THE EFFICACY OF MI VARNISH VERSUS PRO SEAL SEALANT ON PREVENTION OF WHITE SPOT LESIONS IN ORTHODONTIC PATIENTS: A RANDOMIZED CLINICAL TRIAL

A Thesis

by

LAUREN NICOLE FLYNN

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Peter Buschang
Committee Members,	Katie Julien
	Amal Nourel-Din
Head of Department,	Larry Bellinger

May 2020

Major Subject: Oral Biology

ABSTRACT

Purpose: To compare the efficacy of CPP-ACP MI Varnish and ProSeal sealant in preventing white spot lesion (WSL) formation in orthodontic patients.

Materials and Methods: This prospective randomized clinical trial included 40 orthodontic patients between the ages of 12-17 who were randomly allocated to two groups. Group 1 (Sealant Group) received sealant on the maxillary anterior canines, lateral incisors, and central incisors, with reapplication of the sealant every 3 months. Group 2 (Varnish Group) had MI Varnish applied every 4-6 weeks, without sealant placed on the maxillary anterior teeth. White spot lesion formation was evaluated with standardized digital photographs at two timepoints, T1 (initial appointment before bonding), and T2 (12 months later, with brackets removed). The brackets were removed in order to facilitate an adequate photographic exam. Photographs were analyzed side-by-side at the conclusion of the study with the Enamel Decalcification Index (EDI). The location of WSLs were recorded by tooth type and by region. Oral hygiene was evaluated at T1 and T2.

Results: At the start of the study (T1), 32.5% of the subjects and 11.3% of the teeth exhibited WSLs. Approximately 42.5% of the subjects and 14.9% of the teeth developed new WSLs during the course of the study. The lateral incisors showed the highest incidences of WSL formation in terms of number of teeth (9.5%), number of subjects (32.5%), and total new EDI scores. The incidence of WSL formation and new EDI scores was significantly greater in the gingival region, than mesial, distal, or incisal regions. The majority of decalcification scores were minor. Out of all decalcification scores (1-3), 88.3% were scores of “1”, 6.7% were scores of “2”, and 5% were scores of “3”. There were no between-group

differences for development of WSLs by tooth or by region. Poor oral hygiene at T2 showed a high positive predictive value (76%) for development of WSLs.

Conclusions: MI Varnish and ProSeal sealant provide similar protection during fixed orthodontic treatment. Even though the incidence of WSLs is high, the severity of WSLs in patients treated with either sealant or varnish can be minimal. The lateral incisors and the gingival region are more likely to develop new WSLs. Patients who develop WSLs tended to have poorer oral hygiene during treatment.

ACKNOWLEDGEMENTS

I would like to thank my thesis committee, Dr. Buschang, Dr. Julien, and Dr. Noureldin, for the constant support and help throughout the research process. I could not have completed this clinical study without your unwavering support.

I would like to thank my co-residents Dr. Eric Lin, Dr. Amanda Gross, Dr. Brendan Hubbard, Dr. Steven LeRoy, Dr. Brittany Spruiell, Dr. Jackson Savage, and Dr. Lauren Brubaker for allowing your patients to participate in the research study and for helping with scheduling along the way.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis committee consisting of Dr. Buschang and Dr. Katie Julien of the Department of Graduate Orthodontics, and Dr. Noureldin of the Department of Public Health.

All work for the thesis was completed by the student, under the advisement and with assistance of Dr. Buschang of the Department of Orthodontics.

Funding Sources

This work was made possible in part by the Robert E. Gaylord Endowed Chair in Orthodontics.

NOMENCLATURE

WSL(s)	White spot lesions(s)
CPP-ACP	Calcium phosphopeptide amorphous calcium phosphate

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

White spot lesions (WSLs) represent the first sign of the caries process and are a common sequela of orthodontic treatment (Øgaard et al 1989, Kidd et al 2004). While there is a wide range of prevalence reported between 2-96%, it is estimated that WSLs occur in about 23-38% of fixed orthodontic patients (Julien et al 2013, Brown et al 2016). The most common location for developing these lesions is the gingival portion of the labial surface of the teeth (Banks et al 1994, Gorelick et al 1982). The most common anterior teeth affected have been shown to be the anterior maxillary teeth, particularly the lateral incisor and canine (Julien et al 2013, Chapman et al 2010). These white spots are of concern because they do not go away on their own. The presence of WSLs on the anterior teeth poses an esthetic problem for the patient and orthodontist; these lesions compromise the smile esthetics and can lead to medico legal action.

White spot lesions are preventable with good oral hygiene, which is difficult to achieve for orthodontic patients since many patients are not compliant with oral hygiene regimens (Hadler-Olsen et al 2012). Moreover, the risk of developing WSLs in orthodontic patients is increased due to increased plaque accumulation between the appliances and the gingiva, decreased exposure to salivary flow on the facial surfaces of the teeth, and a decreased in ability to clean around the brackets (Gorelick et al 1982). Due to the difficulty removing plaque buildup around the brackets, plaque mass increases along with the level of

mutans streptococci and lactobacilli (Sakamaki et al 1968). These bacteria ingest sugars from the diet and produce acid, which causes enamel demineralization (Klock et al 1979).

Several methods have been developed to prevent WSLs. The most widely used methods for caries prevention include the application of fluoridated products and sealants onto the enamel surfaces (Geiger et al 1988, Sudjalim et al 2004). Fluoride prevents enamel demineralization and can re-mineralize existing white spot lesions. The application of fluoride through toothpaste and mouthrinse is compliance based. The use of fluoride varnish applied at the dental office is less compliance based because it is professionally applied. However, the patient has to go to the dental office for the application. In addition, the protective effects of fluoride varnish remain on the tooth for longer periods of time than toothpaste or mouthrinse (Benson et al 2013). The compound CPP-ACP (casein phosphopeptide-amorphous calcium phosphate) has be added to fluoride products, including varnish, to increase their effectiveness. The fluoride varnish- MI Varnish- contains CPP-ACP and NaF, and has been shown to be superior to fluoride varnish with NaF alone in preventing incipient caries and WSLs in orthodontic patients (Shen et al 2016, Salman et al 2019, Khooshki et al 2019). An in-vitro study by Abufarwa and coworkers demonstrated that MI Varnish can prevent enamel demineralization for at least 4 weeks in-vitro, and limit demineralization up to 12 weeks (Abufarwa et al 2019). Due to these findings, CPP-ACFP varnish would be expected to decrease white spot lesion formation more than traditional fluoride varnish, when applied every 4-6 weeks in vivo (Abufarwa et al 2019).

Sealants act as physical barriers to bacterial acid and plaque (O'Reilly et al 2013). Sealants have been shown to be effective in preventing WSLs, but the sealant has been shown

to come off over time, leaving the enamel surface exposed to plaque and bacterial acid (Knösel et al 2015, Anderson et al 2018). Lightly filled sealant such as ProSeal has been shown to completely inhibit enamel demineralization, as long as the sealant remains on the tooth (Coordes et al 2017). Maintaining a protective coating on the teeth with sealant requires reapplication every few months (Knösel et al 2015).

The purpose of the present study is to test the efficacy of MI Varnish, versus ProSeal sealant in preventing white spot lesion formation in orthodontic patients. MI Varnish and ProSeal sealant are both shown to prevent enamel demineralization in orthodontic patients. If the sealant wears away, causing white spots to still develop, then application of MI Varnish may prove to be a more effective alternative to prevent enamel demineralization by strengthening the enamel.

Literature Review

Enamel

Enamel is the most superficial layer of a tooth's crown, and it is the hardest mineralized tissue in the body (Norman et al 1999, Wang et al 2005). The enamel is composed of both inorganic and organic matter (Norman et al 1999). The inorganic matter is composed of hydroxyapatite crystals (HA) [Ca_{10} $(PO_4)_6$ $(OH)_2$] (Pan et al 2008). The organic matter is made up of water and a protein-rich matrix (Norman et al 1999). Both the inorganic and organic parts of enamel contribute to the maintenance of the enamel. The organic portion facilitates diffusion of molecules and fluids throughout the enamel structure (Norman et al 1999). The inorganic portion of hydroxyapatite has the ability to modify the molecular structures to its chemical composition, which can change the solubility component of enamel (Nelson et al 1983). Hydroxyapatite has a tendency to accept carbonate ions, which have been shown to increase the solubility of enamel, making the enamel more vulnerable to acid demineralization (Nelson et al 1983). However, the hydroxyapatite will also accept fluoride ions to its structure, which decreases the solubility of the enamel, rendering the enamel more resistant to acid erosion (Nelson et al 1983). On that basis, fluoride has been widely accepted as the gold standard in preventing caries and white spots (Featherstone 2000). A more in-depth description of the fluoride mechanism will be discussed later.

WSL Method of Development

White spot lesions (WSLs) are the result of enamel demineralization, and are the first signs of the caries process (Øgaard et al 1989, Kidd et al 2004). Demineralization is the

dissolving of minerals from the enamel and the increase in porosity (Chang et al 1997). The development of white spot lesions is a multifactorial process; factors such as patient's diet, dental plaque, cariogenic bacteria, and saliva quality and quantity all play a role (Chang et al 1997).

Dental tissues are continuously covered by a pellicle that allows bacterial cells to attach within 24 hours. Bacteria begin to colonize the surface, generating extracellular glucans and matrix that increase bacterial colonization of the plaque. By the end of 1 week, an organized community of bacteria exists. This makes up the mature biofilm that can become increasingly cariogenic. This metabolically active biofilm is rich with bacteria, including *mutans streptococci* and *lactobacilli*, which produce acid as a byproduct and decreases the pH of plaque (Chang et al 1997, Kidd et al 2004). The minerals from the surface of the enamel dissolve into the saliva when the enamel is exposed to a critical pH (below 5.5) (Zero 1999). The enamel is able to be repaired via remineralization, where the calcium in the saliva or diet is replaced into the enamel; however, this only occurs when the pH rises above 5.5 (Lynch et al 2012). This dynamic process of remineralization and demineralization of the enamel occurs throughout the day as we eat and drink, and an equilibrium between the two processes is necessary to maintain healthy, strong enamel. When the equilibrium is tipped towards demineralization without enough periods of remineralization, a white spot lesion may develop and start the caries process (Chang et al 1997).

White Spot Lesions get their name due to the chalky white appearance in relation to normal sound tooth enamel (Øgaard et al 1989). To understand why they appear this way, one must evaluate light refractive indexes. The refractive index of normal enamel is 1.62

(Kidd et al 2004). As enamel becomes more demineralized and porous, the enamel's refractive index changes (Sudjalim et al 2006). These pores can fill with water, which has a refractive index of 1.33, which causes the light scattering to make the lesion appear more opaque (Chang et al 1997, Kidd et al 2004). When the lesion is air dried, the water is replaced with air, with a refractive index of 1.0. This is an even greater difference in refractive indexes- sound enamel of 1.62, and water of 1.0 (Kidd et al 2004). This change in optical properties is what makes WSLs of great concern for orthodontists and parents because it's what makes WSLs appear on the visible facial surfaces of teeth (Julien et al 2013). The deeper the depth of WSL penetration through the enamel, the more visible the lesion is clinically (Abbas et al 2018). The refractive index also sheds light on why some white spot lesions can be visible on a dry tooth surface, but not when it is wet (Kidd et al 2004). A lesion that is visible when the enamel is wet means that the lesion has most likely penetrated most of the way through the enamel, whereas a lesion only visible on a dry tooth surface may only have penetrated a superficial layer of the enamel (Kidd et al 2004).

The white spot is histologically split into four zones (Silverstone et al 1973). The surface zone is an intact layer of enamel which overlies the body of the lesion. Underneath is the "body" which is the most demineralized portion of the white spot lesion (Silverstone et al 1968). Underneath the body is the dark and translucent zones (Silverstone et al 1967). The surface zone can protect the underlying lesion from further cavitation and demineralization, but at the same time it prohibits the underlying lesion from remineralizing (Silverstone et al 1968). Few fluoride ions are able to diffuse past the surface layer into the deeper layers of the lesion, causing the lesion to remain optically white (Hicks et al 1984). It is important to note that WSLs can re-mineralize to a smooth, glossy surface, termed "arrested lesions", but

they still pose an esthetic problem due to the white presentation of these lesions (Shivakumar et al 2009).

Prevalence and Incidence

The incidence of formation of white spot lesions varies widely in the literature, from 2-96% among orthodontically treated patients, depending on the methods of detection utilized (Gorelick et al 1982, Mitchell et al 1992, Geiger et al 1988, Mizrahi et al 1982). The highest levels of detection were found in studies using quantitative light-induced fluorescence, which is more sensitive than direct visualization via photographs and clinical exam (Boersma et al 2005). When evaluated using photographs, there is a slight difference in incidence of white spot lesion formation between university and private practice patients. Julien et al demonstrated 23% of orthodontically treated patients in a university setting developed white spots, versus 28% in private practice (Brown et al 2016). To clinically detect a white spot lesion with the naked eye, it has to have a depth of at least 300 micrometers (Zero et al 1999). For other methods of detection, such as Fluorecam, QLF, and Canary systems, it is possible to detect these lesions at lesser depths (Stookey et al 2012, Gomez et al 2013).

In regards to the most frequently affected anterior teeth, there is variation in the literature. A study of 885 university patients showed the most commonly affected anterior teeth were the maxillary anteriors, especially the lateral incisors (Julien et al 2013). Gorelick et al in 1982 found that the maxillary laterals had 3 times as many WSLs as the central incisors. Another study by Chapman et al agrees that the most frequently affected tooth is the maxillary lateral incisor, followed by the maxillary canine, premolar, and central incisor (Chapman et al 2010). The maxillary lateral incisors have also been shown to have the most

severe decalcification out of all the teeth studied (Chapman et al 2010, Geiger et al 1988). Another study shows that maxillary lateral incisors and canines are the most frequently affected teeth (Banks et al 1994).

There are also regional differences. Studies have shown that about two-thirds of white spot lesions occur in the gingival region (Banks et al 1994, Gorelick et al 1982, Mizrahi et al 1983). This has been suggested to be due to a physical plaque trap between the bracket and free gingival margin (Gorelick et al 1982). In contrast, the least frequent location for WSL development is the lingual surfaces of the lower incisors (Gorelick et al 1982). Even with bonded lower lingual retainers, the increased exposure to saliva helps to prevent the start of enamel demineralization (Gorelick et al 1982). The re-mineralization effects of saliva have been noted as one of the most influential factors in white spot and caries prevention (Chang et al 1996). This explains the high prevalence of white spot lesion development on maxillary labial surfaces during orthodontic treatment, which has less exposure to saliva than other regions (Gorelick et al 1982).

Risk factors

The co-existence of numerous factors have been shown to play a role in demineralization, including bacterial plaque, fermentable carbohydrates, time, and a susceptible tooth (Chang et al 1997). Orthodontic treatment has been shown to be a risk factor for developing white spot lesions (Mizrahi et al 1982). Orthodontic patients with braces are 11% more likely to have WSL than those who do not undergo treatment (Mizrahi et al 1982).

Historically in orthodontics, the teeth were all banded with cement (Øgaard et al 1988). As the development of resin bonding progressed, orthodontic brackets were directly

bonded to the teeth. The process of banding with cement does not require etching, but bonding composite requires etching of the enamel. The process of etching increases the bond strength between the enamel and resin via enhanced resin tags. It is difficult to contain the etch to the exact area desired for the bracket base. Therefore, more enamel is etched than is required. Some have postulated that the process of etching weakens the enamel, leading to white spots being formed more easily (Kuhar et al 1997, Lehman et a 1981, Hess et al 2011). Recent studies have shown that etching the enamel for 15 seconds does not increase the susceptibility of the tooth to white spot formation (Abufarwa et al 2018). In addition, a study by Gorelick et al found no difference in incidence of WSL formation between banded and bonded teeth (Gorelick et al 1982). It was found that teeth with full orthodontic bands developed more demineralization, both in terms of area and severity, than non-treated controls, demonstrating that banded teeth also develop white spot lesions (Mizrahi et al 1982).

Oral hygiene status is correlated with development of white spot lesions (Julien et al 2013). The plaque traps between the brackets, wires, and free gingival margin on the facial surfaces of teeth provide an environment for bacteria and acid to demineralize the enamel (Gorelick et al 1982). In a study evaluating orthodontic patients' susceptibility to WSLs, those who had good compliance with oral hygiene developed fewer new WSLs than those with poor oral hygiene compliance (Hadler-Olsen et al 2012). A fair or poor oral hygiene status in patients was found to be associated with more white spots in maxillary anterior teeth (Chapman et al 2010, Julien et al 2013). Patients starting with poor oral hygiene status were 3 times as likely to develop white spots than patients with good oral hygiene, even if their hygiene generally improved throughout treatment (Chapman et al 2010). Another study

by Brown et al found that patients with fair and poor oral hygiene had a 2.7 times and 3.5 times greater chance of developing white spots than good oral hygiene patients, respectively (Brown et al 2016, Julien et al 2013).

Males undergoing orthodontic treatment have been shown to have a higher incidence of white spot lesion development than females (Mizrahi et al 1982). Tufekci et al demonstrated that 76% of males and 24% of females in a study developed white spots (Tufekci et al). Chapman et al found that 40% of males and 22% of females developed white spot lesions throughout orthodontic treatment (Chapman et al 2010). It has also been shown that when males have decalcification, the decalcification tends to be more severe in males than in females (Chapman et al 2010). This could be because adolescent males are less likely to have adequate oral hygiene regimens than their female counterparts (Chapman et al 2010).

Age of the orthodontic patient is also a risk factor for developing white spots (Chapman et al 2010). The lack of tooth brushing dexterity and the lack of attention to personal hygiene have been suggested, as well as increased treatment duration, for increased formation of white spots among young individuals (Chapman et al 2010).

Bacteria

Several bacteria are part of the initiation and progression of the caries process. *Mutans streprococci* are highly involved in the initiation of the caries process (Bjarnason et al 1983). These bacteria prefer an acidic environment and produce extracellular glucans from ingested sugars that increases the amount of plaque around a tooth (Klock et al 1979). The increase in plaque mass increases the acid challenge on a tooth, which increases the growth of *mutans streptococci* and *lactobacilli*, especially around orthodontic bands and

gingival margins (Sakamaki et al 1968). *Lactobacillus* produce acid, contributing to the demineralization of the enamel, and have been found in advanced carious lesions (Van Houte et al 1980). It has been shown that once orthodontic appliances are introduced into the patient's mouth, a shift in bacterial flora occurs in dental plaque favoring these acidogenic and aciduric bacteria (Lundstrom et al 1987). One study showed that the levels of *mutans streptococci* increases 5 times more than normal levels during orthodontic treatment (Sudjalim et al 2006). Once the brackets are removed, the microbial levels have been found to decrease significantly 6-15 weeks into the retention phase (Sudjalim et al 2006).

Saliva

Saliva is important for enamel to repair itself through remineralization. Strong evidence has been shown that caries risk increases with a decrease in salivary flow rate (Papas et al 1993). Studies have shown that tooth surfaces that are exposed to saliva are more likely to undergo mineralization, than labial surfaces less frequently exposed to salivary flow, which are more susceptible to demineralization (Mitchell et al 1992). Salivary flow cleanses the teeth and delivers antibacterial properties to the tooth surface (Chang et al 1996). Saliva also delivers fluoride ions to enamel and plaque, which increases the fluoride reservoir in the mouth (Chang et al 1996). Salivary flow rate and pH buffering are factors which can influence the enamel's chances of repair. The pH of saliva should be maintained between 6 and 8 (Newbrun et al 1989, Andersson et al 1974). Saliva has a buffering capacity, the carbonic acid-bicarbonate system, which counters the acid challenge in plaque (Chang et al 1996). A decreased flow rate results in a lower pH following carbohydrate exposure, and a slower recovery to neutral pH, providing ideal conditions for demineralization.

Time

White spot lesions can take as little as 4 weeks to develop (Øgaard et al 1988), so it comes as no surprise that the highest increase in white spot lesion development occurs within the first 6 months of orthodontic treatment (Tufekci et al 2011). After this point, the rate of white spot lesion formation declines, but white spots continue to form (Tufekci et al 2011). It is important to note that the length of time between orthodontic visits can be 4-8 weeks, which means white spots can form during the 4 weeks before the orthodontist has a chance to address oral hygiene problems. Excessive treatment times over 30-36 months doubles the chances of patients forming white spot lesions (Brown et al 2016, Julien et al 2013).

Presence of fluorosis on the teeth has been shown to be a protective factor from developing white spot lesions. This means that patients with teeth with fluorosis are less likely to develop WSLs (Julien et al 2013).

Measuring WSLs

There are different methods used to evaluate and quantify white spot lesions. The Enamel Decalcification Index (EDI) is used for evaluating demineralized areas on the facial surface of teeth. It uses a number scale, ranging from 0 for no decalcification to 3 for WSLs completely covering the surface (Banks et al 1994). This method incorporates 4 separate areas of the facial surface of the tooth, and each area is assigned a number based on the amount of individual decalcification. This system can be used with photographic analysis of teeth or clinical visual examination. Another method is the Gorelick method, which uses the reference of white spot lesions around the labial gingival margin. This method is also evaluated using photographic analysis or direct clinical examination. The numbers range

from 1 through 4: 1- no white spot lesion, 2- mild white spot, 3- severe white spot, and 4- white spot lesion with cavitation. Another widely used method is the ICDAS system, which utilizes a range of numbers from 0-7 (Shivakumar et al 2009). This is a clinical examination. The numbers most pertinent to white spot lesions are 0 through 3. 0 is sound enamel, 1 is visual white spot lesion only visible after air drying, 2 is visual change in the enamel while wet, 3 is a white spot lesion with enamel surface breakdown (Shivakumar et al 2009).

Treatment Methods

White spot lesions are primarily a demineralization problem, so the ideal treatment would be a form of remineralization. However, the process of completely re-mineralizing lesions has proven to be difficult (Paris et al 2013). Less invasive options are the first line treatment of choice when dealing with these lesions (Paris et al 2013). When thinking long-term, the treatment for white spot lesions should ideally be resistant to discoloration. Color measurements using a spectrophotometer have been shown in previous studies that a Delta E exceeding 3.7 means a color is clinically detectable from another color (Johnston et al 1989). Conversely, less than 3.7 means the lesion is clinically not differentiable.

Remineralization

Remineralization of existing white spot lesions is possible through the repeated application of fluoride (Akin et al 2012). Remineralization ideally restores the affected enamel back to a normal or visually acceptable appearance. Fluoride products such as gels, toothpaste, mousse, and varnish are capable of remineralizing white spots (Abdullah et al 2016). Fluoride gel and toothpaste has been shown to penetrate the porous enamel surface to reach the demineralized body of the white spot lesions and reduce the size of the lesion (Lagerweij et al 2001). However, the amount of fluoride in the product and the method of

administration are important when determining remineralizing capabilities. While high levels of fluoride are desirable to prevent enamel demineralization, application of high concentrations of fluoride on white spot lesions can have undesirable esthetic effects and should not be used**.** High concentrations of fluoride can prevent the remineralization of all but the most superficial layer of enamel. This leaves the deeper layer of enamel unaffected, which means it cannot undergo the slow process of remineralization. Repeated applications of low levels of fluoride allows a slower penetration of the deeper layers by calcium and fluoride, enhanced subsurface remineralization, and lesion regression (Bishara et al 2008).

CPP-ACP

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is derived from the milk protein casein, and has been extensively studied to prevent caries and enhance remineralization of enamel (Salman et al 2019). CPP can stabilize calcium and phosphate ions in solution and saliva, which can diffuse through enamel to repair demineralized areas (Salman et al 2019). CPP-ACP can also help prevent demineralization by incorporating into the salivary pellicle to prevent adhesion of cariogenic bacteria (Abufarwa et al 2019). The CPP-ACP incorporates into plaque to increase the levels of plaque calcium and phosphate ions, making the plaque less cariogenic, and more favorable to remineralization (Sudjalim et al 2006). CPP-ACP is manufactured in the form of gels, chewing gum, paste, and varnish (Sudjalim et al 2006). A frequently studied product is CPP-ACP incorporated in a tooth mousse, MI Paste (GC Company). A clinical trial demonstrated that 31% of white spot lesions regressed with the use of MI Paste over a 12-week period compared to a placebo paste (Bailey et al 2009). White spot lesions that have been remineralized with CPP-ACP have been shown to be more resistant to future demineralization due to the readily available

calcium and phosphate ions which are readily available to remineralize enamel in the form of hydroxyapatite (Sudjalim et al 2006).

The addition of CPP-ACP to products with and without fluoride have shown to have an additive effect on promoting remineralization of white spots (Akin et al 2012). CPP-ACP can be added to toothpaste, mousse, chewing gum, and varnish. The use of CPP-ACP tooth mousse with fluoride toothpaste was shown to be significantly better at remineralizing white spots than fluoride rinse alone (Akin et al 2012). Another study demonstrated CPP-ACP's synergistic effect with fluoride, showing CPP-ACP increased the micro-hardness of a softened enamel by 46% and was significantly more effective in combination with fluoride at 64% (Srinivasan et al 2010). It was also shown that the use of CPP-ACP with fluoride (MI Paste plus) and MI Varnish (CPP-ACPF) significantly increased the efficacy of re-mineralizing white spot lesions (Bakry et al 2018).

Microabrasion

Opalustre is an enamel micro-abrasion slurry made of 6% hydrochloric acid and <45% silicon carbide. Some microabrasion treatments use 18% HCl with pumice. Microabrasion uses the slurry of HCl and silicon carbide on the affected teeth in a rubber cup with a handpiece, with a rubber dam to isolate the teeth from the gingiva and surrounding teeth (Akin et al 2012). The amount of time required for the application of the rubber cup to the tooth varies with each study, with 30 second intervals being common (Akin et al 2012). This process can be repeated as needed for diminishing the appearance of the lesion (Akin et al 2012). Microabrasion alone was found to dramatically reduce the initial appearance of white spots (Yetkiner et al 2014, Akin et al 2012). The use of hydrochloric acid in a slurry removes small amounts of surface enamel each time, ranging

from 12 micrometers 26 micrometers at each application (Waggoner et al 1989). This means that the more microabrasion treatments performed on a tooth surface, the thinner the enamel layer becomes. In addition, this technique has been prone to lead the white spot lesion to discolor. This could be due to a few factors: increased enamel surface roughness leading to increased discoloration, or increased porosity of the enamel surface (Yetkiner et al 2014).

Using microabrasion with subsequent remineralization has been recently studied (Ryan 2019). It was found that the use of microabrasion with remineralization with MI Paste Plus was even more effective than microabrasion alone at diminishing the appearance of white spot lesions (Ryan 2019). Lightness and translucency of enamel was greater in the teeth treated with MI Paste. This is promising treatment for the future, but further research will need to be done on the efficacy of this treatment in vivo.

Infiltration

Icon is a Tri-ethylene-glycol-dimethacrylate (TEGDMA)-based resin matrix, low-viscosity enamel infiltrant (Yetkiner et al 2014). In the process of resin infiltration, the outer surface is etched with a HCl etchant for 60 seconds, which causes the enamel surface to become more porous and permeable. This acid may remove up to 134 +/- 35 microns of surface enamel (Schmidlin et al 2003). The etched surface is then exposed to a low-viscosity resin, Icon (Paris et al 2013, Kielbassa et al 2009). The idea behind this technique is that the resin has a light refractive index similar to enamel. By infiltrating the white spot lesion with the resin with similar light refractivity as enamel, it can improve the appearance of the lesion by masking the differences in light refraction between the materials (Paris et al 2013, Kielbassa et al 2009).

Etching followed by resin infiltration has been shown to partially mask the appearance of WSLs, and in some cases completely mask their appearance clinically (Abbas et al 2018, Yetkiner et al 2014, Paris et al 2013, Borges et al 2016). However, the lesions remain clinically detectable. The resin infiltrant has been measured to extend up to 400 micrometers deep in demineralized areas of enamel (Paris et al 2013). In the short term, it has been shown that resin infiltration prevents the white spot from discoloring when exposed to materials like black tea and citric acid. Polishing the restoration may also improve the resistance to discoloration (Paris et al 2013). However, long term evaluations of the resin infiltration restoration for more than 1 year are needed to be done to evaluate discoloration of the material, margins, and overall esthetics over time (Yetkiner et al 2014, Borges et al 2016). Just like other resin restorative materials, it is known that these materials can break down over time and discolor. More extensive caries may require more invasive procedures such as resin fillings, veneers, or crowns, especially if the caries continues through to dentin (Abbas et al 2018).

Restorative Treatment

The last resort to restoring white spot lesions is the removal of the affected enamel, to replace with a restorative material such as resin, veneers, or crowns. These restorations may be indicated if the desired remineralization effect could not be achieved, if the caries extends into the dentin, or if a significant enamel cavitation is present (Kielbassa et al 2009). These restorations are costly, and involve the removal of significant amounts of enamel. These restorations then enter the cycle of filling and replacing over time, as restorations age, discolor, and encounter secondary caries. Restoration of WSLs should be considered only

when other methods are unsuccessful, or as a last resort to addressing the patient's esthetic concern.

Prevention Methods

White spot lesions are primarily a demineralization problem. Preventing demineralization is the solution to not developing them at all. This includes oral hygiene instruction and awareness of risk in orthodontics, application of sealant to the facial surfaces of teeth, fluoride products, the ingredient CPP-ACP, and the combination of these two products.

Hygiene

Patient education and proper home oral hygiene are the best ways to prevent white spots. When patients have fixed appliances introduced into their mouths, good oral hygiene may worsen due to the difficulty cleaning around braces (Sudjalim et al 2004). Poor oral hygiene has been correlated with the formation of more white spot lesions (Sudjalim et al 2004, Geiger et al 1988). Attempts to help patients obtain better home care include utilizing instructional videos on brushing, flossing, and using rotary toothbrushes (Sudjalim et al 2004). Periodic motivation and reinforcement of proper hygiene practices has also been shown to help change patient oral hygiene trends (Geiger et al 1988). However, changing a patient's behavior permanently is a difficult process (Geiger et al 1988). Brushing the teeth twice a day is a standard and an essential oral hygiene practice for all patients, which can be improved in some patients with the help of an electric toothbrush (Heintze et al 1996). Rotary toothbrushes have been shown to help patients with poor oral hygiene, and may even motivate patients to brush more (Heintze et al 1996). It has also been suggested that more

frequent professional dental cleanings help to keep the dental plaque load at bay (Lundstrom et al 1980). Reducing intake of fermentable carbohydrates in the diet and substituting with noncariogenic sweeteners such as xylitol is also a highly accepted method of reducing caries and white spot lesions (Featherstone 2000). While daily mouth rinse and dentifrice use is helpful in preventing demineralization, these methods are dependent on patient compliance.

Sealant

The application of sealant to the facial surface of the teeth is another approach for WSL prevention. The use of sealants on pit and fissures has been shown to reduce caries and enamel demineralization (Benham et al 2009). Sealants reduce the need to rely on patient compliance for preventing white spot lesions. Sealants can be applied to the facial surfaces of the teeth to act as a physical barrier to bacterial acid and plaque (O'Reilly et al 2013). Bishara et al (2008) found the bond strength of sealed teeth to be comparable to bond strength of unsealed teeth, allowing the clinician to use the sealant on the entire labial surface of the tooth before bonding the bracket. When the sealant ProSeal was compared to traditional fluoride varnish in an in-vitro study, it was shown to be better than traditional varnish in reducing enamel demineralization (Buren et al 2008). Filled sealant has been shown to be more resistant to physical and mechanical wear than unfilled sealant (O'Reilly et al 2013). ProSeal, which is a filled sealant, was shown to be superior to unfilled sealant in an in-vitro study demonstrating resistance to enamel demineralization (Hu et al 2005). In vitro, ProSeal was shown to be superior to numerous other sealants in a test of thermal, mechanical, and chemical loading to completely prevent enamel demineralization (Coordes et al 2017). This suggests that ProSeal should maintain its integrity in vivo. In contrast, ProSeal and a similar sealant, Vanish XT, were shown to be almost completely removed by

a simulated 32-day brushing protocol (Wiewiora et al 2018). This contradicts the previous study, suggesting that the sealant would need to be reapplied periodically to restore the protective features of the sealant.

A downside to the use of sealant is that in vivo, the sealant wears away over time and leaves the enamel exposed to bacteria and acid. Oftentimes, the clinician is unaware of when and where the sealant is missing. Recent studies have shown that in-vivo, area covered by ProSeal diminishes 40% after 2.8 months, thereby leaving the exposed areas of the tooth vulnerable to demineralization and white spot lesions (Anderson et al 2018). It has been recommended that sealants be reapplied every 3-4 months in order to maintain their integrity (Knösel et al 2015). This takes time out of the appointment, and still may allow WSLs to develop as the sealant wears away. When applying sealant with the brackets already bonded to the tooth, the bracket and inflamed gingiva can create a physical barrier to complete sealant reapplication (Buren et al 2008).

Prior sealant studies have left gaps in the literature. There are numerous types of sealants, but it is important to find a sealant with good retention, good wear resistance, and has a method of detecting if it is still on the tooth. If the sealant fluoresces under a blacklight, the presence or absence of the sealant can be determined. If the sealant wears away, the patient is likely to develop white spot lesions. This leads us to question if it is worth the time and effort with sealant, or should other preventive options be studied?

Fluoride

Fluoride has been a proven method to prevent enamel demineralization. The mechanism of fluoride's preventive action is as follows: diffuses into the bacteria as HF molecules to inhibit bacterial metabolism, inhibits demineralization during acidic

environments, and strengthens the surface enamel layer by forming a low solubility enamel layer (Featherstone 2000). In summary, the preventive mechanism relies on "the presence of fluoride in saliva, in the plaque at the tooth surface and in the fluid among the crystals in the subsurface of the enamel" (Featherstone 2000).

Various attempts have been made to utilize fluoride rinses, toothpastes, and varnish to prevent white spot lesions. Numerous studies have tried to ascertain the best fluoride regimen for orthodontic treatment, but the results are inconclusive (Benson et al 2013). Mouthrinse with 450ppm NaF has been shown to increase plaque levels of fluoride ions, which helps prevent demineralization of enamel (Reynolds et al 2008). PreviDent is a 1.1% fluoride gel toothpaste with 5000 ppm fluoride that has been used as an adjunctive agent throughout orthodontic treatment to prevent white spot lesions. The incorporation of fluoride with CPP-ACP in MI Paste Plus has been shown to be effective in reducing white spot lesion formation in orthodontic patients (Robertson et al 2011). It is important to note that home care use of gels, rinses, and toothpastes are patient compliance driven, and can affect whether or not a lesion remineralizes to its full potential (Abdullah et al 2016).

Fluoride varnish applied to the teeth by healthcare providers decreases the need for patient compliance, and have a longer period of efficacy in preventing demineralization. Traditional NaF fluoride varnishes have been shown in vivo to reduce enamel demineralization when used around orthodontic brackets, compared to teeth without fluoride varnish application (Farhadian et al 2008). Traditional fluoride varnishes provide short-term protection, and have been proven to decrease white spot lesions by 70% in-vivo with application every 6 weeks (Benson et al 2013). However, at longer intervals, it has been shown that there is no difference in white spot lesion formation when varnish is applied

every 3 months verses 6 months versus a fluoride rinse control group (Perrini et al 2016). High-level fluoride varnish significantly helps reduce demineralization in anterior teeth versus no fluoride varnish; however, if the patients have excellent oral hygiene, the use of varnish as an adjunctive therapy was shown to have no additional benefit (Perrini at al 2016). This is understandable, since the development of white spot lesions requires a plaque and bacterial acid challenge, typically found in poor hygiene patients.

CPP-ACP has also been incorporated into fluoride varnish, MI Varnish (GC Company) with ~22,600 ppm fluoride, to further increase the effectiveness of the varnish. The resulting compound is termed CPP-ACFP. The varnish has been shown to be effective in enamel remineralization, protection against enamel erosion, and reducing dentin hypersensitivity (Salman et al 2019, Chebel et al 2018, Bayrak et al 2017). MI Varnish was shown to be superior to Duraphat varnish and Clinpro White varnish in preventing enamel erosion (Bayrak et al 2017). MI varnish has also been shown to be superior to Duraphat varnish in increasing enamel surface microhardness and remineralization of incipient caries (Khooshki et al 2019). In-vivo studies have demonstrated that the MI Varnish formulation is superior to normal fluoride varnish in inhibiting demineralization, providing 130% greater inhibition than traditional fluoride varnish controls (Shen et al 2016). MI Varnish has also demonstrated the ability to reduce the depth of enamel white spot lesions, significantly more than Prevident varnish (Salman et al 2019). The greater effect of this formulation can be attributed to the synergistic effect of fluoride in combination with CPP-ACP (Salman et al 2019).

An in-vitro study by Abufarwa and coworkers (2019) demonstrated that MI Varnish can prevent enamel demineralization for at least 4 weeks in-vitro, and limit demineralization

up to 12 weeks. In fact, the MI Varnish produces a net remineralization for 2 weeks, returns back to base-line at 4 weeks, after which progressive demineralization occurs (Abufarwa et al 2019). This means that the MI Varnish prevented demineralization 100% at 4 weeks. The white spot lesion produced at 12 weeks with MI Varnish was equivalent to the control white spot lesion at 2 weeks (Abufarwa et al 2019) Due to these findings, CPP-ACFP varnish would be expected to decrease white spot lesion formation more than traditional fluoride varnish, when applied every 4-6 weeks in vivo (Abufarwa et al 2019). A prospective randomized control trial evaluated MI Varnish application applied every 3 months, but found that this did not significantly prevent white spot lesions as opposed to a daily fluoride rinse and toothpaste regimen (Rechmann et al 2018). This could be because the interval of application is not frequent enough, as the study by Abufarwa et al determined the ideal application period is between 4-6 weeks (Abufarwa et al 2019, Rechmann et al 2018).

There are gaps in the literature in terms of fluoride varnish studies. A prospective randomized control trial by Rechmann et al in 2018 evaluated MI Varnish application applied every 3 months versus a control group. One group had MI Varnish applied every 3 months, had daily MI Paste plus, and brushed twice a day with 1100 ppm fluoride toothpaste. The control group was assigned to a daily fluoride mouthrinse and twice daily 1100 ppm toothpaste regimen. Comparing these groups with EDI scores and ICDAS scores after 12 months, there was no statistically significant difference in white spot lesion formation (Rechmann et al 2018). The problem with this study is that application of the varnish was applied every 3 months (12 weeks), when the ideal application period is between 4-6 weeks (Abufarwa et al 2019, Rechmann et al 2018). They also did not remove

the brackets for their evaluations and therefore could not use photos due to the distortion the brackets created.

Another problematic fluoride varnish study was performed by Perrini et al, who used a split mouth design. They evaluated Duraphat varnish, a traditional fluoride varnish applied every 3-months and 6-months to no fluoride. They found no additional benefit in using fluoride varnish versus the control (Perrini et al 2016). The split mouth design used is problematic because fluoride is incorporated in saliva. There is a cross-over effect making it impossible to say that the control side did not receive any benefit from the fluoride too.

Due to these gaps in the research, a clinical study is warranted to compare the efficacy of the highly studied CPP-ACP MI varnish and ProSeal sealant in prevention of white spot lesions in orthodontic patients. The best clinical practice to prevent white spot lesions needs to be investigated so that costly and invasive white spot lesion treatment can be avoided.

CHAPTER II

MATERIALS AND METHODS

This study was a single center, parallel, randomized clinical trial performed between October 2018 and March 2020 at the Texas A&M University College of Dentistry. The Texas A&M University Institutional Review Board (IRB) approval was obtained (IRB #2018-0724-CD-FB) and the study was registered with the US National Institute of Health – ClinicalTrials.gov. No modifications to the study design were implemented during the course of the study.

Forty patients starting orthodontic treatment at Texas A&M University College of Dentistry orthodontic department were selected based on the following criteria: no significant medical history, no underlying medical problems such as Sjogren's Syndrome or conditions requiring more than 2 medications (to prevent bias of possible dry mouth), less than 17 years old at the start of orthodontic treatment, fully erupted permanent maxillary canines and incisors, starting fixed orthodontic treatment, and ability to come to appointments every 4-6 weeks. Exclusion criteria included the following: professional fluoride application in the last 3 months, allergy to milk, untreated cavitated lesions, heavy initial fluorosis, dry mouth, pregnancy, and any illness/condition that the investigators felt would affect the study outcome.

Sample Size

Sample sizes were determined based on estimates of central tendency and dispersion provided by Rechmann and coworkers in 2013, who compared MI Varnish to MI Paste Plus. Assuming a standard deviation of 3, effect size of 1.2, and a two-tailed test with an alpha

error of .05, a sample of 12 patients in each group were needed to provide 90% power to detect a statistically significant difference between groups. Due to possibility of patient dropout and noncompliance with the study protocol, a total of 40 patients were selected. None of the patients were lost to follow-up, and all of them completed the study (Figure 1).

Randomization

Block randomization of the subjects was performed with Excel (Microsoft, Redmond, Washington) by an investigator (PB) who had no clinical involvement in the trial. After consent was obtained by the principal investigator (LF), the investigator (PB) instructed the principal investigator (LF) which group the patients were assigned to. Patients were assigned to either Group 1) ProSeal applied to the facial surfaces of the maxillary anterior teeth and re-applied every 3 months or Group 2) MI Fluoride Varnish applied to the maxillary anterior teeth every 4-6 weeks.

Interventions

T1 was the initiation of the study and T2 was the final appointment after approximately 12 months of treatment. The length of time of the study from T1 to T2 was .99 +/- .089 years for the sealant group, and .99 +/- .091 years for the varnish group. ProSeal sealant was re-applied to Group 1 (LF) approximately every 3 months. Application of MI Varnish to the maxillary anterior teeth for Group 2 was applied approximately every 5 weeks by orthodontic residents who had previously been standardized. The Group 2 subjects did not have sealant applied to the maxillary anterior teeth. The type of bracket system used for each patient's treatment was not standardized (self-ligating and non-self-ligating).

The subjects in Group 1 had the facial surfaces of their maxillary anterior six teeth, canine to canine, etched for 15 seconds with a 37% phosphoric acid etch gel and rinsed thoroughly. LED ProSeal was applied in a thin layer with a microbrush, and light cured for 3 seconds with OrthoLux Curing Light. Brackets were bonded to the teeth using a thin layer of TransBond XT Composite. Standardized oral hygiene instructions were given to the patient on proper brushing techniques and diet counseling.

The integrity of the sealant on group 1 patients was checked every 3 months with a black light, and sealant was reapplied to the maxillary anterior teeth by the same orthodontic resident (LF) in the missing areas on all teeth. All teeth required reapplication of sealant on at least one area of the tooth every 3 months.

For Group 2, the facial surfaces of their maxillary anterior teeth were etched for 15 seconds with a 37% phosphoric acid etch gel and rinsed thoroughly. Assure (Reliance Orthodontic Products, Inc., Itasca, IL) was applied in a thin layer with a microbrush on the facial surface of the maxillary anterior teeth where the brackets were placed. Brackets were bonded to the teeth using a thin layer of TransBond XT Composite. Standardized oral hygiene instructions were given to the patients on proper brushing techniques and diet counseling. At the end of the appointment, a cheek retractor was used for application of the varnish. The teeth were dried with an air-water syringe, and the MI Varnish was applied to the facial surfaces of the maxillary anterior teeth. Instructions were given to the patients verbally and on a form that they took home, which instructed the patient not to brush for the next 6 hours and avoid hard, crunchy food that would cause the varnish to come off per the manufacturer's instructions.

Group 2 had MI Varnish applied every 4-6 weeks at regular appointments by orthodontic residents who had been calibrated as to the procedure. The varnish was applied at the end of each appointment in the same manner as previously stated.

After about 12 months of treatment, the patients from both groups returned to the clinic for T2 records. The brackets, composite, bonding agent, and sealant were removed from the study teeth at this time to facilitate visibility of the entire facial surface of the teeth. The brackets were immediately rebonded after photos were taken. This concluded the patient's participation in the study.

Evaluations

Oral Hygiene Evaluation

On the same day of bonding (T1), prior to preparing the patient's teeth for bonding, oral hygiene was evaluated based on the accumulation of plaque on the anterior maxillary teeth using the "Turesky modification of the Quigley and Hein plaque index" (Turesky et al 1970). Oral hygiene was recorded in the same manner at the final appointment (T2), before the brackets were removed.

Photographs

Patients were instructed to brush their teeth prior to taking the initial T1 photographs. There was one set of digital photographs taken of the maxillary anterior teeth at T1, immediately prior to initial bonding. The NOLA cheek retractor was placed and the teeth were dried with an air-water syringe prior to photographs being taken. The Canon T5i camera with Macro lens was used, with F stop=29, Focus set to 2. Photographs were taken chairside in the orthodontic clinic. One photo was taken of the upper right canine and lateral incisor, one photo was taken of the central incisors, and one photo was taken of the upper

left lateral incisor and canine. If significant glare was present on these teeth, another photo was taken at a slightly different angle to prevent glare. After approximately 12 months, photos were again taken of the maxillary anterior teeth after the braces, sealant, and bonding agent had been removed.

Outcome Measures

The primary outcome variable was the enamel decalcification index (EDI) score for individual teeth, regions, and subjects. It was used to evaluate prevalence and incidence of WSLs. The secondary outcome variables were the locations of white spot lesion formation, the teeth on which they formed, oral hygiene status, and sex differences.

EDI

The EDI score was used to evaluate the maxillary centrals, laterals, and canines at T1 and T2 via photographic analysis. The EDI method scores four zones on the facial surface of the tooth around the orthodontic bracket (mesial, gingival, distal, occlusal) for decalcification level, from 0 to 3 (Banks et al 1994) (Table 2). For the present study, the scores for the 6 maxillary teeth were added together to create the "EDI Sum" per patient. Scores from T1 were subtracted from T2 to create a "New EDI Scores" variable.

Plaque Index

Before subjects' teeth were cleaned, the Turesky modification of the Quigley and Hein plaque index was used to assess plaque levels of the study teeth at T1 and again at T2 (Table 3) (Turesky et al 1970).

Computer Analysis

Photos for each patient at T1 and T2 were labeled for each tooth for analysis. These patient photo files were randomized and one-blinded investigator (LF) performed all of the assessments. The photos were blown-up to match the corresponding photo in the event that the sizes were not the same. Each patient's T1 and T2 photos of each respective tooth were compared side by side on a computer for analysis. The EDI scoring was used for each tooth and scored for T1 and T2. The location of decalcification was recorded as gingival, mesial, incisal, or distal. The differentiation between a developmental enamel lesion and decalcified white spot followed the recommendations set forth by Kanthasas et al in 2005. After a 2-week interval, the images from 10 patients were re-randomized and the photographic measurements repeated. Reproducibility was assessed by a paired samples *t*-test for systematic error and the intra-class correlation coefficient (ICC) for random error.

Blinding

The principal investigator (LF) and patients were not blinded to the group they were assigned to. For oral hygiene assessment, the principal investigator (LF) could not be blinded. The patients could not be blinded to the treatment they received. Photographic analysis was performed by the same blinded investigator (LF).

Statistical Methods

EDI scores were summed for all 6 teeth at their respective timepoints, T1 and T2. Independent samples t-tests were used to evaluate differences in means between the two groups for oral hygiene scores, EDI sums overall, for each tooth, and for each tooth region.

The Chi Square test was used to determine differences in white spot lesion prevalence, incidence, and differences in EDI scores between different teeth and regions.

CHAPTER III

RESULTS

Entire Sample

40 patients were randomly assigned to either the sealant group or the varnish group, allowing 20 patients per group. 20 patients received the intended treatment in the sealant group, and 20 patients received the intended treatment in the varnish group. All 40 patients' photographs were analyzed. No patients were lost during the study.

At the start of the study (T1), 32.5% of the subjects and 11.3% of the teeth exhibited WSLs (Table 4). Approximately 42.5% of the subjects and 14.9% of the teeth developed new WSLs during the course of the study. The lateral incisors showed higher incidences of WSL formation, in terms of number of teeth (9.5%) and number of subjects (32.5%), than the central incisors and canines. The differences were statistically significant (Table 5; Figure 2). Based on number of teeth (10.4%) and percentage of subjects (32.5%), the incidence of WSL formation was significantly greater in the gingival region, than mesial, distal, or incisal regions (Table 6, Figure 3). There were no statistically significant sex differences for the incidence of WSL formation ($p=.822$) (Table 7).

The EDI scores increased from 36 (T1) to 74 (T2) during the course of the study (Table 8). The lateral incisors showed the greatest increases in EDI scores over time, with the change being significantly greater than the changes for the central incisors ($p=.02$) and canines ($p=.032$) (Table 9, Figure 4). The gingival area showed the greatest increase in EDI scores over time, with the change being significantly greater than the increases observed in the mesial ($p=.009$), distal ($p=.026$) and incisal ($p<.001$) regions (Table 10, Figure 5). The

EDI scores of 22 subjects (57.5%) either remained the same or improved; the EDI scores of 14 subjects (42.5%) worsened (p=.501) (Figure 6, Table 11).

Severity of the number of EDI scores were evaluated for each region. The EDI scores ranged from 0 to 3 for each region of the tooth. 95.8% of all scores were "0". Out of all decalcification scores (1-3), 88.3% were scores of "1", 6.7% were scores of "2", and 5% were scores of "3". It is important to note that the scores of "3" were all found on the same patient. This shows that the majority of decalcifications that developed were minimal. Between groups, 85% of the sealant group decalcification scores were "1"s, and 95% of the varnish group scores were "1s".

Overall oral hygiene scores increased from 49 at T1 to 155 at T2 (Table 12). There was no difference between good oral hygiene and poorer oral hygiene with respect to WSL development at either timepoint. However, the subjects who developed WSLs were more likely to have had poorer OH than if they had good oral hygiene at T2 (p=.049) (Figure 7, Table 13). Poor oral hygiene at T2 shows a high positive predictive value (76%) for development of WSLs.

Group Comparisons

At the start of the study (T1), 45% of the subjects in the sealant group and 19.2% of their teeth exhibited WSLs (Table 14). For the Varnish group (T1), 20% of the subjects and 3.3% of the teeth exhibited WSLs (Table 14). At T2, there were significantly more patients who had WSLs in the sealant group (16) than the varnish group (8) (p=.010) (Table 14). 50% of the subjects in the sealant group and 35% of their teeth developed WSLs during the

course of the study; 35% of the subjects in the varnish group, and 12.5% of their teeth developed WSLs (Table 14).

The sealant group's EDI scores increased from 30 (T1) to 52 (T2) during the course of the study; for the varnish group, the EDI scores increased from 6 (T1) to 22 (T2) (Figure 8, Table 15). The total new EDI scores for the sealant group were 21, and the scores for the fluoride varnish group were 15. This difference was not significant (p=.585) (Figure 8, Table 15). The sealant group had more subjects (10) whose EDI scores increased than the fluoride varnish group (7), but this difference was not statistically significant (p=.337) (Table 16, Figure 9). The EDI scores of 50% of sealant group subjects worsened, compared to 35% of the subjects in the varnish group. The lateral incisors showed the greatest increases in EDI scores over time in both the sealant (12) and the varnish (11) group. Comparing EDI score increases between group, there were no differences for the centrals (p=.592), laterals (p=.878), and canines (p=.292) (Table 17, Figure 10). The gingival region showed the greatest increase in EDI scores over time in both groups, with no between-group difference. While the sealant group showed slightly greater increases in the mesial, distal, gingival, and incisal region EDI scores, none of the differences were statistically significant (Table 18, Figure 11).

The majority of decalcification scores were minor. In the sealant group, 85% of the scores were a score of 1. In the varnish group, 95% of the scores were a score of 1. These differences were not significant (p=.170). One of the patients in the sealant group developed white spot lesion cavitation at T2.

Overall oral hygiene scores at T1 were higher in the sealant (32) than the varnish (17) group, but the difference was not statistically significant (p=.203). Oral hygiene scores at T2 were significantly higher in the sealant group (101) than the varnish group (54) (p=.010) (Table 19, Figure 12). There were no between-group differences in the number of subjects who developed WSLs based on oral hygiene status at either T1 or T2 (Table 20).

CHAPTER IV

DISCUSSION

Substantial numbers of patients should be expected to develop WSLs during orthodontic treatment. In terms of prevalence and incidence of teeth with WSLs, the present study parallels the existing literature closely. It showed an initial prevalence of 11.3% of teeth, and final prevalence of 21.3% of teeth with WSLs. Lovrov et al 2007 reported an initial prevalence of 15% of teeth, and a final prevalence of 26.4% of teeth. In terms of post-treatment incidence of teeth, the literature shows incidences ranging from 16.7%-41.1% of teeth (Rechmann et al 2018, Lovrov et al 2007, Hadler-Olsen et al 2012). The incidence of teeth in the present study (14.9%) falls slightly below that range.

In this study, the incidence of WSL formation among subjects was 42.5%. Previous studies have reported lower incidences among university - (23%) and private practice (26-36%) patients (Brown et al 2016, Chapman et al 2010). The differences between these studies and the present study could be due to several factors.

First, the higher incidence could be related to pre-treatment prevalence of subjects with WSLs. The pre-treatment prevalence of WSLs in the present study (32.5%) was higher than initial prevalences -ranging from 8-15% - previously reported (Julien et al 2013, Lovrov et al 2007). This is important because pre-existing WSLs is a risk factor for the development of WSLs during treatment (Julien et al 2013, Lovrov et al 2007). In other words, the patients were at higher risks of developing WSLs than patients in the other studies reporting lower incidences.

Secondly, differences in method of WSL detection could contribute to differences in incidences reported. The present study analyzed pre- and post- treatment photographs with the Enamel Decalcification Index. While this method is useful in quantifying the extent and location of demineralization, it has not been widely used in the literature to determine prevalence and incidence of WSLs in orthodontic patients. EDI scores might be expected to be higher than Gorelick index scores because a WSL that develops around the bracket may encompass more than 1 region of the tooth (Banks et al 1994). In the EDI scoring, a minor WSL that encompasses more than 1 region of a tooth may score higher than the Gorelick index. The studies utilizing EDI score do not commonly report incidence and prevalence of WSLs, but rather the increase or decrease in EDI scores over time (Rechmann et al 2018, Robertson et al 2011). Other studies have used the Gorelick index for white spot lesion detection, and the incidences were lower than the present study (Julien et al 2013, Hadler-Olsen et al 2012). This index does not specify the particular area the white spot lesion is located. This suggests that the EDI may be better able to detect enamel decalcification changes than the Gorelick index. An even higher prevalence (97%) of WSLs has been reported with QLF, which is a more sensitive instrument to detect enamel demineralization than direct visualization or photographic analysis (Boersma et al 2005).

Third, the higher incidence found in the present study may be due to the fact that the teeth were evaluated immediately after debonding. The newly exposed, dried, and roughened enamel immediately after debonding the brackets often shows white areas of the enamel that may re-mineralize after being exposed to saliva (Øgaard et al 1988). Other studies evaluated teeth at the end of treatment, at some time after the braces have been removed (Julien et al 2013, Brown et al 2016), or mid-treatment with the braces still on

(Rechmann et al 2018). Importantly, the vast majority of EDI scores in the present study indicated minimal decalcification and many of them could have been due to debonding or light reflection of the enamel. These minor decalcifications may contribute to the higher final prevalence and incidence of subjects observed in the present study.

Even though the incidence is high, the severity of WSLs in patients treated with either sealant or varnish can be minimal. At the end of the present study, 93.8% of all scores were "0". Out of all decalcification scores (1-3), 88.3% were scores of "1". This shows that the majority of decalcifications that developed were minimal. The EDI scores in the present study were lower than those found by Rechmann et al in 2018, which found that 55.5% of scores were >0, compared to 6.2% in the present study. This demonstrates that there was less demineralization noted in the present study, which could be due to personal preferences in EDI scoring, or due to a significant clinical reduction in decalcification with the present study treatment interventions.

Lateral incisors are more likely to develop new WSLs than canines or central incisors. In the present study, 32.5% of subjects developed new WSLs on the laterals, and 9.5% of all teeth that developed WSLs were lateral incisors. The lateral incisors had the highest initial, final, and new EDI scores. The finding that the lateral incisor is the most common anterior tooth to develop white spot lesions parallels the existing literature (Julien et al 2013, Gorelick et al 1982, Banks et al 1994, Chapman et al 2010, Lucchese et al 2013, Geiger et al 1988). This is important because the lateral incisor is in a highly visible area, which affects both the patient and parent's perception of the final esthetic orthodontic result. Perhaps the lateral incisors develop more WSLs due to the lateral incisor bracket's thicker profile, and

closer proximity to the gingiva than the central or canine bracket. This creates a more difficult area to clean than the centrals or canines, allowing for increased plaque buildup between the gingiva and bracket pad (Gorelick et al 1982).

The gingival region is more likely to develop WSLs than the other regions of teeth. In the present study, the gingival region developed the highest EDI scores of any region, and 32.5% of all subjects developed at least 1 gingival WSL. 10% of the teeth in the present study developed at least 1 WSL in the gingival area, which was 4x higher than any other area. 63.1% of the new EDI scores developed in the gingival area. The literature indicates that 2/3 of WSLs develop in the gingival region (Banks et al 1994, Mizrahi et al 1983, Julien et al 2013, Gorelick et al 1982). Plaque buildup around brackets is a precipitating factor to developing WSLs, and the plaque trap between the bracket and the gingiva makes the plaque challenge greater (Gorelick et al 1982). These findings are important because it shows where the orthodontist and patient need to focus if WSL are to be prevented. Sealant is a physical barrier which can protect the tooth from decalcification. However, sealant has been shown to come off or erode from the gingival region more than other regions of the tooth (Knösel et al 2015, Anderson et al 2018). A recent unpublished master's thesis by Elizabeth Barnhart revealed the etch pattern in the gingival area of the tooth is not ideal, leading to poorer potential bond strength in this region. Therefore, sealant has been recommended to be reapplied every 3 months to maintain enamel protection (Knösel et al 2015).

MI Varnish and ProSeal provide similar protection during orthodontic treatment. WSLs were not prevented with either intervention studied and there were no statistically significant between-group differences in the incidences of WSL development. However, the sealant

group had higher EDI scores than the varnish group at all timepoints. There are no studies to date that have compared MI Varnish to Pro Seal sealant in vivo. In vitro sealant study results are mixed, with some suggesting that Pro Seal sealant is superior to fluoride varnish in preventing WSLs (Hu et al 2005, Buren et al 2008), whereas others report it to be less effective than fluoride varnish in preventing WSLs in vitro ().

In-vivo sealant studies have shown that the areas covered by ProSeal diminishes 40% after 2.8 months, thereby leaving the exposed areas of the tooth vulnerable to demineralization and white spot lesions (Anderson 2018). It has been recommended that sealants be reapplied every 3-4 months in order to maintain their integrity (Knösel et al 2015). Another in vivo sealant study demonstrated that, patients who received sealant developed the same number of white spot lesions as those that did not receive sealant (Hammad et al 2016). Oral hygiene plays an important role, even when sealant is applied to the teeth, due to the loss of the sealant in critical areas. In the present study, the sealant was evaluated every 3 months with a black light flashlight, and it was found that at least one area on each tooth was missing sealant. Therefore, each patient had every tooth resealed every 3 months during the course of the study. This means that some areas of the teeth may not have been protected during treatment, allowing for the development of WSLs.

Fluoride varnish appears to have a positive preventative effect compared to no treatment, but mixed results when compared to other fluoride protocols in vivo. An in vivo study showed that the application of MI Varnish every 3 months does not significantly decrease WSL development versus a fluoride toothpaste and rinse protocol (Rechmann et al 2018). This same study found that 32.3% of teeth worsened in EDI scores in the MI Varnish group

over a 12-month period, which is higher than the 12.5% of the teeth that developed WSLs in the present study. This suggests that applying MI Varnish at an interval of 5 weeks is more effective at preventing WSLs than every 3 months. In contrast, 6-week applications of Fluor Protect, another fluoride varnish, was shown to significantly reduce the number of WSLs compared to a placebo group (Stecksen-Blicks et al 2007, Sonesson et al 2019). Abufarwa and coworkers demonstrated that MI Varnish was effective at preventing demineralization up to 12 weeks in vitro, but the present study aimed to see if this held true in vivo (Abufarwa et al 2019). Some reasons that the MI Varnish in the present study may not have prevented enamel demineralization could be that the plaque and oral hygiene challenge with braces in vivo is not adequately demonstrated in vitro. The subjects may not have followed the varnish instructions not to brush or eat any hard, crunchy, hot, or sticky foods for six hours after the varnish was applied. If the varnish was pre-emptively removed, then the effects of the varnish would be diminished, rendering the varnish less effective.

Patients who develop WSLs tend to have poorer oral hygiene. At the end of the present study, patients who developed WSLs had poor oral hygiene**.** Oral hygiene in the present study deteriorated overall from T1 to T2, which is expected after starting orthodontic treatment (Julien et al 2013, Chapman et al 2010). While there were no differences in hygiene between groups at T1, hygiene was significantly better in the varnish group at T2 than the sealant group ($p=.014$), which could explain the higher EDI scores in the sealant group. Overall, those patients who developed WSLs were more likely to have poorer oral hygiene (14 subjects) than good oral hygiene (3 subjects) during treatment (T2) ($p=.049$). Other literature has reported poor oral hygiene as a significant risk factor for developing WSLs (Chapman et al 2010, Julien et al 2013, Hadler-Olsen et al 2012). This is important

because it reinforces the idea that prevention of WSLs via improving oral hygiene practices may be the best method of prevention.

Sex differences have been explored in the literature as a risk factor for developing WSLs. In the present study, there were no statistically significant sex differences for the incidence of WSL formation. Previous studies have found that males develop more WSLs than females (Lucchese et al 2013, Boersma et al 2005, Chapman et al 2010, Julien et al 2013), although one study found the opposite (Gorelick et al 1982).The reason males are thought to have more WSLs is due to their significantly poorer oral hygiene during orthodontic treatment (Chapman et al 2010). In the present study, there was no difference in oral hygiene between the females and males.

CHAPTER V

CONCLUSIONS

1. Substantial numbers of patients should be expected to develop WSLs during orthodontic treatment.
2. Even though the incidence is high, the severity of WSLs in patients treated with either sealant or varnish can be minimal.
3. Lateral incisors are more likely to develop new WSLs than canines or central incisors.
4. The gingival region is more likely to develop WSLs than the other regions of teeth.
5. MI Varnish and ProSeal sealant provide similar protection during orthodontic treatment.
6. Patients who develop WSLs tend to have poorer oral hygiene.

REFERENCES

1. Abufarwa M, Noureldin A, Campbell PM, Buschang PH. The longevity of casein phosphopeptide–amorphous calcium phosphate fluoride varnish's preventative effects: Assessment of white spot lesion formation. Angle Orthod 2019;89 (1):10-15.
2. Anderson K, Campbell P, Julien K, Noureldin A. Increasing Sealant Retention During Orthodontic Treatment with Gingival Retraction Cord Isolation: Texas A&M University College of Dentistry; 2018.
3. Banks PA, Richmond S. Enamel sealants: a clinical evaluation of their value during fixed appliance therapy. Eur J Orthod. 1994;16(1):19-25.
4. Benson PE, Parkin N, Dyer F, Millett DT, Furness S, Germain P. Fluorides for the prevention of early tooth decay (demineralised white lesions) during fixed brace treatment. Cochrane Database Syst Rev. 2013(12):CD003809.
5. Buren JL, Staley RN, Wefel J, Qian F. Inhibition of enamel demineralization by an enamel sealant, Pro Seal: an in-vitro study. Am J Orthod Dentofacial Orthop. 2008;133(4 Suppl):S88-94.
6. Farhadian N, Miresmaeili A, Eslami B, Mehrabi S. Effect of fluoride varnish on enamel demineralization around brackets: an in-vivo study. Am J Orthod Dentofacial Orthop. 2008;133(4 Suppl):S95-8.
7. Hadler-Olsen S, Sandvik K, El-Agroudi MA, Øgaard B. The incidence of caries and white spot lesions in orthodontically treated adolescents with a comprehensive caries prophylactic regimen--a prospective study. Eur J Orthod. 2012;34(5):633-9.

8. Hu W, Featherstone JD. Prevention of enamel demineralization: an in-vitro study using light-cured filled sealant. Am J Orthod Dentofacial Orthop. 2005;128(5):592-600.
9. Julien KC, Buschang PH, Campbell PM. Prevalence of white spot lesion formation during orthodontic treatment. Angle Orthod. 2013;83(4):641-7.
10. Knösel M, Ellenberger D, Göldner Y, Sandoval P, Wiechmann D. In-vivo durability of a fluoride-releasing sealant (OpalSeal) for protection against white-spot lesion formation in orthodontic patients. Head Face Med. 2015;11:11.
11. Øgaard B. Prevalence of white spot lesions in 19-year-olds: a study on untreated and orthodontically treated persons 5 years after treatment. Am J Orthod Dentofacial Orthop. 1989; 96(5): 423-7.
12. Perrini F, Lombardo L, Arreghini A, Medori S, Siciliani G. Caries prevention during orthodontic treatment: In-vivo assessment of high-fluoride varnish to prevent white spot lesions. Am J Orthod Dentofacial Orthop. 2016;149(2):238-43.
13. Rechmann P, Bekmezian S, Rechmann BMT, Chaffee BW, Featherstone JDB. MI Varnish and MI Paste Plus in a caries prevention and remineralization study: a randomized controlled trial. Clin Oral Investig. 2018;22(6):2229-39.
14. Reynolds EC, Cai F, Cochrane NJ, Shen P, Walker GD, Morgan MV, et al. Fluoride and casein phosphopeptide-amorphous calcium phosphate. J Dent Res. 2008;87(4):344-8.
15. Robertson MA, Kau CH, English JD, Lee RP, Powers J, Nguyen JT. MI Paste Plus to prevent demineralization in orthodontic patients: a prospective randomized controlled trial. Am J Orthod Dentofacial Orthop. 2011;140(5):660-8.

16. Tufekci E, Dixon JS, Gunsolley JC, Lindauer SJ. Prevalence of white spot lesions during orthodontic treatment with fixed appliances. Angle Orthod 2011;81:206-210.
17. Shen P, Bagheri R, Walker GD, Yuan Y, Stanton DP, Reynolds C, et al. Effect of calcium phosphate addition to fluoride containing dental varnishes on enamel demineralization. Aust Dent J. 2016;61(3):357-65.
18. Turesky S, Gilmore ND, Glickman I. Reduced plaque formation by the chloromethyl analogue of victamine C. J Periodontol. 1970;41(1):41-3.
19. Boersma JG, van der Veen MH, Lagerweij MD, Bokhout B, Prahl-Andersen B. Caries prevalence measured with QLF after treatment with fixed orthodontic appliances: influencing factors. Caries Res 2005;39:41-7.
20. Lundstrom F, Krasse B. Streptococcus mutans and lactobacilli frequency in orthodontic patients: the effect of chlorhexidine treatments. Eur J Orthod 1987;9:109-116.
21. Kuhar M, Cevc P, Schara M, Funduk N. Enhanced permeability of acidetched or ground dental enamel. J Prosthet Dent 1997;77:578-582.
22. Lehman R, Davidson CL, Duijsters PP. In vitro studies on susceptibility of enamel to caries attack after orthodontic bonding procedures. Am J Orthod 1981;80:61-72.
23. Hess E, Campbell PM, Honeyman AL, Buschang PH. Determinants of enamel decalcification during simulated orthodontic treatment. Angle Orthod 2011;81:836-842.
24. Abufarwa, M., Voorhees, R., Varanasi, V., Campbell, P., & Buschang, P. White spot lesions: Does etching really matter? J Investig Clin Dent 2018;9:1-6.

25. Lovrov S, Hertrich K, Hirschfelder U. Enamel Demineralization during Fixed Orthodontic Treatment Incidence and Correlation to Various Oral-hygiene Parameters. J Orof Orthop. 2007;68(5):353-63.
26. Chapman JA, Roberts WE, Eckert GJ, Kula KS, González-Cabezas C. Risk factors for incidence and severity of white spot lesions during treatment with fixed orthodontic appliances. Am J Orthod Dentofacial Orthop 2010;138(2):188-94.
27. Chang HS, Walsh LJ, Freer TJ. Enamel demineralization during orthodontic treatment. Aetiology and prevention. Aust Dent J 1997;42(5):322-7.
28. Gorelick L, Geiger AM, Gwinnett AJ. Incidence of white spot formation after bonding and banding. Am J Orthod. 1982;81(2):93-98.
29. Geiger AM, Gorelick L, Gwinnett AJ, Griswold PG. The effect of a fluoride program on white spot formation during orthodontic treatment. Am J Orthod 1988;93(1):29-37.
30. Øgaard B, Rølla G, Arends J, ten Cate JM. Orthodontic appliances and enamel demineralization Part 2. Prevention and treatment of lesions. Am J Orthod 1988;94(2):123-28.
31. Benham AW, Campbell PM, Buschang PH. Effectiveness of Pit and Fissure Sealants in Reducing White Spot Lesions during Orthodontic Treatment. Angle Orthod 2009;79(2):338-45.
32. Brown MD, Campbell PM, Schneiderman ED, Buschang PH. A practice-based evaluation of the prevalence and predisposing etiology of white spot lesions. Angle Orthod 2015;86(2):181-86.

33. Mizrahi E. Enamel demineralization following orthodontic treatment. Am J Orthod 1982;82(1):62-67.
34. Mizrahi E. Surface distribution of enamel opacities following orthodontic treatment. Am J Orthod 1983;84(4):323-31.
35. Mitchell L. Decalcification during orthodontic treatment with fixed appliances – an overview. Br J Orthod 1992;19:199-205.
36. Newbrun E. Cariology. 3rd edn. Chicago: Quintessence, 1989:29-61.
37. Bjarnason S, Kohler B, Wagner K. A longitudinal study of dental caries and cariogenic microflora in a group of young adults from Goteborg. Swed Dent J 1993;17:191-9.
38. Klock B, Krasse B. A comparison between different methods for prediction of caries activity. Scand J Dent Res 1979;87:129-39.
39. Van Houte J. Bacterial specificity in the etiology of dental caries. Int Dent J 1980;30:305-26.
40. Papas AS, Joshi A, MacDonald SL, Maravelis-Splagounias L, Pretara-Spanedda P, Curro FA. Caries prevalence in xerostomic individuals. J Can Dent Assoc 1993;59:171-9.
41. Andersson R, Arvidsson E, Crossner CG, Holm AK, Mansson B, Grahnen H. The flow rate, pH and buffer effect of mixed saliva in children. J Int Assoc Dent Child 1974;5:5-12.
42. Sakamaki ST, Bahn AN. Effect of orthodontic banding on localised oral lactobacilli. J Dent Res 1968;47:275-9.

43. Pan H, Tao J, Yu X, Fu L, Zhang J, Zeng X et al. Anisotropic demineralization and oriented assembly of hydroxyapatite crystals in enamel: smart structures of biominerals. J Phys Chem B 2008;112:7162-7165.
44. Nelson DG, Featherstone JD, Duncan JF, Cutress TW. Effect of carbonate and fluoride on the dissolution behaviour of synthetic apatites. Caries Res 1983;17:200-211.
45. Zero DT. Dental caries process. Dent Clin North Am 1999;43:635-664.
46. Lynch RJ, Smith SR. Remineralization agents - new and effective or just marketing hype? Adv Dent Res 2012;24:63-67.
47. Silverstone LM. Observations on the dark zone in early enamel caries and artificial caries-like lesions. Caries Res 1967;1:260-274.
48. Silverstone LM. The surface zone in caries and in carieslike lesions produced in vitro. Br Dent J 1968;125:145-157.
49. Silverstone LM. Structure of carious enamel, including the early lesion. Oral Sci Rev 1973;3:100-160.
50. Hicks MJ, Silverstone LM. Acid-etching of caries-like lesions of enamel: a polarized light microscopic study. Caries Res 1984;18:315-326.
51. Sudjalim TR, Woods MG, Manton DJ. Prevention of white spot lesions in orthodontic practice: a contemporary review. Aust Dent J 2006;51:284-289; quiz 347.
52. Shivakumar K, Prasad S, Chandu G. International Caries Detection and Assessment System: A new paradigm in detection of dental caries. J Conserv Dent 2009;12:10-16.

53. Gomez J, Tellez M, Pretty IA, Ellwood RP, Ismail AI. Non-cavitated carious lesions detection methods: a systematic review. Community Dent Oral Epidemiol 2013;41:54-66.

54. Johnston WM, Kao EC. Assessment of appearance match by visual observation and clinical colorimetry. Journal of Dental research 1989. 68:819-822.

55. Schmidlin PR, Gohring TN, Schug J, Lutz F. Histological, morphological, profilometric and optical changes of human tooth enamel after microabrasion. American Journal of Dentistry 2003. 16 Spec No: 4A-8A.

56. Lundstrom F, Hamp SE, Nyman S. Systematic plaque control in children undergoing long-term orthodontic treatment. Eur J Orthod 1980;2:27-39.

57. Waggoner WF, Johnston WM, Schumann S, Schikowski S. Microabrasion of human enamel in vitro using hydrochloric acid and pumice. Pediatr Dent. 1989;11:319-323.

58. Srinivasan N, Kavitha M, Loganathan SC. Comparison of the remineralization potential of CPP-ACP and CPP-ACP with 900 ppm fluoride on eroded human enamel: An in situ study. Arch Oral Biol 2010; 55:541-544.

59. Bishara SE, Ostby AW. White Spot Lesions: Formation, Prevention, and Treatment. Semiin Orthod 2008;14(3):174-82.

60. Abbas BA, Marzouk ES< Zaher AR. Treatment of various degrees of white spot lesions using resin infiltration- in vitro study. Prog Orthod 2018;19:27.

61. Yetkiner E, Wegehaupt F, Wiegand A, Attin R, Attin T. Colour improvement and stability of white spot lesions following infiltration, micro-abrasion, or fluoride treatments in vitro. Eur J Orthod 2014;36:595-602.

62. Bailey DL, Adams GG, Tsao CE, Hyslop A, Escobar K, Manton DJ et al. Regression of post-orthodontic lesions by a remineralizing cream. J Dent Res 2009;88:1148-1153.

63. Paris S, Schwendicke F, Keltsch J, Dörfer C, Meyer-Lueckel H. Masking of white spot lesions by resin infiltration in vitro. J Dent 2013;41:e28-e34.

64. Lagerweij MD, ten Cate JM. Remineralisation of Enamel Lesions with Daily Applications of a High-Concentration Fluoride Gel and a Fluoridated Toothpaste: An in situ Study. Caries Research 2002;36(4):270-74.

65. Kidd EAM, Fejerskov O. What Constitutes Dental Caries? Histopathology of Carious Enamel and Dentin Related to the Action of Cariogenic Biofilms. J Dent Res 2004;83(1_suppl):35-38.

66. Heintze SD, Jost-Brinkmann P-G, Loundos J. Effectiveness of three different types of electric toothbrushes compared with a manual technique in orthodontic patients. Am J Orthod Dentofacial Orthop 1996;110(6):630-38.

67. Borges AB, Caneppele TMF, Masterson D, Maia LC. Is resin infiltration an effective esthetic treatment for enamel development defects and white spot lesions? A systematic review. J Dent 2017;56:11-18.

68. Akin M, Basciftci FA. Can white spot lesions be treated effectively? Angle Orthod 2012;82(5):770-5.

69. Bakry AS, Abbassy MA. Increasing the efficiency of CPP-ACP to remineralize enamel white spot lesions. J Dent 2018;76:52-57.

70. Bayrak S, Tuloglu N, Bicer H, Tunc ES. Effect of fluoride varnish containing CPP-ACP on preventing enamel erosion. Scanning 2017;1-7.

71. Salman NR, ElTekeya M, Bakry N, Omar SS, El Tantawi ME. Comparison of remineralization by fluoride varnishes with and without casein phosphopeptide amorphous calcium phosphate in primary teeth. Acta Odontol Scand 2019; 77:9-14.
72. Chebel FB, Zogheib CM, Baba N, Corbani KA. Clinical comparative evaluation of Nd:YAG laser and a new varnish containing casein phosphopeptides-amorphous calcium phosphate for the treatment of dentin hypersensitivity: a prospective study. J Prosth 2018;27:860-867.
73. Khooshki F, Pajoohan S, Kamarch S. Effects of treatment with three types of varnish remineralizing agents on the microhardness of demineralized enamel surface. J Clin Exp Dent 2019;11(7):e630-5.
74. O'Reilly MT, De Jesus Vinas J, Hatch JP. Effectiveness of a sealant compared with no sealant in preventing enamel demineralization in patients with fixed orthodontic appliances: a prospective clinical trial. Am J Orthod Dentofacial Orthop 2013;143:837-844.
75. Wang L, Tang R, Bonstein T, Orme CA, Bush PJ, Nancollas GH. A new model for nanoscale enamel dissolution. J Phys Chem B 2005;109:999- 1005.
76. Ryan J, Campbell P, Noureldin A, Buschang P. Effects of Microabrasion-With or Without MI Paste Plus- on the Treatment of White Spot Lesions. Texas A&M University College of Dentistry; 2019.
77. Stecksen-Blicks C, Renfors G, Oscarson ND, Bergstrand F, Twetman S. Caries-preventive effectiveness of a fluoride varnish: a randomized controlled trial in adolescents with fixed orthodontic appliances. Caries Res 2007;41:455-459.

78. Featherstone JD. The Science and Practice of Caries Prevention. J Am Dent Assoc 2000; 131:887-899.
79. Kielbassa AM, Muller J, Gernhardt CR. Closing the gap between oral hygiene and minimally invasive dentistry: a review on the resin infiltration technique of incipient (proximal) enamel lesions. Quintessence Int 2009;40:663-681.
80. Kanthasas K, Willmot DR, Benson PE. Differentiation of developmental and post-orthodontic white lesions using image analysis. Eur J Orthod 2005; 27:167-172.
81. Lucchese A, Gherlone E. Prevalence of white-spot lesions before and during orthodontic treatment with fixed appliances. Eur J Orthod 2013; 35:664–668.
82. Sonesson M, Brechter A, Abdulraheem S, Lindman R, Twetman S. Fluoride varnish for the prevention of white spot lesions during orthodontic treatment with fixed appliances: a randomized controlled trial. Eur J Orthod 2019.

APPENDIX A

FIGURES

Figure 1. Study Flow Diagram

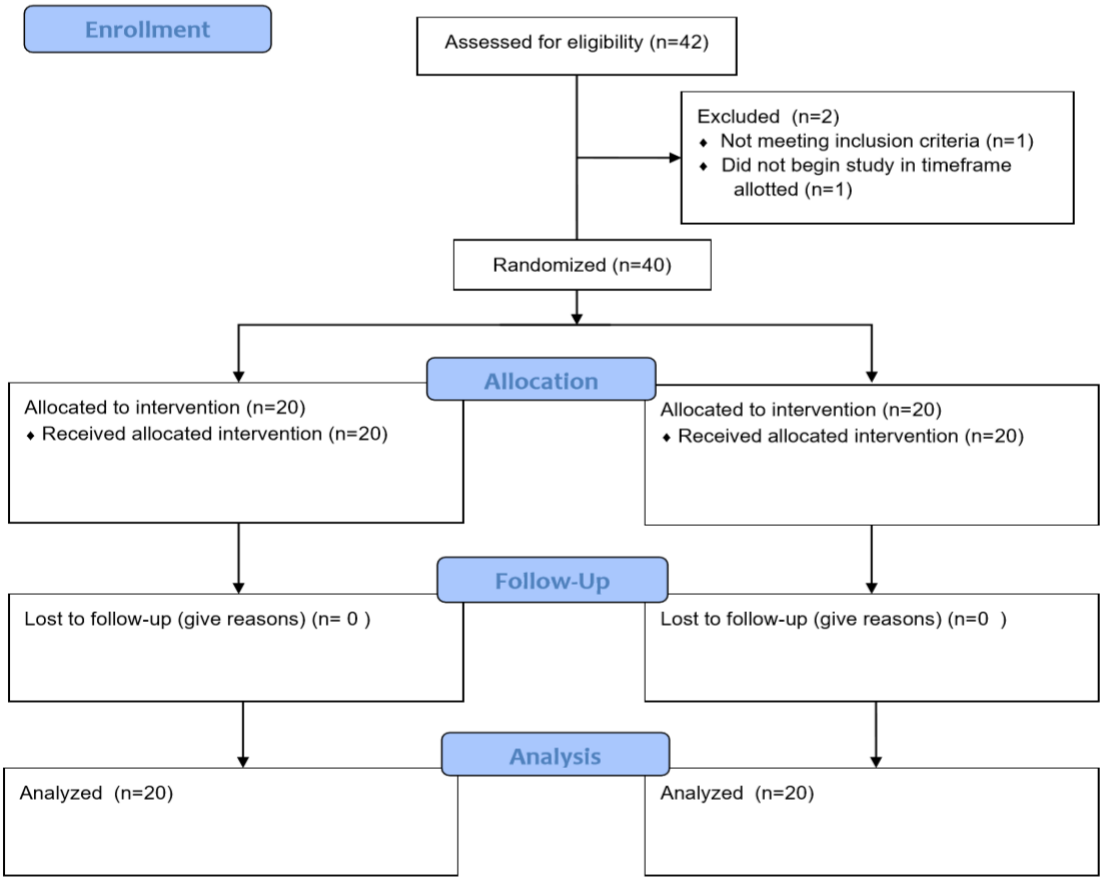


Figure 2. Percentage of Subjects who Developed WSLs based on Tooth Type

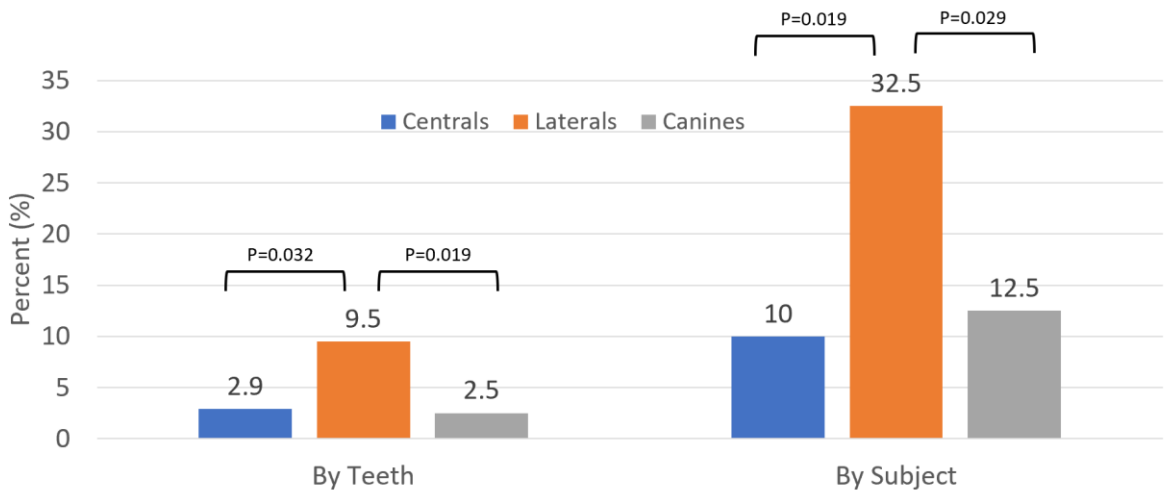


Figure 3. Percentage of Subjects who Developed WSLs Based on Tooth Region

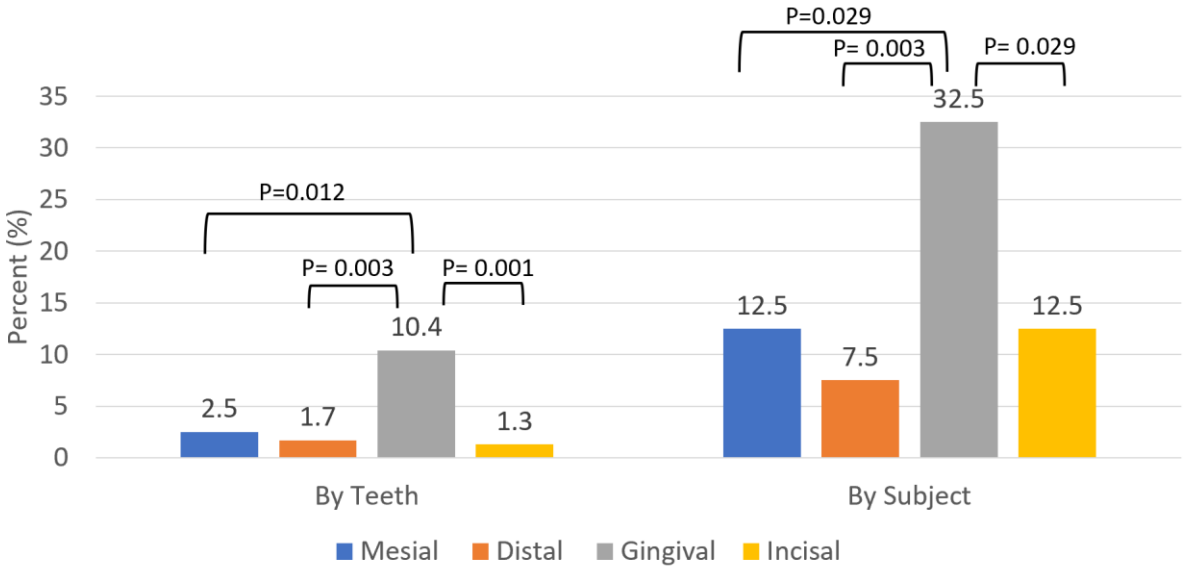


Figure 4. New EDI Scores by Tooth Type

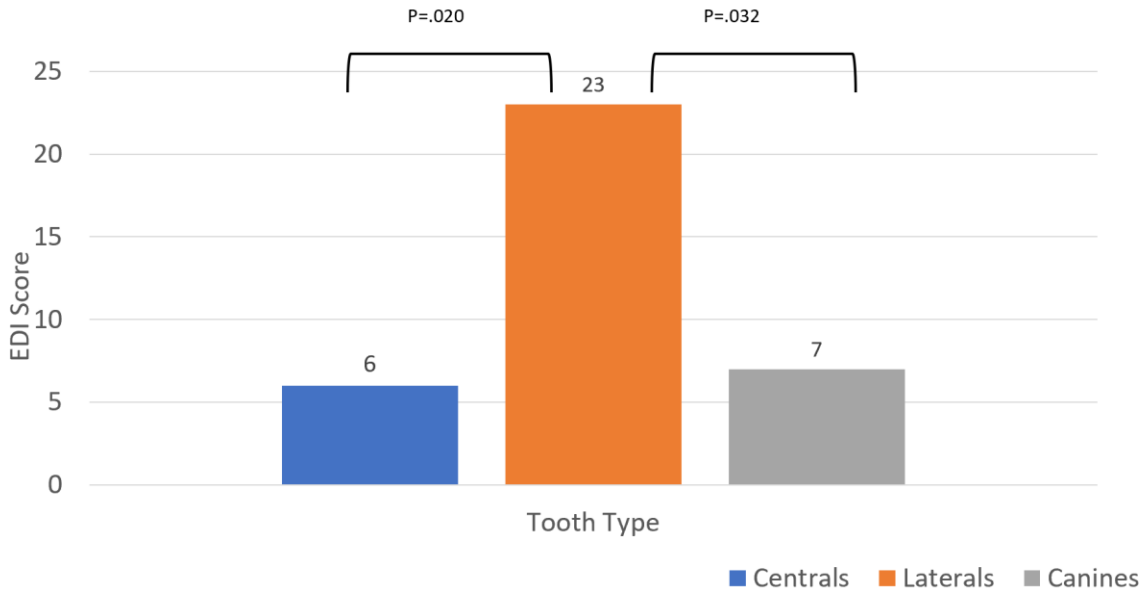


Figure 5. New EDI Scores by Tooth Region

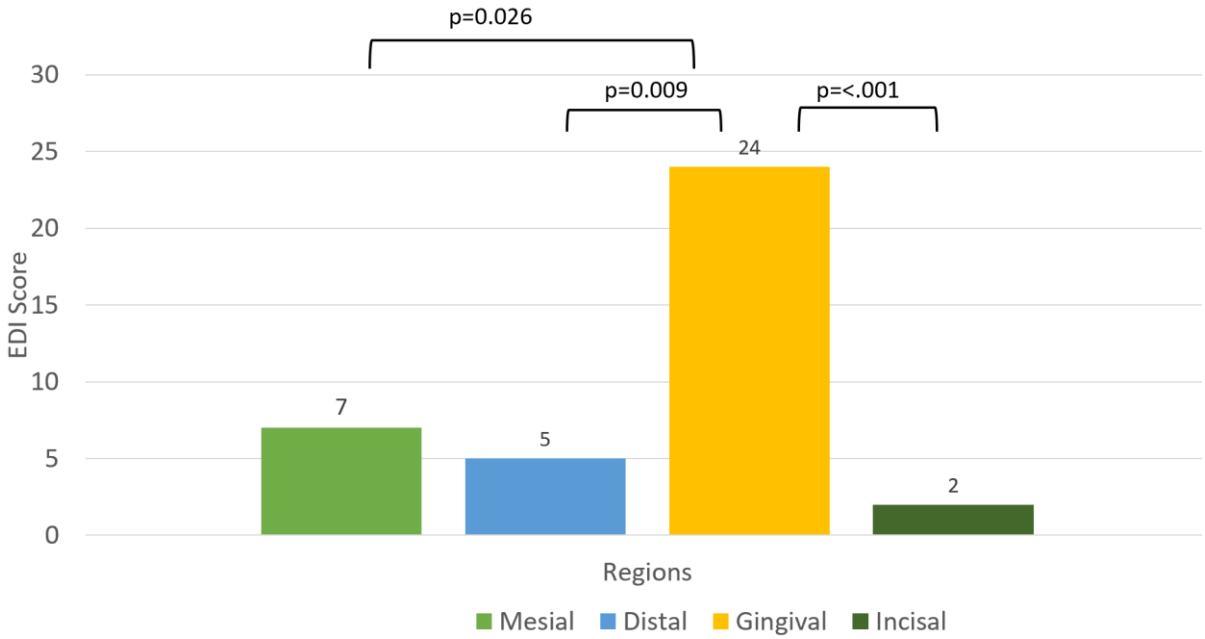


Figure 6. Percentage of Subjects that Remained the Same or Improved, versus Those that Worsened from T1 to T2

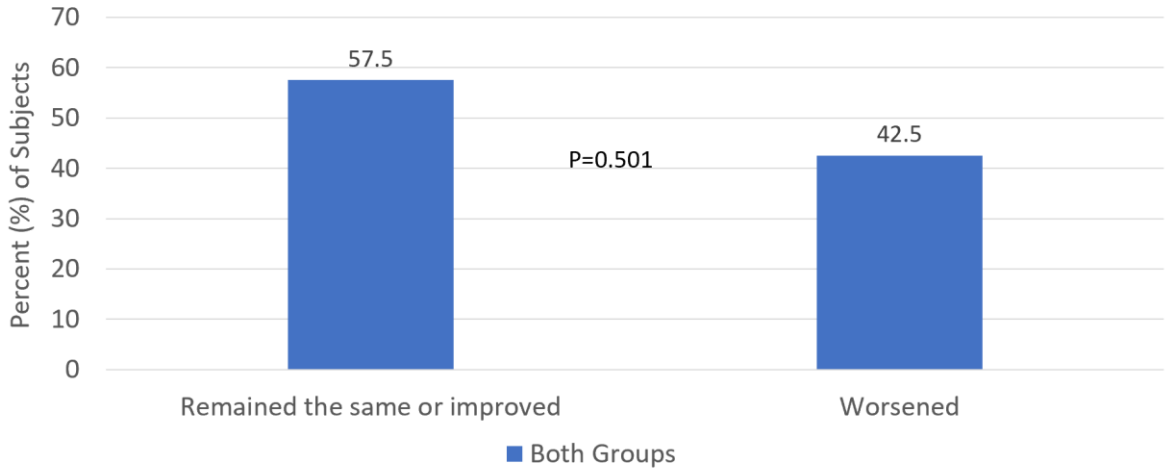


Figure 7. Overall Number of Subjects Who Developed WSLs Based on Oral Hygiene Status at T1 and T2

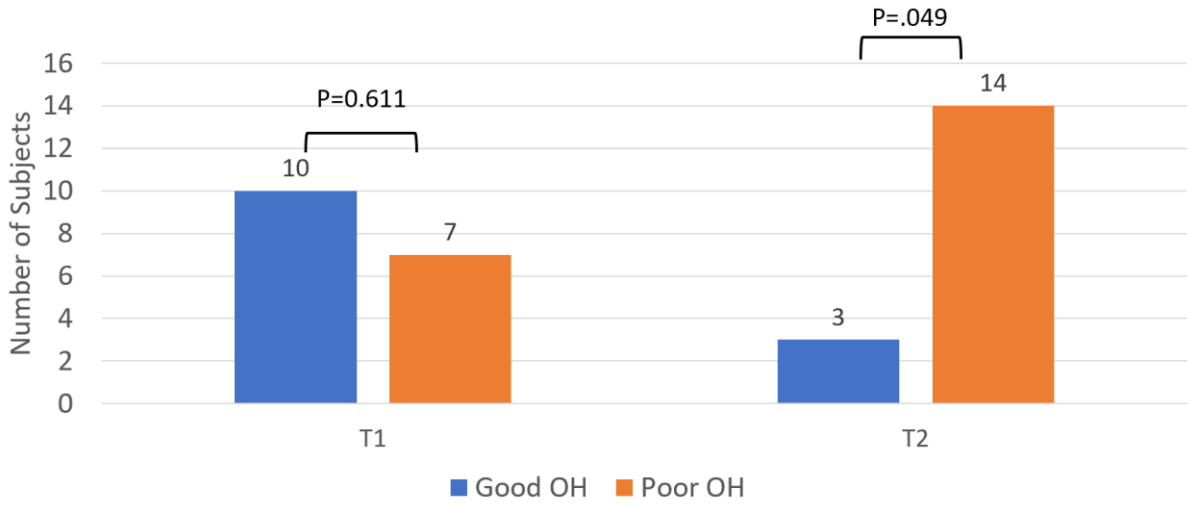


Figure 8. Total EDI Sums for Sealant and Varnish Group for T1, T2, and New Scores

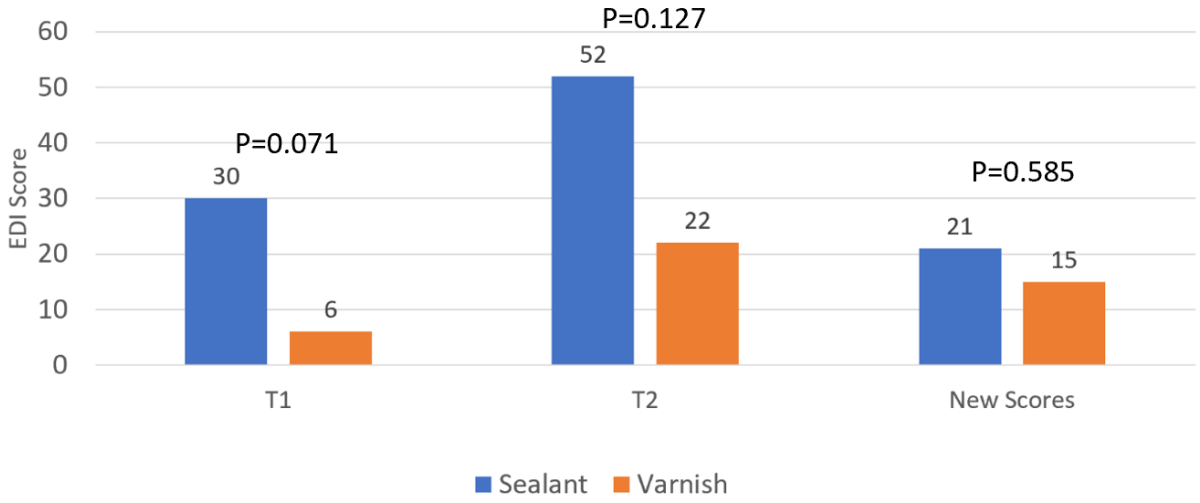


Figure 9. Number of Subjects Who Remained the Same or Improved, versus Those who Worsened from T1 to T2

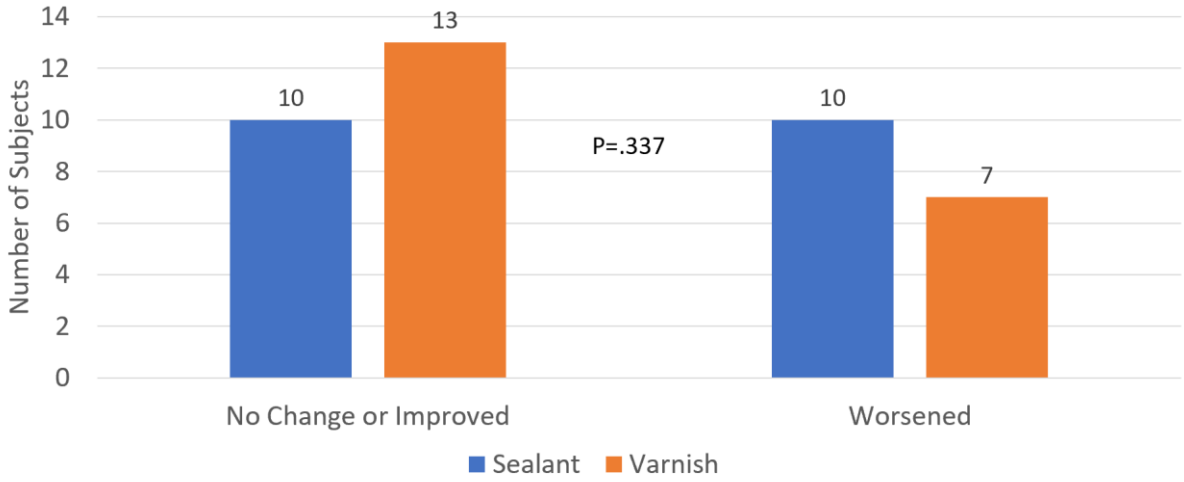


Figure 10. New EDI Scores by Tooth Type for Sealant Group and Varnish Group

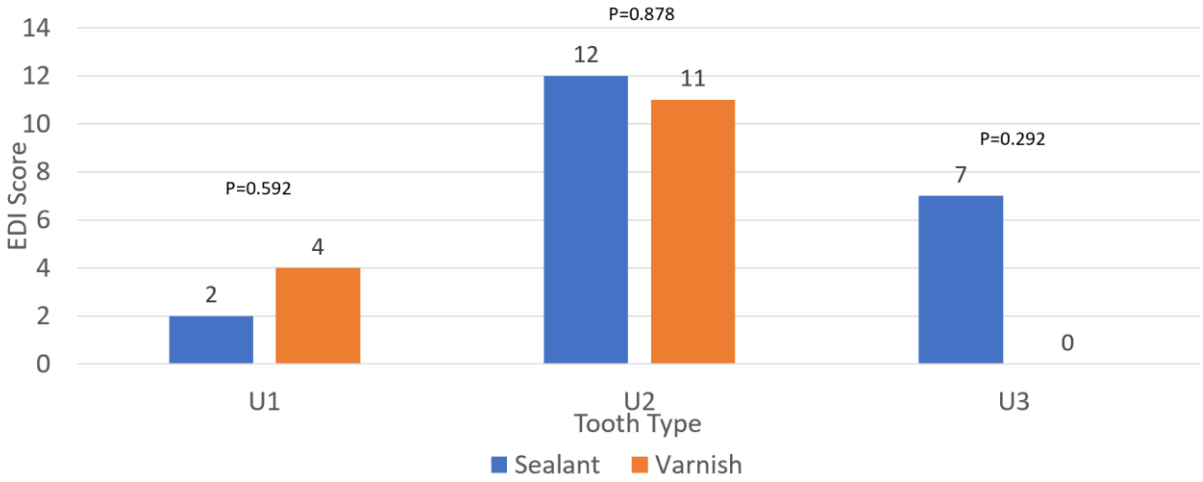


Figure 11. New EDI Scores by Tooth Region for Sealant Group and Varnish Group

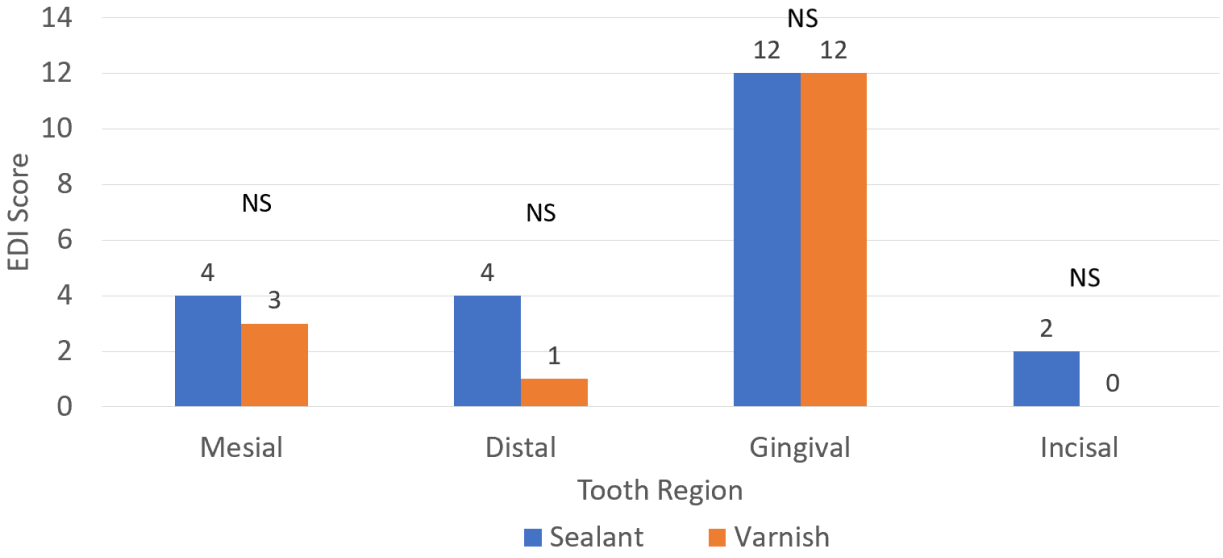
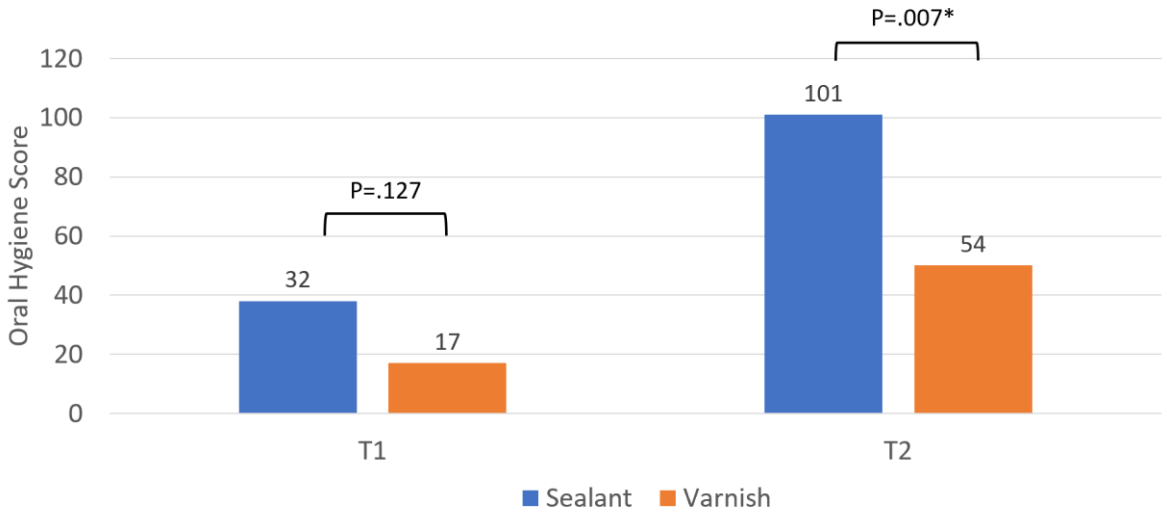


Figure 12. Oral Hygiene Scores at T1 versus T2 for Sealant Group and Varnish Group



APPENDIX B

TABLES

Table 1. Group Characteristics

	Sealant Group	Varnish Group
# of Subjects	20 subjects	20 subjects
Average Age	14.0 years	14.1 years
Male/Female	10 M, 10 F	8 M, 12 F
Duration of Treatment	.99 +/- .089 years	.99 +/- .091 years

Table 2. Enamel Decalcification Index (EDI) Score

EDI Score	Description
0	No decalcification
1	Decalcification covering <50% of the area
2	Decalcification covering >50% of the area
3	Decalcification covering 100% of the area or severe decalcification with cavitation

(Banks et al 1994)

Table 3. Turesky Modification of the Quigley and Hein Plaque Index

Score	Description of Plaque Index
0	No plaque
1	Isolated flecks of plaque at the gingival margin
2	A continuous band of plaque up to 1mm at the gingival margin
3	Plaque greater than 1mm in width and covering up to one third of the tooth surface
4	Plaque covering from one thirds to two thirds of the tooth surface
5	Plaque covering more than two thirds of the tooth surface

Table 4. Prevalence and Incidence of WSLs for All Subjects Overall

	Prevalence				Incidence	
	T1		T2		T1-T2	
	Subjects	Teeth	Subjects	Teeth	Subjects	Teeth
Percentage (%)	32.5%	11.3%	60%	21.3%	42.5%	14.9%
N/Total N	13/40	27/240	24/40	51/240	17/40	35/240

Table 5. Incidence of WSLs by Tooth Type for All Subjects

	Incidence by Subject		Incidence by Teeth	
	Percentage (%)	N/Total N	Percentage (%)	N/Total N
Centrals	10%	4/40	2.9 %	7/240
Laterals	32.5%	13/40	9.5 %	23/240
Canines	12.5%	5/40	2.5%	6/240

Table 6. Incidence of WSLs by Tooth Region for All Subjects

	Incidence by Subject		Incidence by Teeth	
	Percentage (%)	N/Total N	Percentage	N/Total N
Mesial	12.5%	5/240	2.5%	6/240
Distal	7.5%	3/240	1.7%	4/240
Gingival	32.5%	13/240	10.4%	25/240
Incisal	2.5%	1/240	1.3%	3/240

Table 7. Females Versus Males Who Developed WSLs for All Subjects

	Patients who Developed WSLs	
	Percentage (%)	N/Total N
Females	41.9%	9/22
Males	44.4%	8/18

Table 8. Overall EDI Sums at T1, T2, and New Scores

	Sum	Mean	Standard Deviation
T1	36	.9	2.06
T2	74	1.85	3.09
New	38	.95	1.71

Table 9. EDI Sums at T1, T2, and New Scores by Tooth Type

		All Groups		
	Tooth	Sum	Mean	SD
T1	Centrals	9	.23	.58
	Laterals	16	.4	.9
	Canines	11	.28	.96
T2	Centrals	15	.375	.70
	Laterals	39	.98	1.37
	Canines	20	.5	1.85
New	Centrals	6	.15	.58
	Laterals	23	.575	1.0
	Canines	7	.23	1.03

Table 10. EDI Sums at T1, T2, and New Scores by Tooth Region

		Sealant		
		Sum	Mean	SD
T1	Mesial	4	.1	.06
	Distal	5	.13	.404
	Gingival	22	.55	1.6
	Incisal	5	.125	.404
T2	Mesial	11	.28	.64
	Distal	10	.25	.84
	Gingival	46	1.15	2.06
	Incisal	7	.18	.68
New	Mesial	7	.175	.08
	Distal	5	.125	.52
	Gingival	24	.6	1.19
	Incisal	2	.05	.32

Table 11. Number of Subjects Who Got Better or Remained the Same, versus Those that Worsened from T1 to T2

	No Change or Better	Worsened	Probability
Total	23 Subjects (57.5%)	17 Subjects (42.5%)	.501

Table 12. Overall Oral Hygiene Means, Standard Deviations, and Probability at T1 and T2

	Oral Hygiene		
	Total Score	Mean	SD
T1	49	1.22	1.85
T2	155	3.88	2.97

Table 13. Number of Subjects who Developed WSLs Based on Oral Hygiene at T1 versus Oral Hygiene at T2

	# of subjects that Developed WSLs according to hygiene status at (T1)	# of Subjects that developed WSLs according to hygiene status at T2
Good Hygiene (0)	10	3
Poor Hygiene (>0)	7	14

Table 14. Prevalence and Incidence of WSLs for Sealant and Varnish Group

		Prevalence				Incidence	
		T1		T2		Change	
		Subjects	Teeth	Subjects	Teeth	Subjects	Teeth
Sealant	Percentage (%)	45%	19.2%	80%	28.3%	50%	17.5%
	N/Total N	9/20	23/120	16/20	34/120	10/20	21/120
Varnish	Percentage (%)	20%	3.3%	40%	14.2%	35%	12.5%
	N/Total N	4/20	4/120	8/20	17/120	7/20	15/120

Table 15. EDI Sums for Sealant and Varnish Group at T1, T2, and New Scores

		Sealant			Varnish		
EDI Sums	Total	Mean	SD	Total	Mean	SD	Probability
T1	30	1.5	2.74	6	.3	.66	.071
T2	52	2.6	3.99	22	1.1	1.59	.127
New	21	1.1	1.93	15	.80	1.48	.585

Table 16. Number of Subjects Who Got Better or Remained the Same, versus Those that Worsened from T1 to T2 in the Sealant Group and Varnish Group

	Sealant		Varnish	
	%	N	%	N
No Change or Better	50%	10	65%	13
Worsened	50%	10	35%	7

Table 17. EDI Sums by Tooth Type for Sealant and Varnish Group at T1, T2, and New Scores

		Sealant			Varnish			
	Tooth	Sum	Mean	SD	Sum	Mean	SD	Probability
T1	Centrals	8	.4	.76	1	.05	.22	.059
	Laterals	11	.55	1.10	5	.25	.64	.299
	Canines	11	.55	1.31	0	0	0	.077
T2	Centrals	10	.5	.76	5	.25	.64	.267
	Laterals	23	1.15	1.53	16	.8	1.20	.426
	Canines	19	.95	2.56	1	.05	.22	.134
New	Centrals	2	.10	.55	4	.20	.62	.592
	Laterals	12	.6	.94	11	.55	1.10	.878
	Canines	7	.4	1.43	0	.5	.22	.292

Table 18. EDI Sums by Tooth Region at T1, T2, and New Scores for Sealant Group and Varnish Group

		Sealant			Varnish			
		Sum	Mean	SD	Sum	Mean	SD	Probability
T1	Mesial	4	.15	.49	1	.05	.22	.411
	Distal	5	.2	.52	1	.05	.22	.249
	Gingival	22	.95	2.19	3	.15	.37	.122
	Incisal	5	.2	.52	1	.05	.22	.249
T2	Mesial	11	.35	.67	4	.2	.62	.466
	Distal	10	.4	1.14	2	.1	.31	.264
	Gingival	46	1.55	2.58	15	.75	1.29	.223
	Incisal	7	.3	.92	1	.05	.22	.252
New	Mesial	4	.2	.52	3	.15	.49	.757
	Distal	4	.2	.70	1	.05	.22	.365
	Gingival	12	.6	1.10	12	.6	1.31	1
	Incisal	2	.1	.45	0	0	0	.330

Table 19. Oral Hygiene Means, Standard Deviations, and Probability at T1 and T2 for Sealant Group and Varnish Group

	Sealant			Varnish			
EDI Sums	Total Score	Mean	SD	Total Score	Mean	SD	Probability
T1	32	1.6	2.13	17	.85	1.46	.203
T2	101	5.05	2.67	54	2.7	2.85	.010*

Table 20. Number of Subjects Who Developed WSLs based on Oral Hygiene at T1 versus Oral Hygiene at T2 for Sealant Group and Varnish Group

		Oral Hygiene (0)	Oral Hygiene (>0)	Probability
Sealant	T1	6	4	.178
	T2	1	9	.531
Varnish	T1	4	3	.589
	T2	2	5	.279