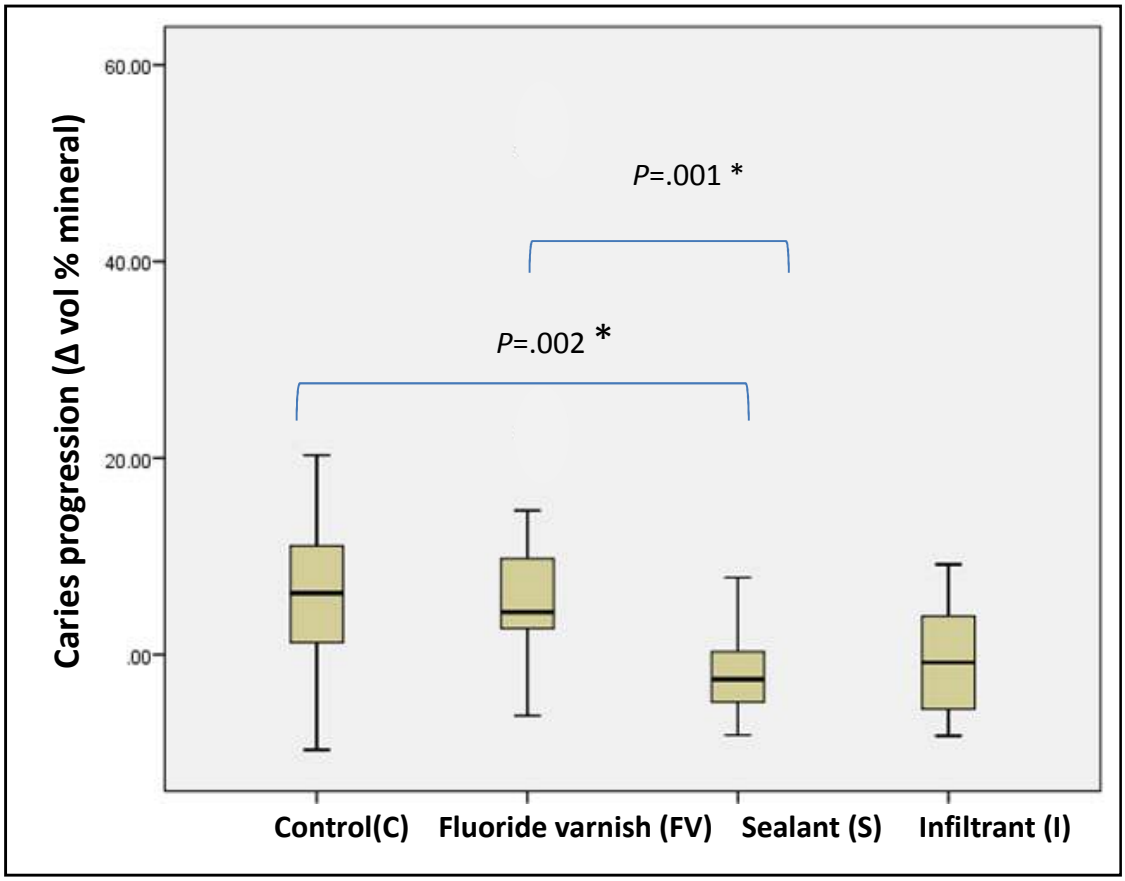
*** Statistically Significant Difference between the Two Groups (P< .008).**

Post hoc test's *P*-values at depth point 125µm

(C) and (FV)=0.892	(C) and (I)=0.03
(FV) and (I)=0.004*	(C)and (S)=0.15
(FV) and(S)=0.007*	(S) and (I)=0.905

***Statistically significant *P*< .008**

Figure 11. Box Plot Representing Lesion Progression Data of the Four Groups at Depth Point 175µm.



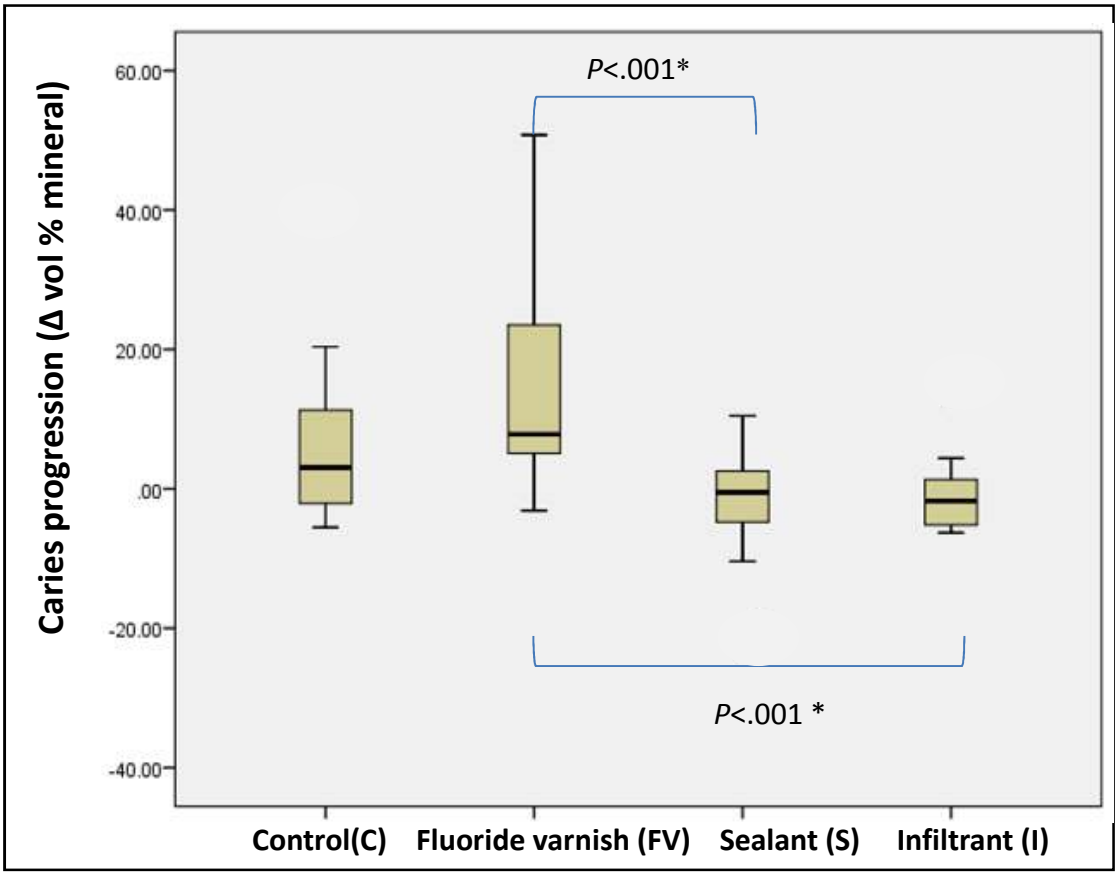
*** Statistically Significant Difference between the Two Groups (*P*< .008).**

Post hoc test's *P*-values at depth point 175µm

(C) and (FV)=0.118	(C) and (I)=0.06
(FV) and (I)<0.001*	(C)and (S)=0.072
(FV) and(S)<0.001*	(S) and (I)=0.94

***Statistically significant *P* < .008**

Figure 12: Box Plot Representing Lesion Progression Data of the Four Groups at 200µm Depth Point.



*** Statistically Significant Difference between the Two Groups (*P*< .008).**

Post hoc test's *P*-values at depth point 200µm

(C) and (FV)=0.96	(C) and (I)=0.046
(FV) and (I)=0.041	(C)and (S)=0.002*
(FV) and(S)=0.001*	(S) and (I)=0.614

***Statistically significant *P* < .008**

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