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An In Vitro Study on the Permeability of Human Dental Enamel That is Unetched, Etched, Treated With Self-Etching Primer, or Sealed

by

Nicole Pui-Ming Chiu, DMD

THESIS

Submitted in partial satisfaction of the requirements for the degree of

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of the

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[redacted]

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ABSTRACT

PURPOSE: The purpose of this study is determine if there are differences in the permeability of untreated enamel, etched enamel, enamel that has been treated with self-etching primer, and enamel that has been treated with Pro Seal and then subjected to varying amounts of thermocycling. The specific aims were 1) to test the hypothesis that etched enamel is more permeable than untreated enamel, and enamel treated with Pro Seal is less permeable than untreated enamel; and 2) to test the hypothesis that enamel treated with Pro Seal followed by thermocycling is more permeable than sealed enamel that has not been thermocycled

METHODS: Sixty enamel samples were obtained from human molars in this *in vitro* study. Samples were divided into three experimental groups of twenty: etched, self-etching primer, and Pro Seal. Each sample had a control half and an experimental half. The samples were then placed in 50% aqueous silver nitrate solution, sectioned, mounted, and the amount of dye penetration measured using a light microscope to determine permeability. Twenty new Pro Seal samples were then divided into four experimental groups of five: 500, 1000, 2000, and 3000 thermocycles. Each sample had an untreated half and a Pro Seal half. Each sample was sectioned so that one section underwent thermocycling, and the other did not. Permeability was then determined in the same manner as the first part of the study.

RESULTS: Etched enamel was significantly the most permeable, enamel treated with Pro Seal was significantly the least permeable, and enamel treated with self-etching primer was significantly more permeable than untreated enamel but less permeable than etched enamel.

CONCLUSION: Etching the enamel or treating it with self-etching primer increases enamel permeability and thereby increases the susceptibility to white spot lesions immediately after orthodontic bonding. Pro Seal is an effective means of decreasing enamel permeability and thus white spot lesion susceptibility as long as it is able to stay on the tooth intraorally.

TABLE OF CONTENTS

List of Tables

List of Figures

Introduction

White Spot Lesions

Enamel decalcification can occur whenever bacterial plaque is retained on the enamel surface for a prolonged period of time. The bacteria cause dissolution of calcium and phosphate ions from the enamel surface (Ghiz *et al.,* 2009). Early enamel caries are first observed clinically as white spot lesions. The demineralized area beneath the body of the enamel lesion can lose as much as 50% of its original mineral content (Hughes *et al.,* 1979). The resulting "white spot" appearance is caused by an optical phenomenon owing to subsurface tissue loss and is exaggerated by drying. The outermost layer of enamel remains relatively intact, and in the presence of fluoride, is a zone of potential remineralization within certain limits (Gorelick *et al.,* 1982; Silverstone, 1977a; 1980). Unfortunately, by the time white spot lesions are clinically visible, the damage is usually unsightly and irreversible (Gorton and Featherstone, 2005). Therefore, the emphasis needs to be on prevention, not treatment.

Permeability of Etched Enamel

The first step in bonding orthodontic brackets is to acid etch the enamel surface. This is commonly done with 37% phosphoric acid. Etching with phosphoric acid changes the enamel surface in two distinct ways. First of all, it dissolves a shallow layer of enamel. Secondly, it makes the enamel surface porous by partially dissolving the ends of enamel prisms. The microscopic appearance of etched enamel has frequently been described as having a honeycomb pattern (Olsen *et al.,* 1996). After the enamel is etched, acrylic resin is applied which flows into the histologic porosities (resin tags), forming a mechanical bond. Various studies have reported an average depth of penetration ranging from 8 to 15 microns, with a maximum tag length of up to 50 microns (Buonocore *et al.,* 1968; Jorgensen and Shimokobe, 1975; Pahlavan *et al.,* 1976;

Retief, 1974; Wickwire and Rentz, 1973). A previous study has shown that etched enamel has a porous surface and a higher solubility rate than normal enamel (Silverstone, 1977b). Therefore, acid etching appears to make the enamel surface more permeable.

A newer etching technique that has been increasing in popularity among orthodontists is the use of a self-etching primer, which combines the etching and priming of enamel into one step. Previous studies have shown adequate bond strength with self-etching primers (Bishara *et al.,* 2002; Fritz *et al.,* 2001). Simultaneous etching and priming allows the primer to penetrate the entire depth of the etch, ensuring good mechanical interlock. However, the primer provides no resistance to enamel demineralization when exposed to an *in-vitro* acidic challenge (Tanna *et al.,* 2009). A potential reason for the self-etching primers popularity could be that the elimination of separate etching and rinsing steps has made the bonding protocol easier to perform (Pivetta *et al.,* 2008) and saves chair time (Miller, 2001). This, in turn, minimizes the contamination risks and the sources of errors (Ramires-Romito *et al.,* 2004). The self-etching primers differ from the traditional acid etches in that the smear layer is not removed, but is incorporated into the hybridized complex (Perdigao J, 2003). However, certain self-etching primers such as L-Pop (3M Unitek, Monrovia, CA) produce an etching pattern similar to that achieved by phosphoric acid (Ghiz *et al.,* 2009; Pivetta *et al.,* 2008). Therefore, self-etching primers should also make the enamel surface more permeable.

Measuring Permeability

All of the techniques that have been used to determine permeability in previous studies have involved the application of a tracer, and then a method to obtain an image of the extent of tracer penetration. One common technique is the use of an aqueous solution of silver nitrate dye as the tracer. Images are then obtained using a light microscope. This has been used in studies looking at the margin of porcelain and resin veneers (Lacy *et al.,* 1992), the resin-dentin interface

(Neelakantan *et al.,* 2009; Sauro *et al.,* 2008), and composite restorations (Heintze *et al.,* 2008). Previous studies have only looked at the permeability of cavosurface margins (D'Alpino *et al.,* 2006; Hevinga *et al.,* 2007; Paris *et al.,* 2006; Srinivasan *et al.,* 2005). This will be the first study to look at the permeability of a noncarious enamel surface after it receives various treatments.

Orthodontic Appliances and Oral Hygiene

Orthodontic treatment with fixed appliances predisposes patients to larger accumulations of bacterial plaque (Beyth *et al.,* 2003; Geiger *et al.,* 1988; Millett *et al.,* 2005). Increased levels of mutans streptococci and of lactobacilli have been detected in the oral cavity after bonding orthodontic attachments (Lundstrom and Krasse, 1987). Metallic brackets have been found to induce specific changes in the oral environment, such as decreases in pH and increases in plaque accumulation, that further increase the risk of decalcification (Mitchell, 1992b). The affinity of bacteria to a metallic surface is probably due to electrostatic, hydrophobic, and specific interactions (Beyth *et al.,* 2003). An almost linear correlation between plaque accumulation and development of white spot lesions has been demonstrated in orthodontic patients (Zachrisson and Zachrisson, 1971). There are inherent mechanical difficulties to removing plaque with orthodontic brackets in place because of the bands and brackets, themselves, as well as the different elements that are used such as elastics, plastic leaves, and springs. Therefore, compliance with proper oral hygiene is critical. However, this compliance is often severely lacking (Farrow *et al.,* 2007). As a result, white spot lesions are a very common undesirable complication of orthodontic therapy. This is of great concern because the lesions are unaesthetic, unhealthy, and irreversible (Artun and Thylstrup, 1989; Ogaard, 1989). The positive effects of orthodontic treatment can be overshadowed by these lesions. It has been found that orthodontically-treated people have significantly more teeth with white spot lesions than those who are untreated (Ogaard, 1989), and that teenagers are at a higher risk of demineralization than adults (Kukleva *et al.,* 2002). There is equal susceptibility to white spot formation whether teeth are banded or bonded (Geiger *et al.,* 1988). The prevalence of white spot lesions in patients who seek orthodontic treatment is in the range of 50% to 96% (Geiger *et al.,* 1988; Gorelick *et al.,* 1982; Vivaldi-Rodrigues *et al.,* 2006). The development of white spots during treatment is also an extremely rapid process. It has been reported that the lesions can develop as quickly as 4 weeks (Gorton and Featherstone, 2003; O'Reilly and Featherstone, 1987; Ogaard *et al.,* 1988).

Prevention of White Spot Lesions

There have been major improvements in the materials and techniques used during orthodontic treatment, in order to attempt to reduce the incidence of white spot lesions. Most of the materials that have been developed involve fluoride in some form. Fluorides provide a balance between demineralization and remineralization of the enamel surface. They act by inhibiting demineralization and stimulating remineralization. Generally, the remineralized surface is more resistant to demineralization than the original enamel surface. The presence of fluoride minimizes the ionic loss from the tooth structure until the pH of the plaque becomes as low as 4.5. A dose response relationship has been found between the frequency of fluoride application and the degree of enamel protection (Alexander and Ripa, 2000). Several fluoride regimens with varying fluoride concentrations, pH, and delivery systems such as in dentrifices, mouth rinses, gels, and varnishes, have been shown to be effective in preventing demineralization (Geiger *et al.,* 1988; O'Reilly and Featherstone, 1987). The daily use of a mouthwash containing sodium fluoride in addition to daily tooth-brushing has shown positive results in preventing decalcification (Gorton and Featherstone, 2003). However, in order for these regimens to be effective, patient cooperation is essential. Studies have shown that full compliance with fluoride regimens is unlikely even when extensive educational efforts are expended (Geiger *et al.,* 1988), and that partial or sporadic compliance can result in only limited benefit (Stratemann and Shannon, 1974). Therefore, in spite of new technologies, white spot lesions continue to be an unwanted side effect of orthodontic treatment with high clinical relevance. This is largely due to the continued lack of patient compliance in terms of oral hygiene. In order to eliminate white spot lesions, a fluoride regimen must be used in which patient compliance is no longer a factor.

One such technique is the professional application of fluoride varnishes. They allow the orthodontist to control the timing and amount of fluoride used. It has been shown to effectively reduce the prevalence of demineralization (Demito *et al.,* 2004; Gillgrass *et al.,* 2001). Ogaard et al. found that the use of fluoride varnish alone was as effective at reducing white spot lesion formation as the combined use of chlorhexidine and fluoride varnishes (Ogaard *et al.,* 2001). Also, fluoride varnishes result in an increased enamel fluoride uptake compared to mouth rinses (Petersson, 1993). However, varnishes require several in-office applications with which some patients may not be pleased. Also, this method has not been effective in preventing caries nor in the remineralization of existing lesions because the high dose of fluoride only has a brief period of release (Gorton and Featherstone, 2003). Studies have shown that a continuous application of a low-dose of fluoride has a greater cariostatic effect than an individual high-dose application (Corry *et al.,* 2003).

Another technique in which this cariostatic effect could be achieved is through the use of glass ionomer cements. Because glass ionomer cements release fluoride, they have the potential to reduce demineralization. Usually the fluoride is maximally released during the first few days, and in rare cases, can still be measured in minor quantities after 2 or 3 months (Basdra *et al.,* 1996). A previous study has shown that resin-modified glass ionomer cements can reduce enamel demineralization around bonded brackets, mainly in a tooth area at high caries risk (Pascotto *et al.,* 2004). This may be the result of the availability of low concentrations of fluoride ion, or it may be the result of initial changes in the enamel surface that occurred when the fluoride ion concentration was high (Basdra *et al.,* 1996). However, there are many disadvantages to these cements. Conventional glass ionomer cements have a low adhesive strength which limits their clinical use (Cook, 1990). Resin-modified glass ionomer cements have a greater adhesive strength than the conventional ones (Silverman *et al.,* 1995) and do not promote enamel surface changes after debonding (Komori and Ishikawa, 1997). However, their fluoride releasing potential is dependent on both material and local factors (Monteith *et al.,* 1999). Another disadvantage is that their clinical handling properties are less than ideal. Also, long-term studies have shown that glass ionomer cements are often unsuccessful in preventing white spot lesions around the brackets (Millett *et al.,* 1999; Mitchell, 1992a).

Another potential technique is the application of a fluoride-releasing resin sealant on the enamel surface around and underneath the orthodontic bracket to prevent demineralization. Several factors can affect the success of a sealant: duration of protection, material thickness, distribution on the tooth surface, composition of the sealant material, and endurance to oral stresses such as abrasion and thermal changes (Farrow *et al.,* 2007). Placement of sealant after acid etching can provide several benefits: increased bond strength, sealing of etched enamel, and protection against demineralization around the bracket during treatment. Also, sealants are able to act as a fluoride reservoir which provides a distinct advantage in caries resistance. They have the ability to be recharged with fluoride from the daily use of readily available fluoride sources such as fluoridated dentrifices, fluoride mouth rinses, and fluoride gels. This allows for extended fluoride release long after the exogenous source of fluoride has been cleared from the oral environment by salivary flow (Salar *et al.,* 2007). However, previous studies have shown that most chemically-cured sealants do not effectively seal smooth enamel surfaces. This is due to the oxygen inhibition of polymerization when the sealant is in contact with the air in a thin layer (Zachrisson *et al.,* 1979). Conversely, light-cured sealants have been shown to cure completely on smooth enamel surfaces and prevent enamel demineralization effectively *in vitro* (Joseph *et al.,* 1994). However, unfilled or lightly filled light-cured sealants, which have the desired low viscosity and high flowability to facilitate application, are highly susceptible to mechanical (tooth brushing) and chemical (acid attack) wear *in vivo*. Wearing off or breaks in the sealant layer can lead to decalcification under the sealant. Therefore, light-cured sealants cannot provide more protection than chemically-cured sealants (Hu and Featherstone, 2005).

One sealant that is able to overcome all of the aforementioned shortcomings is Pro Seal (Reliance Orthodontic Products, Itasca, IL). It is a fluoride-releasing, highly filled, light-cured sealant. The manufacturer claims that it has 100% polymerization and no oxygen-inhibited layer, can protect enamel against demineralization during orthodontic treatment with fixed appliances, withstand toothbrush abrasion and erosion by oral fluids for up to 2 years due to the filler particles, and be combined with every adhesive thus allowing universal application. The 100% polymerization creates a smooth hard coating that prevents leakage, protects the enamel, and makes it easier to remove any excess adhesive paste during and after bonding. Because Pro Seal is transparent, it can be applied to the facial enamel surfaces of anterior teeth and still yield an esthetic result. Previous studies have shown that Pro Seal results in a significant reduction of enamel demineralization *in vitro*, and offers adequate resistance against wear during tooth brushing (Benham *et al.,* 2009; Buren *et al.,* 2008; Hu and Featherstone, 2005). It has been shown that Pro Seal releases fluoride ions in sustained but significantly decreasing amounts (Soliman *et al.,* 2006). It has also been found that there is no significant decrease in shear-peel bond strength following the use of Pro Seal (Paschos *et al.,* 2006). Bishara et al. found that the application of Pro Seal did not affect the shear bond strengths of the adhesive used within the first half hour after initial bonding (Bishara *et al.,* 2005). Therefore, Pro Seal provides a clinically applicable method to reduce the incidence of white spot lesions, presumably by sealing the enamel surface (decreasing the permeability).

However, one question that has not been answered is how much of the Pro Seal layer applied during the bonding procedure remains on the tooth when it is subjected to other rigors of the oral environment besides toothbrush abrasion. Clinically, the absence of Pro Seal from the surface of the tooth has been noted as early as 1 to 2 months after the initial bonding procedure.

One factor could be intraoral temperature changes which may be induced by routine eating, drinking, ambient temperature and humidity, smoking, and mouth-breathing (Boehm, 1972). Temperatures at various sites in the mouth can vary considerably for an individual over a 24-hour period (Moore *et al.,* 1999). It has been proposed that the oral environment might go through 20 to 50 thermocycles per day (Gale and Darvell, 1999). Thermal stresses can be pathogenic because mechanical stresses induced by differential thermal changes can directly induce crack propagation through bonded interfaces (Nelsen *et al.,* 1952; Torstenson and Brannstrom, 1988). If the sealant layer is no longer intact, then it is no longer effective in the prevention of white spot lesions.

Purpose

The purpose of this study is to determine if there are differences in the permeability of untreated enamel, etched enamel, enamel that has been treated with self-etching primer, and enamel that has been treated with Pro Seal and then subjected to varying amounts of thermocycling.

Specific Aims

The specific aims are to:

- Test the hypothesis that etched enamel is more permeable than untreated enamel, and enamel treated with Pro Seal is less permeable than untreated enamel
- Test the hypothesis that enamel treated with Pro Seal followed by thermocycling is more permeable than sealed enamel that has not been thermocycled

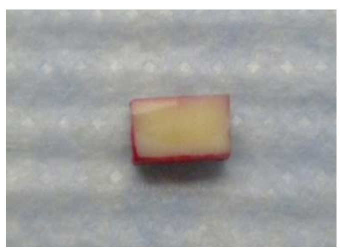
Materials and Methods

A. Permeability of enamel treated with etch, self-etching primer, or sealant

1. Sample Preparation

i. Sample Acquisition

Sixty non-carious human molars were selected, sterilized with gamma irradiation, and then stored in deionized water at 4°C until use. Each molar was sectioned with a low-speed diamond saw (Isomet Low Speed Saw, Buehler, Lake Bluff, IL) yielding one enamel sample, either from the buccal or lingual surface. The dimensions of the samples were ~3mm in width, ~6mm in length, and the depth was into dentin but not the pulp. All surfaces of the sample except for the enamel surface were coated with 1 layer of nail polish (Cherries in the Snow Nail Enamel, Revlon, New York, NY) as shown in Figure 1.



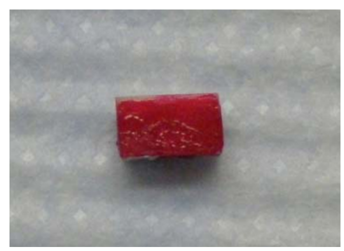


Figure 1 Enamel surface and one of the five nail polish covered surfaces of the enamel sample.

ii. Division of Samples into Experimental Groups

The sixty enamel samples were then divided into three experimental groups of twenty. Each sample had two treatment halves so that the groups consisted of: unetched – etched, unetched – self-etching primer, etched – light cure sealant as shown in Figure 2.

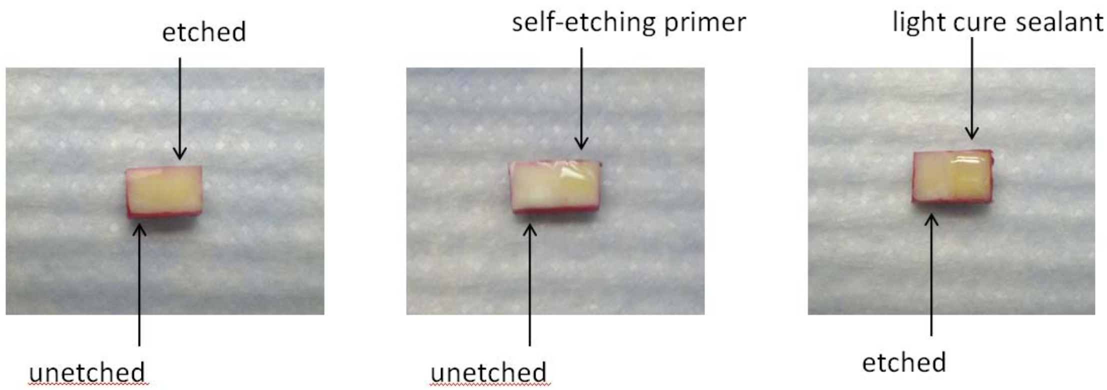


Figure 2 The three experimental groups (each enamel sample is divided into treatment halves).

Self-etching primer was selected because it is becoming a popular orthodontic bonding technique and may affect enamel permeability. For the etched halves, the enamel surface was etched for thirty seconds with 37% phosphoric acid solution (Ormco Etching Solution, Ormco, Glendora, CA) and then rinsed thoroughly with water for 10 seconds. For the self-etching primer halves, the enamel surface was rubbed with Transbond^{TM} Plus Self Etching Primer (3M Unitek, Monrovia, CA) for two seconds, and then an air syringe was used to create an even, uniform layer. For the light cure sealant halves, the enamel surface was first etched with 37% phosphoric acid solution for thirty seconds, and rinsed thoroughly with water for 10 seconds. Then, one layer of light cure sealant (Pro Seal, Reliance Orthodontic Products, Itasca, IL) was applied and light-cured for 10 seconds. The bottom of each sample was marked with a white permanent marker to differentiate the etched halves in the etched group, the self-etching primer halves in the self-etching primer groups, and the sealant halves in the sealant group as shown in Figure 3.

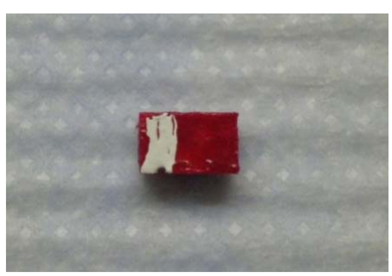


Figure 3 White marking indicating etched half of unetched – etched sample.

iii. Application of Photographic Solutions

All samples were placed in a 50% aqueous silver nitrate solution for 2 hours. The samples were thoroughly rinsed with water and then placed in developer for 2 hours, rinsed again, and placed in fixer for 4 hours before being thoroughly rinsed one last time.

iv. Sample Mounting

The enamel samples were then sectioned lengthwise with a low-speed diamond saw (Isomet Low Speed Saw, Buehler, Lake Bluff, IL). The cut surface of each side was polished using polishing strips which progressed from 600 grit, 400 grit, 320 grit, and 240 grit (Strip Grinder, Buehler, Lake Bluff, IL). It was then given a final polish with a 3 µm diamond suspension (Meta Di Monocrystalline Diamond Suspension, Buehler, Lake Bluff, IL). Each side was mounted onto a microscope slide with epoxy (Loctite Epoxy, Henkel Consumer Adhesives, Avon, OH) as shown in Figure 4.

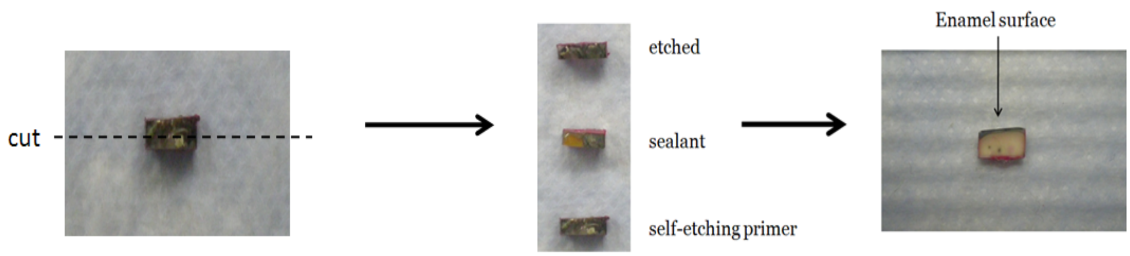


Figure 4 Sample mounting

2. Imaging and Analysis

Images of the enamel samples were obtained using a light microscope (Olympus BX51, Olympus, Center Valley, PA) as shown in Figure 5. Image analysis was performed with the software program Image-Pro Plus 7.0 (Media Cybernetics, Bethesda, MD). The depth of silver nitrate dye penetration was measured from the enamel surface to the deepest point (in microns). Five arbitrarily chosen measurements were taken on each treatment half as shown in Figure 6.

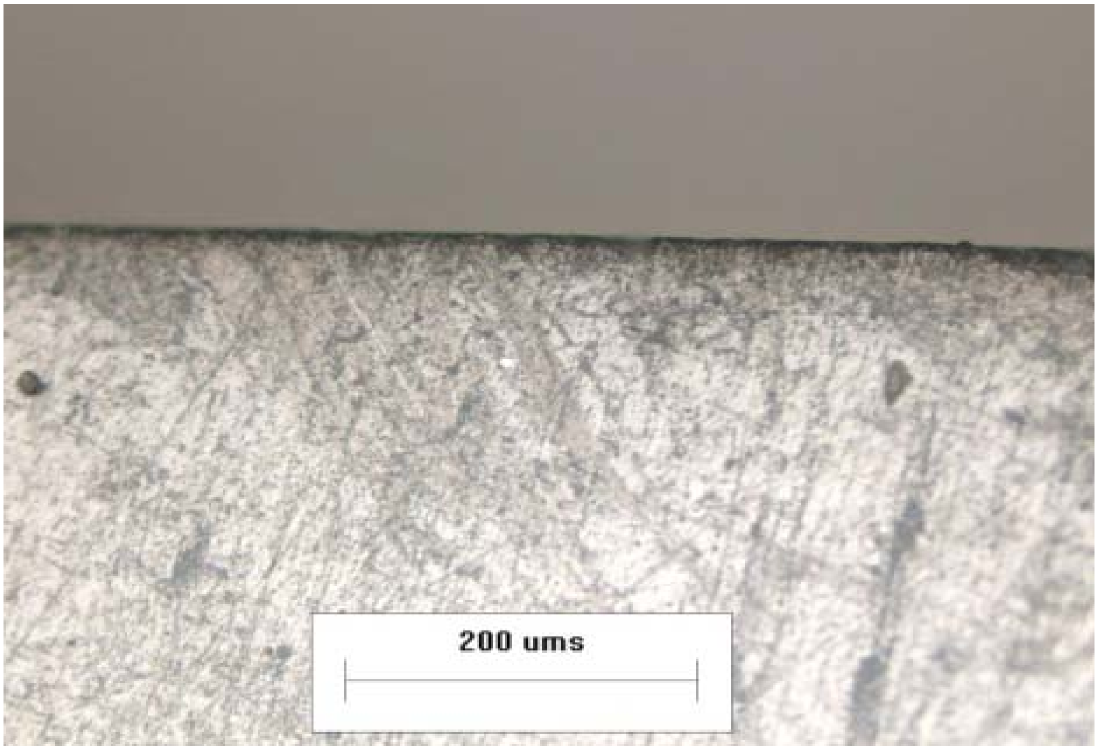


Figure 5 Image of enamel sample at 10X magnification.

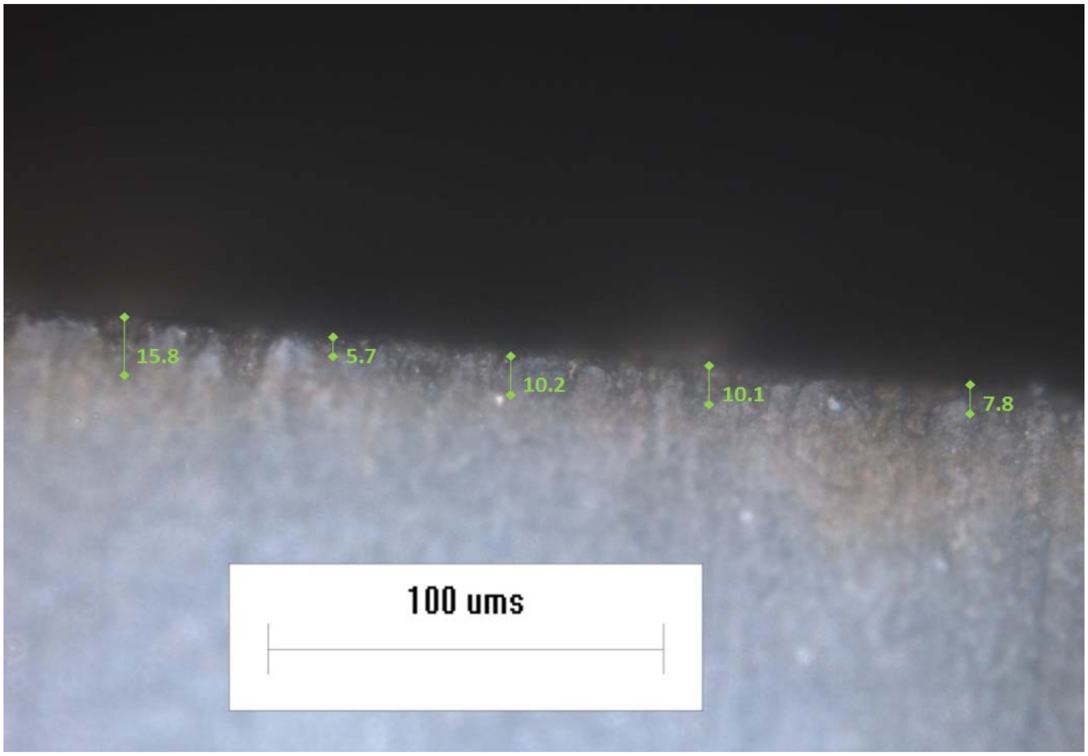


Figure 6 Image of enamel sample at 40X magnification with measurements of silver nitrate dye penetration.

All measurements were taken by one investigator, and then repeated by a second investigator for inter-observer reliability. Both were blinded to the experimental group to which each sample belonged. Measurements were then repeated by the first investigator on 10 treatment halves for intra-observer reliability.

B. Effect of thermocycling on permeability of enamel treated with sealant

1. Sample Preparation for Thermocycling

i. Sample Acquisition

Twenty non-carious human molars were selected, sterilized with gamma irradiation, and then stored in deionized water at 4°C until use. Each molar was sectioned with a low-speed diamond saw (Isomet Low Speed Saw, Buehler, Lake Bluff, IL) yielding one enamel sample, either from the buccal or lingual surface. The dimensions of the samples were ~3mm in width, ~6mm in length, and the depth was into dentin but not pulp. All surfaces of the sample except for the enamel surface were coated with 1 layer of nail polish (Cherries in the Snow Nail Enamel, Revlon, New York, NY).

ii. Application of sealant

The enamel surface of each sample was divided into treatment halves so that one half remained unetched while the other half was etched for thirty seconds with 37% phosphoric acid solution (Ormco Etching Solution, Ormco, Glendora, CA), and then rinsed thoroughly with water for 10 seconds. These halves were then coated with one layer of light cure sealant (Pro Seal, Reliance Orthodontic Products, Itasca, IL), and light-cured for 10 seconds. The bottom of each sample was marked with a white permanent marker to indicate the sealant half.

2. Thermocycling

The samples were divided into four experimental groups of five. The groups consisted of 500, 1000, 2000, and 3000 thermocycles. This number of thermocycles was chosen because previous studies have shown that the average human mouth goes through 50 chewing cycles per day (Gale and Darvell, 1999). Therefore, 2000 cycles is equivalent to 40 days which is the typical amount of time between orthodontic appointments. Each sample was sectioned lengthwise. One section was set aside (control) while the other was put into the thermocycler as shown in Figure 7.

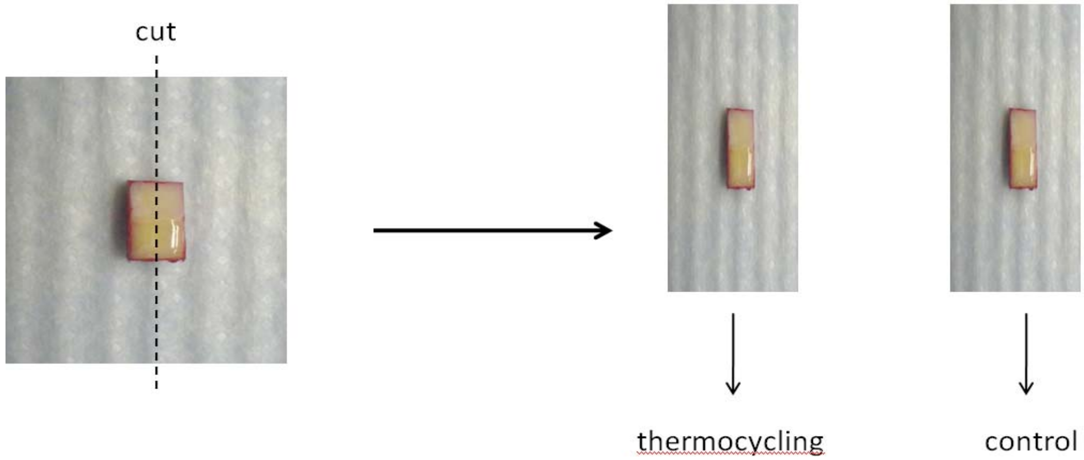


Figure 7 Sample sectioned into control and thermocycling halves.

The thermocycler consisted of three water baths at temperatures of 5°C, 21°C (room temperature), and 55°C as shown in Figure 8.

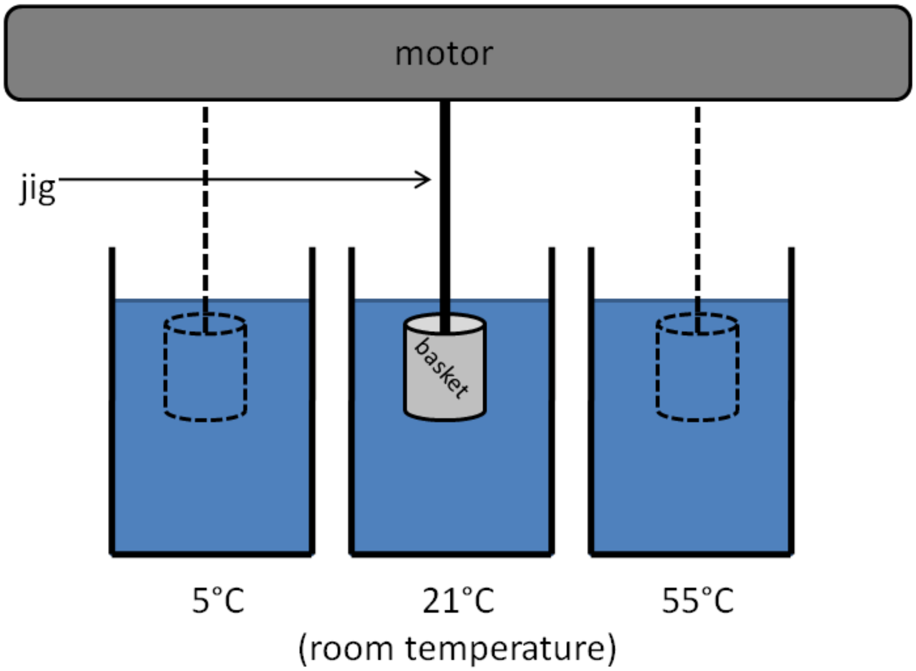


Figure 8 Thermocycler

The samples were placed in a basket and cycled between the water baths so that 1 cycle consisted of: 20 seconds in the room temperature bath, 5 seconds in the hot bath, 20 seconds in the room temperature bath, 5 seconds in the cold bath, 20 seconds in the room temperature bath.

3. Sample Preparation for Imaging

i. Application of Photographic Solutions

All samples were placed in a 50% aqueous silver nitrate solution for 2 hours. The samples were thoroughly rinsed with water and then placed in developer for 2 hours, rinsed again, then placed in fixer for 4 hours before being thoroughly rinsed one last time.

ii. Sample Mounting

One side of each sample was then polished using polishing strips which progressed from 600 grit, 400 grit, 320 grit, and 240 grit (Strip Grinder, Buehler, Lake Bluff, IL) to remove all nail polish.

It was then given a final polish with a 3 μm diamond suspension (Meta Di Monocrystalline Diamond Suspension, Buehler, Lake Bluff, IL). Each sample was mounted onto a microscope slide with epoxy (Loctite Epoxy, Henkel Consumer Adhesives, Avon, OH).

4. Imaging and Analysis

Images of the enamel samples were obtained using a light microscope (Olympus BX51, Olympus, Center Valley, PA). Image analysis was performed with the software program Image-Pro Plus 7.0 (Media Cybernetics, Bethesda, MD). The depth of silver nitrate dye penetration was measured from the enamel surface to the deepest point (in microns). Five arbitrarily chosen measurements were taken on each treatment half. All measurements were taken by one investigator, and then repeated by a second investigator for inter-observer reliability. Both were blinded to the experimental group to which each sample belonged.

C. Statistics

All statistics were calculated using Bootstrap resampling with 1000 replications. The sample number was used as the basis for clustering.

Results

A. Inter- and intra-investigator reliability

The following table, Table 1, compares the silver nitrate depth measurements of the two investigators.

Investigator	1
2	0.278 (-0.351, 0.906) *p*=0.39

Table 1 Estimated mean difference in depth measurements between investigators with 95% confidence interval; *p*-value is for the test of a significant difference between groups (*p* < *0.05*).

Because the measurements of the two investigators were not significantly different, they were pooled together in future analyses. Therefore, the study has high inter-investigator reliability.

Table 2 compares the silver nitrate depth measurements that were repeated by one of the investigators.

Reliability	
	0.22 (-0.16, 0.61) *P*=0.25

Table 2 Estimated mean difference between replicate measurements with 95% confidence interval and *p*-value for the test that the actual difference is non-zero (*p* < 0.05).

The non-significance indicates that the difference between the replicate measurements was not significantly different from zero. Therefore, the study has high intra-investigator reliability and measurements were repeatable.

B. Permeability of enamel with various treatments.

The following figures, Figures 9 to 13, show the silver nitrate depth measurements that were taken for the sixty enamel samples in the different treatment groups by Investigator 1. The five measurements that were taken for each treatment half were averaged. The average depth of penetration for each treatment half was plotted on a scattergram.

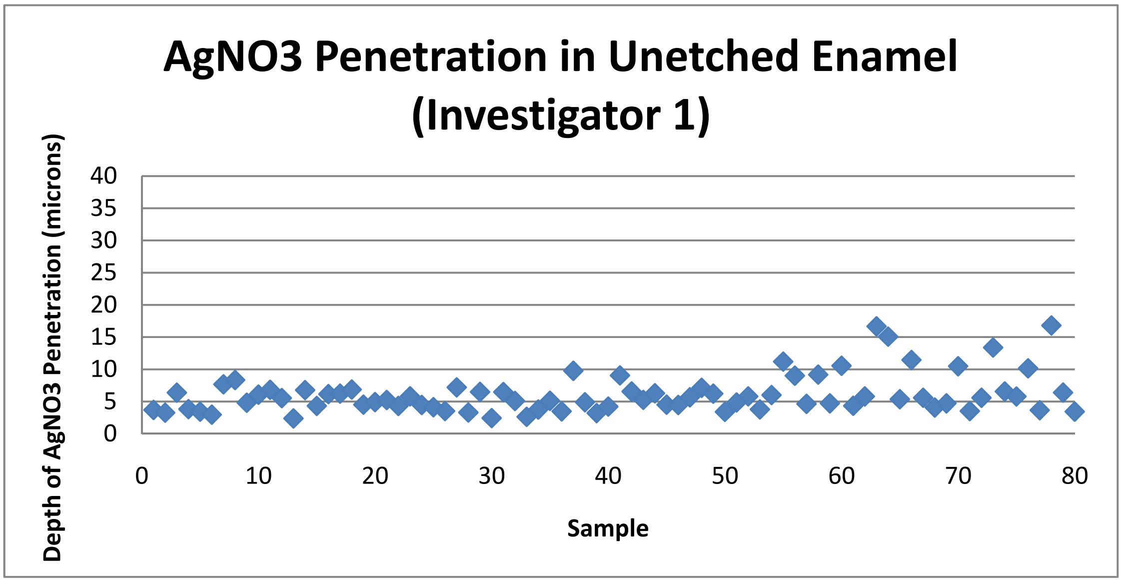


Figure 9 Scattergram showing the average silver nitrate depth measurements for each enamel sample half that was unetched (Inv 1).

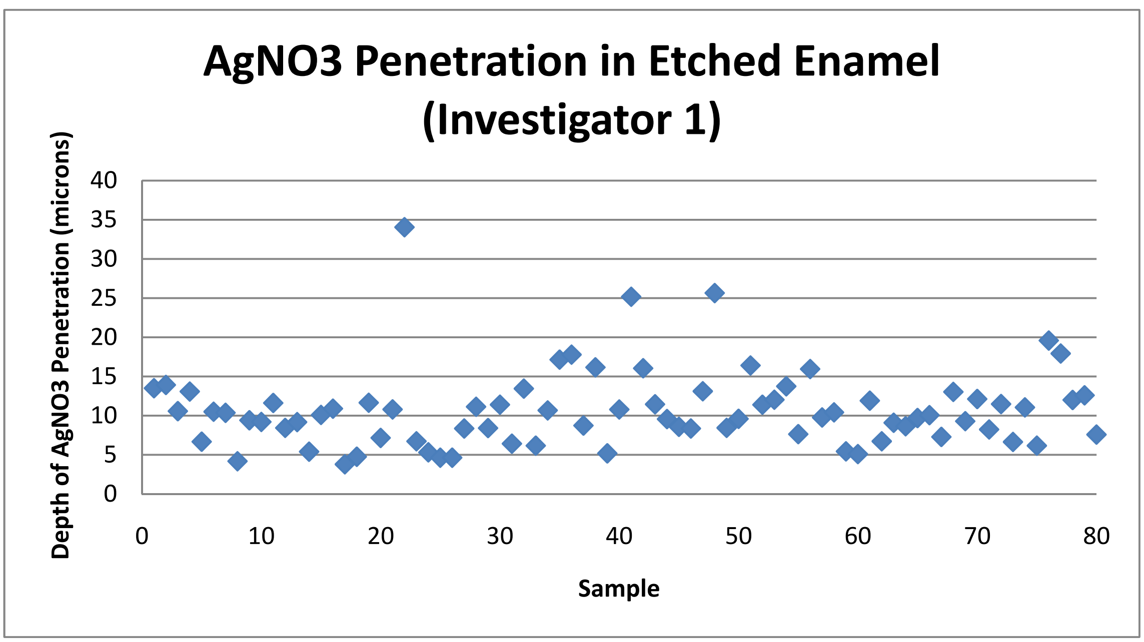


Figure 10 Scattergram showing the average silver nitrate depth measurements for each enamel sample half that was etched (Inv 1).

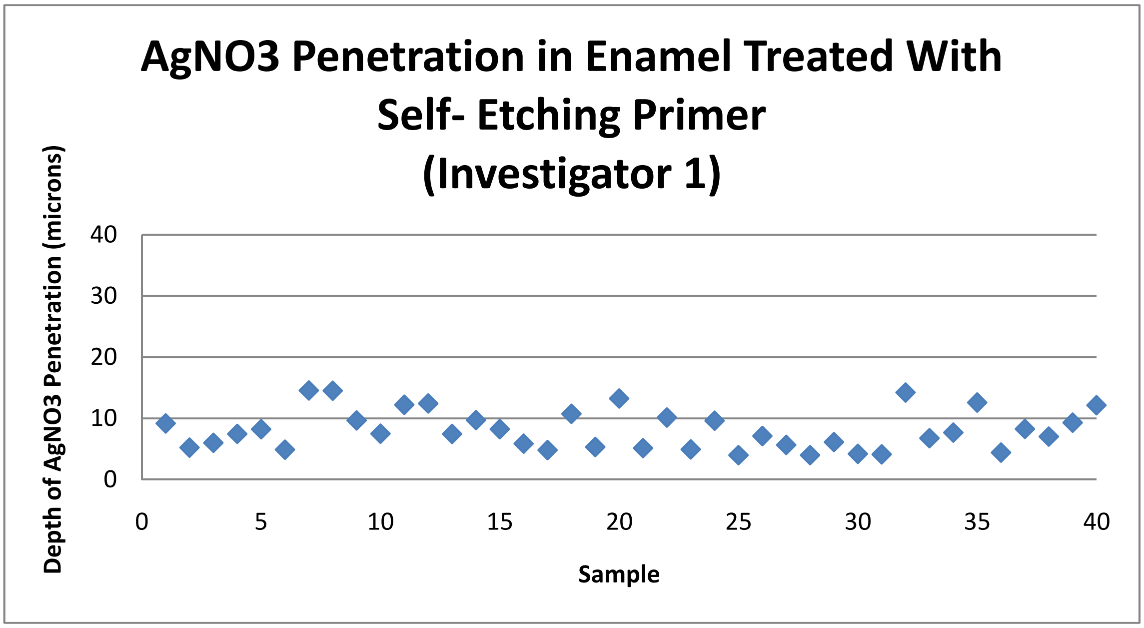


Figure 11 Scattergram showing the average silver nitrate depth measurements for each enamel sample half that was treated with self-etching primer (Inv 1).

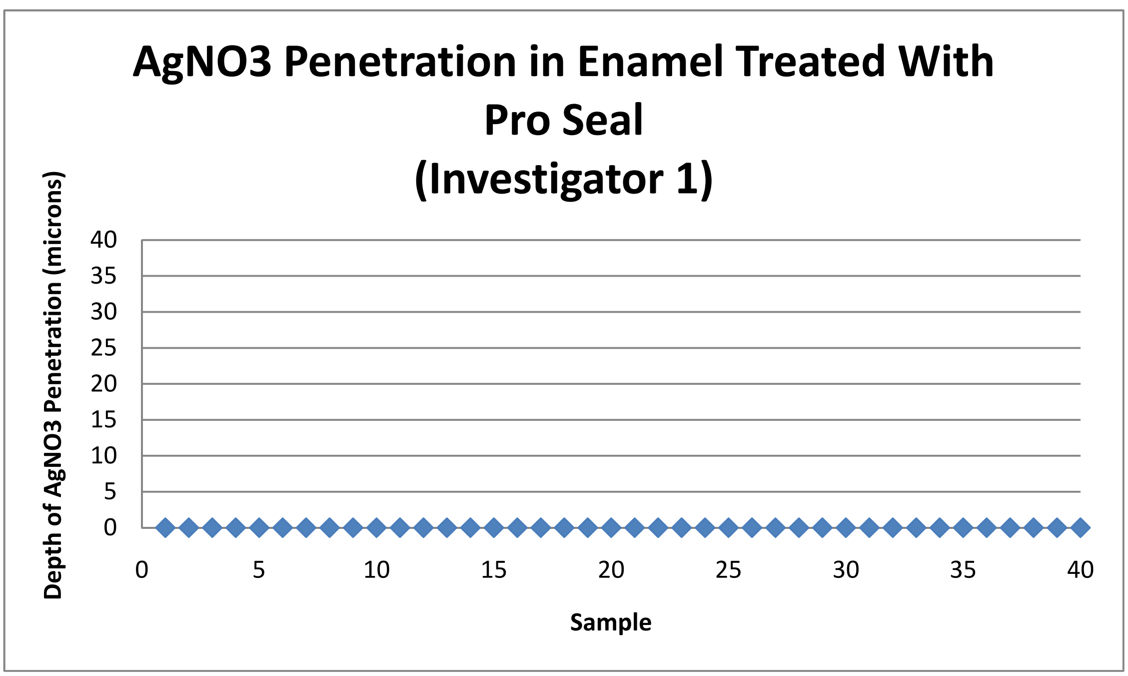


Figure 12 Scattergram showing the average silver nitrate depth measurements for each enamel sample half that was treated with Pro Seal (Inv 1).

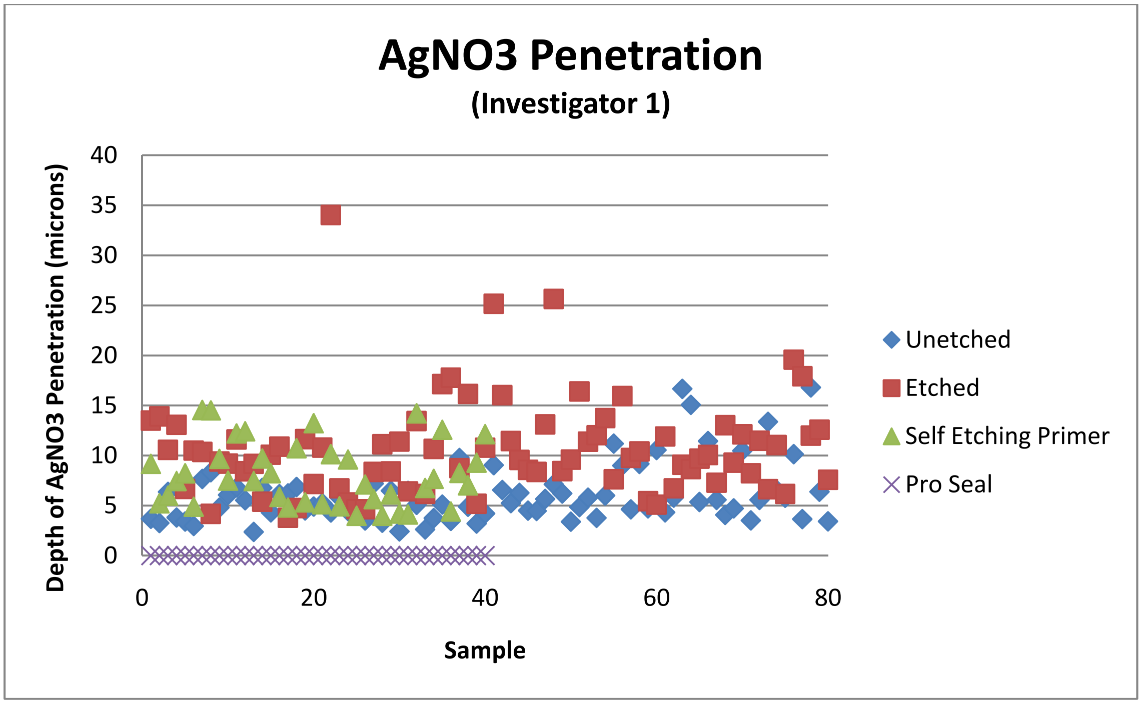


Figure 13 Scattergram showing the average silver nitrate depth measurements for each enamel sample half in all treatment groups (Inv 1).

Figures 14-18 also show silver nitrate measurements that were taken for the sixty enamel samples in the different treatment groups, but by Investigator 2.

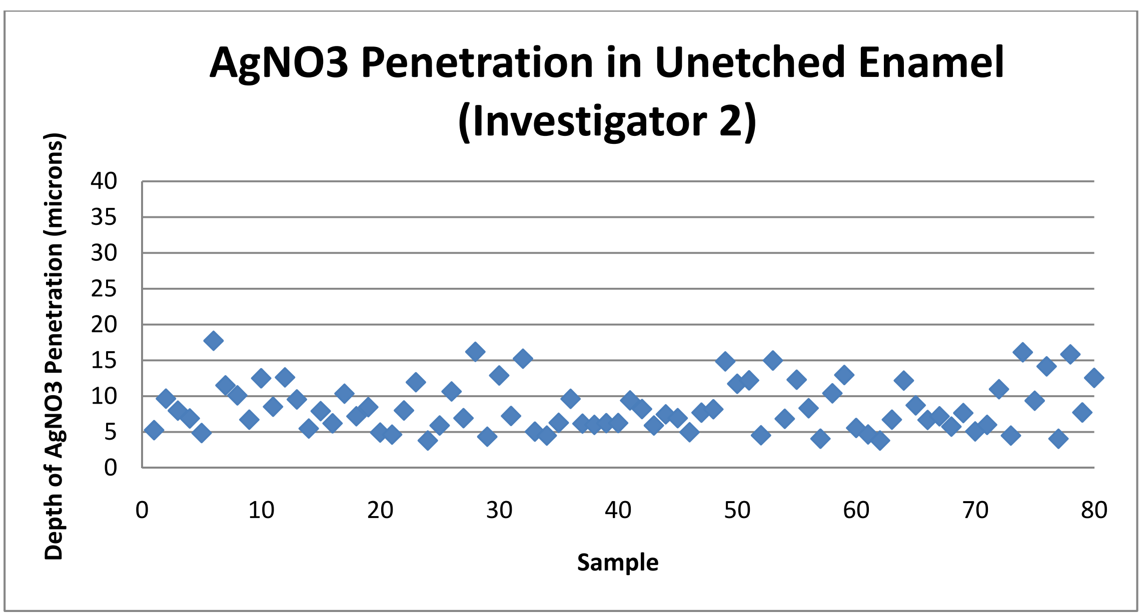


Figure 14 Scattergram showing the average silver nitrate depth measurements for each enamel sample half that was unetched (Inv 2).

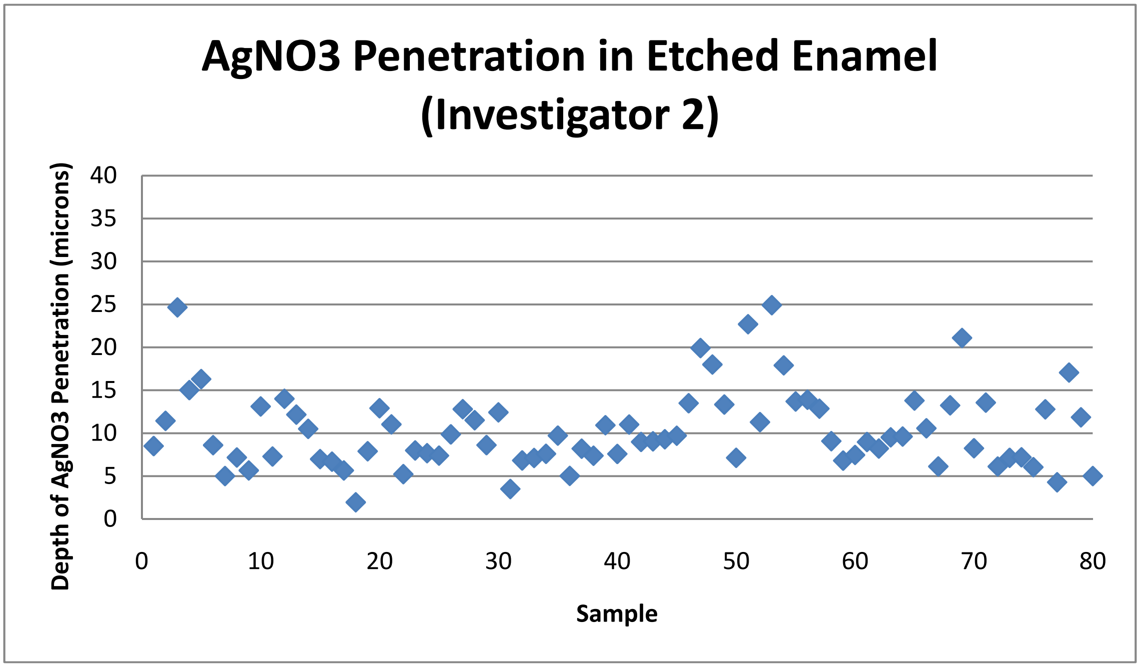


Figure 15 Scattergram showing the average silver nitrate depth measurements for each enamel sample half that was etched (Inv 2).

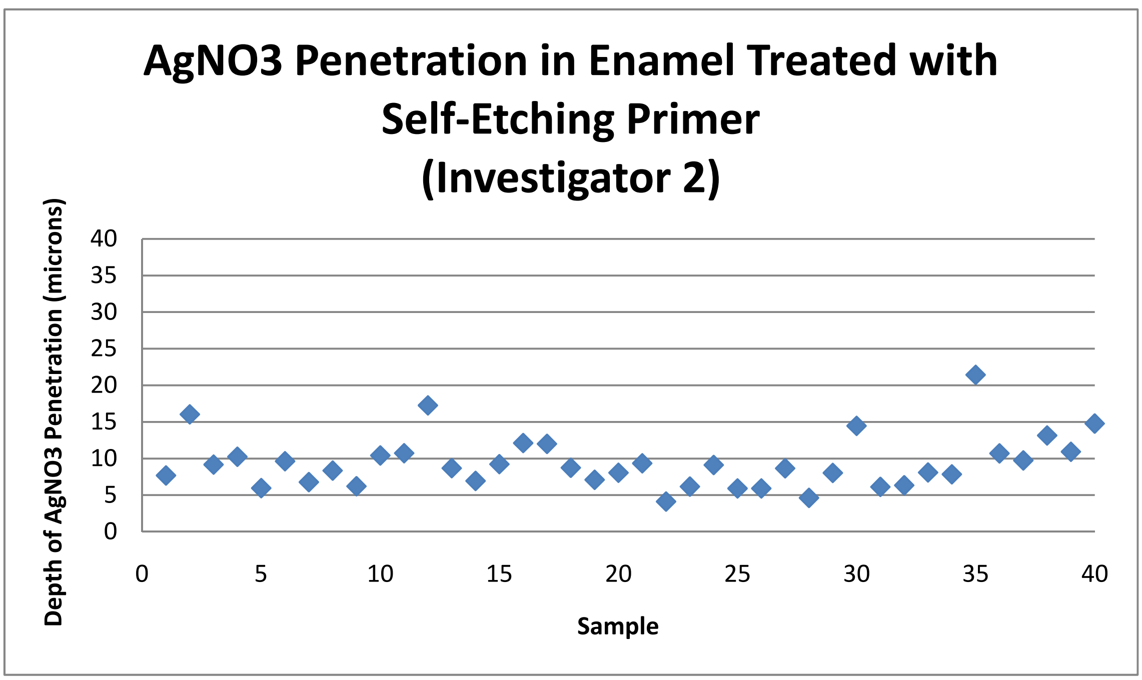


Figure 16 Scattergram showing the average silver nitrate depth measurements for each enamel sample half that was treated with self-etching primer (Inv 2).

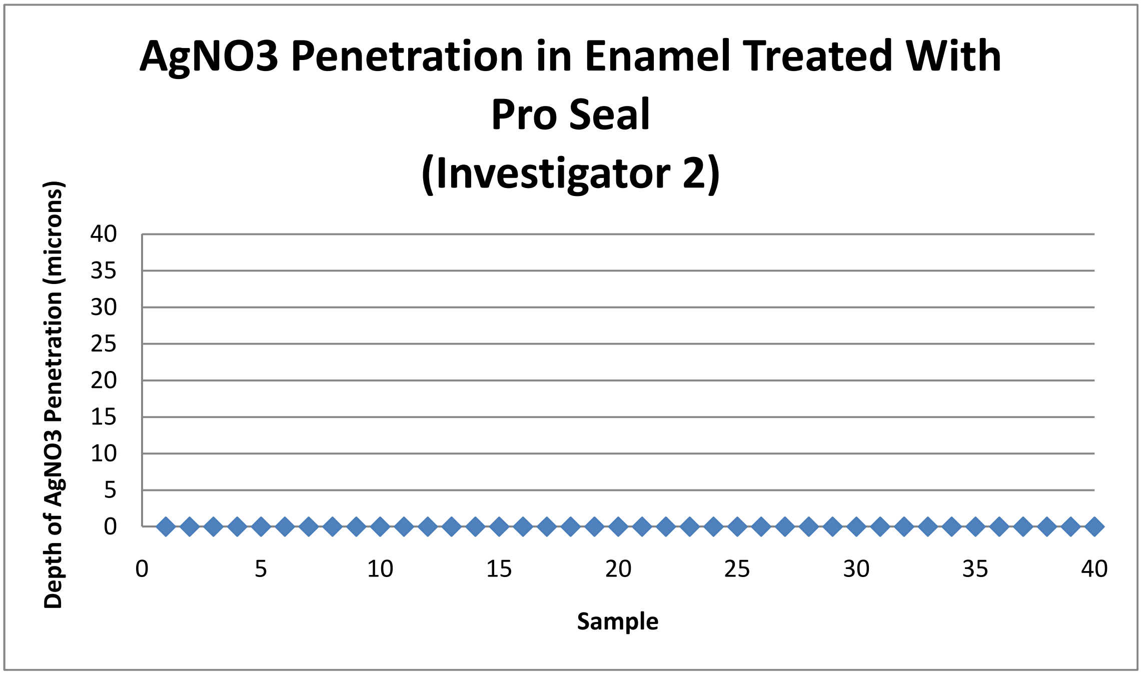


Figure 17 Scattergram showing the average silver nitrate depth measurements for each enamel sample half that was treated with Pro Seal (Inv 2).

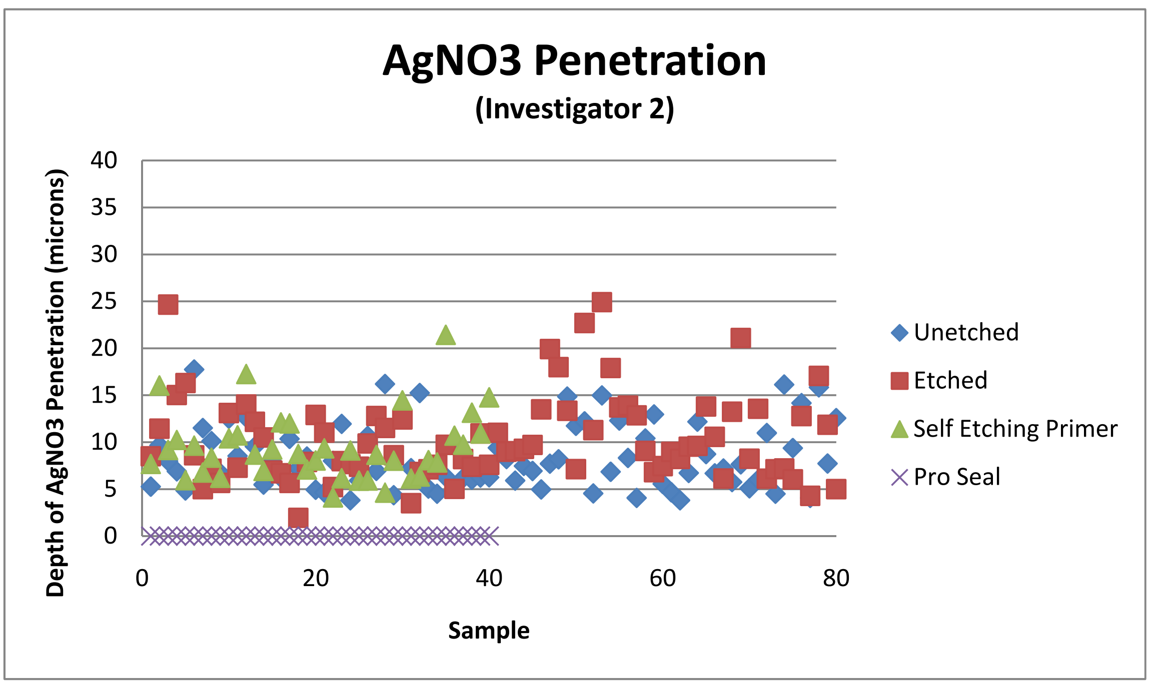


Figure 18 Scattergram showing the average silver nitrate depth measurements for each enamel sample half in all treatment groups (Inv 2).

The means and standard deviations of the silver nitrate depth measurements were then calculated for each treatment group as seen in Figures 19 and 20. For Investigator 1, the mean and standard deviation were 6.08 and 3.00 microns, respectively, for unetched enamel, 10.71 and 4.80 microns for etched enamel, 8.11 and 3.20 microns for enamel treated with self-etching primer, and 0 and 0 microns for enamel treated with Pro Seal (Figure 19).

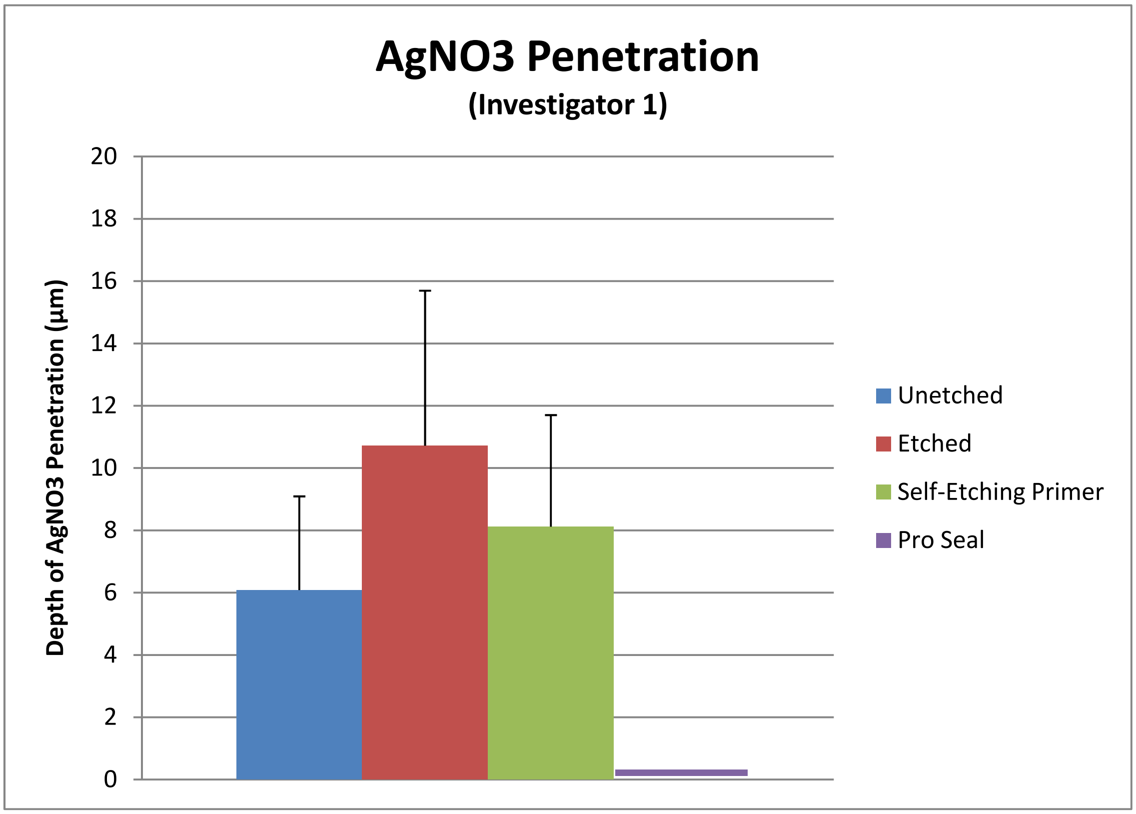


Figure 19 Bar graph showing means and standard deviations of all treatment groups (Inv 1).

For Investigator 2 the mean and standard deviation were 8.50 and 3.49 microns, respectively, for unetched enamel, 10.38 and 4.63 microns for etched enamel, 9.42 and 3.58 microns for enamel treated with self-etching primer, and 0 and 0 microns for enamel treated with Pro Seal (Figure 20).

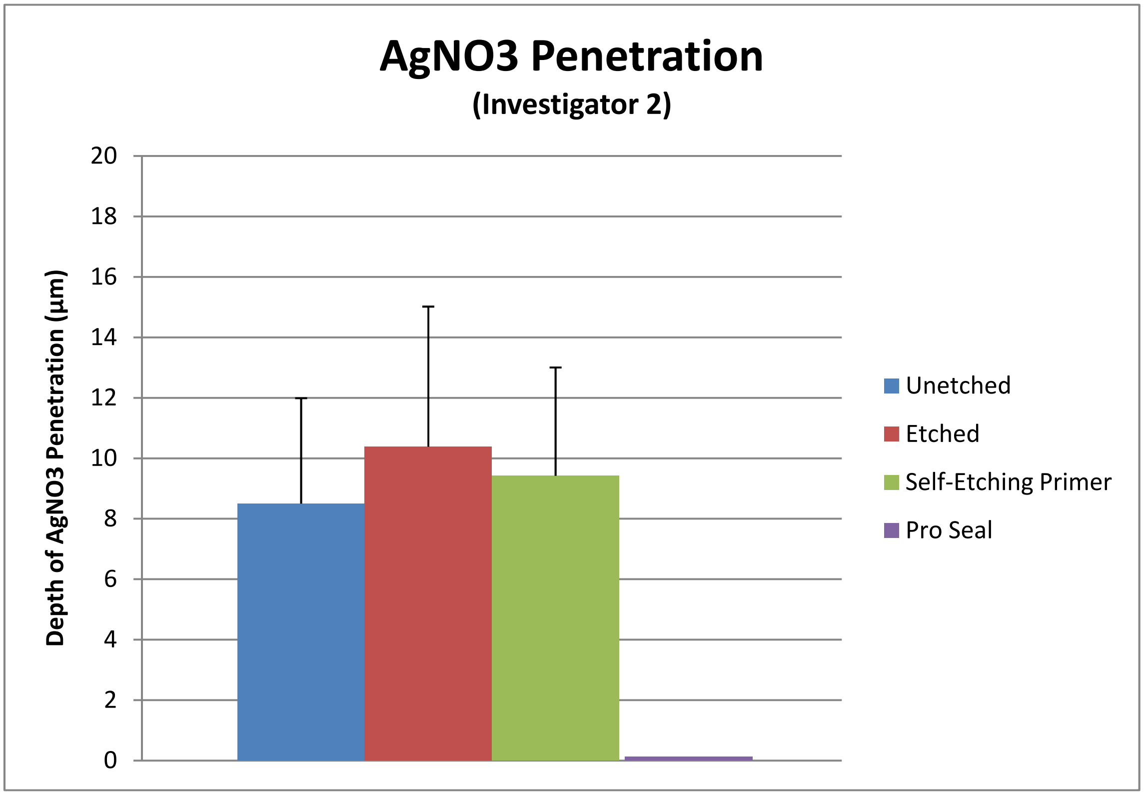


Figure 20 Bar graph showing means and standard deviations of all treatment groups (Inv 2).

Because the inter-investigator reliability for the study is high, the silver nitrate depth measurements for the two investigators can be combined, as was done in Figures 21 to 25. An overall mean and standard deviation was calculated (Figure 26).

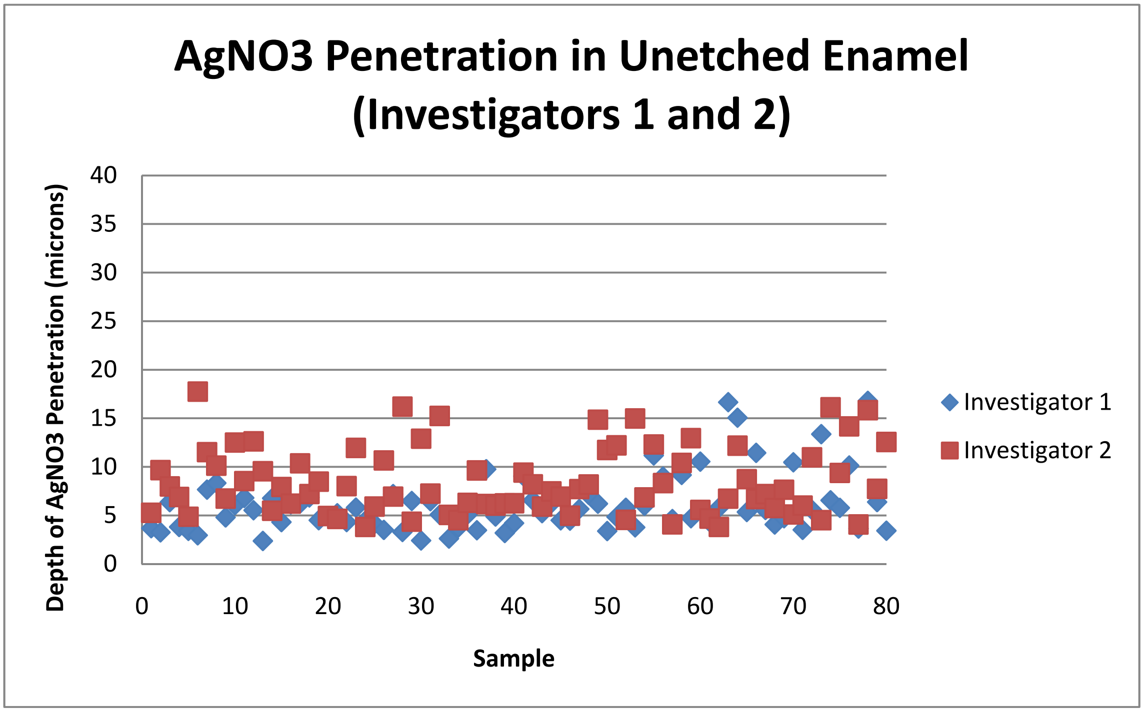


Figure 21 Scattergram showing the average silver nitrate depth measurements for each enamel sample half that was unetched (Inv 1 and 2).

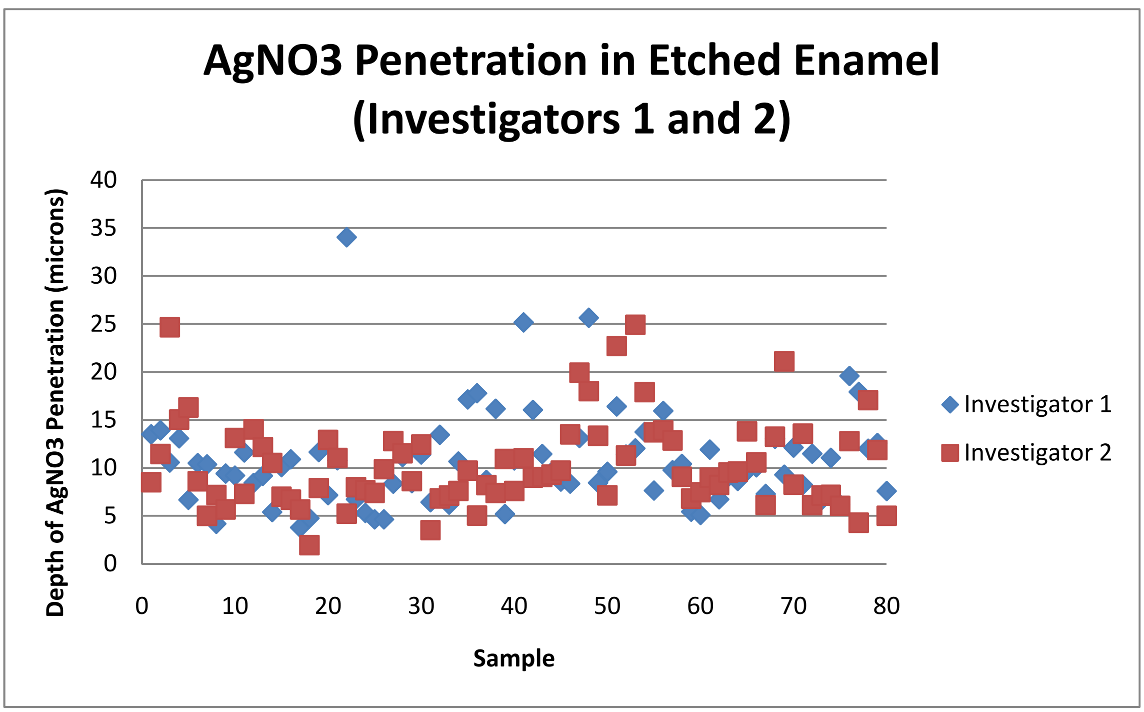


Figure 22 Scattergram showing the average silver nitrate depth measurements for each enamel sample half that was etched (Inv 1 and 2).

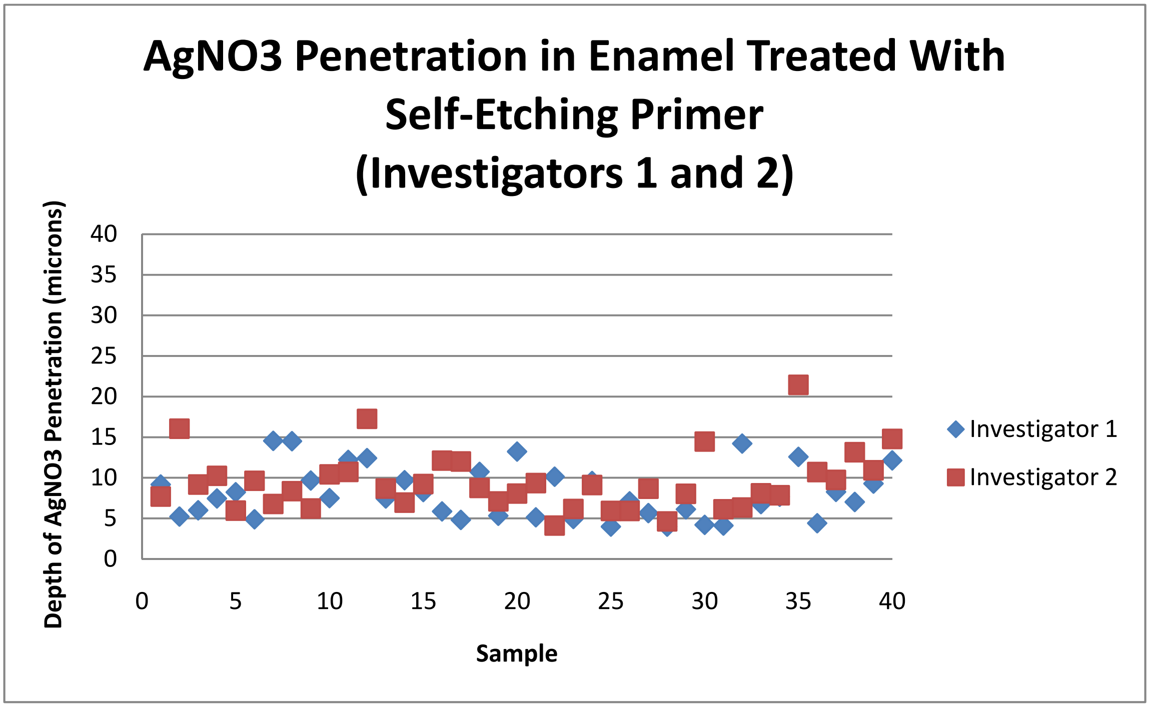


Figure 23 Scattergram showing the average silver nitrate depth measurements for each enamel sample half that was treated with self-etching primer (Inv 1 and 2).

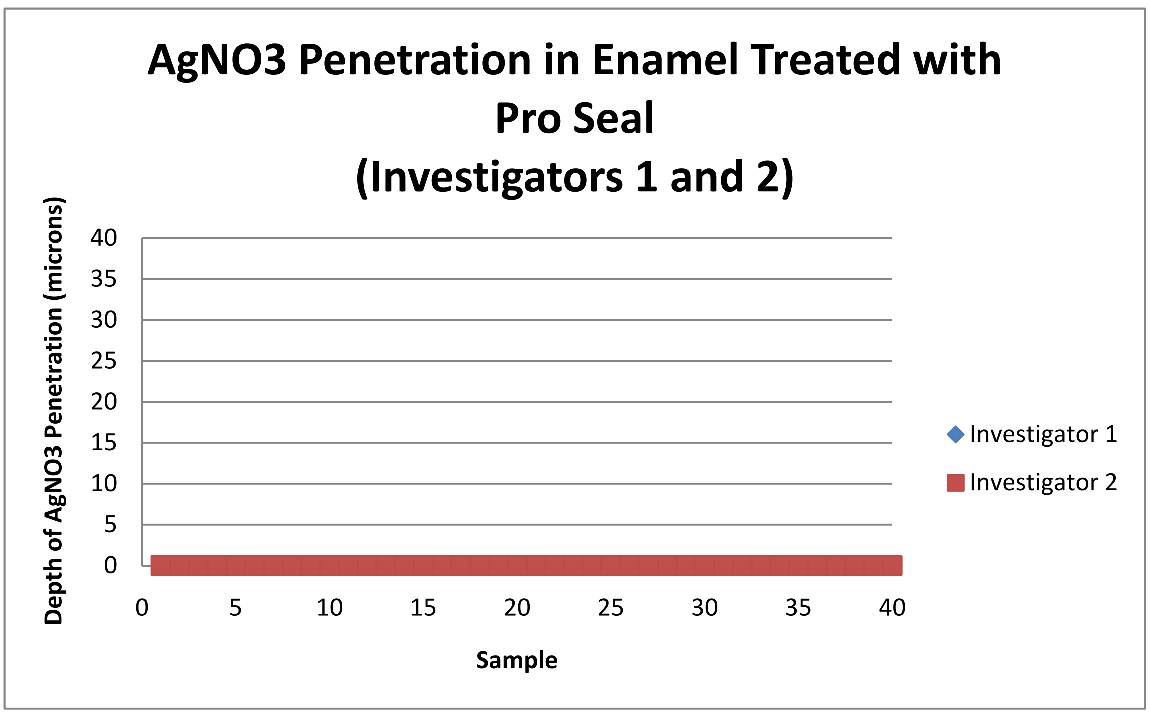


Figure 24 Scattergram showing the average silver nitrate depth measurements for each enamel sample half that was treated with Pro Seal (Inv 1 and 2).

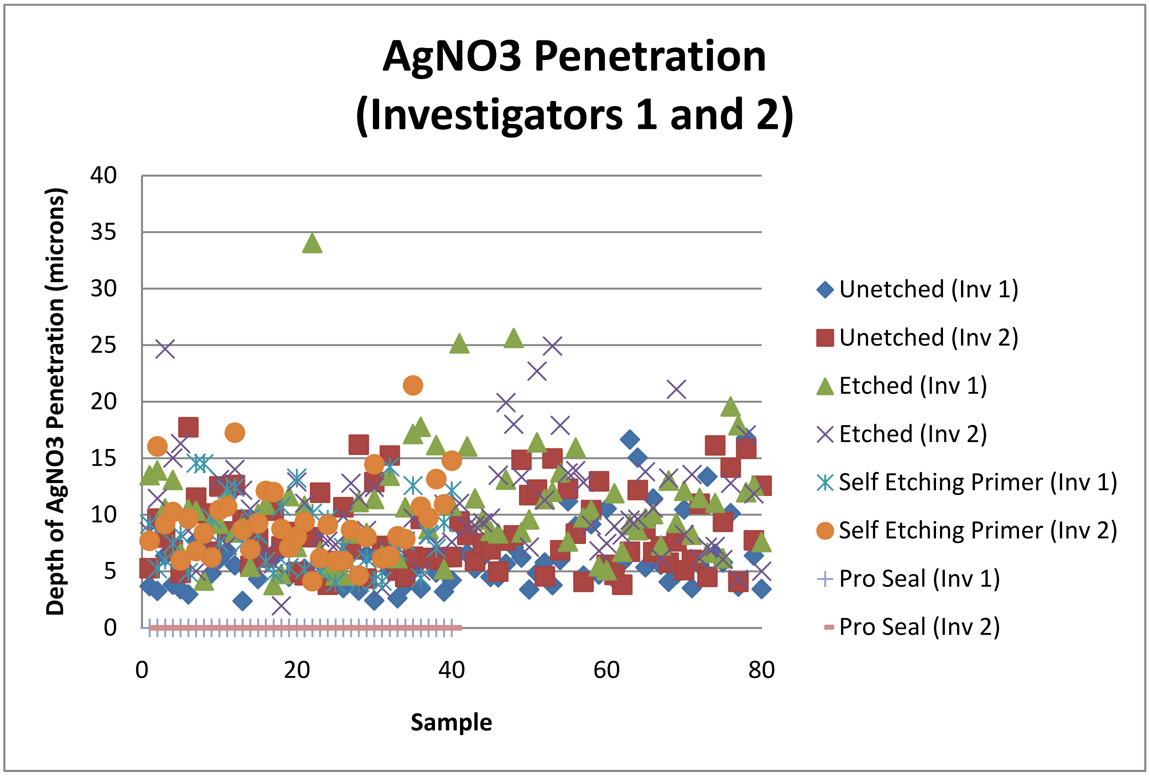


Figure 25 Scattergram showing the average silver nitrate depth measurements for each enamel sample half in all treatment groups (Inv 1 and 2).

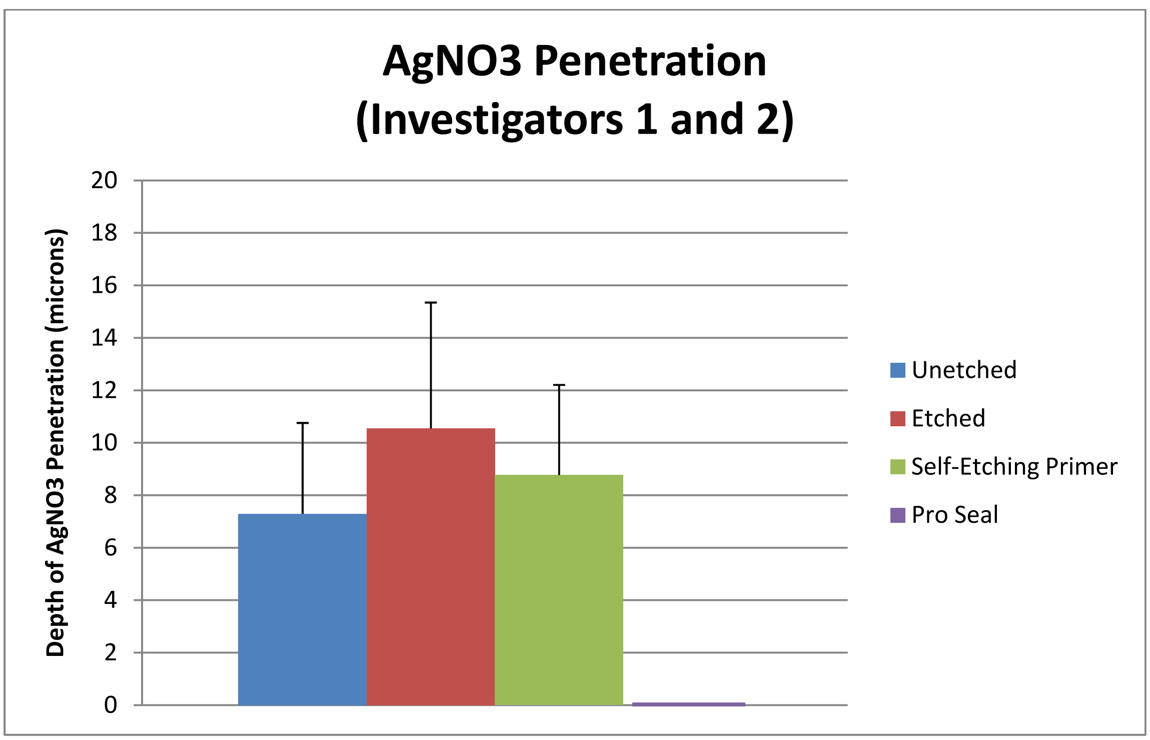


Figure 26 Bar graph showing means and standard deviations of all treatment groups (Inv 1 and 2).

As seen in Figure 26 above, the overall mean and standard deviation were 7.29 and 3.46 microns, respectively, for unetched enamel, 10.55 and 4.80 microns for etched enamel, 8.77 and 3.44 microns for enamel treated with self-etching primer, and 0 and 0 microns for enamel treated with Pro Seal.

Table 3 shows that all of the treatment groups are significantly different from each other. The Pro Seal group is significantly less permeable than all other groups and is, in fact, virtually impermeable. The etched group is significantly more permeable than all other groups. The self-etching primer group is significantly more permeable than the unetched group, but significantly less permeable than the etched group.

Treatment Group	Unetched	Etched	Self-etching primer
Etched	3.26 (2.33, 4.18) *P*<0.001*		
Self-etching primer	1.47 (0.59, 2.36) *P*=0.001*	-1.78 (-3.11, -0.46) *P*=0.008*	
Pro Seal	-7.30 (-7.84, -6.75) *P*<0.001*	-10.55 (-11.53, -9.57) *P*<0.001*	-8.77(-9.64,-7.90) *P*<0.001*

Table 3 Estimated mean difference in silver nitrate depth measurements between treatment groups with 95% confidence interval, *p*-value is for the test of a significant difference between groups (*$p < 0.01$*).

C. Permeability of enamel after thermocycling.

The following figures, Figures 27 and 28, show the silver nitrate depth measurements that were taken for the twenty enamel samples that underwent thermocycling. Each enamel sample was half unetched, half treated with Pro Seal. As was done for the first part of the study, the average depth measurement for each treatment half was plotted on a scattergram. All measurements in the following figures were taken by Investigator 1. The control group underwent zero thermocycles.

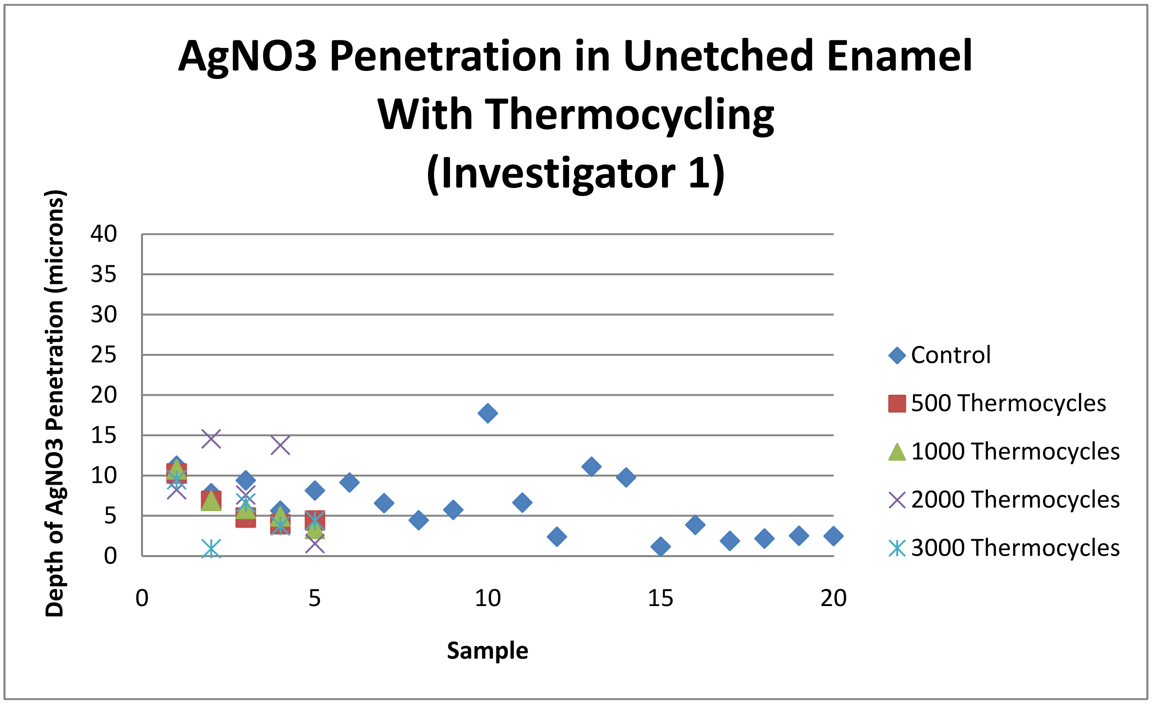


Figure 27 Scattergram showing the average silver nitrate depth measurements for each unetched enamel sample half that was put through various amounts of thermocycling (Inv 1).

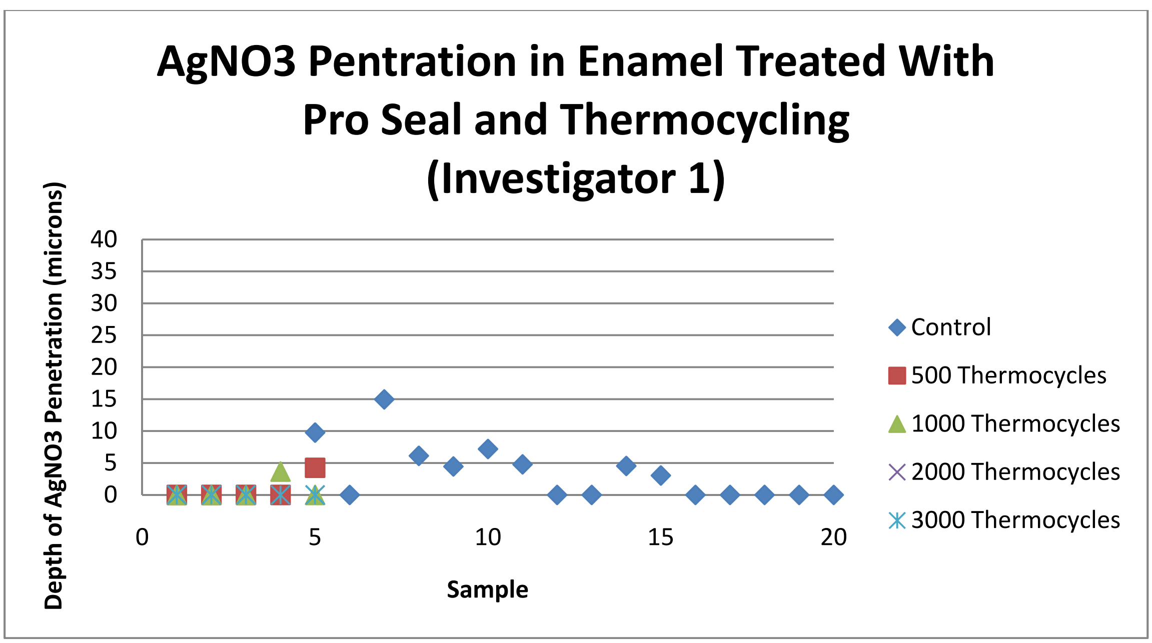


Figure 28 Scattergram showing the average silver nitrate depth measurements for each enamel sample half treated with Pro Seal that was put through various amounts of thermocycling (Inv 1).

As seen in the following figure, Figure 29, for unetched enamel, the overall mean and standard deviation were 6.49 and 4.17 microns, respectively, after 0 thermocycles, 6.05 and 2.60 microns after 500 thermocycles, 6.36 and 2.78 microns after 1000 thermocycles, 9.13 and 5.28 microns after 2000 thermocycles, and 5.03 and 3.22 microns after 3000 thermocycles. For enamel treated with Pro Seal the overall mean and standard deviation were 2.74 and 4.17 microns, respectively, after 0 thermocycles, 0.85 and 1.90 microns after 500 thermocycles, 0.73 and 1.63 microns after 1000 thermocycles, 0 and 0 microns after 2000 thermocycles, and 0 and 0 microns after 3000 thermocycles.

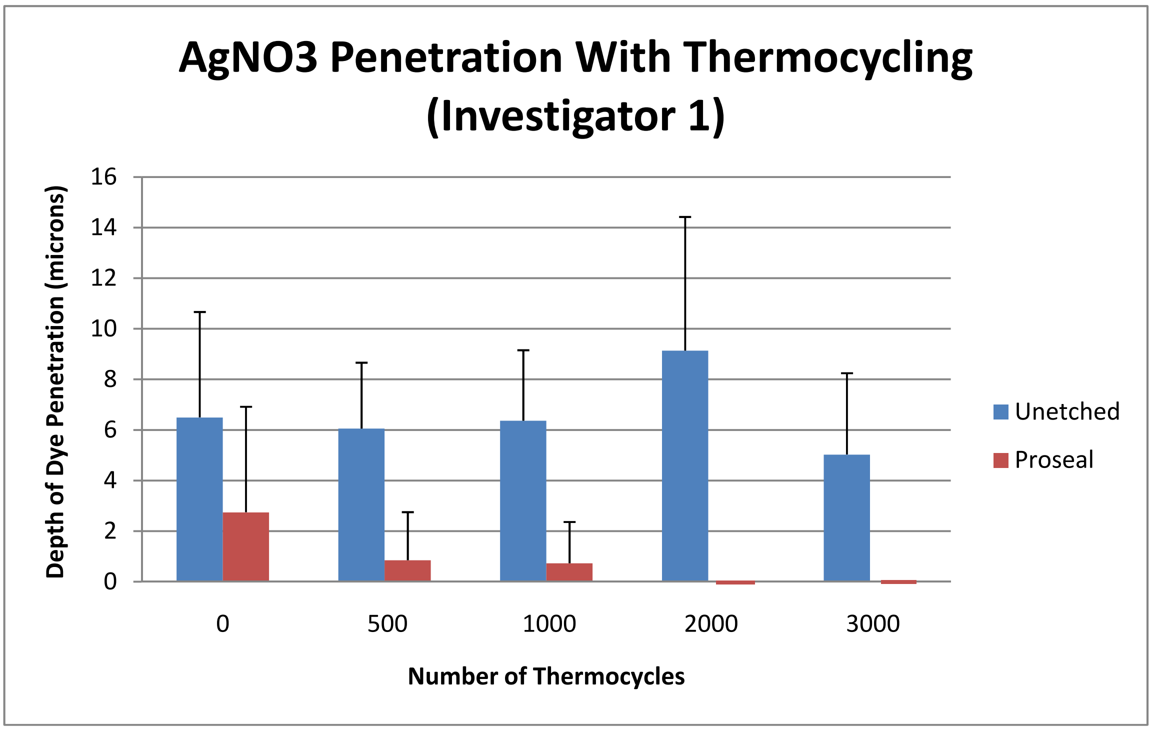


Figure 29 Bar graph showing means and standard deviations for unetched enamel and enamel treated with Pro Seal after various amounts of thermocycling (Inv 1).

Figures 30 and 31 show the silver nitrate depth measurements that were taken for the twenty enamel samples that underwent thermocycling, but by Investigator 2.

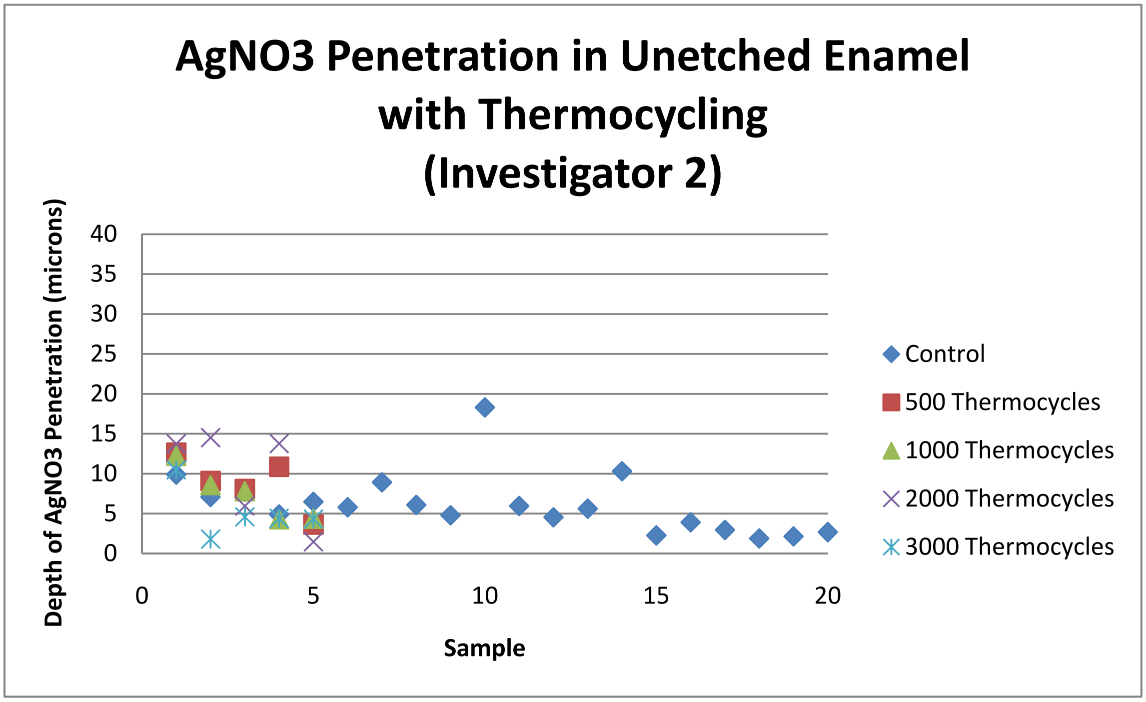


Figure 30 Scattergram showing the average silver nitrate depth measurements for each unetched enamel sample half that was put through various amounts of thermocycling (Inv 2).

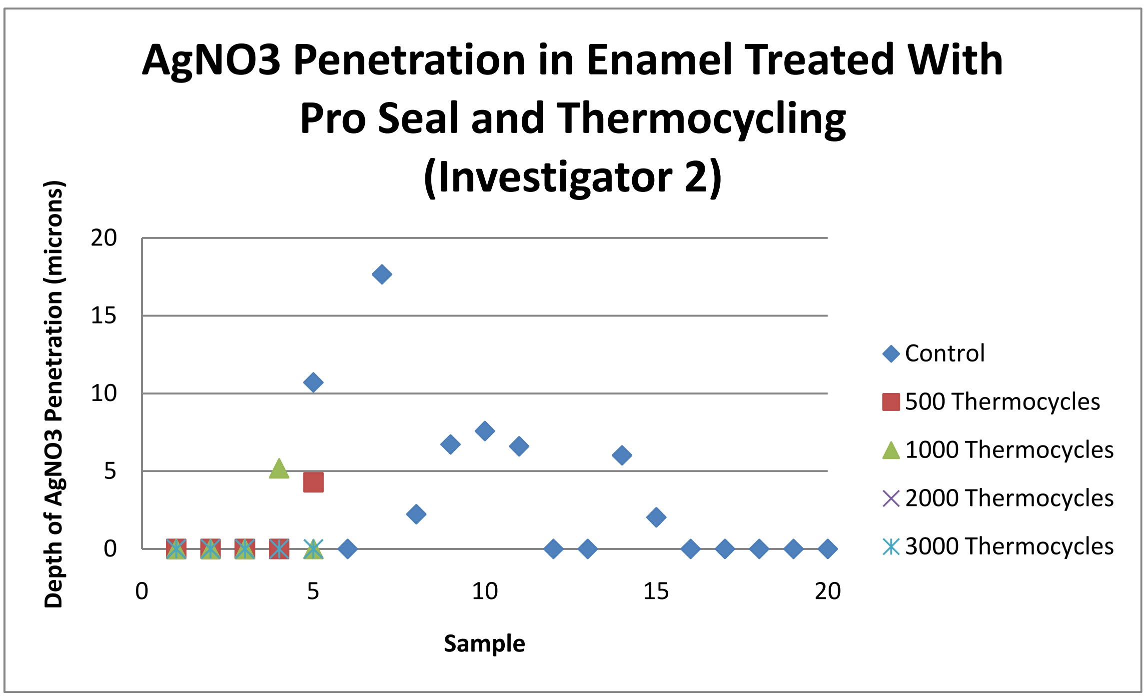


Figure 31 Scattergram showing the average silver nitrate depth measurements for each enamel sample half treated with Pro Seal that was put through various amounts of thermocycling (Inv 2).

As seen in the following figure, Figure 32, for unetched enamel, the overall mean and standard deviation were 6.11 and 3.80 microns, respectively, after 0 thermocycles, 8.87 and 3.40 microns after 500 thermocycles, 7.46 and 3.32 microns after 1000 thermocycles, 9.89 and 5.87 microns after 2000 thermocycles, and 5.12 and 3.20 microns after 3000 thermocycles. For enamel treated with Pro Seal the overall mean and standard deviation were 2.97 and 4.82 microns, respectively, after 0 thermocycles, 0.86 and 1.91 microns after 500 thermocycles, 1.04 and 2.32 microns after 1000 thermocycles, 0 and 0 microns after 1000 thermocycles, and 0 and 0 microns after 3000 thermocycles.

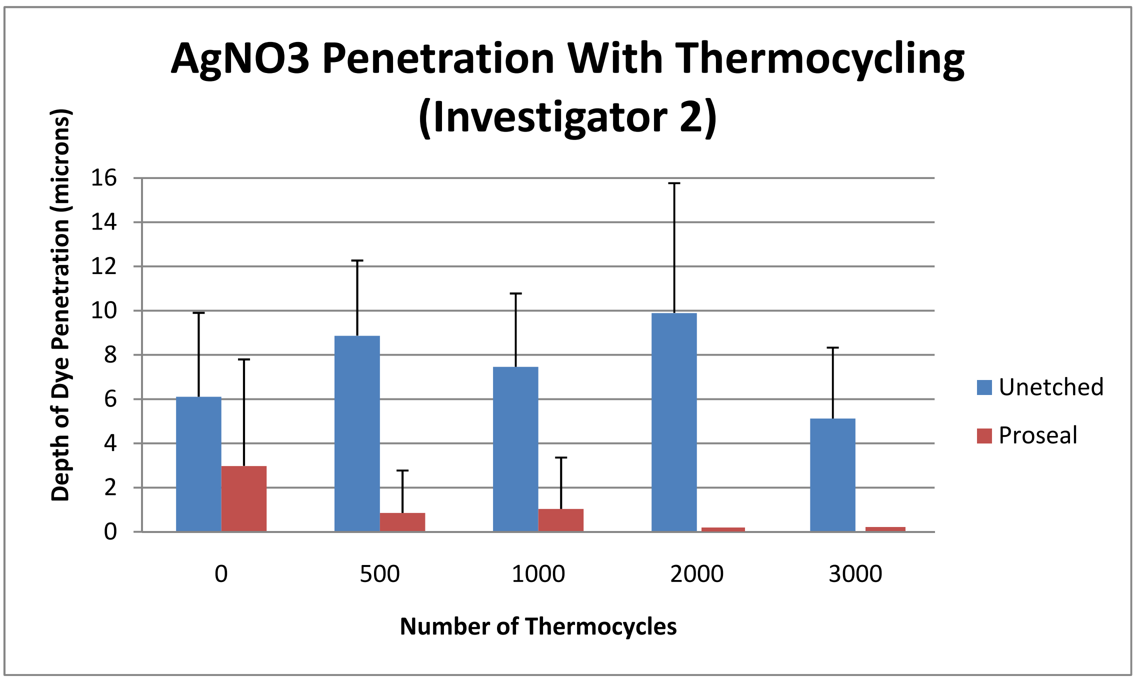


Figure 32 Bar graph showing means and standard deviations for unetched enamel and enamel treated with Pro Seal after various amounts of thermocycling (Inv 2).

As was done in the first part of the study, the silver nitrate depth measurements for the two investigators were combined because the inter-investigator reliability was high (Figures 33 and 34). An overall mean and standard deviation could then be calculated (Figure 35).

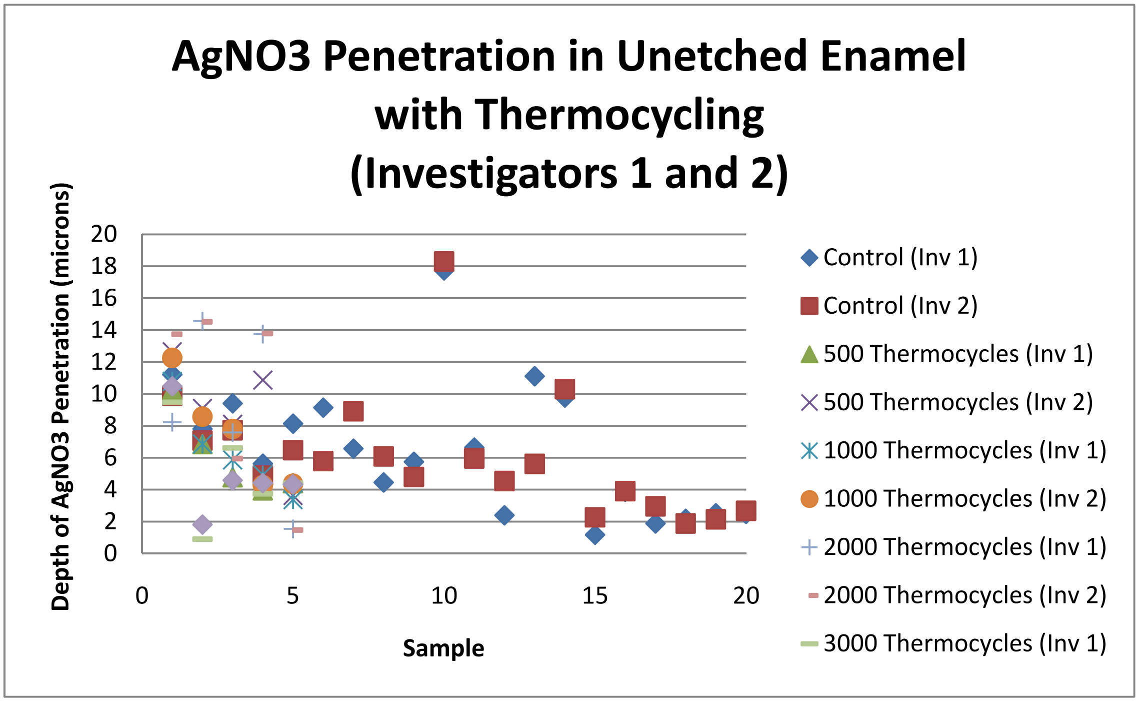


Figure 33 Scattergram showing the average silver nitrate depth measurements for each unetched enamel sample half that was put through various amounts of thermocycling (Inv 1 and 2).

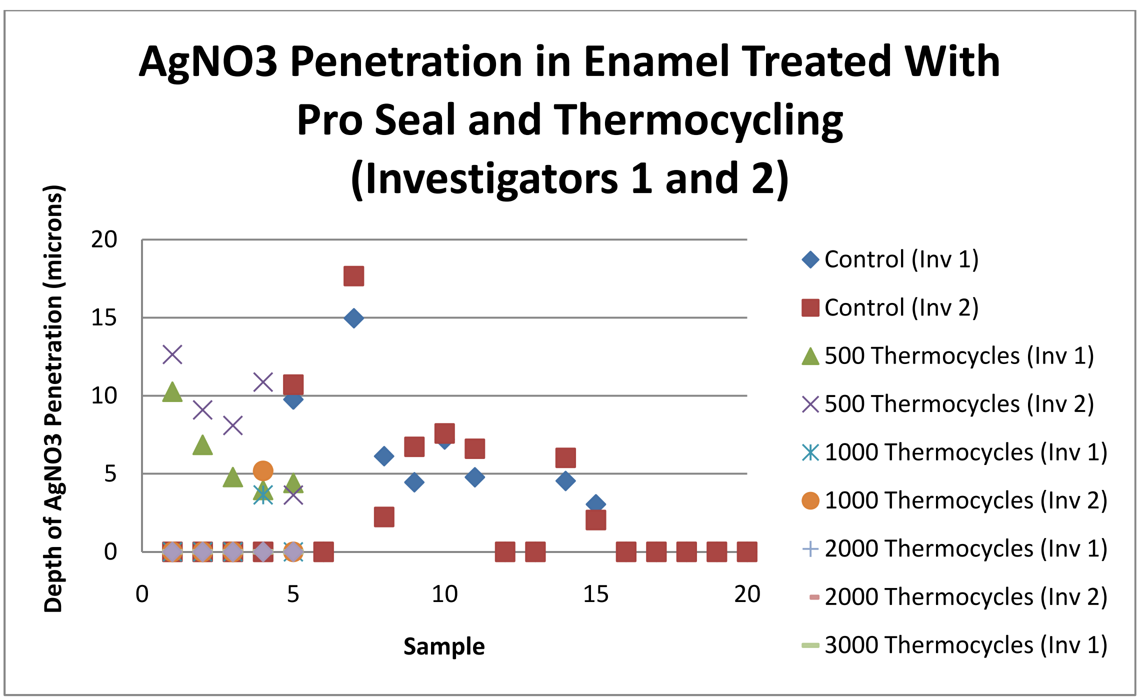


Figure 34 Scattergram showing the average silver nitrate depth measurements for each enamel sample half treated with Pro Seal that was put through various amounts of thermocycling (Inv 1 and 2).

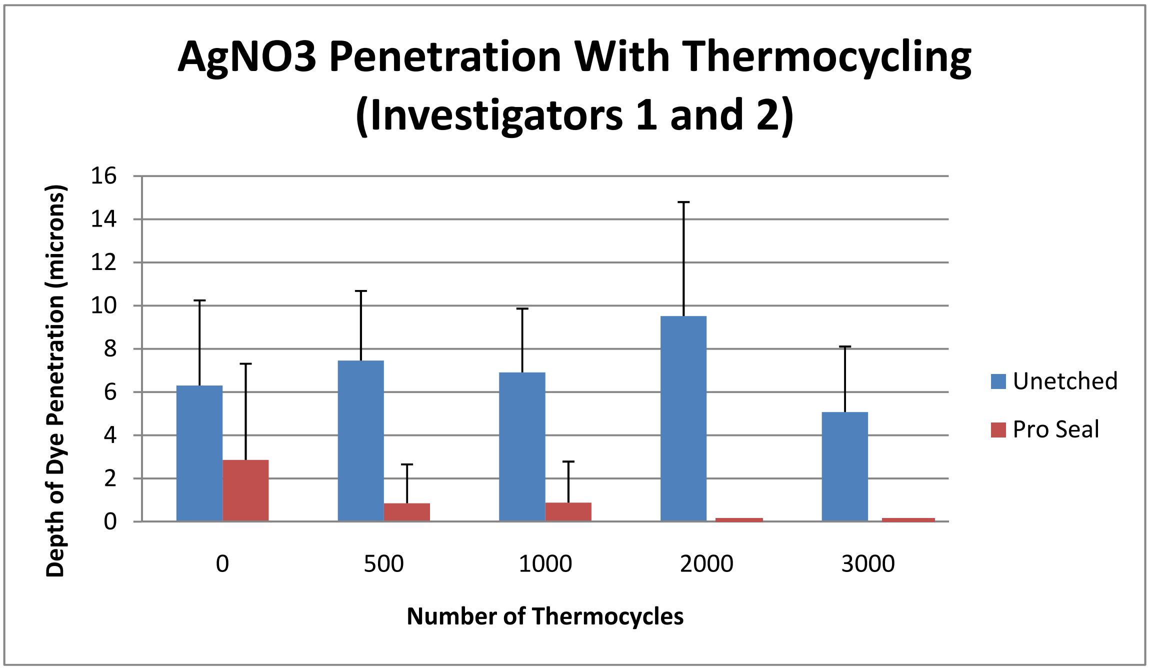


Figure 35 Bar graph showing means and standard deviations for unetched enamel and enamel treated with Pro Seal after various amounts of thermocycling (Inv 1 and 2).

As seen in the previous figure, Figure 35, for unetched enamel the overall mean and standard deviation were 6.30 and 3.94 microns respectively after 0 thermocycles, 7.46 and 4.22 microns after 500 thermocycles, 6.91 and 2.95 microns after 1000 thermocycles, 9.51 and 5.28 microns after 2000 thermocycles, and 5.07 and 3.03 microns after 3000 thermocycles. For enamel treated with Pro Seal the overall mean and standard deviation were 2.86 and 4.45 microns, respectively, after 0 thermocycles, 0.85 and 1.80 microns after 500 thermocycles, 0.88 and 1.90 microns after 1000 thermocycles, 0 and 0 microns after 2000 thermocycles, and 0 and 0 microns after 3000 thermocycles.

Table 4 shows that for unetched enamel, none of the thermocycling groups were significantly different from each other. Therefore, thermocycling does not have an effect on enamel permeability for unetched enamel.

Number of thermocycles	0 (control)
3000	1.16 (-1.91, 4.22) *P*=0.45
2000	0.63 (-2.63, 3.89) *P*=0.71
1000	3.22 (-1.51, 7.93) *P*=0.18
500	-1.23 (-4.56, 2.11) *P*=0.47

Table 4 Estimated mean difference in silver nitrate depth measurements between thermocycling groups for unetched enamel with 95% confidence interval, *p*-value is for the test of a significant difference between groups ($p < 0.05$).

Table 5 shows that for enamel treated with Pro Seal, only the 2000 and 3000 thermocycles groups were significantly different from the control group (0 thermocycles). Permeability significantly decreased compared to the control group for the 2000 and 3000 thermocycles groups. However, thermocycling did not have a significant effect on the 500 and 1000 thermocycles groups when compared to the control group.

Number of thermocycles	0 (control)
500	-2.01 (-4.34, 0.32) *P*=0.09
1000	-1.98 (-4.09, 0.14) *P*=0.07
2000	-2.87 (-4.75, -0.97) *P*=0.003*
3000	-2.87 (-4.75, -0.97) *P*=0.003*

Table 5 Estimated mean difference in silver nitrate depth measurements between thermocycling groups for enamel treated with Pro Seal with 95% confidence interval, *p*-value is for the test of a significant difference between groups ($p < 0.01$).

Discussion

The purpose of this study was to first test the hypothesis that etched enamel is more permeable than untreated enamel, and that enamel treated with Pro Seal is less permeable than untreated enamel. Secondly, we wanted to test the hypothesis that enamel treated with Pro Seal and then thermocycled is more permeable than sealed enamel that has not been thermocycled. Since the mouth goes through up to 50 thermocycles per day (Gale and Darvell, 1999), it is important for Pro Seal to be able to withstand thermal stresses in order to remain on the tooth for a clinically acceptable period of time under intraoral conditions.

As expected, the etched enamel samples were significantly more permeable than the unetched, self-etching primer, and Pro Seal samples. This is likely due to the fact that acid etching the tooth surface removes a thin layer of enamel, making the enamel surface more porous by partially dissolving the enamel prism ends. Because the surface is more porous, the permeability is increased accordingly. Therefore, etched enamel surfaces such as those present immediately after orthodontic bracket bonding are most susceptible to white spot lesions. On average the permeability of etched enamel was 10.55 microns, which is in agreement with previous studies that have found that etched enamel permeability ranges from 8-15 microns Buonocore *et al.,* 1968; Jorgensen and Shimokobe, 1975; Pahlavan *et al.*).

Another finding that corroborated our hypothesis was that the samples that had been treated with Pro Seal were significantly less permeable than the untreated, etched, and self-etching primer samples. The sealant was able to effectively coat the enamel surface thereby reversing the effects of acid etching and reducing the permeability to negligible amounts. We found an average permeability of 0 microns. We can then hypothesize that as long as Pro Seal remains on the tooth surface it is an effective means of defence against acid attack and white spot lesion formation. This is in agreement with previous studies, such as Hu and Featherstone (Hu and Featherstone, 2005).

Of note, was the finding that enamel treated with self-etching primer was significantly more permeable than untreated enamel. This makes sense because self-etching primers produce a similar etching pattern to those produced by traditional acid etching (Ghiz *et al.,* 2009; Pivetta *et al.,* 2008). It should follow then that self-etching primers would have a comparable effect on enamel permeability. We found that the self-etching primer samples did exhibit increased permeability (average of 8.77 microns), but significantly not as much as the etched samples (average of 10.55 microns). A possible reason for this could be that instead of completely removing the smear layer like traditional acid etches, self-etching primers simply incorporate the smear layer into the hybridized complex (Perdigao J, 2003). Since the self-etching primers were significantly more permeable than the untreated samples, it can be postulated that enamel surfaces treated with self-etching primer are more susceptible to white spot lesion formation than untreated enamel surfaces.

Previous studies have shown that Pro Seal is very resistant to mechanical abrasion such as that from tooth brushing (Hu and Featherstone, 2005). The manufacturer claims that the sealant layer should be able to last over 2 years. However, according to anecdotal evidence, Pro Seal only appears to be able to stay on the tooth surface for a matter of months. Therefore, another factor must be involved. We hypothesized that the inherent difference between the coefficients of thermal expansion of Pro Seal and the enamel surface might be the culprit.

As expected, we found that thermocycling had no significant effect on the permeability of untreated enamel. However, the results for the Pro Seal samples that underwent thermocycling were not what we hypothesized. We thought that with increasing amounts of thermocycles, there would be increasing mechanical stresses induced by the differential thermal changes. This would lead to microcracks in the layer of Pro Seal which would eventually result in an erosion of the Pro Seal from the enamel surface and, subsequently, an increase in permeability. Instead, we found that with increased numbers of thermocycles, the permeability of enamel treated with Pro Seal actually decreased. Thermocycling had no significant effect on the permeability of the Pro Seal samples that underwent 500 and 1000 thermocycles (equivalent to 10 and 20 days respectively).

However, thermocycling significantly decreased the permeability of the Pro Seal samples that underwent 2000 and 3000 thermocycles (equivalent to 40 and 60 days respectively).

A potential reason for this finding could be that instead of causing microcrack propagation in the Pro Seal layer, the thermal stresses actually had the opposite effect and caused it to harden. This would result in an increased sealing effect on the pores of the enamel surface and a subsequent decrease in permeability. However, there are no previous studies that investigate this finding. Therefore, this is mere postulation at this point in time.

Another potential reason for this finding could be that the thermocycler that was used in this study had a middle water bath of 21°C (room temperature) instead of 37°C (body temperature). Since intraoral conditions return to body temperature after thermal insults, this would have been the preferred temperature for the middle water bath. With a 37°C middle water bath, the temperature differentials would have been 18°C with the hot water bath and 32°C with the cold water bath. However, with the 21°C middle water bath that was used in this study, the temperature differentials were 34°C with the hot water bath and 16°C with the cold water bath. This is almost a complete reversal of the thermal differentials for a body temperature middle water bath. Perhaps having a larger "hot" differential and a smaller "cold" differential could have had a significant effect on our thermocycling results. However, there are no previous studies that investigate the influence of different thermal differentials on thermocycling effects. Further studies would need to be performed.

Another potential reason for this finding could be that for this part of the study, we had a very small sample size. Each thermocycling group only had five samples. Because of this, the power of the study was quite low. Outliers were able to exert a significant influence on the study statistics. Also, because the buccal and lingual surfaces of human molars are quite small, we were only able to get 1 enamel sample from each molar. It was not possible to get all of the samples from one molar. Therefore, inherent differences between the enamel from different subjects were introduced. These differences could be due to subject age, diet, fluoridation history, etc. Because the sample size was so small, these differences may have been able to have an effect. The effect of these differences would be negligible in larger sample sizes.

However, the theory behind this second part of the study is sound. It needs to be determined why Pro Seal only has the intraoral lifespan that it demonstrates clinically according to anecdotal evidence, in order to be an effective means of preventing white spot lesions in orthodontic patients. The study needs to be repeated but with a significantly larger sample size.

Conclusion

This study clearly demonstrates that etched enamel is significantly more permeable than untreated enamel, enamel treated with Pro Seal is significantly less permeable than untreated enamel, and enamel treated with self-etching primer is significantly more permeable than untreated enamel but significantly not as permeable as etched enamel. These findings are relevant because it shows that etched enamel and enamel treated with self-etching primer are at an increased risk of white spot lesion formation in the days following orthodontic bracket bonding until the enamel has been fully remineralized via intraoral conditions. Therefore, patients should be instructed to be the most cognisant in avoiding cariogenic foods and beverages in this time period. Pro Seal is an effective means of decreasing enamel permeability and thus white spot lesion susceptibility as long as it is able to stay on the tooth intraorally. Further studies need to be performed in order to determine if thermal stresses play a role in the intraoral lifespan of Pro Seal.

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APPENDICES

Appendix A: Depth Measurements (1st Part of Study)

Legend

Tx Indicator	**Reader ID**
1=unetched	**1=Nicole**
2 = etched	**2=Daniele**
3 = L-pop	
4 = Pro Seal	

Reader ID	Tooth ID	Side	Tx Indicator	Replicate	Depth (microns)	Reliability
1	1	1	1	1	3.64	
1	1	1	1	2	3.64	
1	1	1	1	3	2.57	
1	1	1	1	4	3.21	
1	1	1	1	5	5.35	
1	1	1	3	1	6.85	
1	1	1	3	2	10.3	
1	1	1	3	3	7.28	
1	1	1	3	4	13.1	
1	1	1	3	5	8.35	
2	1	1	1	1	5.14	
2	1	1	1	2	6.21	
2	1	1	1	3	4.93	
2	1	1	1	4	6	
2	1	1	1	5	4.07	
2	1	1	3	1	7.49	
2	1	1	3	2	8.57	
2	1	1	3	3	5.57	
2	1	1	3	4	5.8	
2	1	1	3	5	11	
1	1	2	1	1	2.78	
1	1	2	1	2	3.22	
1	1	2	1	3	3.21	
1	1	2	1	4	5.14	
1	1	2	1	5	1.93	
1	1	2	3	1	4.28	
1	1	2	3	2	3.85	
1	1	2	3	3	5.14	
1	1	2	3	4	9.42	
1	1	2	3	5	3.43	
2	1	2	1	1	10.9	
2	1	2	1	2	7.71	

2	1	2	1	3	11.3
2	1	2	1	4	10.9
2	1	2	1	5	7.49
2	1	2	3	1	15
2	1	2	3	2	12.8
2	1	2	3	3	13.6
2	1	2	3	4	17.6
2	1	2	3	5	21.2
1	2	1	1	1	3.7
1	2	1	1	2	8.68
1	2	1	1	3	3.59
1	2	1	1	4	11.7
1	2	1	1	5	4.16
1	2	1	3	1	7.71
1	2	1	3	2	5.14
1	2	1	3	3	6.21
1	2	1	3	4	2.57
1	2	1	3	5	8.35
2	2	1	1	1	6.06
2	2	1	1	2	6.48
2	2	1	1	3	7.29
2	2	1	1	4	7.29
2	2	1	1	5	12.8
2	2	1	3	1	7.29
2	2	1	3	2	10.3
2	2	1	3	3	9.43
2	2	1	3	4	10.7
2	2	1	3	5	8.14
1	2	2	1	1	4.71
1	2	2	1	2	1.93
1	2	2	1	3	3.21
1	2	2	1	4	3.85
1	2	2	1	5	5.35
1	2	2	3	1	6.33
1	2	2	3	2	9.07
1	2	2	3	3	7.01
1	2	2	3	4	5.46
1	2	2	3	5	9.41
2	2	2	1	1	6.42
2	2	2	1	2	7.71
2	2	2	1	3	4.93

2	2	2	1	4	8.57
2	2	2	1	5	6.87
2	2	2	3	1	9.64
2	2	2	3	2	14.6
2	2	2	3	3	13.7
2	2	2	3	4	6.21
2	2	2	3	5	7.07
1	3	1	1	1	2.59
1	3	1	1	2	3.53
1	3	1	1	3	3.39
1	3	1	1	4	3.74
1	3	1	1	5	3.79
1	3	1	3	1	8.82
1	3	1	3	2	10.4
1	3	1	3	3	5.6
1	3	1	3	4	10.3
1	3	1	3	5	6.01
2	3	1	1	1	5.18
2	3	1	1	2	6.02
2	3	1	1	3	4.63
2	3	1	1	4	4.99
2	3	1	1	5	3.49
2	3	1	3	1	5.35
2	3	1	3	2	5.78
2	3	1	3	3	3.65
2	3	1	3	4	7.93
2	3	1	3	5	7.07
1	3	2	1	1	2.78
1	3	2	1	2	2.14
1	3	2	1	3	3.43
1	3	2	1	4	3.21
1	3	2	1	5	3.21
1	3	2	3	1	3.12
1	3	2	3	2	5.46
1	3	2	3	3	3.53
1	3	2	3	4	5.62
1	3	2	3	5	6.71
2	3	2	1	1	10.4
2	3	2	1	2	15.4
2	3	2	1	3	12.9
2	3	2	1	4	29.1

2	3	2	1	5	20.9
2	3	2	3	1	9.64
2	3	2	3	2	12.2
2	3	2	3	3	9.85
2	3	2	3	4	8.14
2	3	2	3	5	8.35
1	4	1	1	1	9.64
1	4	1	1	2	10.9
1	4	1	1	3	4.28
1	4	1	1	4	4.28
1	4	1	1	5	9.21
1	4	1	3	1	14.9
1	4	1	3	2	17.8
1	4	1	3	3	12.7
1	4	1	3	4	12
1	4	1	3	5	15.4
2	4	1	1	1	19.3
2	4	1	1	2	7.76
2	4	1	1	3	8.61
2	4	1	1	4	10.3
2	4	1	1	5	11.6
2	4	1	3	1	4.72
2	4	1	3	2	7.93
2	4	1	3	3	6.21
2	4	1	3	4	9.64
2	4	1	3	5	5.39
1	4	2	1	1	11.8
1	4	2	1	2	9.86
1	4	2	1	3	9.03
1	4	2	1	4	5.77
1	4	2	1	5	5.21
1	4	2	3	1	8.27
1	4	2	3	2	10.6
1	4	2	3	3	20.3
1	4	2	3	4	17.1
1	4	2	3	5	16.3
2	4	2	1	1	10.3
2	4	2	1	2	8.8
2	4	2	1	3	8.99
2	4	2	1	4	10.7
2	4	2	1	5	11.8

2	4	2	3	1	7.49
2	4	2	3	2	7.71
2	4	2	3	3	10.5
2	4	2	3	4	8.78
2	4	2	3	5	7.28
1	5	1	1	1	4.07
1	5	1	1	2	3.43
1	5	1	1	3	8.78
1	5	1	1	4	3.64
1	5	1	1	5	4.07
1	5	1	3	1	9.42
1	5	1	3	2	7.71
1	5	1	3	3	9.64
1	5	1	3	4	12.8
1	5	1	3	5	8.78
2	5	1	1	1	6
2	5	1	1	2	6
2	5	1	1	3	7.08
2	5	1	1	4	7.71
2	5	1	1	5	6.85
2	5	1	3	1	7.93
2	5	1	3	2	5.78
2	5	1	3	3	5.57
2	5	1	3	4	3
2	5	1	3	5	8.78
1	5	2	1	1	8.44
1	5	2	1	2	2.79
1	5	2	1	3	8.33
1	5	2	1	4	6
1	5	2	1	5	4.73
1	5	2	3	1	9.21
1	5	2	3	2	9.64
1	5	2	3	3	7.71
1	5	2	3	4	6.85
1	5	2	3	5	4.07
2	5	2	1	1	9.62
2	5	2	1	2	11
2	5	2	1	3	14
2	5	2	1	4	10.6
2	5	2	1	5	17.3
2	5	2	3	1	7.71

2	5	2	3	2	12.7
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2	5	2	3	5	12.3
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2	6	1	3	4	7.48
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1	6	2	1	2	3.85
1	6	2	1	3	6.21
1	6	2	1	4	6.43
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2	9	1	2	1	6.21
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1	9	2	2	3	9.08
1	9	2	2	4	9.93
1	9	2	2	5	18.5
2	9	2	1	1	7.71
2	9	2	1	2	5.78
2	9	2	1	3	6.64
2	9	2	1	4	5.57
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2	9	2	2	3	13.3
2	9	2	2	4	12
2	9	2	2	5	11.3

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1	10	1	1	4	5.35
1	10	1	1	5	3.01
1	10	1	2	1	11.1
1	10	1	2	2	18.1
1	10	1	2	3	9.93
1	10	1	2	4	5.18
1	10	1	2	5	8.53
2	10	1	1	1	9
2	10	1	1	2	10.1
2	10	1	1	3	5.3
2	10	1	1	4	9
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1	51	1	2	1	11.1
1	51	1	2	2	11.4
1	51	1	2	3	9.42
1	51	1	2	4	15.6
1	51	1	2	5	12
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1	51	1	4	3	0
1	51	1	4	4	0
1	51	1	4	5	0
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2	51	1	2	2	9.89
2	51	1	2	3	8.61
2	51	1	2	4	7.82
2	51	1	2	5	10.7
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2	51	1	4	3	0
2	51	1	4	4	0
2	51	1	4	5	0
1	51	2	2	1	6.85
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1	51	2	2	3	5.14
1	51	2	2	4	5.78
1	51	2	2	5	6.21
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1	51	2	4	3	0
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2	51	2	2	1	7.51
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2	51	2	2	3	8.57
2	51	2	2	4	6.65
2	51	2	2	5	9.33
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2	51	2	4	3	0
2	51	2	4	4	0
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1	52	1	2	1	8.35

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1	52	1	2	4	9.64
1	52	1	2	5	6.85
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1	52	1	4	3	0
1	52	1	4	4	0
1	52	1	4	5	0
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2	52	1	2	3	9.85
2	52	1	2	4	8.58
2	52	1	2	5	7.97
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2	52	1	4	2	0
2	52	1	4	3	0
2	52	1	4	4	0
2	52	1	4	5	0
1	52	2	2	1	8.78
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1	52	2	2	3	9.22
1	52	2	2	4	11.3
1	52	2	2	5	8.14
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1	52	2	4	4	0
1	52	2	4	5	0
2	52	2	2	1	11.3
2	52	2	2	2	10.7
2	52	2	2	3	5.83
2	52	2	2	4	9.42
2	52	2	2	5	10.8
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2	52	2	4	3	0
2	52	2	4	4	0
2	52	2	4	5	0
1	53	1	2	1	8.14
1	53	1	2	2	10.1

1	53	1	2	3	10.7
1	53	1	2	4	7.92
1	53	1	2	5	11.6
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1	53	1	4	3	0
1	53	1	4	4	0
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2	53	2	2	4	11.2
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2	53	2	4	3	0
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2	54	1	4	3	0
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1	54	2	2	3	6.64
1	54	2	2	4	10.1
1	54	2	2	5	15.8
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1	54	2	4	3	0
1	54	2	4	4	0
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2	54	2	2	3	17.8
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2	54	2	4	3	0
2	54	2	4	4	0
2	54	2	4	5	0
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1	55	1	2	4	8.14

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1	55	1	4	3	0
1	55	1	4	4	0
1	55	1	4	5	0
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2	55	1	2	3	21.8
2	55	1	2	4	25.9
2	55	1	2	5	13.9
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2	55	1	4	3	0
2	55	1	4	4	0
2	55	1	4	5	0
1	55	2	2	1	10.1
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1	55	2	2	3	10.5
1	55	2	2	4	10.3
1	55	2	2	5	11.3
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1	55	2	4	2	0
1	55	2	4	3	0
1	55	2	4	4	0
1	55	2	4	5	0
2	55	2	2	1	11.1
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2	55	2	2	3	8.14
2	55	2	2	4	8.58
2	55	2	2	5	7.82
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2	55	2	4	2	0
2	55	2	4	3	0
2	55	2	4	4	0
2	55	2	4	5	0
1	56	1	2	1	8.78
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1	56	1	2	3	6.42
1	56	1	2	4	7.71
1	56	1	2	5	11.6

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1	56	1	4	3	0
1	56	1	4	4	0
1	56	1	4	5	0
2	56	1	2	1	10.7
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2	56	1	2	3	14.1
2	56	1	2	4	15.2
2	56	1	2	5	12.8
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2	56	1	4	2	0
2	56	1	4	3	0
2	56	1	4	4	0
2	56	1	4	5	0
1	56	2	2	1	10.9
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1	56	2	2	3	10.5
1	56	2	2	4	12
1	56	2	2	5	10.9
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1	56	2	4	2	0
1	56	2	4	3	0
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1	56	2	4	5	0
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2	56	2	2	3	4.28
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2	56	2	2	5	6.48
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2	56	2	4	3	0
2	56	2	4	4	0
2	56	2	4	5	0
1	57	1	2	1	5.57
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1	57	1	2	4	7.28
1	57	1	2	5	5.57
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1	57	1	4	3	0
1	57	1	4	4	0
1	57	1	4	5	0
2	57	1	2	1	5.78
2	57	1	2	2	7.49
2	57	1	2	3	9.06
2	57	1	2	4	4.71
2	57	1	2	5	8.57
2	57	1	4	1	0
2	57	1	4	2	0
2	57	1	4	3	0
2	57	1	4	4	0
2	57	1	4	5	0
1	57	2	2	1	13.7
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1	57	2	2	3	10.3
1	57	2	2	4	8.35
1	57	2	2	5	12.8
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1	57	2	4	3	0
1	57	2	4	4	0
1	57	2	4	5	0
2	57	2	2	1	7.93
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2	57	2	2	3	7.49
2	57	2	2	4	6.21
2	57	2	2	5	7.92
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2	57	2	4	3	0
2	57	2	4	4	0
2	57	2	4	5	0
1	58	1	2	1	4.93
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1	58	1	2	3	7.28
1	58	1	2	4	3.64
1	58	1	2	5	6.64
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1	58	1	4	2	0

1	58	1	4	3	0
1	58	1	4	4	0
1	58	1	4	5	0
2	58	1	2	1	4.93
2	58	1	2	2	4.07
2	58	1	2	3	7.07
2	58	1	2	4	8.35
2	58	1	2	5	5.78
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2	58	1	4	2	0
2	58	1	4	3	0
2	58	1	4	4	0
2	58	1	4	5	0
1	58	2	2	1	22.9
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1	58	2	2	4	16.5
1	58	2	2	5	16.5
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1	58	2	4	3	0
1	58	2	4	4	0
1	58	2	4	5	0
2	58	2	2	1	14.6
2	58	2	2	2	9.42
2	58	2	2	3	10.1
2	58	2	2	4	12
2	58	2	2	5	17.8
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2	58	2	4	3	0
2	58	2	4	4	0
2	58	2	4	5	0
1	59	1	2	1	23.6
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1	59	1	2	3	16.9
1	59	1	2	4	11.8
1	59	1	2	5	13.1
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1	59	1	4	3	0

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1	59	1	4	5	0
2	59	1	2	1	4.28
2	59	1	2	2	3.43
2	59	1	2	3	4.07
2	59	1	2	4	4.07
2	59	1	2	5	5.57
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2	59	1	4	2	0
2	59	1	4	3	0
2	59	1	4	4	0
2	59	1	4	5	0
1	59	2	2	1	9.21
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1	59	2	2	3	16.7
1	59	2	2	4	14.6
1	59	2	2	5	10.9
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1	59	2	4	2	0
1	59	2	4	3	0
1	59	2	4	4	0
1	59	2	4	5	0
2	59	2	2	1	21.5
2	59	2	2	2	18.9
2	59	2	2	3	16.7
2	59	2	2	4	15.9
2	59	2	2	5	12.3
2	59	2	4	1	0
2	59	2	4	2	0
2	59	2	4	3	0
2	59	2	4	4	0
2	59	2	4	5	0
1	60	1	2	1	16.6
1	60	1	2	2	5.42
1	60	1	2	3	7.22
1	60	1	2	4	17.9
1	60	1	2	5	15.8
1	60	1	4	1	0
1	60	1	4	2	0
1	60	1	4	3	0
1	60	1	4	4	0

1	60	1	4	5	0
2	60	1	2	1	9.59
2	60	1	2	2	9.78
2	60	1	2	3	12.1
2	60	1	2	4	17.7
2	60	1	2	5	10.1
2	60	1	4	1	0
2	60	1	4	2	0
2	60	1	4	3	0
2	60	1	4	4	0
2	60	1	4	5	0
1	60	2	2	1	6.6
1	60	2	2	2	8.61
1	60	2	2	3	16.4
1	60	2	2	4	2.65
1	60	2	2	5	3.66
1	60	2	4	1	0
1	60	2	4	2	0
1	60	2	4	3	0
1	60	2	4	4	0
1	60	2	4	5	0
2	60	2	2	1	2.71
2	60	2	2	2	5.37
2	60	2	2	3	9.11
2	60	2	2	4	4.68
2	60	2	2	5	3.16
2	60	2	4	1	0
2	60	2	4	2	0
2	60	2	4	3	0
2	60	2	4	4	0
2	60	2	4	5	0

Appendix B: Depth Measurements (2nd Part of Study)

Legend

Tx Group	**Tx Indicator**	**Reader ID**
1 = control	**1= unetched**	**1 = Nicole**
2 = 3000 cycles	**2 = ProSeal**	**2 = Daniele**
3 = 2000 cycles		
4 = 1000 cycles		
5 = 500 cycles		

Reader ID	Tooth ID	Tx Group	Tx Indicator	Replicate	Depth Measurement (μm)
1	2	1	2	1	0
1	2	1	2	2	0
1	2	1	2	3	0
1	2	1	2	4	0
1	2	1	2	5	0
1	2	1	1	1	9.64
1	2	1	1	2	9.64
1	2	1	1	3	12.4
1	2	1	1	4	12.6
1	2	1	1	5	11.8
1	11	1	1	1	11.3
1	11	1	1	2	4.27
1	11	1	1	3	10
1	11	1	1	4	9.95
1	11	1	1	5	3.53
1	11	1	2	1	0
1	11	1	2	2	0
1	11	1	2	3	0
1	11	1	2	4	0
1	11	1	2	5	0
1	1	1	1	1	11.6
1	1	1	1	2	10.4
1	1	1	1	3	4.79
1	1	1	1	4	7.74
1	1	1	1	5	12.5
1	1	1	2	1	0
1	1	1	2	2	0
1	1	1	2	3	0
1	1	1	2	4	0
1	1	1	2	5	0

1	18	1	1	1	7.49
1	18	1	1	2	6.91
1	18	1	1	3	9.02
1	18	1	1	4	1.94
1	18	1	1	5	2.79
1	18	1	2	1	0
1	18	1	2	2	0
1	18	1	2	3	0
1	18	1	2	4	0
1	18	1	2	5	0
1	12	1	2	1	9.65
1	12	1	2	2	11.1
1	12	1	2	3	6.72
1	12	1	2	4	9.62
1	12	1	2	5	11.7
1	12	1	1	1	8.99
1	12	1	1	2	4.5
1	12	1	1	3	15
1	12	1	1	4	7.92
1	12	1	1	5	4.28
1	9	1	2	1	0
1	9	1	2	2	0
1	9	1	2	3	0
1	9	1	2	4	0
1	9	1	2	5	0
1	9	1	1	1	5.79
1	9	1	1	2	7.71
1	9	1	1	3	10.1
1	9	1	1	4	12.2
1	9	1	1	5	9.91
1	16	1	1	1	4.5
1	16	1	1	2	11.2
1	16	1	1	3	9.67
1	16	1	1	4	4.93
1	16	1	1	5	2.57
1	16	1	2	1	8.14
1	16	1	2	2	8.35
1	16	1	2	3	19.9
1	16	1	2	4	11.6
1	16	1	2	5	26.8
1	10	1	1	1	4.28
1	10	1	1	2	6.64
1	10	1	1	3	3.21

1	10	1	1	4	3
1	10	1	1	5	5.14
1	10	1	2	1	3.85
1	10	1	2	2	5.35
1	10	1	2	3	9.43
1	10	1	2	4	6.21
1	10	1	2	5	5.78
1	3	1	2	1	3.85
1	3	1	2	2	5.14
1	3	1	2	3	4.07
1	3	1	2	4	5.14
1	3	1	2	5	4.07
1	3	1	1	1	8.57
1	3	1	1	2	5.57
1	3	1	1	3	4.94
1	3	1	1	4	1.3
1	3	1	1	5	8.35
1	14	1	1	1	17.8
1	14	1	1	2	19.1
1	14	1	1	3	15.4
1	14	1	1	4	22.3
1	14	1	1	5	14.1
1	14	1	2	1	9.21
1	14	1	2	2	7.5
1	14	1	2	3	5.57
1	14	1	2	4	7.49
1	14	1	2	5	6.21
1	4	1	2	1	6.12
1	4	1	2	2	6.27
1	4	1	2	3	6.81
1	4	1	2	4	2.98
1	4	1	2	5	1.71
1	4	1	1	1	5.35
1	4	1	1	2	6.21
1	4	1	1	3	5.8
1	4	1	1	4	7.28
1	4	1	1	5	8.57
1	8	1	2	1	0
1	8	1	2	2	0
1	8	1	2	3	0
1	8	1	2	4	0
1	8	1	2	5	0
1	8	1	1	1	6.42

1	8	1	1	2	5.57
1	8	1	1	3	0
1	8	1	1	4	0
1	8	1	1	5	0
1	15	1	1	1	13.9
1	15	1	1	2	12
1	15	1	1	3	11.1
1	15	1	1	4	10.1
1	15	1	1	5	8.45
1	15	1	2	1	0
1	15	1	2	2	0
1	15	1	2	3	0
1	15	1	2	4	0
1	15	1	2	5	0
1	6	1	2	1	6.22
1	6	1	2	2	5.79
1	6	1	2	3	2.78
1	6	1	2	4	5.78
1	6	1	2	5	2.14
1	6	1	1	1	14
1	6	1	1	2	9.56
1	6	1	1	3	7.9
1	6	1	1	4	6.09
1	6	1	1	5	11.3
1	17	1	2	1	0
1	17	1	2	2	5.79
1	17	1	2	3	4.5
1	17	1	2	4	4.93
1	17	1	2	5	0
1	17	1	1	1	1.77
1	17	1	1	2	0
1	17	1	1	3	1.93
1	17	1	1	4	0
1	17	1	1	5	2.15
1	20	1	1	1	3.67
1	20	1	1	2	0
1	20	1	1	3	3.88
1	20	1	1	4	7.31
1	20	1	1	5	4.5
1	20	1	2	1	0
1	20	1	2	2	0
1	20	1	2	3	0
1	20	1	2	4	0

1	20	1	2	5	0
1	19	1	1	1	1.28
1	19	1	1	2	2.14
1	19	1	1	3	2.14
1	19	1	1	4	2.14
1	19	1	1	5	1.71
1	19	1	2	1	0
1	19	1	2	2	0
1	19	1	2	3	0
1	19	1	2	4	0
1	19	1	2	5	0
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2	5	5	1	3	11.3
2	5	5	1	4	12.2
2	5	5	1	5	9.21
2	5	5	2	1	0
2	5	5	2	2	0
2	5	5	2	3	0
2	5	5	2	4	0
2	5	5	2	5	0
2	19	5	2	1	0

2	19	5	2	2	0
2	19	5	2	3	0
2	19	5	2	4	0
2	19	5	2	5	0
2	19	5	1	1	0
2	19	5	1	2	0
2	19	5	1	3	2.36
2	19	5	1	4	2.61
2	19	5	1	5	4.07
2	17	5	1	1	3
2	17	5	1	2	4.28
2	17	5	1	3	3.43
2	17	5	1	4	6.21
2	17	5	1	5	6
2	17	5	2	1	0
2	17	5	2	2	0
2	17	5	2	3	0
2	17	5	2	4	0
2	17	5	2	5	0
2	18	5	2	1	0
2	18	5	2	2	0
2	18	5	2	3	0
2	18	5	2	4	0
2	18	5	2	5	0
2	18	5	1	1	7.71
2	18	5	1	2	3.21
2	18	5	1	3	2.79
2	18	5	1	4	3.01
2	18	5	1	5	5.35
2	6	5	2	1	0
2	6	5	2	2	0
2	6	5	2	3	0
2	6	5	2	4	0
2	6	5	2	5	0
2	6	5	1	1	5.79
2	6	5	1	2	4.28
2	6	5	1	3	6.21
2	6	5	1	4	5.36
2	6	5	1	5	0

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