Potential Inhibition Of Enamel Demineralization In Vitro By a New Filled Orthodontic Sealant

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Abstract

Introduction: Enamel demineralization during orthodontic treatment is an unpleasant problem. Resin-based orthodontic sealants have been developed to protect enamel from demineralization. The purpose of this in-vitro study was to compare between the integrity of two highly filled orthodontic resin sealants (OPAL®SEAL and ProSeal™) after extensive tooth-brushing simulation and thermocycling, and to compare the efficacy of the two sealants (OPAL®SEAL and ProSeal™) on the inhibition of enamel demineralization during orthodontic treatment.

Methods: Forty-five non-carious specimens, either buccal or lingual surface from human premolars were divided into 3 groups (15 per group) and received 1 of the following treatments: no treatment (control), ProSeal™ (by Reliance Orthodontic Products, Itasca, IL) and OPAL®SEAL (Opal Orthodontics by Ultradent). The teeth were subjected to extensive tooth-brushing simulation and thermocycling followed by acidic challenge for 96 hours. They were examined macroscopically to identify any lost sealant and then sectioned for examination with polarized light microscopy.

Results: Mann Whitney U test revealed a non-significant effect for type of sealant on the integrity of the sealant after subjecting the specimens to extensive mechanical wear & temperature fluctuation ($P = 0.217$). Kruskal-Wallis test revealed a p value < 0.001 for lesion depth comparison among the three study groups. Accordingly, the null hypothesis was rejected. Post-hoc Mann Whitney U indicated that the median lesion depth observed in ProSeal™ and OPAL®SEAL were significantly lower (P-value < 0.001) than the control

group. There were no statistical difference in lesion depth between ProSeal™ and OPAL®SEAL groups. ProSeal™ and OPAL®SEAL performed significantly better, decreasing lesion depth by 92.49% and 84.68% respectively compared with the controls and completely inhibiting lesion formation in 8 and 5 specimens respectively. Spearman's rank correlation coefficient test for sealant integrity and lesion depth revealed a substantial correlation in ProSeal™ group ($r_s = 0.866$, $P < .001$), a weak correlation in OPAL®SEAL ($r_s = 0.153$, $P = .587$) and a moderate correlation for the two groups combined ($r_s = 0.537$, $P = .002$).

Conclusions: ProSeal™ and OPAL®SEAL show promise as an effective method of preventing enamel demineralization during orthodontic treatment without patient compliance. Both sealants survived the extensive tooth brushing and thermocycling well and expected to have adequate integrity throughout the average orthodontic treatment time. The exact correlation between the sealant integrity and the resultant enamel demineralization could not be identified from this study.

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Potential inhibition of enamel demineralization in vitro by a new filled Orthodontic sealant

Introduction

White spot formation is a common undesirable side effect of fixed orthodontic treatment. Since most of the orthodontic patients seek orthodontic treatment to achieve desirable esthetics, this common side effect should be of priority to orthodontists to minimize & control throughout the course of treatment.

White spot formation is the start of caries formation process. If left uncontrolled, it can progress to dental caries and cause cavitations. Dental caries is a bacterial disease that should be preventable (at early stages) and curable (at advanced stages). Scientifically and ethically, simply restoring teeth does not, in the long term, stop the disease process. Orthodontic appliances increase plaque accumulation on the teeth, thus creating a favorable environment for dental diseases and increasing the patient's susceptibility to caries development. Intuitively, orthodontists should place more emphasis on caries risk assessment and incorporate maximum efforts to prevent caries development in the orthodontic patient population.

Recently, dentistry is shifting toward a more conservative and preventive approach. Many efforts and clinical studies have focused on preventive dentistry. Today, CAMBRA[1,2], which stands for "**CA**ries **M**anagement **B**y **R**isk **A**ssessment" is the leading organizational method of establishing universal guidelines in caries risk assessment and prevention. CAMBRA includes, first, caries risk assessment that is performed before the treatment is initiated through the evaluation of a number of factors including clinical evidence of plaque control, oral hygiene, use of fluoride, tooth anomalies, dietary habits, salivary flow, medical history, and social history. The assessment will categorize the patient into different risk

levels (High, Moderate or Low). According to the risk level, customized preventive measures will be recommended including home dental care and/or in-office preventive measures. At the World Congress of Minimally Invasive Dentistry annual meetings dental schools from all over the nation, dental schools from California, Arizona, Oregon, Washington, Nevada, … etc, gather to work on improving CAMBRA. The schools along with members from insurance companies, state funding organizations, and dental research organizations usually hold panels of discussion on CAMBRA at such meetings. Now, most dental schools in the United States have implemented CAMBRA. Dental students are being trained to practice preventive dentistry.

Recently Featherstone et al.[1] completed a blinded, randomized clinical trial funded by the National Institute of health. They tested the validity of CAMBRA. In summary, this study tested high risk caries patients with simple interventions such as chlorhexidine and fluoride as needed based on the results of the caries risk assessment. The control group received traditional restorative care. Results showed that the CAMBRA group had lower caries incidence. Our principle is that conventional restorative procedures alone do not effectively treat dental caries. It is time to change the way we think about and how we treat this common disease.

Different terms have been given to enamel demineralization and are used interchangeably creating confusion. White spots or enamel decalcification or enamel demineralization have been used in the literature and imply the same meaning. Orthodontists prefer to use the term enamel demineralization and/or white spots. Enamel demineralization describes the

scientific process. The term white spots reflect the clinical picture of the demineralization process. In this study enamel demineralization and white spots will be used interchangeably.

Background

Incidence of enamel demineralization associated with orthodontic treatment:

Enamel demineralization (white spots) is a potential problem that can undermine the esthetic results of orthodontic treatment. Typically, the maxillary lateral incisors and mandibular canines are the most affected[3]. The gingival thirds of the facial surfaces are the most common sites for white spots[4]. Patient cooperation with preventive measures is frequently inadequate to solve this issue. Because patients' compliance can be a limiting factor, the orthodontist should search for alternative methods to achieve these goals with out relying on patient cooperation. Gorelick *et al* found[5] enamel demineralized lesions in 50% of orthodontically treated patients in contrast to 25% of untreated patients. O'Reilly et al[6], as well as Ogaard et al.[7,8], reported that these lesions could develop within four weeks time which is the average time between orthodontic appointments. This high incidence of enamel demineralization among orthodontic patients gives this side-effect a significant importance that requires attention. A recent study[9] quantified the percentage of the affected patients with white spots from the overall orthodontic patients population. The authors reported that new white spots developing on the maxillary front teeth during fixed orthodontic treatment remain a frequent undesired side effect, affecting 60.9% of patients.

Incipient caries lesions or early enamel demineralization:

Incipient lesions represent the earliest phase of dental caries. Histologically, incipient lesion consists of four zones (figure 1). The first zone, "the translucent zone", is the deepest, acts

as the defensive layer of the lesion, and is characterized by disorganized appearance microscopically. The second zone is the “dark zone” which does not transmit polarized light and is opaque. The third zone, “body of the lesion”, is the thickest zone of the lesion and has the largest volume of pores and bacteria and appears transleucent under the light microscope. The fourth zone, “surface zone”, surprisingly is the area that is not affected by the caries distruction. It has a low pores volume and similar radiopacity to adjacent intact enamel. The surface zone is most crucial zone, must be kept intact for remineralization to occur.

Demineralization/remineralization:

The tooth has on its enamel surface an acquired bio-film, called the pellicle, which plays a role in the ongoing physical-chemical equilibrium with the enamel and oral enviroment[10,11]. When the acidity of oral fluids gets below the physiologic norm (at pH 7.4), from the acids that is produced by cariogenic bacteria mainly Mutans streptococci and Lactobacilli, calcium and phosphate ions in the enamel dissolve from hydroxyapatite crystals and diffuse through the pellicle and into the oral cavity. This later mechanism is called demineralization. When the pH of oral fluids reaches the norm again, calcium and phosphate ions in the saliva are transmitted through the pellicle into the enamel following the laws of chemical equilibrium. This later mechanism is called remineralization (figure 2). In order for remineralization to take place effectively, a prerequisite of a healthy saliva is required. The saliva provides buffers, and extra calcium and phosphate ions. Since this concept has been fully understood, dental researchers, who are interested in cariology, are investigating ways to slowdown or eliminate enamel demineralization and at the same time enhance or initiate enamel remineralization. Remineralization and demineralization

mechanisms occur routinely within the mouth, following each episodes of food and drink consumption. An Incipient lesions can remineralized, depending on a number of factors including diet, availability of fluoride, and the buffering action of saliva. If the outer layer of enamel continues to demineralize, cavitations occur[12].

Enamel Demineralization prevention:

Enamel protection against white spots/demineralization can be achieved through two main approaches, either by application of topical fluoride and/or enamel protective sealants.

Topical Fluoride application:

The remineralization process is significantly enhanced by low levels of fluoride in the saliva and plaque[10]. Therefore, the first caries preventive approach was through fluoride application. Fluoride can diffuse into the crystalline lattice of all tooth mineralized structures, forming a stronger crystalline structure in the form of fluorhydroxyapatite. Fluorhydroxyapatite is less soluble in acidic enviroment[13].

Therefore, Fluorides act by inhibiting demineralization and stimulating remineralization and can be applied in the form of toothpastes, mouth rinses, gels, solutions, and varnishes. Orthodontists became interested in the benefit of fluoride varnishes for their potential to reduce enamel demineralization that develop during treatment with fixed appliances. Excellent patient diet and oral hygiene practices and the use of topical fluoride supplements such as toothpastes and mouth rinses can prevent, or minimize, the formation of enamel demineralization lesions during orthodontic treatment[14]. However, patients with poor diet and oral hygiene practices can develop demineralization lesions within four weeks in the

absence of fluoride supplementation[14] and these lesions can progress to cavities[14]. Topical fluoride can be applied in different consistencies and ways.

A- Home use Fluoride:

Fluoride can be supplemented in the form of toothpastes and mouth rinses. Geiger et al.[15] found a relationship between the dose and frequency of rinsing, with 10 ml of neutral 0.05% sodium fluoride solution, and the extend of enamel demineralization. Patients who rinsed at least once every other day had 49% fewer lesions than those who rinsed less frequently. Other authors[16] reported greater results with regular use of a high concentrated sodium fluoride (1.1%) dentifrice or gel. Even though this method of application is effective, it requires patient compliance & cooperation. Geiger et al. observed that 52.5% of the patients did not use the home fluoride solutions as prescribed[17]. This means that 50% of the orthodontic patients are not good candidates for this type of fluoride application. In another study on a different patient's population, Geiger et al. found that only 12-13% of patients reported reliable compliance with a home fluoride rinse program. Also, this protocol of prevention is not effective without acceptable oral hygiene[15].

B- Fluoride varnishes:

Because of the large percentage of uncooperative patients, an alternative method of fluoride application has been developed using varnishes. Application of fluoride varnishes is a preventive measure that does not require patient compliance and allows the clinician to control dose & frequency of application. Fluoride varnishes contain the natural resin colophony, which provide the adhesive behavior that extends the contact of fluoride to

enamel. The first fluoride varnish was developed in the 1960s (Duraphat®, 5% sodium fluoride; Colgate Oral Pharmaceuticals Inc., Canton, MA) and in the 1970s (Fluor ProtectorTM, a clear, transparent polyurethane lacquer containing 0.1% weight fluoride ion as difluorosilane; Ivoclar Vivadent, Inc., Amherst, NY). Meta-analyses of controlled clinical trials concerning Duraphat®[10] confirmed the clinical effectiveness of this varnish in reducing the incidence of dental caries. While, results for Fluor ProtectorTM have been open to doubt. An in vivo study[18] showed that toothpaste with 250 ppm of fluoride and a single application of high-concentration fluoride varnish around the brackets reduced enamel demineralization significantly by 40% in contrast to the controlled teeth.

In orthodontic patients, fluoride varnishes usually applied around the brackets on the facial surfaces of the teeth. Fluoride varnishes are effective & allow clinicians to control the timing and amount of fluoride use. However, the drawback to the use of fluoride varnishes is that they require several in-office applications.

A recent Cochrane systematic review[19] evaluating the effectiveness of fluoride in preventing white spots during orthodontic treatment and comparing the different forms of delivery of fluoride, concluded “There is some evidence that the use of topical fluoride or fluoride-containing bonding materials during orthodontic treatment reduces the occurrence and severity of white spot lesions, however there is little evidence as to which method or combination of methods to deliver the fluoride is the most effective”. Based on current evidence based practice in other areas of dentistry, the authors recommended that patients with fixed braces rinse daily with a 0.05% sodium fluoride mouthrinse.

Orthodontic bonding systems & orthodontic sealants:

Since most of the forms by which fluoride can be delivered to the teeth require patient cooperation and/or multiple applications throughout orthodontic treatment. Dental material researchers have been trying to develop the ideal sealants/bonding system that can be applied to the facial surfaces of the teeth during orthodontic treatment which contribute both a long lasting physical barrier and fluoride release. Their efforts were as follow in chronological order.

A- Fluoride-releasing resin bonding systems:

Composite resins are commonly used to bond orthodontic brackets to teeth. Although the revolutionary technology of being able to bond small metallic brackets to teeth, rather than the old fashion way of banding the teeth has been in vogue, plaque adheres more to composite resin adhesive than to enamel[20]. Following the introduction of fluoride varnishes, fluoride was incorporated into the bonding material used to bond orthodontic brackets to the teeth. The first resin to have fluoride incorporated in it was developed in 1983. Clinical evaluation of fluoride-releasing adhesives showed remarkable reduction in enamel demineralization by 93%[21]. The limited effect of such a preventive measure is that it does not cover the entire facial surfaces. The enamel surface beyond the bracket location is still at risk of demineralization.

B- Fluoride-releasing glass-ionomer and resin modified glass ionomer adhesives:

Bond strength of pure glass ionomer adhesives is lower than conventional composite resins and less than standard for clinical treatment[22-26] even though some studies showed reduced white spots[27]. Eventually, these materials lost popularity in clinical practice. Acceptable clinical performance as well as reduction in enamel demineralization were reported with the use of resin modified glass ionomer adhesives[28].

C- Chemical cure Resin sealants:

This category of sealants did not last long. It vanished from clinical use due to the failure to reach complete polymerization as a result of the formation of an oxygen inhibited layer on the outer surface[29].

D- Light cure Resin sealants:

Sealants serve two functions; they enhance & facilitate the bond between the brackets and the enamel, and theoretically provide a protective barrier to the enamel from the surrounding cariogenic factors. This category of sealants has no oxygen-inhibited layer. Unfortunately, the unfilled or lightly filled resins, with the required low viscosity and high flowability to facilitate application, is not strong enough to resist mechanical abrasion over an extended period of time. Many of the mostly used orthodontic sealants fall in this category & most have been studied in vitro to determine their ability to protect enamel from demineralization. It has been found that the incidence of lesions was 50% in the sealant group (unfilled Light Bond by Reliance Orthodontic) and 100% in the self-etching primer group (Transbond Plus by 3M). The lesions in the sealant group were present wherever the sealant integrity was broken. This study concluded “the application of sealant provided

protection in 50% of the samples, whereas the SEP provided no resistance to enamel demineralization. Protection from acid demineralization depends on the integrity of the sealant"[30]. Pit and fissure sealant (light cured unfilled resin) has been suggested to be used on the facial surfaces of teeth in conjunction with orthodontic treatment. In in-vitro[31] experiment found 80% of the treated teeth had no enamel demineralization. In vivo study[32] supported the former in vitro study. Enamel demineralization found to be almost 4 times less in teeth sealed with 58% filled pit and fissure sealant. The sealant resistance to wear depends on filler content, however the filler content is not the only factor. The wear resistance does not increase after a certain saturation level. Van Bebber et al.[33], in vitro study, found that the available filler saturation level of Proseal (18%) is sufficient to resist the acidic environment and the mechanical abrasion simulation.

Since the sealant integrity depends on the ability of the sealant to resist mechanical abrasion, then one of the main characteristics of an ideal orthodontic sealant would be resistance to mechanical abrasion over extended period of time.

The above limitation existed until the advent of "ProSeal™" (Reliance Orthodontic Products, Itasca, IL). This limitation was overcome by increasing the filler content in the sealant.

ProSeal™ background & literature review:

ProSeal™ was released to the market in 2004. It is the first highly filled light-cured fluoride-releasing sealant. It is advertised, by the manufacturer, as being resistant to

toothbrush abrasion (up to 2 years). The sealant can be used with light-cure, chemical-cure, or dual-cure bonding systems. ProSeal™ contains ethoxylated bisphenol A diacrylate (10-50%), urethane acrylate ester (10-40%), and polyethyleneglycol diacrylate (10-40%). The exact percentages are a trade secret (Paul Gange, Owner of Reliance orthodontic products INC). The amount of filler content in Proseal that is available in the market is 18%[33].

ProSeal™ achieves 100% polymerization without incorporating a residual oxygen-inhibited layer. This in turn creates a smooth hard coating that prevents leakage and protects the enamel. However, at the end of orthodontic treatment, this layer requires mechanical removal by polishing burs. Currently, ProSeal™ is the gold standard and the most efficient way to prevent or minimize the occurrence of white spot lesions during orthodontic treatment without relying on patient's compliance.

Since the release of ProSeal™, this material has gotten the attention of orthodontists. In many studies, both in vitro and in vivo, ProSeal™ has demonstrated high laboratory and clinical performance. Hu et al.[34] reported a remarkable decrease in enamel demineralization on teeth treated with Pro Seal™ when subjected to extensive mechanical abrasion in vitro. This study used enamel microhardness methodology to evaluate ProSeal™ performance. Teeth treated with ProSeal™ demonstrating better profiles than those treated with a fluoride varnish, etchant only, or an unfilled resin. This study's methodology did not test the effect of thermocycling on ProSeal™. Loucks Buren et al.[35] results agreed with the former study. Pro Seal™ reduced enamel demineralization by 92 % and the sealant resisted toothbrush abrasion for 24 months. Other investigations were in agreement with the above findings[36-39].

On the other hand, few studies have found ProSeal™ to be equivalent to a non-fluoride releasing unfilled orthodontic sealant (Transbond MIP, 3M Unitek, Monrovia, Calif) when both were tested in vivo to evaluate white spots inhibition. The authors concluded, "The additional time and expense of using the fluoride-releasing sealant to prevent white spots does not appear to be justified"[40].

Few studies have found Pro Seal™ performance to be inferior to other orthodontic sealants. Fuji Ortho LC showed a significantly smaller lesion depth and less mineral loss compared to ProSeal™ and other bonding systems[41].

ProSeal™ was also evaluated from the standpoint of its other characteristic, namely the effect of ProSeal™ on the bond strength of brackets to enamel. Bishara *et. al.*[42], concluded that the application of ProSeal™ did not affect the sheer bond strength (SBS) of the adhesive used within the first half hour after initial bonding. Also, they found that the SBS was not significantly different whether the ProSeal™ was light cured separately before the application of the adhesive or light cured simultaneously with the adhesive. The mean SBS of their tested groups ranged from 4.0 to 4.9 MPa, which is lower than what is considered to be the minimum acceptable bond strength (8 MPa) for orthodontic bonding[43,44]. In another study[45], the application of ProSeal™, before the adhesive resin, appears to slightly weaken the adhesive-enamel bond compared to traditional orthodontic sealant. Although, the bond strength (above 10 MPa) remained well above the clinically acceptable level. Recently in 2009[46], an in vivo study was conducted comparing the bond failure rate between brackets bonded with conventional sealant and ProSeal™. The results were insignificant and the study concluded that "ProSeal™ did not adversely affect the failure

rate and time of metal brackets when it was used instead of conventional sealant in a composite resin bonding system". Another characteristic of ProSeal™ is its ability to release and recharge fluoride ions. ProSeal™ found, in vitro, to have the ability to release fluoride in a sustained manner but significantly in decreasing amounts. Also, it had the ability to recharge with fluoride ions after it was exposed to fluoridated solution[47]. Analyzing their results carefully, the rate of fluoride-releasing in the first 3 weeks declined from a mean of 0.074 ppm/week/mm^2 to 0.037 ppm/week/mm^2 and, at the end of the 17th week, to 0.01 ppm/week/mm^2. In conclusion, the mean release of fluoride ions drastically decreased within 2 months.

Since the release and clinical success of ProSeal™, other dental manufacturers have prepared similar sealants that can provide long lasting prevention against enamel demineralization. Many of these products have just been released and have not been tested clinically.

Recently, Opal Orthodontics by Ultradent has introduced a new filled orthodontic sealant to the market under the name of "OPAL®SEAL". Opal seal is marketed as having the following characteristics:

- Releases and recharges fluoride.
- 38% filled with substantial glass ionomer plus nano-fillers for long-lasting strength
- Non-yellowing and stain resistant.
- Drying agent seeks out moisture and draws resin in, ensuring superior bonding and fluoride uptake.

- Fluorescent properties make reapplication and removal easy and convenient.

A review of the literature seems to be deficient in studies investigating “OPAL®SEAL” performance. From a clinical standpoint, clinicians might be hesitant to either incorporate or exclude “OPAL®SEAL” in their daily practice until there is research indicating its effectiveness.

We only found one study[48] that included OPAL®SEAL in their tested materials. The main objective was to compare, in vitro, how acid etching, composite resin, and orthodontic brackets influence demineralization of enamel surfaces. A secondary aim was to evaluate whether OPAL®SEAL prevents enamel demineralization. They reported significantly less demineralization of teeth that were sealed.

Objectives and Study hypothesis

The study was designed to compare, in vitro, the efficacy of the orthodontic resin sealants OPAL®SEAL and ProSeal™ on the inhibition of enamel demineralization during orthodontic treatment.

Objectives of this In vitro study were:

- To compare between the integrity of ProSeal™ and OPAL®SEAL after extensive tooth abrasion and thermocycling.
- To compare between ProSeal™ and OPAL®SEAL in inhibiting enamel demineralization and compare them to the untreated control group.
- To investigate if there is any correlation between the integrity of the sealant and resultant enamel demineralization.

The Study Hypotheses:

1. The integrity of ProSeal™ and OPAL®SEAL can be equivalently maintained after extensive tooth brushing (mechanical wear) and thermocycling (temperature fluctuation).

2. OPAL®SEAL and ProSeal™ equivalently inhibit enamel demineralization. Both sealants significantly reduce enamel demineralization when compared to the control group (no sealant).
3. As the integrity of the sealant reduces, the ability of the sealant to inhibit enamel demineralization decreases.

Materials and Methods

Tooth preparation:

Human premolars, that were to be extracted for orthodontic treatment purposes and to be discarded, were collected & stored in a glass container containing normal saline. 0.1% (wt/vol) Thymol crystals was added to the ionized water to inhibit bacterial growth.

The extracted teeth were selected according to the following inclusion criteria:

- Caries & white spot lesions free.
- No restorations.
- No surface fracture or cracks due to the pressure from the extraction forceps.

Both the buccal and lingual surfaces of the premolars' crowns were examined under 10 times magnification for enamel defects or decalcifications. Then, the selected teeth were cleaned of any remaining soft tissue, calculus and bone with a scaler and a razor blade. The roots were amputated 2-4 mm apical to cemento-enamel junction (CEJ) using a bur (# 170 Brasseler carbide bur) on a high speed hand piece under water coolant. The remaining part of the toot was sectioned mesio-distally to provide two specimens. The specimens were kept in deionized water at 37^{o} C throughout the study to keep the specimens moist and prevent desiccation. Each half of the crowns served as a specimen.

Specimens were cleaned with non-fluoridated pumice slurry and rubber prophylactic cups for 3 seconds. All teeth were thoroughly washed and dried. Commercial acid resistant nail

varnish was applied to the specimens to provide approximately 2x5 mm window of exposed enamel surface (figure 3). This enamel window served as the treatment window. Each group of the sample was designated a specific nail varnish color to easily distinguish between the specimens throughout the study.

The specimens were mounted in clear orthodontic acrylic (Densply Caulk, Milford, DE, USA), prepared according to the manufacturer instructions. The specimen's buccal or lingual surface was facing upward. An ice cube-forming mold was used to mount the specimens. Each mold is around 1 inch x 1 inch x1 inch.

Forty-five specimens were randomly assigned to three equal test groups:

Group 1 (n = 15): Served as the control and did not receive any enamel surface treatment.

Specimens in group 2 & 3 (as described below) were etched with 35% phosphoric acid gel (Ultra-Etch, Ultradent products, Inc, South Jordan, UT) for 30 seconds, rinsed with a water spray for 20 seconds and dried with an oil-free air source for 20 seconds until the enamel surface of the etched teeth appeared chalky white.

Group 2 (n = 15): Received ProSeal™ (by Reliance Orthodontic Products, Itasca, IL), according to manufacturer instructions. The sealant was applied in a thin, uniform layer on the etched enamel surface with a brush and then light cured for 20 seconds with a curing light (SmartLite maX LED curing light, Densply, Milford, DE). SmartLite MaX has a high intensity light with an output of at least 1500 mW/cm^2.

Group 3 (n = 15): Received OPAL®SEAL (Opal Orthodontics by Ultradent), according to manufacturer instructions. The sealant was applied in a thin, uniform layer on the etched enamel surface with a brush and then light cured for 20 seconds with a curing light (SmartLite maX LED curing light, Densply, Milford, DE). SmartLite MaX has a high intensity light with an output of at least 1500 mW/cm^2. Because ProSeal™ and OPAL®SEAL have fluorescent particles, the integrity of the applied sealant was confirmed with a black light (ultraviolet light) before proceeding in to the next step. The manufacturer usually supplies the black light in the sealant kit. Any missing areas that are visible by the black light were spot treated with an additional sealant. The integrity of the sealant was assumed 100% for the group an served as a baseline. This baseline was compared to the integrity of the sealant after extensive tooth abrasion and thermocycling.

Extensive tooth abrasion simulation:

A toothpaste slurry was prepared by mixing 9 g of nonfluoridated toothpaste (Crest; Procter and Gamble, Cincinnati, Ohio) and 50 ml of water. Tooth brushing simulation was performed by an automated toothbrush (BRAUN/Oral-B, professional smart series 5000, Germany). The automated toothbrush was stabilized by a surveyor (DENSPLY NEYTECH, Yucaipa, CA) and adjustable ring. The ice cube mold, with the specimens still not separated, was held & stabilized on top of a digital weight scale (Mettler PM2000. Mettler-Toledo, Inc, Columbus, OH). The reading on the scale was zeroed before laying the toothbrush head perpendicular to the treatment window. The automated toothbrush was moved up & down by the adjustable surveyor's arm to maintain a constant range of

pressure (85-100 g)[30]. Adequate toothpaste slurry was added manually using a 10 cm^3 syringe. Each specimen received 1 cm^3 of the toothpaste slurry during the brushing simulation time. The toothbrush head (Oral-B soft brush) was changed after each study group. To simulate a 2-year time span of extensive toothbrush abrasion, each specimen will be brushed for 2 minutes[30,35] to simulate a patient's hygiene maintenance. Previous studies[35] have calculated the average number of brushing strokes; they estimated that 20 strokes per tooth are similar to the daily patient's brushing practice. That means in one year, the tooth is subjected to 7,300 strokes. The automated toothbrush generates around 7,600 oscillating movements per minute. So a minute of brushing is equal to approximately one year of brushing and two minutes of brushing by the automated toothbrush resembles 2-year time span. This calculation is consistent with 15,000 brush strokes that was used by Loucks Buren et al.[35] to represent 2 years of toothbrush abrasion.

Thermocycling:

After the designated time for brushing simulation, the specimens were washed thoroughly from the toothpaste slurry. All specimens were stored in sealed containers containing deionized water at 37°C for thermocycling. The specimens were thermocycled (Thermocycling Test Apparatus, Sabri Dental Enterprises, Downers Grove, IL, USA) at 5°C (41°F) in 30 seconds dwell time and 55°C (131°F) in 30 seconds dwell time with 10 seconds interval between cycles (approximately 7000 cycles)[45,49] to simulate the fluctuating temperature in the oral environment in 2-year time span.

There is a disagreement in the literature with regard to how many exposure cycles occur in the oral cavity in a one-year time span. There is a great variation of regimens in the

literature, making comparison of reports difficult. In 1999[50], a recommendation of 10,000 cycles was suggested after a systematic review of 130 studies of laboratory thermal cycling of teeth. The authors who did the review concluded that "No evidence of the number of cycles likely to be experienced in vivo was found and this requires investigation, but a provisional estimate of approximately 10,000 cycles per year is suggested". Since there was not a convincing basis for choosing the 10,000 cycle number, it was disregarded. In a study[45] that was concerned with testing the effect of ProSeal™ on the bond strength of the brackets to the enamel had used 1,500 cycles. There was no explanation or referencing to support choosing this number of cycles. The frequency of the thermocycling regime proposed in our study is based on an assumption documented in another study[49]. The authors estimated that 10 extreme thermocycles would occur per day. As a result, the 3500 cycles would replicate approximately one year of experience in temperature variation. This thermocycling protocol corresponds well with the experimental designs of previous investigators[51].

Testing the effect of thermocycling is a unique step in our study. None of the previous studies concerning the effect of ProSeal™ on the incidence of enamel demineralization thermocycled their specimens.

Enamel demineralization by acidic challenge:

After thermocycling, each group was placed in a separate glass container. Each group of specimens was submerged in Ten Cate demineralizing solution[52,53] (PH 4.4) consisting of 2.20 mmol/L calcium, 2.20 mmol/L phosphate and 0.05 mol/L acetic acid in one liter of

deionized water for 96 hours[31,35]. The 3 groups were placed in a water bath at 37°C. The PH of the solution was checked daily by PH meter (Orion Dual Star series, Thermo Scientific, USA) and adjusted with NaOH buffer solution if necessary.

Most previous studies with a similar protocol performed the demineralization process at room temperature. To verify if the temperature plays any effect on the demineralization process, a preliminary study was conducted on four specimens without any enamel treatment. Two specimens were submerged in Ten Cate solution at room temperature and another two specimens were submerged in Ten Cate solution at 37°C water bath. The specimens were checked daily for signs of enamel demineralization (White chalky appearance). The specimens at room temperature required 144 hours to initiate the white chalky appearance, while the other two specimens at 37°C required only 96 hours.

After the designated time for the demineralization process, the specimens were washed thoroughly from the Ten Cate solution and stored in deionized water at 37°C.

Testing the specimens:

(1) The overall integrity of the ProSeal™ (group 2) and OPAL®SEAL (group 3) groups:

After mechanical abrasion, thermocyclying and demineralization process, and before specimens sectioning in groups 2 and 3, the integrity of the sealant was examined to identify how well the sealant could withstand mechanical wear & temperature fluctuation in 2 year time span.

The integrity of the sealant was examined by the black light (ultraviolet light) and under polarized light microscopy, at 40 times magnification and compared to the baseline. Areas that lack the fluorescence appearance indicated loss of the sealant. A photomicrography was captured by the computer & saved. Software named OmniMet (Version 9.0, BUEHLER, Lake Bluff, IL) was used to calculate the surface area (in μm^2). The outline of the treatment window served as the baseline assuming the integrity after application of the sealant was ideal and covered 100% of the treatment window (figure 3). As mentioned before, the baseline integrity for the sealant was confirmed by the black light illumination. After mechanical abrasion, thermocyclying and demineralization process, the areas that did not illuminate under the black light was outlined. A calculation of the areas that lost the sealant (in μm^2) was performed by the computer software and converted to a percentage from the overall treatment window (figures 4, 5, 6, 7, 8 and 9). Subsequently, an average percentage of the lost sealant was calculated for each sealant group (ProSeal™ and OPAL®SEAL groups).

(2) Measuring enamel demineralization lesions of all groups:

Each tooth was visually examined for evidence of demineralization (white chalky spots) under polarized light microscope. Then, an ortho resin coat over the treatment windows was added to protect the enamel and any fragile demineralized lesions during the sectioning process afterward.

Demineralization of enamel was measured by calculating the average depth of demineralized lesions resulted on the specimens.

Depth of demineralized lesions was evaluated in longitudinal buccolingual sections. Buccolingual longitudinal sections, of approximately 600µm, were made of each specimen using a low-speed diamond saw (Isomet 1000 Precision saw Buehler; Buehler Ltd., Lake Bluff, IL). The disc (blade) was rotating at speed of 175 rpm and with a 100-gram load under water coolant. Three sections were selected for each specimen. Each specimen's sections were stored separately in a sealed, labeled plastic bag in deionized water. Sections made through the treatment window' edges were excluded due to the fact that the quality of the bond between the sealant and the nail varnish was questionable. Also, any demineralized lesions at the junction between the nail varnish and the sealant were not considered. The sections were dried and placed on histological slides for evaluation under polarized light microscopy using an Olympus SZX16 microscope with a Ueye Series digital camera attached to it. Photomicrographs were made at 0.8 objective lens magnification with maximum illumination. OmniMet (Version 9.0, BUEHLER, Lake Bluff, IL) software was used to analyze the digital photomicrographs. This software projects a reference line on a computer screen. Two lines (L1, L2) perpendicular to the outer surface of the tooth structure were drawn. L1 represented the deepest point of the lesion, while L2 represented the shallowest point of the lesion (Figures 10). An average depth of each section was calculated. Average lesion depth for each specimen and then for each study group were calculated.

For the purpose of assessing the intra-examiner reliability, lesion depth measurement was repeated for three specimens from each study group (nine longitudinal cross-sections in total from each group). At least an interval of 7 days between the first and second

measurements was elapsed. The mean absolute difference between the first and second lesion depth readings was calculated. Then a mean absolute difference for each group was calculated.

The primary outcomes:

1. Percentage of the sealant surface area lost after extensive tooth abrasion & thermocycling for ProSeal™ (group 2) and OPAL®SEAL (group 3) study groups.
2. Average lesion depth for the three study groups.

Data Analysis

A sample size calculation was performed using nQuery Advisor (version 7.0) based on previous similar studies. Assuming an effect size of $\Delta^2 = 3.13$, 15 specimens per group were adequate to achieve Type I error rate of 5% and a power of 99%.

All statistical analyses were performed by a statistical software program (SPSS Version 19, Chicago, Ill). All data were tested for normality (comparing histograms to normal curve); because the data for both outcomes exhibited substantial non-normality, statistical analyses were conducted by non-parametric statistical tests.

Descriptive statistics were compiled from the results of the study. Medians, interquartile ranges, means, standard deviations (SD), minima and maxima were recorded for the first outcome (sealant surface area lost) for the two sealant groups and the second outcome (lesion depth) for the three study groups.

The Mann Whitney U test was used to determine whether there was a significant difference in the sealant lost between the two sealant types. The Mann Whitney U test employed a 0.05 level of statistical significance.

The Kruskal-Wallis test followed by post-hoc Mann Whitney U tests were used to determine whether there was a significant difference in lesion depth between study groups. The Kruskal-Wallis test employed a 0.05 level of statistical significance. Three post-hoc Mann Whitney U tests with Bonferroni correction (at a predetermined significance level of

0.05 / 3 = 0.017) were performed to determine more specifically where significant differences existed between the groups.

Additionally, intra-examiner reliability was evaluated for as was described in the material and methods section.

Correlation coefficient (Spearman's rank correlation coefficient, r_s) for the two outcomes (sealant lost and lesion depth) was assessed for each sealant group, as well as the two sealants groups combined together.

Results

Forty-five non-carious specimens, either buccal or lingual surface from human premolars were randomly selected and included in the study. They were divided into three study groups, comprising 15 specimens per group.

For the first outcome (percentage of the sealant surface area lost after extensive tooth abrasion & thermocycling for ProSeal™ *(group 2) and* OPAL®SEAL *(group 3) groups:*

Descriptive statistics from the results of the study (medians, interquartile ranges, means, standard deviations (SD), minima and maxima) are reported in Table 2.

From Table 2, ProSeal™ group demonstrated a median sealant loss of 0.47%, while the OPAL®SEAL group demonstrated a median sealant loss of 1.03%. Side-by-side box plot demonstrates the distribution of measurements across the two sealant types (Fig 20).

The Mann Whitney U test revealed a non-significant effect for type of sealant on the integrity of the sealant after subjecting the specimens to extensive mechanical wear & temperature fluctuation. The p-value was 0.217 (significance level was predetermined at 0.05). Accordingly, the null hypothesis was not rejected.

For the second outcome (lesion depth for the 3 study groups):

Descriptive statistics from the results of the study (medians, interquartile ranges, means, standard deviations (SD), minima and maxima) are reported in Table 3. Lesions were observed in all 15 samples in the control group. Seven specimens in the ProSeal™ group and ten in the OPAL®SEAL group had demineralization, but eight in the ProSeal™ group and five in the OPAL®SEAL group showed no lesions under the polarized light microscopy. When compared with the controls, substantial reduction in lesion depth was obtained by ProSeal™ and OPAL®SEAL, 92.49% and 84.68% reductions, respectively.

From Table 3, the median lesion depth of OPAL®SEAL group (11.24 µm) and ProSeal™ group (0.00 µm) were far below the control group (95.34 µm). Side-by-side box plot demonstrates the distribution of measurements across the different groups (Fig 21).

The Kruskal-Wallis test revealed a p value < 0.001. Accordingly, the null hypothesis was rejected. Post-hoc Mann Whitney U tests with Bonferroni correction (at a predetermined significance level of 0.017) indicated that the differences observed between the control group and the other two groups (ProSeal™ and OPAL®SEAL) were statistically significant (P-value < 0.001). However, no significant difference was found between ProSeal™ and OPAL®SEAL groups (P-value = 0.202).

The intra-examiner reliability was also assessed. Lesion depth measurement was repeated for three specimens from each study group (nine longitudinal cross-sections in total from each group). At least an interval of 7 days between the first and second measurements was elapsed. The mean absolute difference between the first and second lesion depth readings for the control group was 6.36 µm (SD=3.08), for ProSeal™ group was 2.40 µm(SD=1.33)

and for OPAL®SEAL group was 4.22 µm (SD=3.75). The overall mean absolute difference in lesion depth between the first and second measurements for the nine specimens was 4.33 µm (SD=1.98).

Spearman's rank correlation coefficient (r_s) between the two outcomes (sealant lost and lesion depth) was calculated. A positive correlation ($r_s = 0.537$, $P = 0.002$) was found when the two sealant groups (ProSeal™ and OPAL®SEAL) were combined. As shown in Table 4, this correlation was statistically significant. Also, a highly significant correlation coefficient ($r_s = 0.866$, $P< 0.001$) between the two outcomes was found for the ProSeal™ group alone. Spearman's test failed to show a statistical significant correlation between the two outcomes in the OPAL®SEAL group ($r_s = 0.153$, $P = 0.587$). Scatterplots in figures 22, 23 and 24 demonstrate the above.

Discussion

The goal of this study was to compare, *in vitro*, the effectiveness of highly filled resin sealants ProSeal™ and OPAL®SEAL on the inhibition of enamel demineralization after subjection to extensive mechanical toothbrush abrasion and thermocycling. Forty-five non-carious specimens, either buccal or lingual surface from human premolars were used in the study. Fifteen teeth were used in each of the three groups.

A procedure that can inhibit enamel demineralization under and around orthodontic appliances, independent of patient's compliance, would be highly valuable for clinical orthodontics. Both the thickness and abrasion resistance of the sealant affect the duration of protection. Previous studies have reported that chemically cured sealants did not polymerize completely due to the oxygen inhibition of the reaction[29,54] and subsequently did not last long enough. Light cured sealants solve the problem of incomplete polymerization, and some in vitro studies[29,55,56] showed that these sealants reduced enamel demineralization but most failed in clinical trials due to poor mechanical abrasion resistance. The poor mechanical abrasion resistance is related to the filler content. Most likely, the tested sealants were unfilled or lightly filled that could not withstand the mechanical abrasion long enough. This is why; many investigators[29,55] have suggested adding more filler particles to the orthodontic sealants to make them more resistant to mechanical wear. Many studies[29,34,55,56] have found no significant difference between enamel demineralization rates of the unfilled sealant group or the control group. It can be explained that, once the sealant wears off, the enamel is exposed to acid and

demineralization could develop. Since a fundamental requirement for a long lasting orthodontic sealant is to be a highly filled, that has been proven by previous studies, highly filled or unfilled sealant was not among our comparison groups.

The literature was found deficient in studies testing the performance of OPAL®SEAL in inhibiting enamel demineralization. However, the literature is rich in studies concerned with laboratory and clinical performance of ProSeal™. Hence, our results will be compared against studies that investigated the effectiveness of ProSeal™ on enamel demineralization. The solo OPAL®SEAL study by Hess[48] that tested the effects of phosphoric acid etching, metal brackets and composite resin adhesives on enamel demineralization, found OPAL®SEAL to be effective in reducing enamel demineralization. They used Scanning electron photomicrographs (SEMs) to qualitatively evaluate the enamel surface and DIAGNOdent laser fluorescence to quantitatively evaluate enamel demineralization. This study used OPAL®SEAL as a sealing agent for half of the buccal surface to serve as the baseline and compare it to the other half that received a different treatment. It was not the primary intention of the study to test the effect of this particular orthodontic sealant on enamel demineralization. Although our results are in agreement with their conclusion, we considerably differ in the procedure of the experiment. In our study, OPAL®SEAL reduced enamel demineralization by 85% compared to the unsealed teeth. While in Hess's study, sealed teeth had 5% less demineralization than the unsealed teeth.

In our study we used a quantitative method to assess and measure enamel demineralized lesions (lesion depth) by polarized light microscopy. The major benefit with polarized light

microscopy is that it allows both qualitative and quantitative evaluation due to its ease of visualization of the color spectrum (Hicks, 1981). Our methodology was in consistent with previous studies that tested the ability of various orthodontic products to inhibit enamel demineralization[35,38,41] by measuring the lesions depth under polarized light microscopy. Enamel demineralization or white spots can be evaluated in vitro by either qualitative or quantitative methods[57]. Qualitative methods include evaluating the lesions by polarized light microscopy and scanning electron microscopy. Quantitative methods include polarized light microscopy, transversal microtomography, cross-sectional microhardness analysis, confocal laser scanning microscopy, DIAGNOdent laser fluorescence and polarization sensitive optical coherence tomography. Among all the methods, transversal microtomography is considered the most accurate and sensitive method. That is why microtomography is the golden standard method to assess enamel demineralization and remineralization in vitro studies[58]. This method requires special expensive equipments not readily available to all researchers. Investigators[58,59] found that microhardness analysis method is an accurate assessment of enamel demineralization, provided that sufficient numbers of samples are used for comparison. Other authors reported[60] that there is a statistical significant correlation between the transversal microtomography and the confocal microscopy methods.

The first hypothesis of this study was that OPAL®SEAL and ProSeal™ equivalently inhibit enamel demineralization. The statistical significant of the results indicates that the two groups with orthodontic sealants demonstrated much less demineralization than the group without orthodontic sealant.

This study showed results that were comparable with similar studies. Hu and Featherstone in 2005[34] showed significant decrease in enamel demineralization on teeth treated with ProSeal™. This later study used enamel microhardness profiles to evaluate enamel demineralization. Microhardness technique measures the mineral content and compares it to that of intact enamel. Teeth treated with ProSeal™ demonstrated superior profiles (similar to intact enamel) than those treated with a fluoride varnish, etchant only, or an unfilled resin. We used polarized light microscopy to directly measure the demineralized lesions. They used 14 days of acidic challenge cycles instead of one acidic challenge. Mechanical wear abrasion was similar to our protocol. Their results seem similar to our study, although the two studies' methodologies were different.

Chong et al.[39] compared between two tools to evaluate enamel demineralization in vitro, the nondestructive method by using polarization-sensitive optical coherence tomography (PSOCT) and polarized light microscopy to measure lesion depth in cross-sections. Also, they tested the efficacy of a fluoride-releasing adhesive (glass ionomer), a fluoride sealant (ProSeal™), and fluoride in solution in inhibiting demineralization around brackets. They found that the two methods were equally comparable and ProSeal™ significantly reduced enamel demineralization.

Salar et al., in 2007, [37] was interested in evaluating the effect of fluoride in ProSeal™ on the adjacent unsealed enamel. They evaluated the effect of fluoride indirectly. Rather than quantifying fluoride ions discharge, they examined the lesion depth in the enamel adjacent

to ProSeal™. We were interested to examine the enamel underneath ProSeal™, mainly testing the benefit of the physical barrier that is provided by the sealant. The investigators restored 1.5 mm cavity preparations on the buccal surfaces with a conventional non-fluoride releasing sealant or fluoride releasing sealant (ProSeal™) or glass ionomer sealant. The entire cavity preparation was filled with the sealant, without any restorative material. The area of interest was the 1 mm rim around the cavity preparations. The design of the study has few weaknesses. First, the authors did not follow the manufacturer recommendation. These sealants were not manufactured for restorative purposes and should not be applied in thick layers. Second, the area of interest was not directly protected by the sealant but adjacent to the sealant. The primary line of action for ProSeal™ is to provide a physical barrier between enamel and surrounding environment. All other benefits as fluoride release are secondary benefits. The authors reported the greatest lesions depth in the conventional non-fluoride releasing sealant, followed by ProSeal™ then glass ionomer sealant. Since there is a fundamental difference between Salar's methodology and ours, there results could not be compared.

Our study shares many similarities with Loucks Buren et al[35] study. In both studies, the specimens were subjected to extensive mechanical abrasion and a single acidic challenge as recommended by Ten Cate[52,53]. Enamel demineralization was assessed by measuring the lesion depths under polarized light microscopy. Loucks Buren evaluated the effectiveness of ProSeal™, on enamel demineralization and compared it to untreated teeth, fluoride varnish (Fluor Protector) and an unfilled resin sealant (Delton). They reported a similar reduction in lesion depth by 92% compared to the controls.

Paschos et al.[41] examined, in vitro, Transbond Plus SEP (self etching primer), ProSeal™, Clearfil Protect Bond SEP, Light Bond (lightly filled sealant) and Ortho Conditioner and Fuji Ortho LC (resin modified glass ionomer). Enamel demineralization was evaluated by commercial cone-beam microtomography (calculating relative mineral loss) and polarized light microscopy (measuring lesion depth). They found that Fuji Ortho LC was the most effective material in inhibiting enamel demineralization. Fuji Ortho LC contains a unique antibacterial monomer. ProSeal™ was found statistically insignificant compared to the control group. It should be pointed out that in this particular study, ProSeal™ was applied only to the area underneath the bracket and not as recommended by the manufacturer to cover the entire facial surface. That justified their results, the benefit of having a physical barrier between enamel and the acidic challenge was not provided by ProSeal™. Our results could not be compared with Paschos's study. A crucial difference existed in the methodology.

Pratt et al.[38] Compared between ProSeal™ and Light Bond, both are fluoride releasing sealants. They used polarized light microscopy to measure lesions depth that were created artificially in vitro. Half of ProSeal™ and Light Bond samples were exposed to 0.05% NaF solution. They reported that ProSeal™ and Light Bond reduced lesions depth by 38% and 41% respectively and even more, 53% and 55% respectively, when combined with fluoride solution.

Behnan et al.[36] tested the ability of 4 different products (amorphous calcium phosphate

containing cement, MI paste plus, fluoride varnish and ProSeal™) to prevent enamel demineralization. The authors assessed enamel demineralization by measuring two outcomes, fluorescence loss (using light-induced fluorescence) and lesion depth (using confocal laser scanning microscopy). They found that ProSeal™ and fluoride varnish (Vanish) prevented enamel demineralization in 100% of the samples. Amorphous calcium phosphate containing cement and MI paste plus were statistically not different than the control group. Although their methodology to measure enamel demineralization is different than our study, the results regarding the effect of orthodontic sealant is in agreement.

The second hypothesis of this study was that the integrity of ProSeal™ and OPAL®SEAL can be equivalently maintained after extensive tooth brushing (mechanical wear) and thermocycling (temperature fluctuation). It has been shown in this in vitro study that there was no statistical difference in the percentage of the sealant loss between these two sealed groups. No previous studies investigated the integrity of any orthodontic sealant. For this reason, we could not compare our results to any other studies.

The use of toothbrushing to simulate mechanical wear in vitro is a common method to assess wear of dental materials. In our study, an automated toothbrush (BRAUN/Oral-B, professional smart series 5000, Germany) and nonfluoridated toothpaste were used to simulate abrasion by everyday toothbrushing. This automated toothbrush generate 7,600 oscillations / minute. The hypothesis was that 20 strokes per smooth surface per day would be considered good oral hygiene practice for orthodontic patients[30,35]. At an average treatment time of 2 years, 15,000 strokes of tooth brushing would be equivalent to a 2-year

of toothbrush abrasion in vivo.

A unique aspect in our methodology is the thermocycling of the specimens. Thermocyclying is used in laboratory studies to simulate the temperature fluctuation that routinely occurs in the oral cavity. Tooth structures as well as all kind of dental materials undergo dimensional changes on heating and cooling[61]. This dimensional change is measured by coefficient of thermal expansion (CTE). The coefficient of thermal expansion is defined as "a measure of the dimensional change on heating or cooling, expressed as length change per degree of temperature change"[61]. Since both ProSeal™ and OPAL®SEAL are resin based sealants, it is expected that both experience thermal dimensional changes with temperature fluctuation[61]. The issue lies when the dental material has different CTE than the tooth structures. This can result in microleakage and tensile stresses across the tooth structures. The major factor that affects the CTE is the filler content[61]. Higher filler content results in less contraction / expansion[62]. Both tested sealants are highly filled sealants. We expect less dimensional changes if they were to be compared to unfilled or lightly filled sealants. There were no previous in vitro studies, that evaluated the effect of orthodontic sealants on enamel demineralization, have subjected the teeth to thermocyclying. Our results indicate that both sealants withstood the thermocycling well. The enamel demineralization that resulted in the sealant groups might have resulted in areas that were affected by this phenomenon and resulted in a break in the sealant integrity. Despite any side effect of thermocycling that might have happened, it did not affect the overall performance of either sealant. Both sealants provided a significant protection for the enamel.

The result of this study showed that the median demineralization lesion depth as well as the median percentage sealant loss in the ProSeal™ group is slightly less than the OPAL®SEAL group, although the difference is statistically insignificant. That could be explained by an observed phenomenon during the conduct of the study. When the specimens were examined under the polarized light microscope, before sectioning, it was noticed that OPAL®SEAL specimens exhibited more air bubbles/voids and cracks/craze lines than the ProSeal™ group. It is suggested that the voids were trapped during the application of the sealant by the microbrush. The voids were sites for unsupported sealant and considered potential areas for wear, leading to a break in the sealant integrity. We observed that most of the areas that lost the sealants were perfectly round, this correlate well to the shape of air bubbles. We suggest for the manufacturer to revise the design of the material's dispenser to minimize the trapping of air bubbles during application. While, the cracks have resulted from the contraction and expansion behavior of the resin when subjected to sudden temperature changes during thermocycling "thermal expansion". It is well understood the fact that resin undergoes dimensional changes when subjected to temperature fluctuation[61]. Another possible cause could be due to the polymerization shrinkage that occurs during the setting of any resin like material. The two tested orthodontic resin sealants are highly filled. Less dimensional changes expected to happen compared to unfilled or lightly filled sealants, as polymerization shrinkage decreases when the filler content increases[62]. The above mentioned phenomena were only an observation that were not quantified or evaluated in this study. Our results suggested that this observation had no significant effect on the performance of either sealant. Pro seal and

Opal seal provided 92.49% and 84.68% reduction in lesion depth compared with the controls. Further investigation is recommended to better understand the effect on the clinical outcome.

Another observation, from the handling standpoint of ProSeal™ and OPAL®SEAL, was that the difference in the flowability and consistency of the two materials. ProSeal™ showed an even, shiny and smooth glassy surface. OPAL®SEAL showed dull and opaque surface. The microbrush bristles marks were visible in the OPAL®SEAL specimens. We suggest that the difference might be related to the filler particle size. Although we do not know for sure if these differences have any significant consequences on the performance of the sealant, our results suggest that there isn't any.

Recently, there has been a greater than before interest in calcium phosphate based remineralization technology. The incorporation of amorphous calcium phosphate (ACP) in to orthodontic resin bonding materials claims to prevent and reverse white spots. The acidic environment triggers the release of calcium and phosphate from the cement to remineralize enamel. Another material, MI paste plus, casein phosphopeptide-ACP (CPP-ACP), offers a similar line of defense against enamel demineralization/white spots and promote remineralization of existing white spots. MI paste plus contains casein phosphopeptide-ACP (CPP-ACP). In randomized clinical trial, MI paste plus was found to reduce enamel decalcification index scores by 53.5%. MI paste plus not only prevented the formation of new white spots but also found to decrease the number of white spot lesions already present[63]. From this standpoint, the low possibility of enamel demineralization that might

occur with the use of enamel sealants, and if it is clinically visible, can be taking care of with this line of non-invasive measure. On the other hand, MI paste plus failed to prevent enamel demineralization around orthodontic brackets when it was applied daily for 15 days in laboratory testing[36]. Research indicates that MI paste plus is a promising material for remineralization rather than demineralization prevention tool.

A substantial positive linear correlation between the integrity of the sealant and lesion depth was found in Pro Seal™ group and a weak correlation was found in OPAL®SEAL group. The strong correlation that was found for ProSeal™ group indicates that as the percentage of the sealant loss increases, the lesion depth for that particular specimen increases as well. In another word, the isolated areas that had "breaks" in the sealant layer, lead to the associated demineralization. The weak correlation in the OPAL®SEAL group may be explained based on the findings of Davidson et al.[64] who suggested that properly sealed enamel exerts cariostatic effect for up to two years after the placement of the resin. Even with some mechanical removal of sealant, the remaining surface of enamel was resistant to demineralization as long as the resin tags were present, and the resistance of this sealed enamel to demineralizing factors is superior to that of sound enamel. Resin tags extend to a depth of 25-50 µm into the etched enamel[33]. Whether the presence of these resin tags inside enamel rods has any adverse effect is unknown. Resin tags might interfere with the diffusion of the bleaching material through the enamel rods.

Conclusion and clinical considerations

With the limitations of the study and based on its results, we conclude that

(1) The integrity of the two orthodontic sealants studied was well maintained after extensive mechanical wear simulation & temperature fluctuation, and were not significantly different. Both sealants were expected to have adequate integrity throughout the average orthodontic treatment time.

(2) The application of orthodontic sealants over the facial surfaces of teeth reduces enamel lesion depth significantly when compared to non-sealed teeth.

(3) The lesion depth in teeth treated with ProSeal™ and OPAL®SEAL were not significantly different,

(4) This study could not identify the exact correlation between the sealant integrity and the resultant enamel demineralization.

Based on the results of this study, we recommend the routine use of highly filled orthodontic sealants in the orthodontic patients. The added cost & extra chair side time are well justified over the benefits generated from the application of those sealants.

Limitations:

This study provides a basic foundation about the performance of a new highly filled orthodontic sealant “OPAL®SEAL”. OPAL®SEAL Seems to be a promising solution for the most commonly encountered problem in clinical orthodontics. At this point we must be conservative in generalizing the results and expanding it to the real clinical practice.

(1) This study is an in vitro investigation with inherited limitations. Future research should attempt to determine clinically whether OPAL®SEAL can prevent or decrease the incidence of white spots formation and resist mechanical and chemical wear in randomized clinical trials.

(2) Some factors of the oral environment can not be simulated in vitro. Factors such as drink, diet and acidity might discolor the orthodontic sealant and affect its appearance.

(3) The relative small sample size could not show exactly the pattern of correlation between the sealant integrity and extend of enamel demineralization. Although, the sample size was adequate to test the effectiveness of the sealants on enamel demineralization based on previous studies.

Future suggestions:

(1) Other aspects of OPAL®SEAL such as fluoride discharge and recharge, and the effect on brackets bond need to be investigated.

(2) The performance and benefits of OPAL®SEAL from a clinical standpoint must be carried out in clinical trials.

(3) The effect of the remaining resin tags after removal of the sealant at the end of the orthodontic treatment should be explored. A negative impact on bleaching outcome can be

identified.

(4) The effect of the mechanical removal of the sealant, as recommended by the manufacturer, on the enamel surface at the end of the orthodontic treatment must be considered and studied in future research.

(5) A larger sample size could help to show if there is any correlation between sealant integrity and the resultant enamel demineralization.

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Figures:

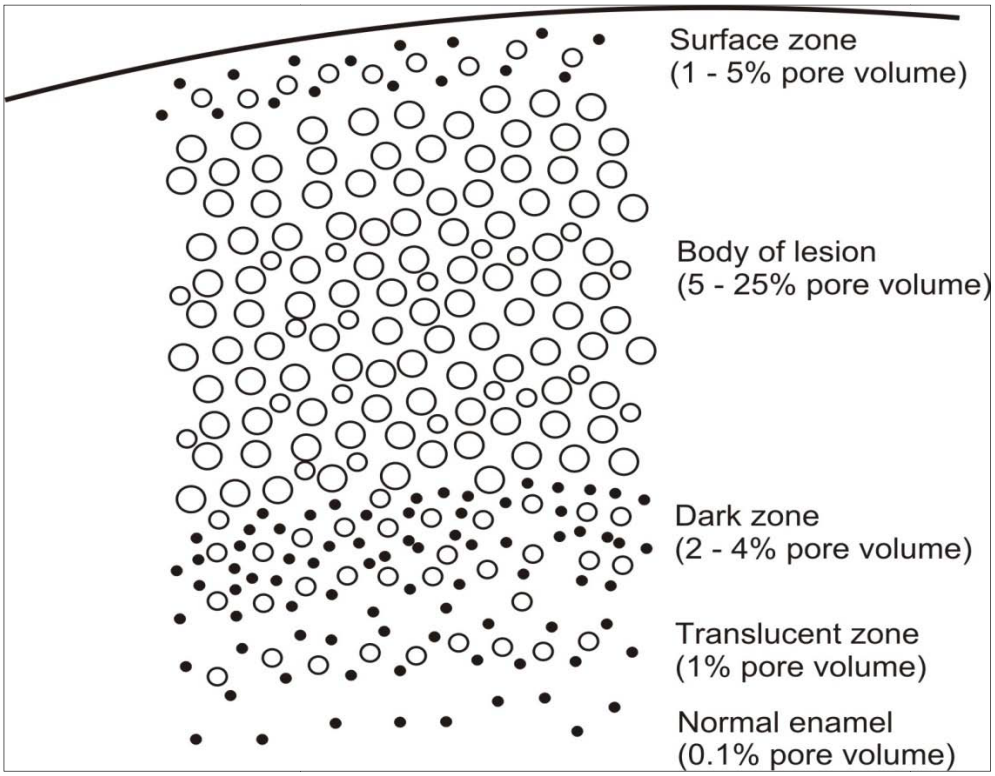

Figure 1: The four zones of incipient lesion

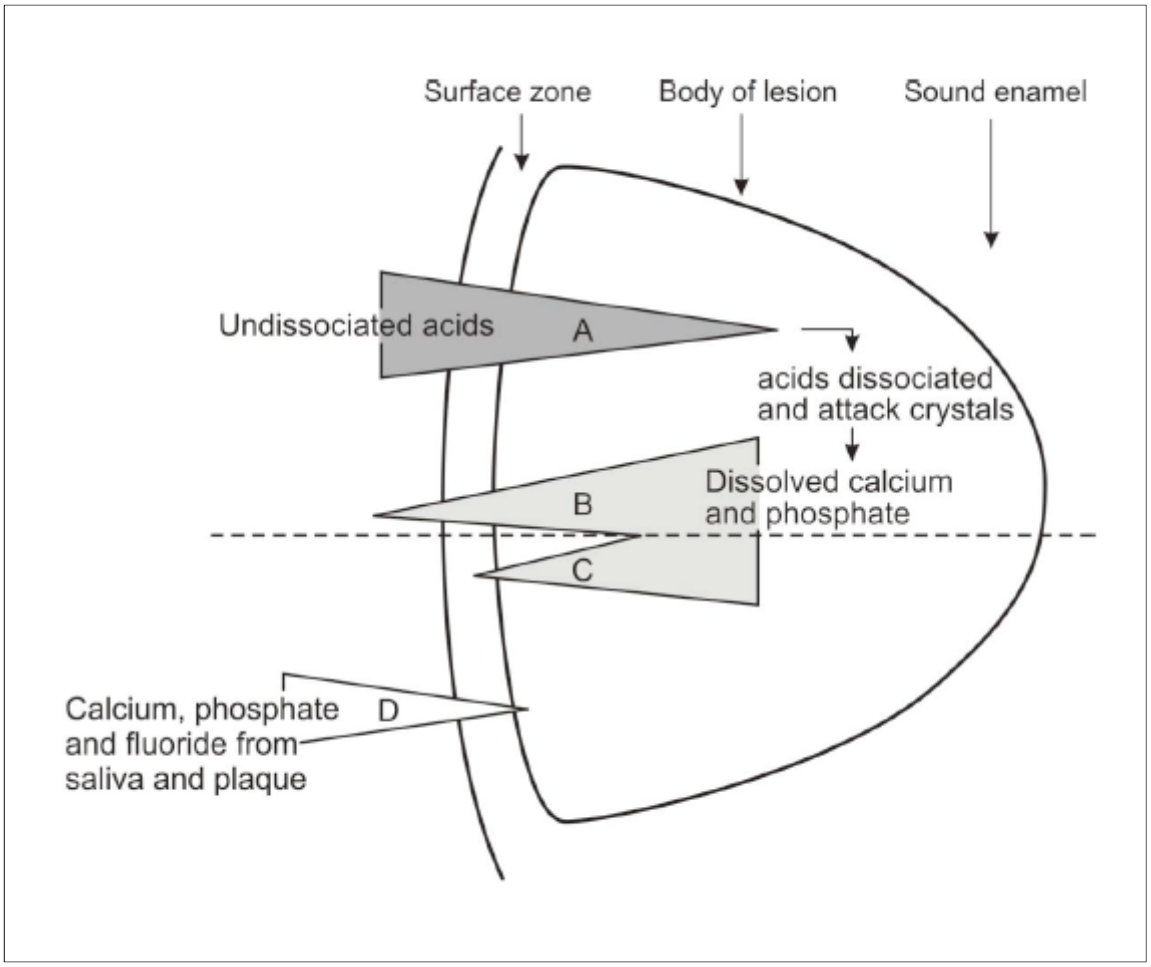

Figure 2: Diagram representing the processes of demineralization and remineralization (Harris and Christen, 1995).

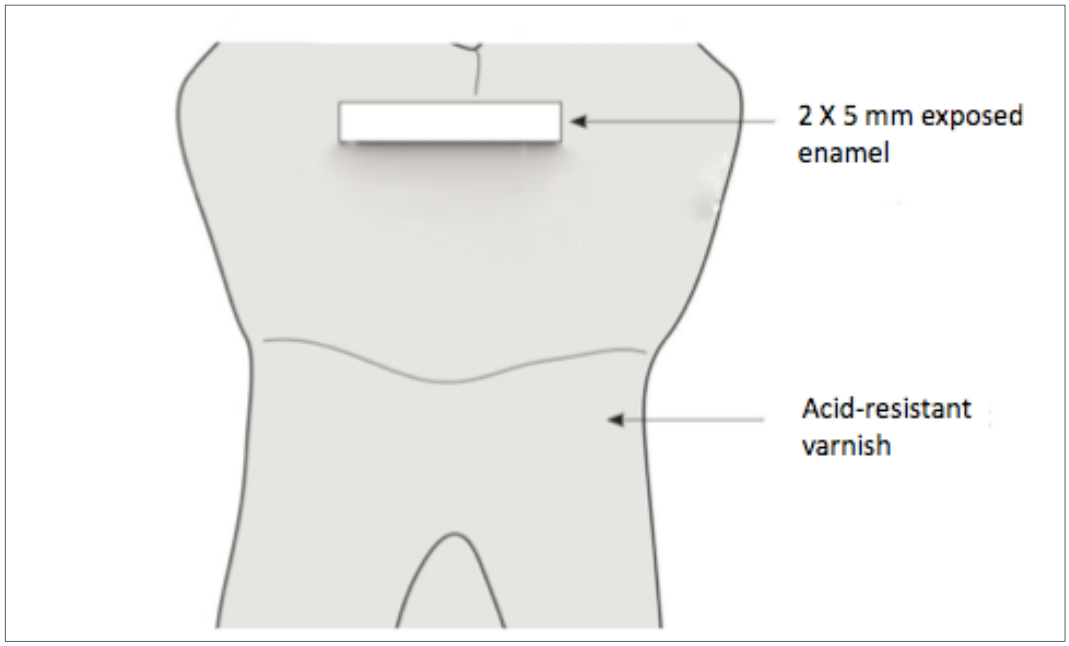

Figure 3: Diagram showing the treatment window in specimens.

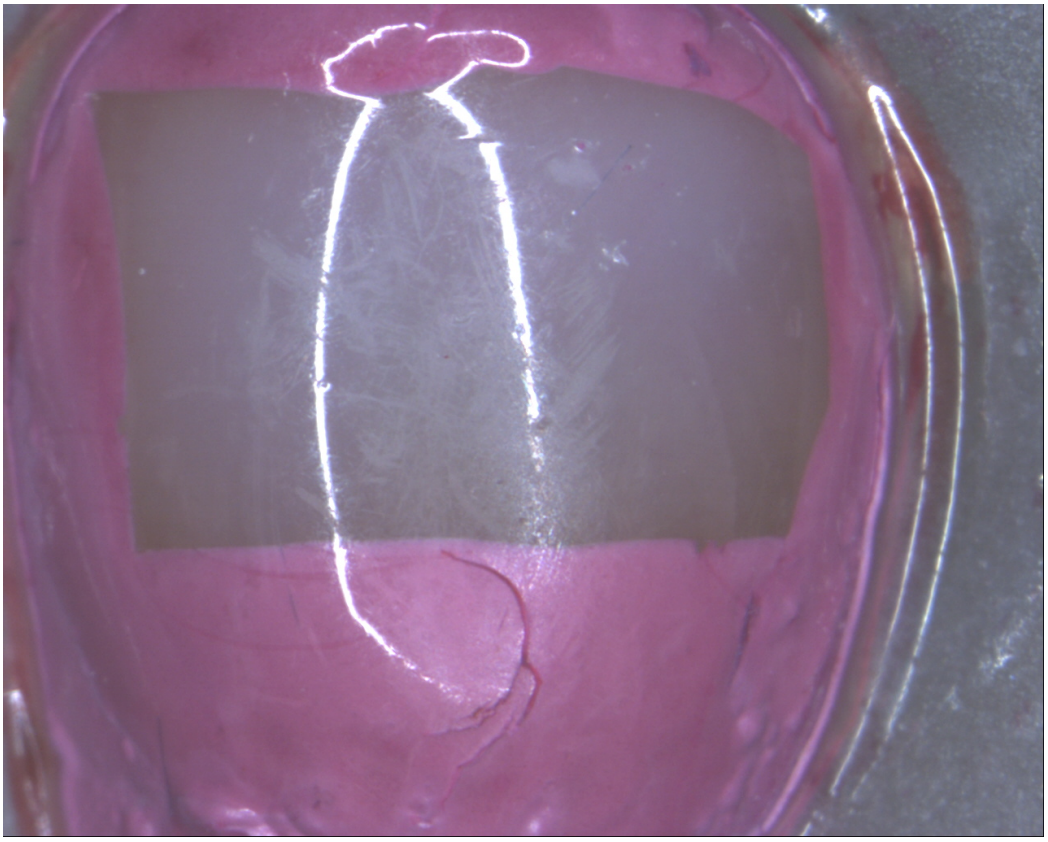

Figure 4: Treatment window of a sample from ProSeal™ group.

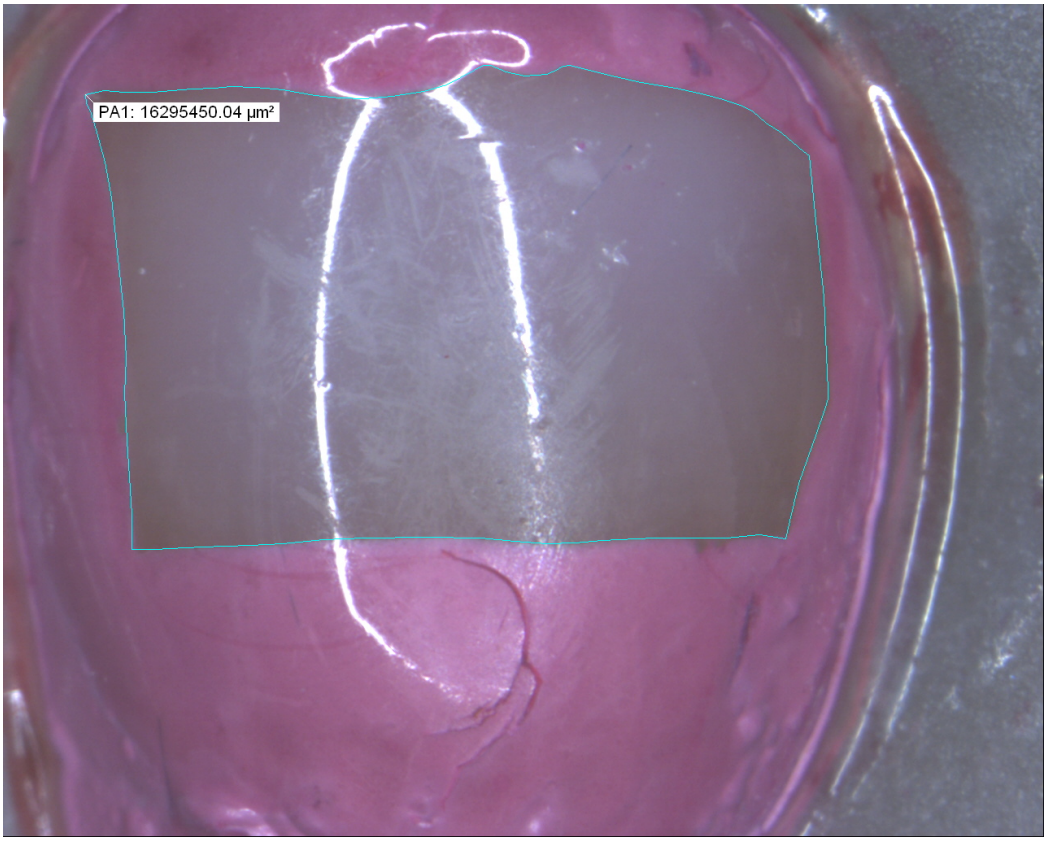

Figure 5: Treatment window of a sample from ProSeal™ group outlined by the computer software and the total surface area calculated.

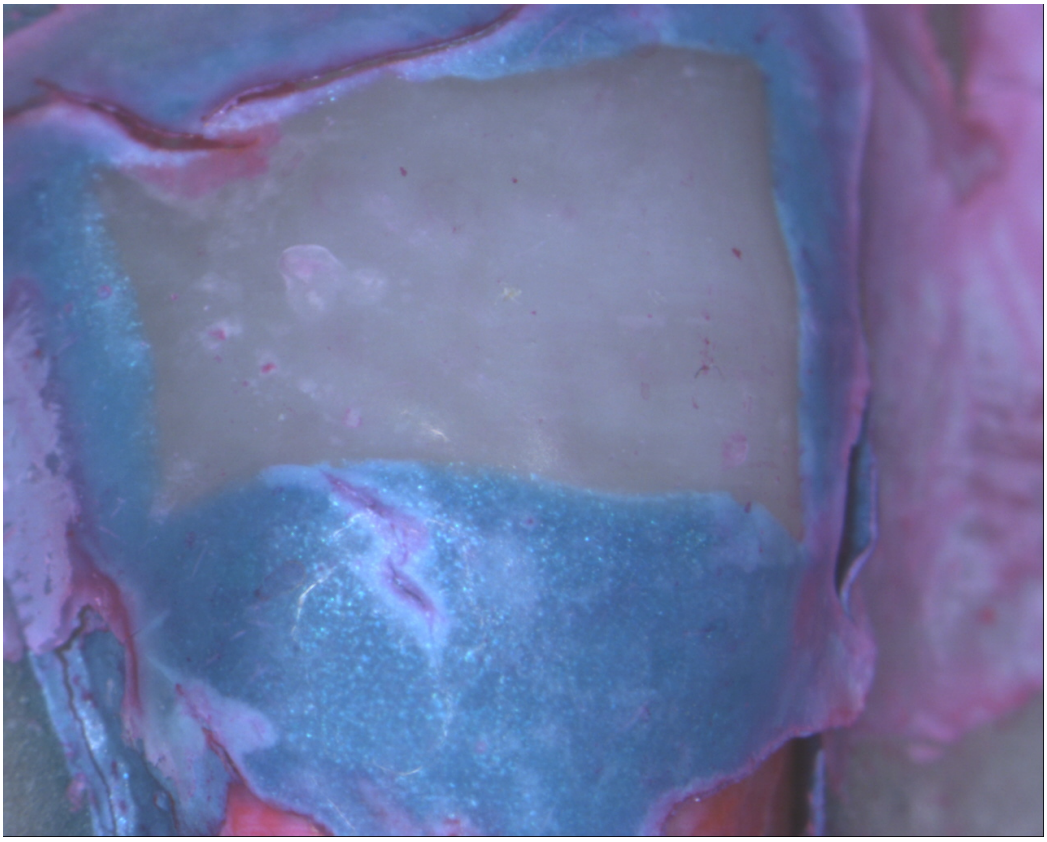

Figure 6: Treatment window of a sample from OPAL®SEAL group.

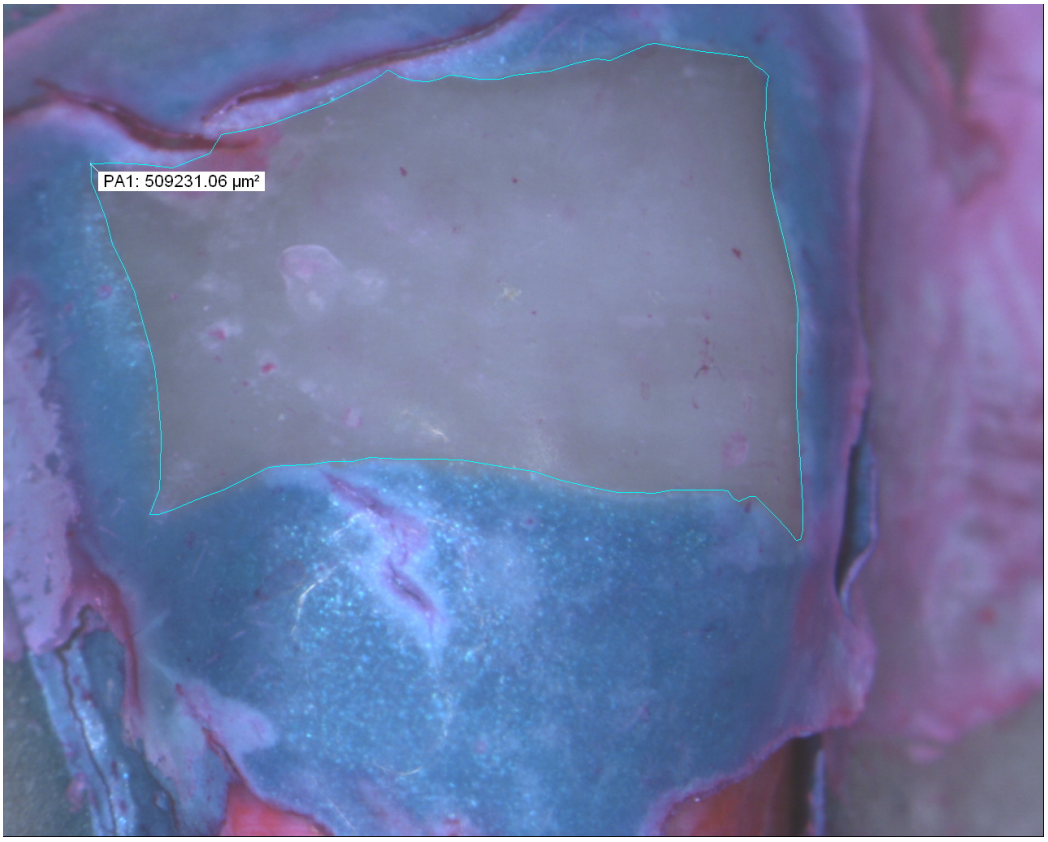

Figure 7: Treatment window of a sample from OPAL®SEAL group outlined by the computer software and the total surface area calculated.

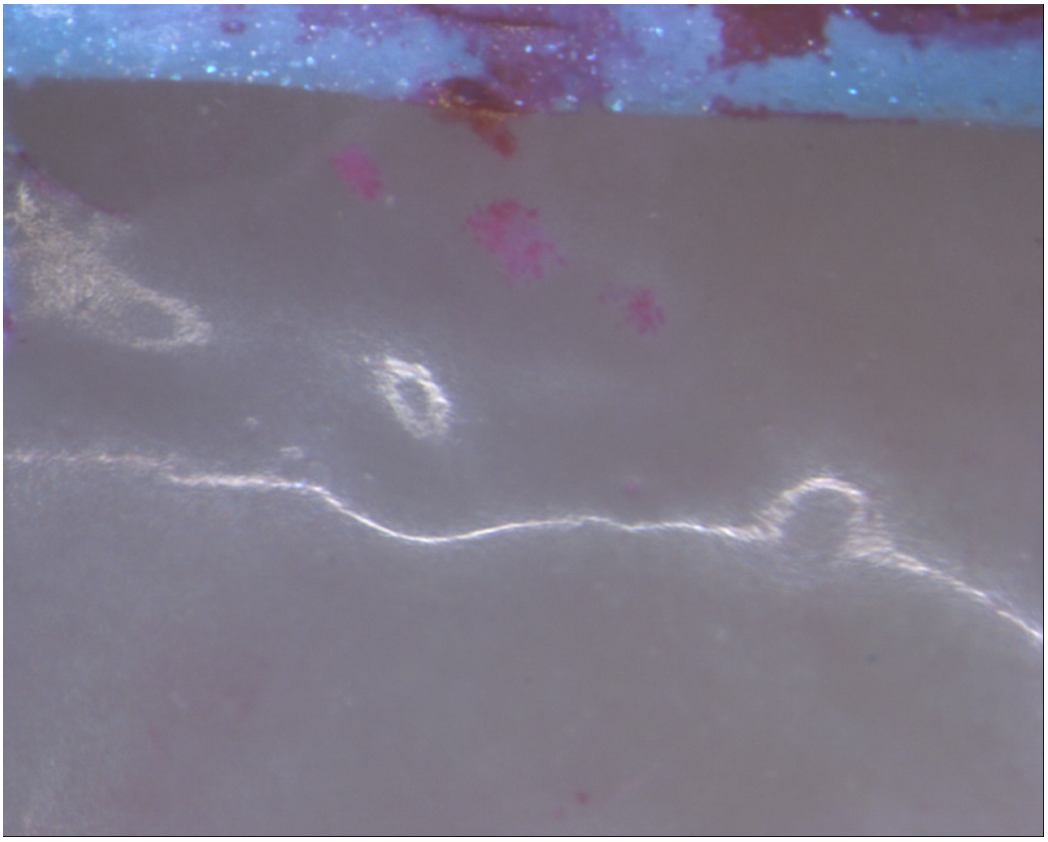

Figure 8: Areas of lost sealants in a sample from the OPAL®SEAL group under polarized light microscopy magnification.

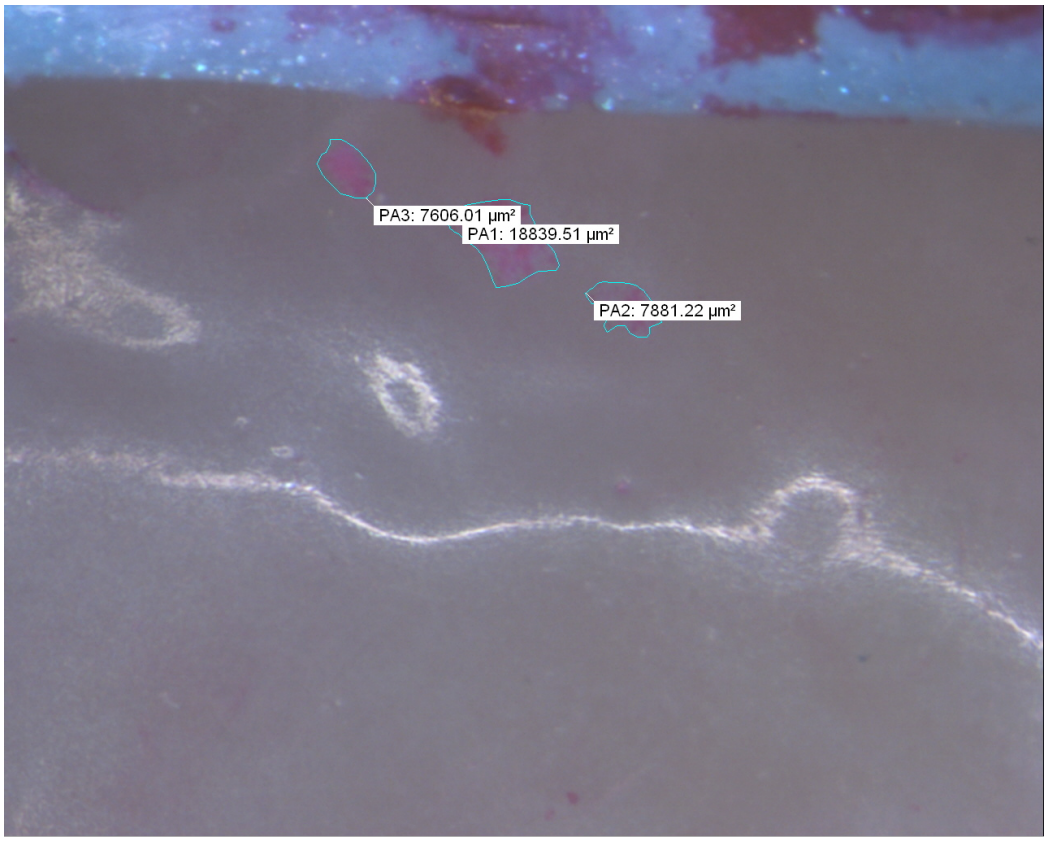

Figure 9: Areas of lost sealant outlined and calculated in a sample from the OPAL®SEAL group by the computer software under polarized light microscopy magnification.

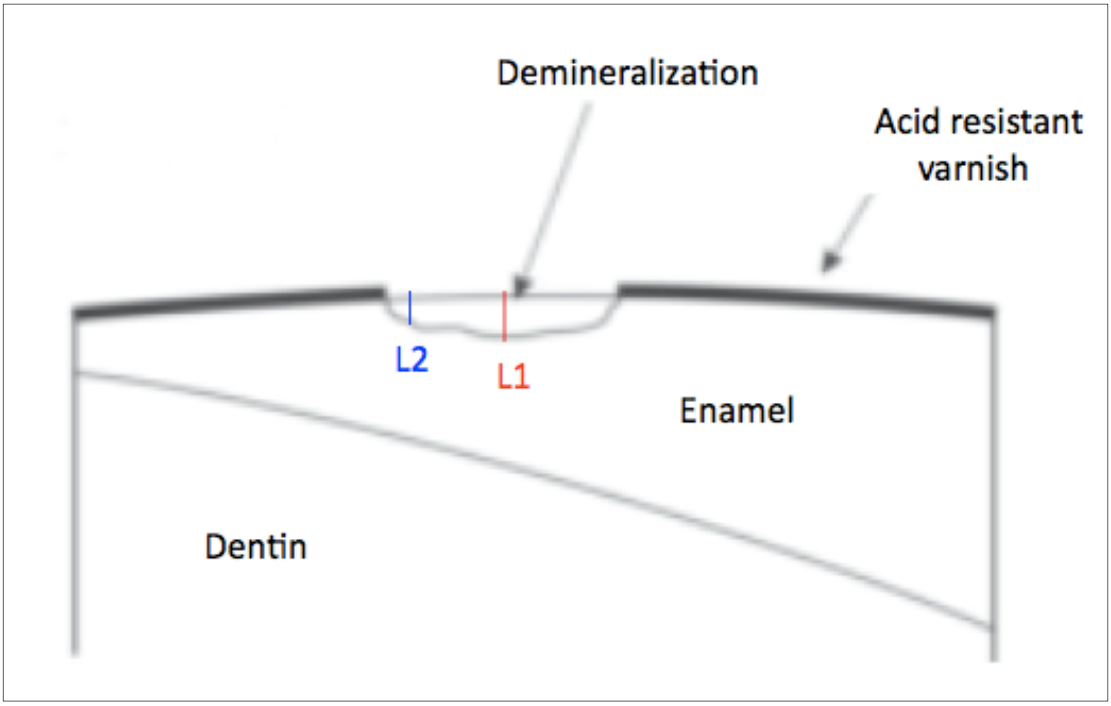

Figure 10: Diagram showing how the deepest (L1) and shallowest (L2) lesion depths are measured in the longitudinal cross-sections.

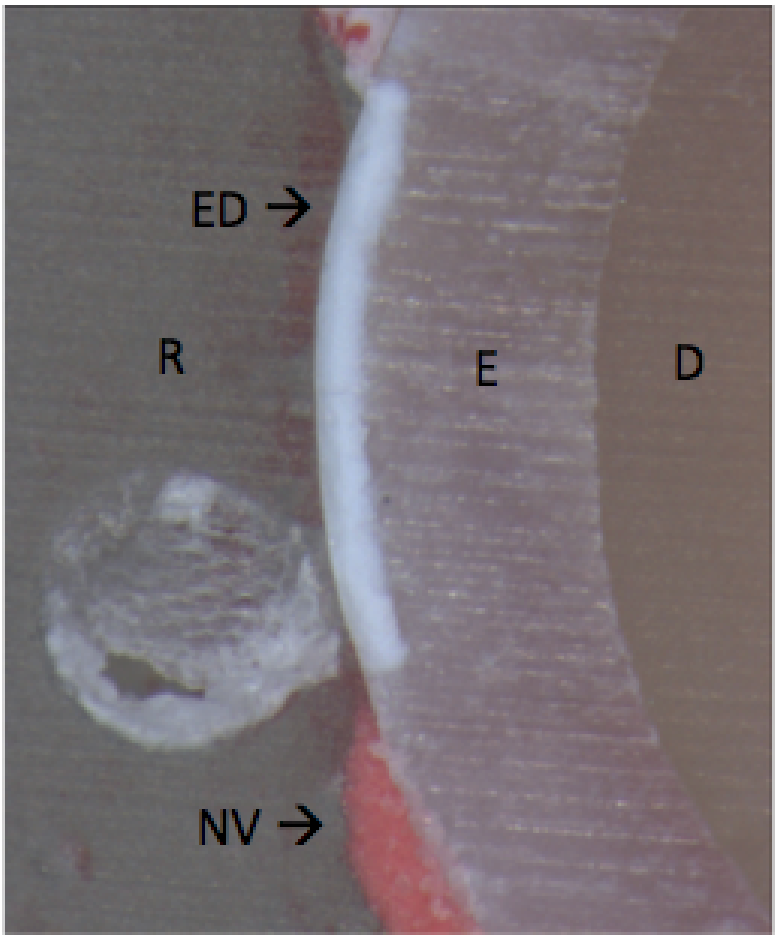

Figure 11: Polarized light microscopy image of a cross-section from the control group showing demineralization (R: orthodontic resin, ED: enamel demineralization, NV: nail varnish, E: enamel, D: dentin).

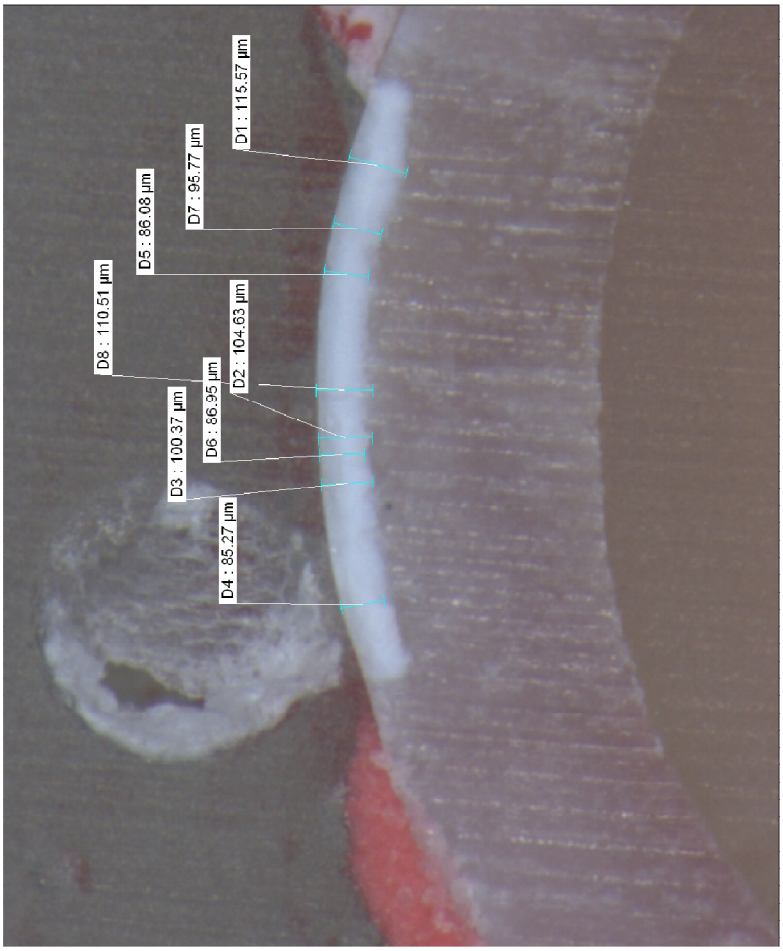

Figure 12: Polarized light microscopy image of a cross-section from the control group showing lesion depth measurements

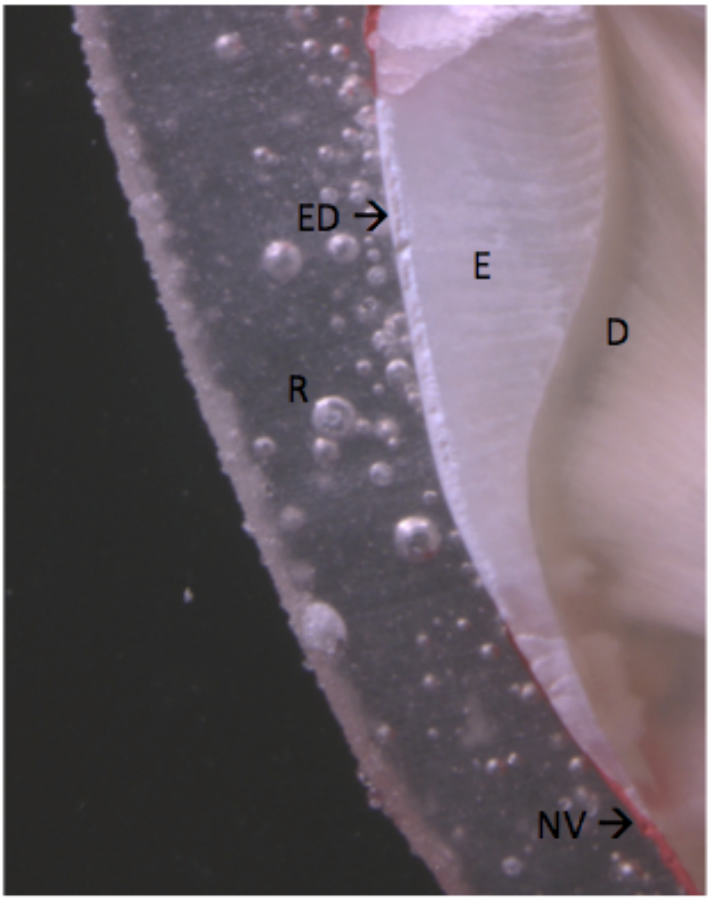

Figure 13: Polarized light microscopy image of a cross-section from the control group (using different power of illumination and filters for enhanced view of the demineralized lesion). (R: orthodontic resin, ED: enamel demineralization, NV: nail varnish, E: enamel, D: dentin).

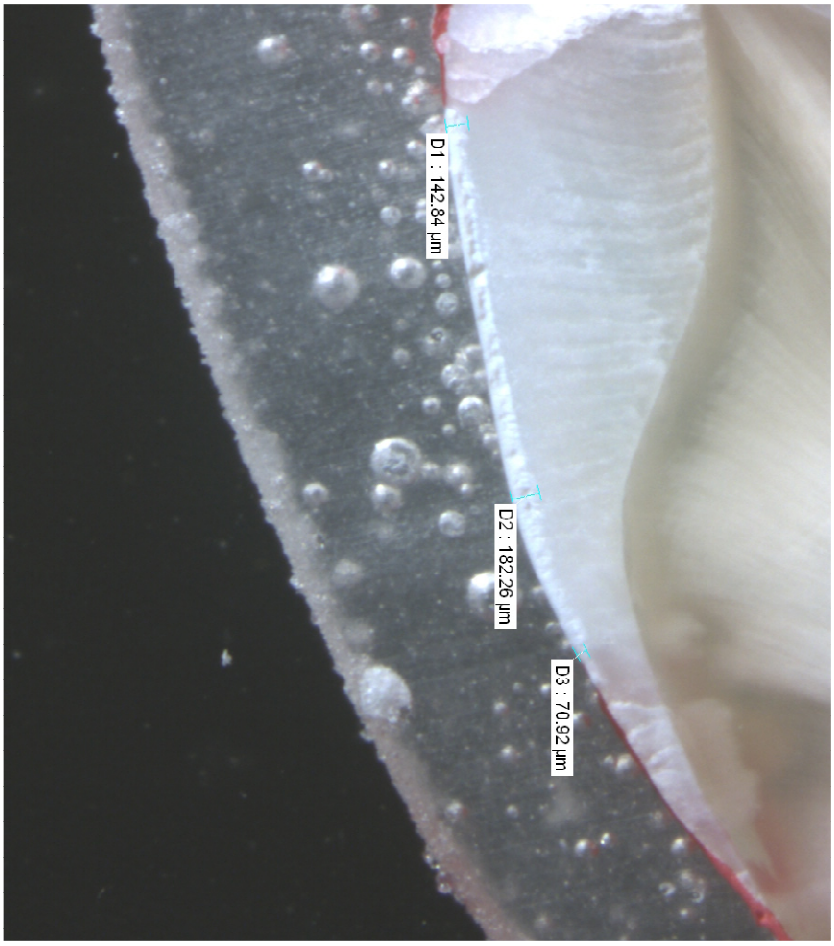

Figure 14: Same cross-section as in figure 13 from the control group showing lesion depth measurements (using different power of illumination and filters for enhanced view of the demineralized lesion).

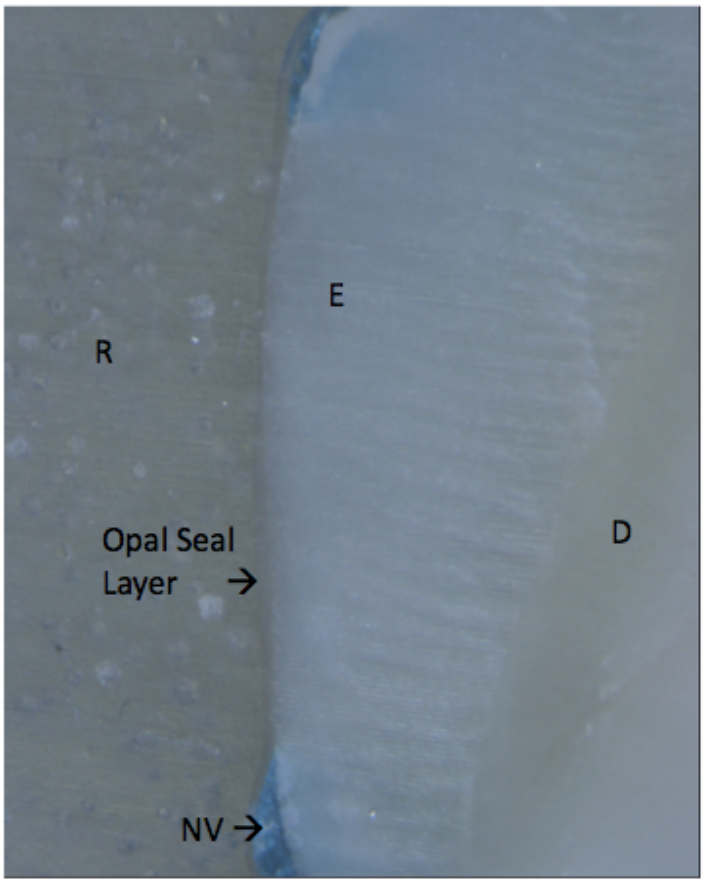

Figure 15: Polarized light microscopy image of a cross-section from the OPAL®SEAL group showing intact sealant layer over enamel (R: orthodontic resin, NV: nail varnish, E: enamel, D: dentin).

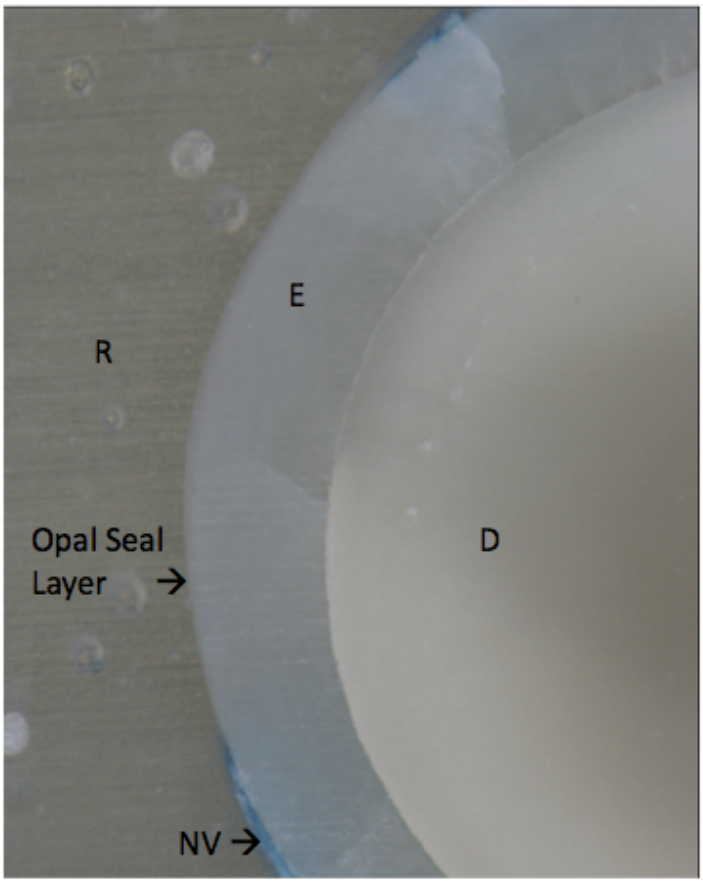

Figure 16: Polarized light microscopy image of a cross-section from the OPAL®SEAL group showing intact sealant layer over enamel (R: orthodontic resin, NV: nail varnish, E: enamel, D: dentin).

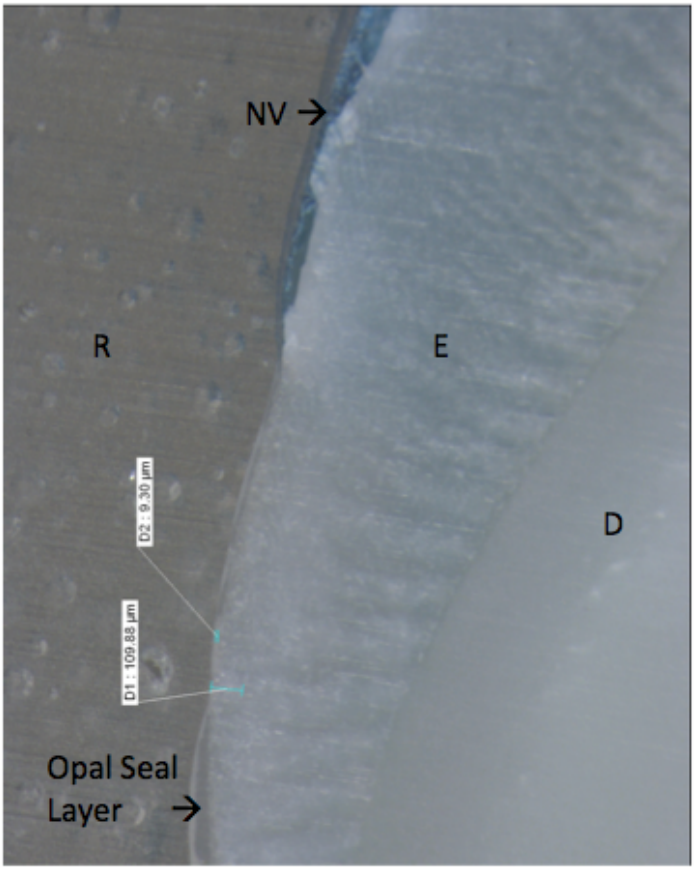

Figure 17: Polarized light microscopy image of a cross-section from the OPAL®SEAL group showing lesion depth measurements in area of missing sealant (R: orthodontic resin, NV: nail varnish, E: enamel, D: dentin).

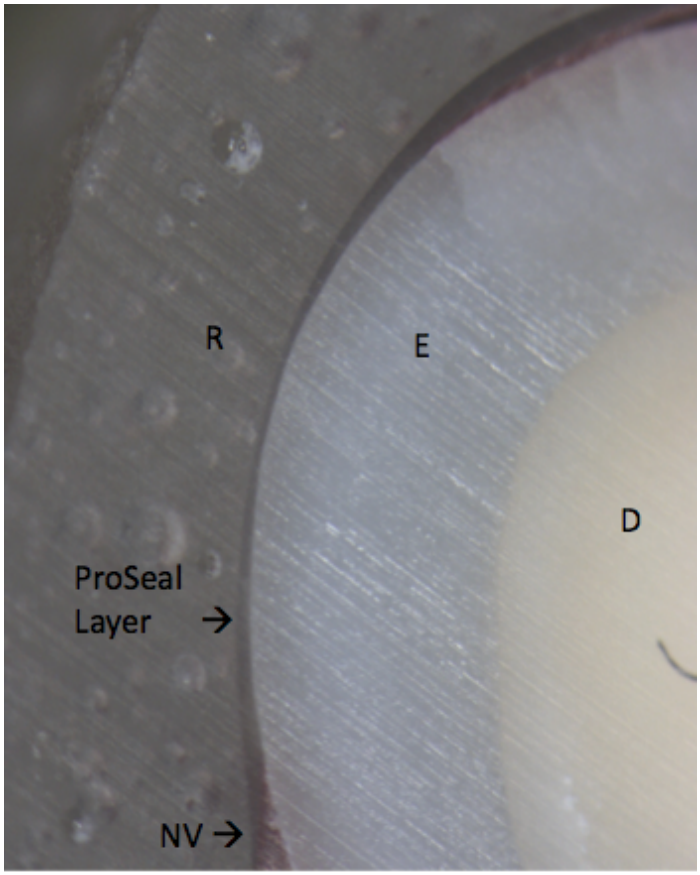

Figure 18: Polarized light microscopy image of a cross-section from the ProSeal™ group showing intact sealant layer over enamel (R: orthodontic resin, NV: nail varnish, E: enamel, D: dentin).

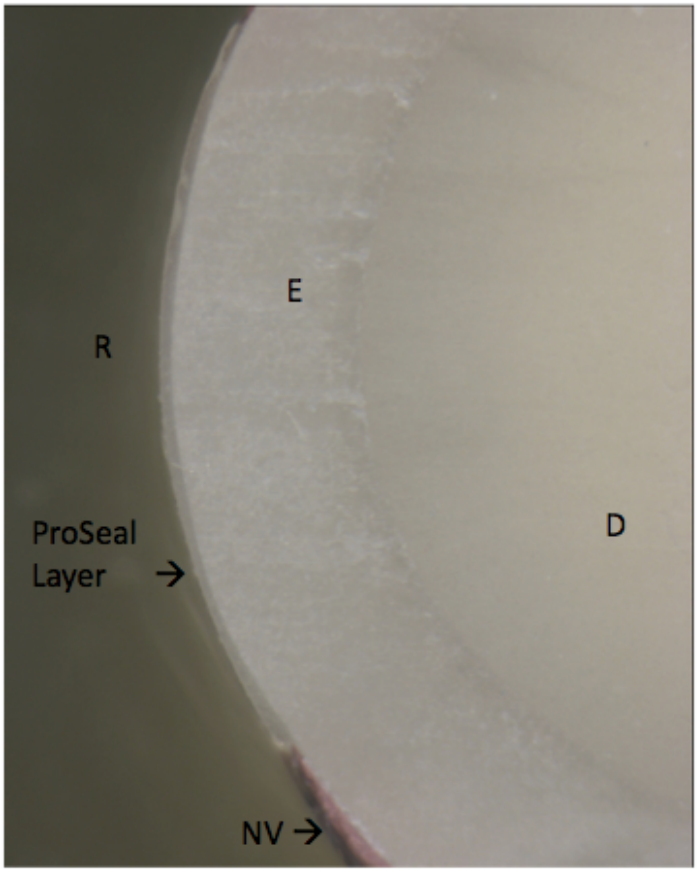

Figure 19: Polarized light microscopy image of a cross-section from the ProSeal™ group showing intact sealant layer over enamel (R: orthodontic resin, NV: nail varnish, E: enamel, D: dentin).

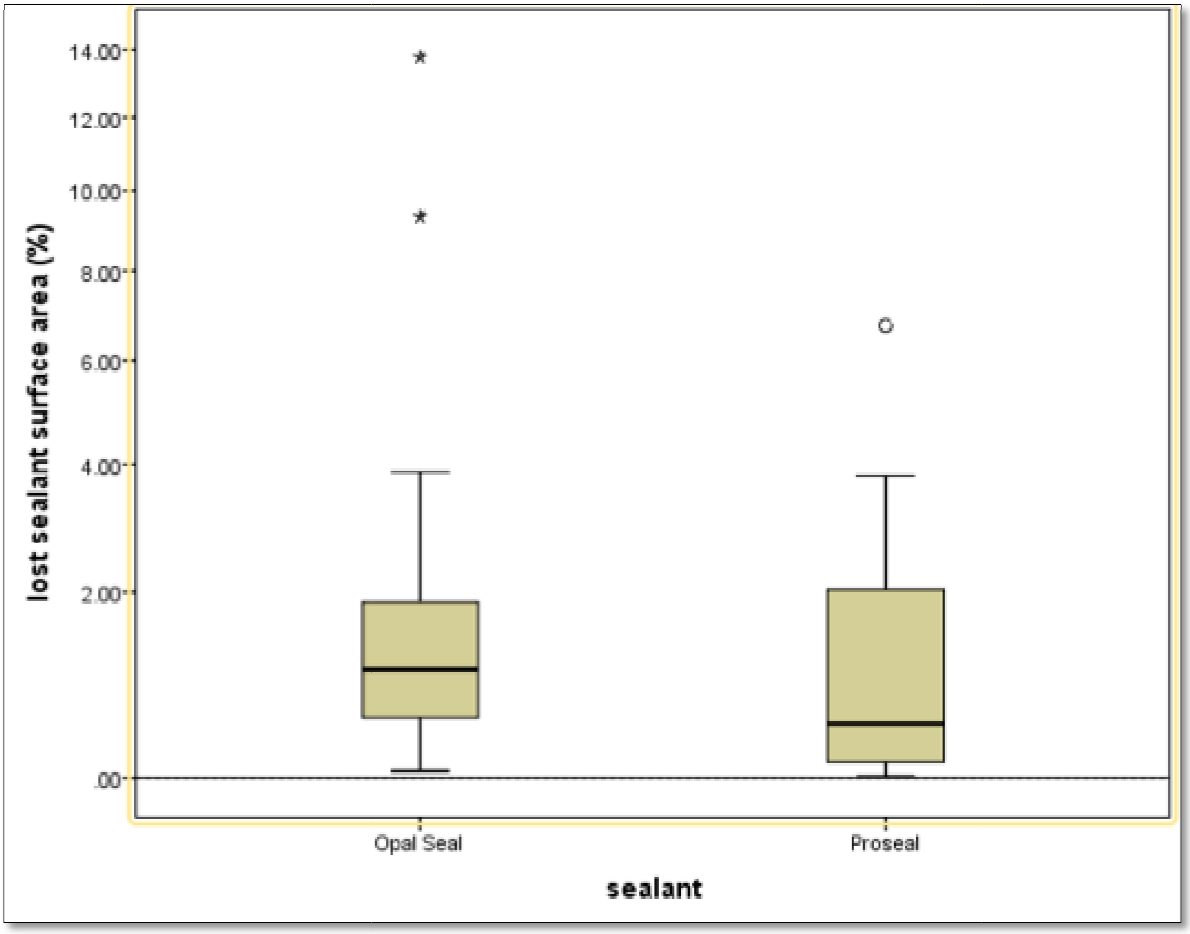

Figure 20: Side-by-side box plot for sealant lost in the sealant groups: ProSeal™ and OPAL®SEAL

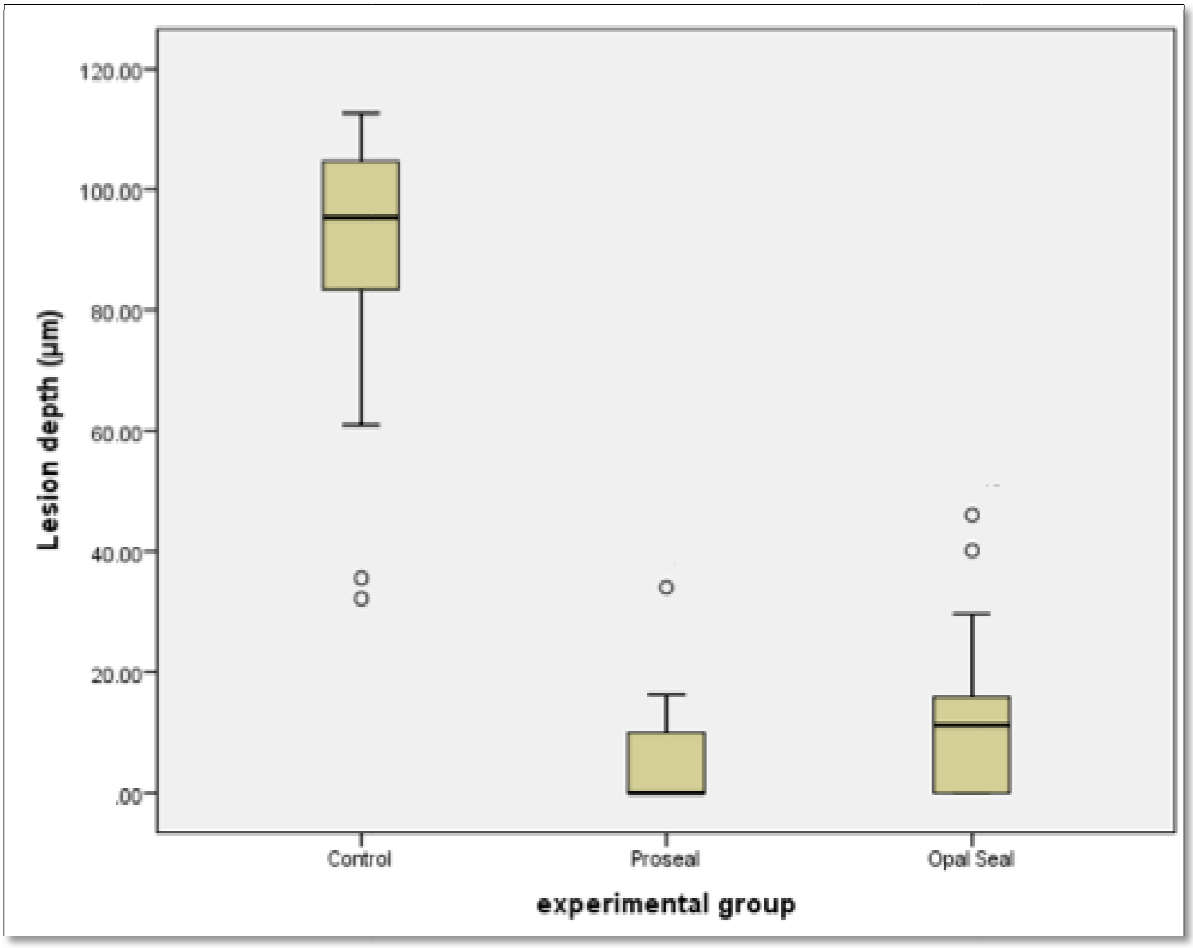

Figure 21: Side-by-side box plot for Lesion depth (µm) in the study groups: Control, ProSeal™ and OPAL®SEAL.

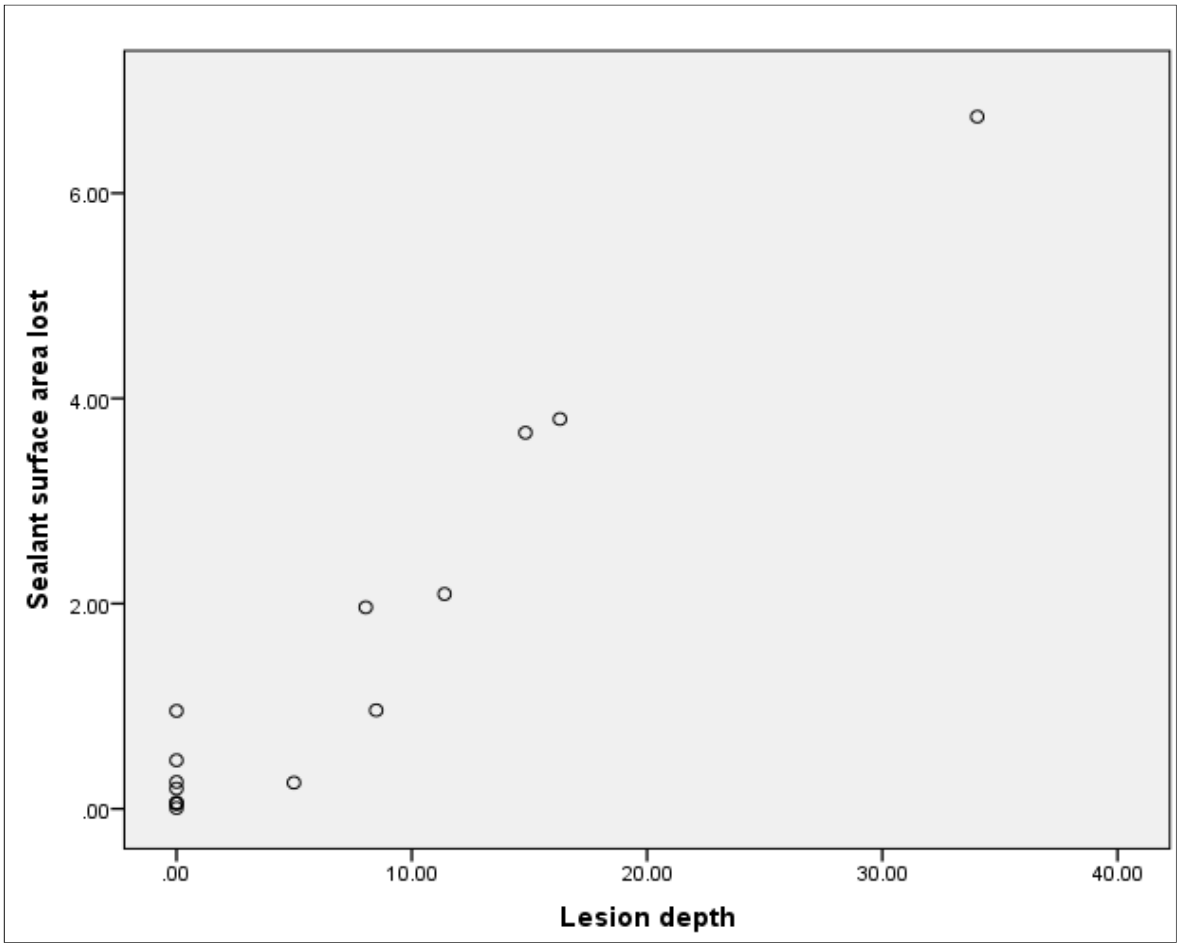

Figure 22: Scatterplot for correlation in ProSeal™ group

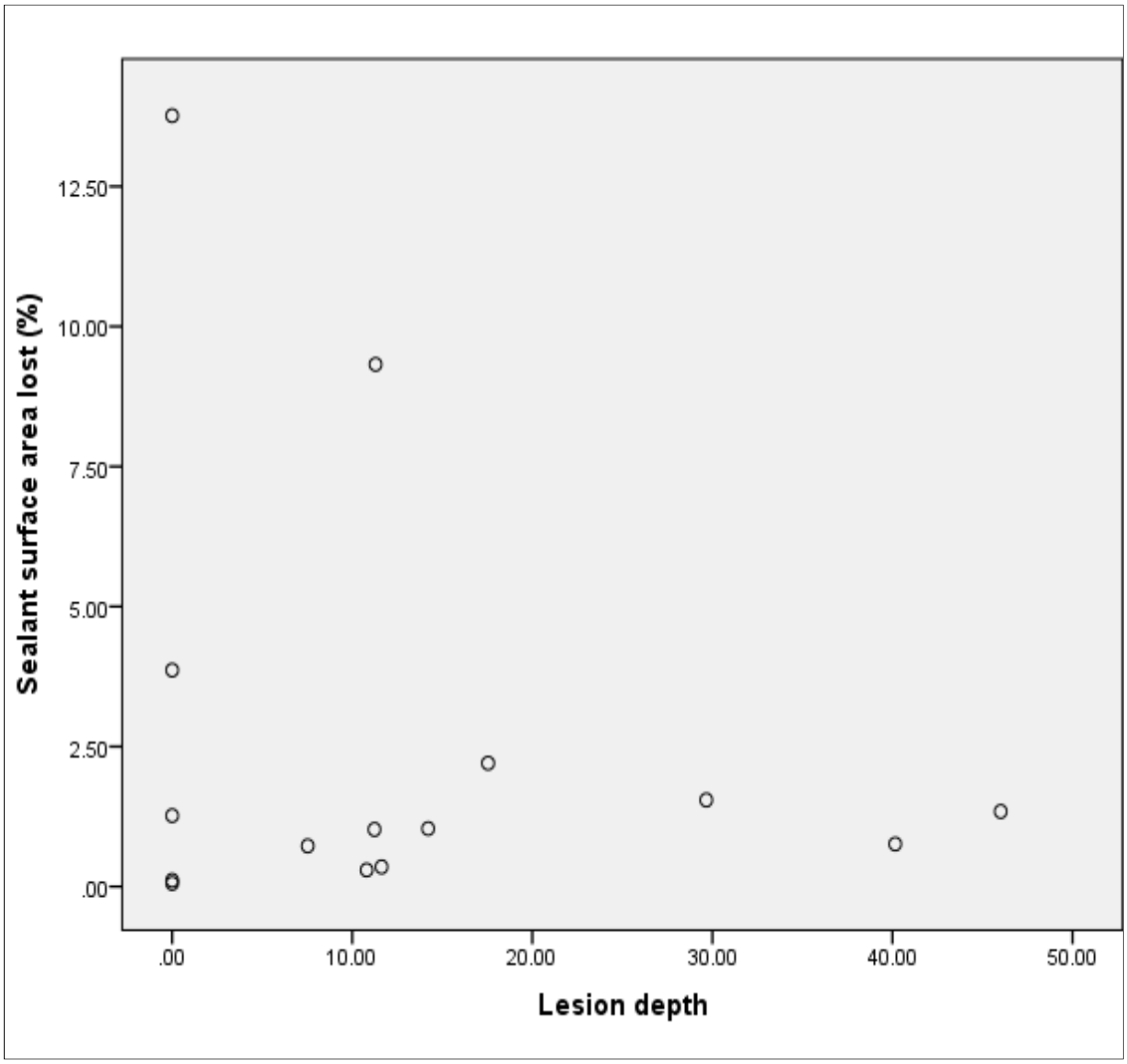

Figure 23: Scatterplot for correlation in OPAL®SEAL group

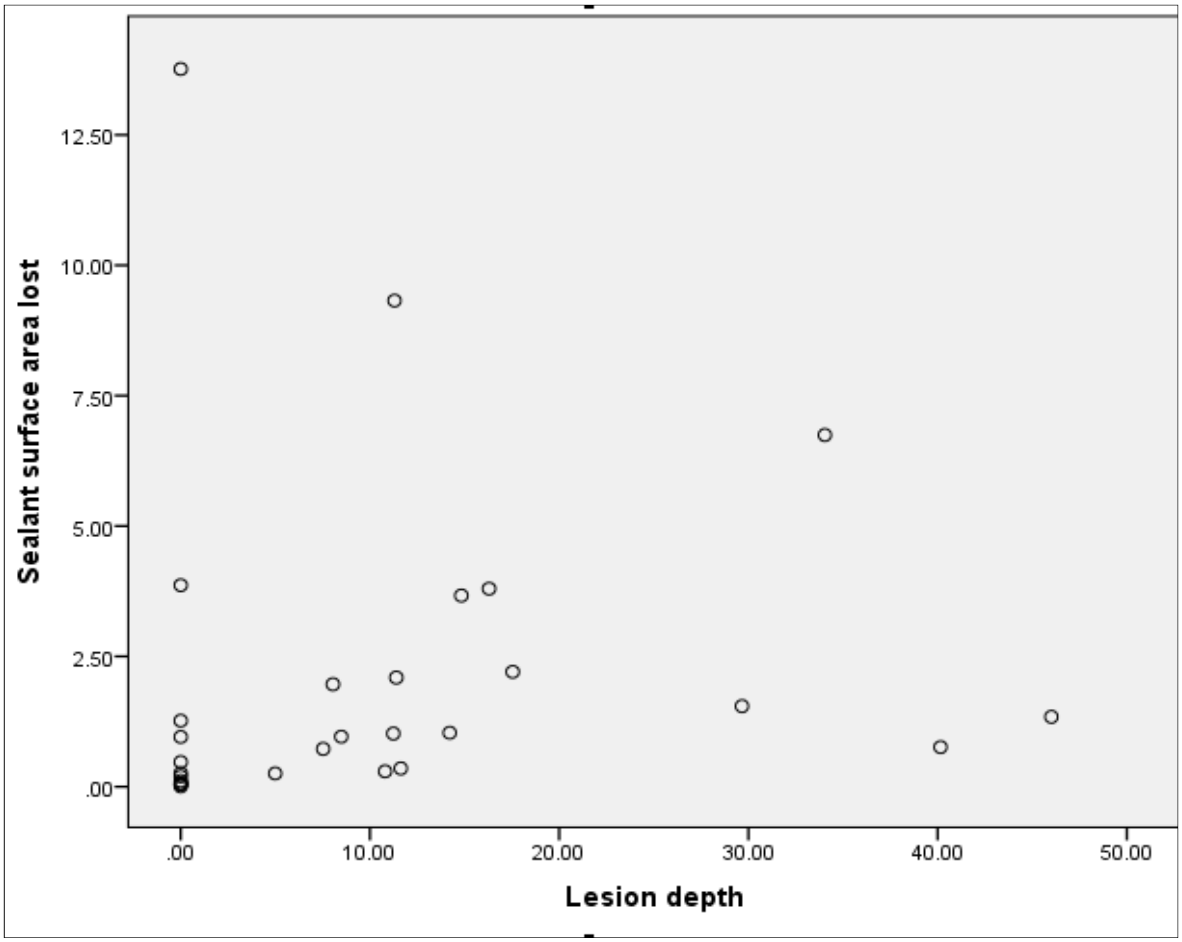

Figure 24: Scatterplot for correlation in the two sealants groups combined

TABLES:

Group	N (specimens)	Orthodontic sealant type
1	15	No sealant
2	15	ProSeal™
3	15	OPAL® SEAL

Table 1: Groups to which teeth were assigned

Sealant type	N (teeth)	Median sealant lost*	Interquartile Range	Mean sealant lost*	SD	Minimum	Maximum
ProSeal™	15	0.47	2.03	1.44	1.94	0.01	6.75
OPAL®SEAL	15	1.03	1.85	2.51	3.88	0.06	13.76

Table 2: Descriptive statistics for sealant lost by sealant type

All numbers represent percentages except N.

* Percentage of the total treatment window

Group	N (teeth)	Median lesion depth	Interquartile Range	Mean lesion depth	SD	Minimum	Maximum
Control	15	95.34	22.69	87.08	25.19	32.21	112.71
ProSeal™	15	0	11.39	6.54	9.61	0	34.04
OPAL®SEAL	15	11.24	17.54	13.34	14.67	0	46.01

Table 3: Descriptive statistics for lesion depth (µm) by study group.

All numbers represent µm except N.

Group	N (Specimens)	r_s ‡	P-value*
ProSeal™ group	15	0.866	< 0.001
OPAL®SEAL group	15	0.153	0.587
Both treatment groups combined	30	0.537	0.002

Table 4: Correlation between sealant lost and lesion depth

‡ Spearman's rank correlation coefficient
* Correlation is significant at the 0.05 level