EFFECT OF ACIDULATED PHOSPHATE FLUORIDE GEL ON THE PREVENTION OF WHITE SPOT LESIONS IN PATIENTS UNDERGOING ACTIVE ORTHODONTIC TREATMENT

A Thesis

by

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ABSTRACT

Introduction: The purpose of this study was to evaluate the effect of 1.23% acidulated phosphate fluoride (APF) gel on white spot lesion (WSL) formation utilizing a typical orthodontic treatment interval for the applications.

Methods: In this double-blind, placebo controlled trial, 55 patients starting orthodontic treatment, either at Texas A&M University, Baylor College of Dentistry or at a private practice, were randomly divided into two groups (a treatment and placebo). Every eight weeks, the treatment group received an application of Nupro 1.23% APF gel (Dentsply®) for one minute, while the placebo group received a gel treatment for one minute that was exactly the same composition, but lacked fluoride. Patients were followed for 6 or 8 months depending on when their second set of brackets was placed. Pre and post treatment photographs and Fluorecam® readings were used to quantify the development of WSLs.

Results: There was no statistically significant difference in the percentage of patients who developed WSLs, the number of new WSLs that developed on each tooth during treatment, or in the number of new WSLs developed per patient when using photographic analysis or Fluorecam® readings. Based on photographic assessments, 37.5% of patients in the fluoride group and 32.3% of patients in the placebo group developed new WSLs ($p=0.685$), while with Fluorecam assessments 37.5% of patients in the fluoride group and 58.1% of patients in the placebo group developed a new WSL ($p=.130$). Five patients had pre-existing WSLs (1 patient in the treatment group and 4

patients in the placebo group). Only patients in the placebo group demonstrated worsening of white spot lesions (p=.375). When using the Fluorecam® to quantify WSL development by measuring the size, intensity, and impact of each lesion, the only measurement which demonstrated a significant difference between the fluoride and placebo group was the intensity of the lower left first premolar (P<.05). The remaining readings on all of the other teeth were not statistically significant between the groups.

Conclusion: Application of 1.23% APF gel for one minute every eight weeks does not have an effect in the prevention of WSL development.

DEDICATION

This thesis is dedicated to my husband, Casey, my son, Hudson, and my new little one who will arrive in September. Casey, thank you for your constant encouragement, love, and support through this journey. Thank you for the sacrifices you have made to allow me to fulfill my dreams. Hudson, you have brought so much joy into our lives. I never could have imagined that I could love someone so much. I look forward to watching you grow and to the many wonderful memories to come in the future!

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

White spot lesions (WSLs)/enamel decalcifications are a persistent problem in clinical orthodontics. These lesions develop in areas surrounding orthodontic appliances and remain after treatment is completed, forming permanent scars. White spot lesions compromise orthodontic esthetic results, in addition to the health of the dentition. White spot lesions are the result of accumulation of plaque and bacteria on the enamel surface, which in turn results in an increase in acidic by-products and demineralization of the enamel. [1] The overall prevalence in patients undergoing orthodontic treatment has been reported to range between 2-96%, depending on the method of detection.[1-5] In a recent study evaluating 885 orthodontic patients, Julien et al. determined that white spot lesions developed in 23% of patients.[6] While these lesions have been reported to occur on all teeth, the maxillary laterals and mandibular canines and premolars are the teeth most affected by these permanent scars. [4] Øgaard et al. found that white spot lesions can occur as early as four weeks. [7]

Many methods have been utilized to prevent white spot lesions from developing during orthodontic treatment. These include, but are not limited to, filled resin-sealants, glass ionomer cements, fluoride-releasing cements, fluoride varnishes, fluoride rinses, and fluoride gels. [2] Excellent oral hygiene can prevent white spot lesions from developing, but this is complicated by the presence of orthodontic appliances that cause increased plaque retention. Also, most orthodontic patients are adolescents, many of

whom have poor oral hygiene. The present study will focus on the use of acidulated phosphate fluoride gel to prevent white spot lesions. Only a few clinical studies have been conducted to evaluate the effectiveness of 1.23% APF application in reducing enamel demineralization during orthodontic treatment. O'Reilly and Featherstone, found that 1.2% acidulated phosphate fluoride applied once a week for a month prevented demineralization in patients undergoing active orthodontic treatment. [8] While their study was conducted in a clinical setting, orthodontic patients are not typically seen on a weekly basis. More recently, Jiang et al. studied 1.23% acidulated phosphate foam's effect in preventing white spot lesion development. One hundred orthodontic patients received either a fluoride or placebo treatment every two months during the course of treatment. It was shown that there was a 76 percent reduction in the incidence of white spot lesions in the 1.23% acidulated phosphate fluoride foam. More research is required to evaluate the extent of enamel demineralization with fluoride treatment applied at regular orthodontic appointments (which generally occur every 4 to 6 weeks).

The literature review will begin with a description of the demineralization process, the causative factor of white spot lesions. In order to understand why orthodontic patients are at an increased risk for developing white spot lesions, the etiology of white spot lesions will be outlined, as well as the prevalence of these lesions among orthodontic patients. Different methods for detection and treatment of white spot lesions will be reviewed. Finally, the various methods for WSL prevention will be discussed.

Definition of White Spot Lesions

A white spot lesion is defined as a "subsurface enamel porosity from carious demineralization", and its formation is the initial stage of the caries process. [9] Lesions appear white due to the loss of translucency of the enamel, as a result of demineralization. No cavitation is usually evident, but the surface may feel rougher than normal when checked with a sharp instrument. Sliverstone described four distinct zones of a carious lesion in enamel: the intact surface area, the body of the lesion, the dark zone, and the translucent zone.[10] A white spot lesion has a lesion body covered by an intact surface layer. The surface layer varies in width from 20-50 micrometers and has a pore volume (amount of mineral loss) of less than 5%. Underneath the intact surface area is the body of the lesion, the translucent zone, and a dark zone. In the body of the lesion, the pore volume exceeds 5%. The translucent zone is at the leading edge of the lesion. It varies in width from 5 to 100 micrometers and has a pore volume of slightly greater than 1%. The dark zone is located between the body of the lesion and translucent zone, and has a pore volume between 2 and 4%. Pore volume is greatest in the body of the lesion and decreases towards the advancing front of the lesion. [11]

The subsurface lesion develops through a cyclic demineralization/ remineralization process. Demineralization results from calcium and phosphate ions diffusing out of the enamel and into the surrounding environment. The minerals from the subsurface then replenish the mineral content of the surface enamel. [2] Under normal conditions, the environment surrounding enamel is supersaturated with hydroxyapatite

and fluorapetite. As the pH in the oral cavity decreases, the solubility of the enamel surface increases. At the critical pH of 5.5, the oral environment is undersaturated with respect to hydroxyapatite, leading to demineralization of enamel. [11] Long periods of demineralization followed by shorter periods of remineralization, will result in cavitation of the enamel surface.[2]

Etiology of White Spot Lesions

WSLs are caused by the accumulation of plaque and bacteria on the enamel surfaces of teeth, and are affected by salivary function, orthodontic appliances, diet, and poor oral hygiene. [2]

Bacterial Accumulation

Dental caries are the result of the metabolic activity of bacteria found in dental plaque. There are several stages in plaque development. First, an organic pellicle forms on a tooth. Attachment of bacterial cells occurs, followed by growth of attached bacteria, leading to the formation of microcolonies within 24 hours. A mature biofilm develops after approximately 1 week. *S. sanguinis*, *S. oralis* and *S. mitis* are all early colonizers of dental plaque. As the microcolonies age, there is a shift from a *Streptococcus*-dominated dental plaque to one dominated by *Actinomyces*. Mature plaque, associated with caries, has high numbers of *S. mutans,* lactobacilli and some *Actinomyces*. [11]

Bloom and Brown, using saliva samples, demonstrated an increase in overall oral bacterial count after the placement of orthodontic appliances.[12] Demineralization of

enamel, hydroxyapatite, is initiated by organic acids released by acidogenic bacteria, such as *S. mutans* and lactobacilli, located in the dental plaque.[13] *S. mutans* is an acidogenic and acid tolerant bacteria. It creates extracellular glucans from dietary sucrose, which can enlarge the plaque mass and enhance the colonization of bacteria, increasing the cariogenicity of plaque.[2] Scheie et al. determined that orthodontic treatment may result in increased *S mutans*.[14] It was found that after 3 months of treatment, the number of S. mutans exceeded the initial numbers in 13 patients. Lang et al. found that the smooth surfaces of first permanent premolars with non-cavitated lesions were colonized with *S. mutans*.[15] Their study also found that the proportion of *S. mutans* increased 10-12% 6-9 months prior to smooth surface lesion detection. In some instances the lesions remineralized, in which case the levels of *S. mutans* fell from around 20% to 2-5% of the total streptococcal count. Multiple studies have reported increased adhesion of bacteria to orthodontic appliances, especially in the presence of excess resin at the bracket/ tooth interface. [16-18]

Salivary Function

Saliva has many functions, including a role in the demineralization/re-mineralization process associated with caries progression.[13] While the presence of bacterial plaque, fermentable carbohydrates, a susceptible tooth surface and adequate time is necessary for demineralization of the enamel, saliva also can contribute this process. The flow rate of saliva, the pH, and the buffer capacity can impact the amount, rate, and progression of demineralization of enamel following an acid challenge, as well as the likelihood of remineralization.[2]

Normal saliva flow rate is 0.3 ml/min for unstimulated whole saliva and 1.5 ml/min for the stimulated saliva.[19] When the salivary glands are stimulated, the composition of saliva changes from a more mucoid to a more serous fluid, allowing for increased clearance of ingested materials.[13] Decreases in saliva flow rates have been shown to result in an increased caries incidence. Gorelick et al. noted that the most common sites of demineralization during orthodontic treatment are the maxillary anterior teeth, which have little exposure to saliva.[1] Papas et al. showed that xerostomic individuals have an increased caries incidence when matched with non-xerostomic individuals.[20]

The resting pH of saliva is indicative of the caries level of an individual, as well as the salivary buffering capacity.[13] The pH of saliva as well as its buffering capacity affects its ability to neutralize the plaque produced acid. The critical pH is described as the pH in which the ion activity product is equal to the solubility product of hydroxyapatite. At this pH (pH=5) the solution is saturated and no demineralization or remineralization will occur. After the intake of sugary foods, the pH in plaque will drop and remain lowered until the sugar is cleared from the mouth and the acid produced by bacteria is buffered.[19] The carbonic acid-bicarbonate system is the primary salivary buffer, while phosphates and proteins play a minor role. The carbonic acid-bicarbonate system acts as the primary salivary buffer, while phosphates and proteins also contribute.[2]

Oral Hygiene

Poor oral hygiene predisposes individuals to the development of white spot lesions. Improper cleaning techniques lead to increased plaque accumulation, which in turn creates an environment that is more susceptible to demineralization. Removal of plaque daily has been shown to limit the amount of reaccumulated plaque. This allows saliva to remove and buffer the acid that is formed from bacteria.[21] Orthodontic appliances make oral hygiene practices more difficult, leading to accelerated plaque accumulation on the tooth surfaces, especially between the attachments and at the gingival margins.[2] Gorelick et al. stated that most orthodontic patients are adolescents with poor oral hygiene, which increases plaque accumulation, and predisposes enamel to demineralization.[1] In a study evaluating 885 orthodontic patients, Julien et al. determined that poor oral hygiene is a risk factor for developing white spot lesions.[6] Their study evaluated hygiene before treatment and hygiene changes during treatment. Patients with fair or poor pretreatment oral hygiene had significantly more white spot lesions than those with good pretreatment oral hygiene. They also determined that patients whose oral hygiene decreased during orthodontic treatment were more likely to develop white spot lesions. Of patients whose oral hygiene worsened during treatment, 59% developed white spot lesions, whereas only 20% of patients whose hygiene remained the same developed white spot lesions.

Orthodontic Appliances

Orthodontic appliances have been shown to increase the risk of developing white spot lesions due to the preparation of the tooth that is necessary, the cement that is

present, and the increased difficulty in achieving good oral hygiene. The preparation of a tooth (acid etching) required for orthodontic bracket placement has been shown to increase the occurrence of WSLs. Teeth that are acid etched have been shown to exhibit 34% more decalcification than teeth that are not acid etched.[22] Sukontapatipark et al. reported that excess cement adjacent to the bracket base is a major site for plaque accumulation. Two to three weeks after bracket placement, mature plaque was present on the cement, while the plaque on gingival enamel remained in the immature state. [23] Orthodontic brackets also make it more difficult to remove plaque from the enamel surface. It has also been shown that there is an increase in bacteria among orthodontic patients. Bloom and Brown demonstrated an increase in overall oral bacterial count in salivary samples after the placement of orthodontic appliances.[12] Rosenbloom and Tinanoff, as well as Scheie et al., found increases in levels of *S. mutans* among patients undergoing orthodontic treatment.[14, 24] As discussed earlier, *S. mutans* increases the cariogenicity of plaque.

Diet

The development of white spot lesions has also been associated with the frequency of carbohydrate consumption. Demineralization has been shown to primarily occur in areas with a high carbohydrate exposure and a low salivary flow. Ingestion of a fermentable substrate leads to a decrease in the pH of plaque due to increased production of acids. This decrease in pH typically recovers as a result of salivary buffering. With a greater frequency of consumption, the enamel surface is subjected to longer periods of acidic pH resulting in a net demineralization over time.[2] Marsh demonstrated that the

frequency of carbohydrate intake is more harmful than total carbohydrate consumption.[25] Frequent consumption of sugary foods and drinks has been shown to decrease the pH below the critical level of 5.5 for 20 minutes.[26]

All dietary sugars diffuse into plaque and are converted to lactic acid, or stored as intracellular polysaccharides by residing bacteria. Sucrose, however, is unique in that it is the substrate for production of extracellular polysaccahrides (fructan and glucan) and matrix polysaccharides (mutan). This can lead to the colonization of microoganisms and increases the stickiness of plaque.[21]

Methods for White Spot Lesion Detection

The conventional method of detecting WSLs is visual, which is subjective. Objective methods for assessing smooth surface lesions have been introduced. They include the DIAGNOdent, transverse microradiography (TMR), hardness tests, polarizing light microscopy (PLM), scanning electron microscopy (SEM), and quantitative laser fluorescence.

Visual Analysis

Gorelick et al. first described a method for visually scoring white spot lesions in 1982.[1] This was modified by Øgaard in 1989, who scored the lesions as follows[4]:

Score 0= No white spot lesion

Score 1= White spot lesion covering less than one third of the surface

Score 2= White spot lesions covering more than one third of the surface

Score 3= White spot lesion with cavitation

Mizrahi also developed a method for classifying white spot lesions. The demineralization was scored for the labial and lingual surfaces of each tooth[3]. Lesions were scored as:

Score 0= No enamel opacity. An opacity of less than an estimated 1 mm in length or diameter was considered absent

Score 1= An opacity covering up to one-third of the surface area

Score 2=An opacity covering from one-third to two-thirds of the surface area

Score 4=An opacity covering from two-thirds to the full surface area

Although the above methods do not give any indication of mineral loss, they can be used to classify and report the prevalence of lesion occurrence. They provide quick and relatively easy ways to detect WSLs.

An attempt has also been made to visually classify the depth of a lesion. Ekstrand et al. performed a visual examination of cleaned occlusal surfaces of extracted teeth and demonstrated that changes on the occlusal surfaces were related to the depth of the lesions. Lesions were classified as follows: [27, 28]

Score 0= No or slight change in enamel translucency after prolonged air-drying (5 seconds)

Score 1= Opacity or discoloration hardly visible on the wet surfaces, but distinctly visible after air-draying

Score 2= Opacity or discoloration distinctly visible without air-drying

Score 3= Localized enamel breakdown in opaque or discolored enamel and/or grayish discoloration from the underlying dentin

Score 4= Cavitation in opaque or discolored enamel exposing dentin

While classification based on visual examination is a quick and easy method for identifying white spot lesions, it is a subjective approach that does not account for actual mineral loss or indicate the true depth of the existing lesion.

Photographic Analysis

Photographs have been utilized in a number of studies to detect the presence of white spot lesions.[1, 5, 6, 29-31] Chapman et al. determined that photographs are a reliable method of evaluating the presence of white spot lesions.[32] Based on the maxillary 8 anterior teeth of 10 patients (evaluated both clinically and photographically), they calculated an intraclass correlation of 0.88 between the two methods. Photographs are a convenient, but subjective, method for detecting white spot lesions in orthodontic patients because they are typically included in a patient's initial and final records.[32]

Detection of white spot lesions from photographs can be divided into two methods: scaled visual analysis and quantitative visual analysis. The original scaled visual analysis was developed by Gorelick et al.[1] and modified by Øgaard[4] as discussed above. It is based on a subjective assessment of the extent of demineralization. While this method does not account for the actual mineral loss or depth of the lesion, it allows for the classification of a white spot lesion. The second method of photographic analysis makes it possible to quantify the area of the lesion. Chapman et al. recently used this method to describe white spot lesions.[32] They used the photographs to determine the

percentage of facial surface of the tooth affected. Teeth with white spot lesions had their facial surfaces as well as the actual lesions outlined, and the areas were calculated with imaging software. The area of the white spot lesion was divided by the total area of the tooth to determine the percentage of facial surface affected. Huang et al. utilized a similar method to detect changes in white spot lesions after orthodontic treatment.[33] Instead of calculating the percentage of facial surface affected for each individual tooth, they calculated the total surface area of the 4 maxillary incisors affected. The total white spot area of the 4 maxillary incisors was divided by the total surface area of the 4 maxillary incisors, and an average of the 4 teeth was calculated. They were able to detect a change by subtracting the pre-treatment value from the post-treatment value.

When using photographs to detect the presence of white spot lesions, it is important to control for photographic conditions. Benson et al. conducted a study that evaluated the effect of camera angulation on the detection of white spot lesions.[34] The study concluded that photos that were taken within 20 degrees of perpendicular will have a slight reduction in the area of the white spot lesions being measured.

Transverse Microradiography

Transverse microradiography (TMR) is a way to measure the mineral loss and depth of a lesion of extracted teeth. TMR requires sectioning the specimen, obtaining a microradiograph of the sectioned specimen, and analyzing the image under a microscope with a special computer program to determine the mineral content. While this method is very accurate, it cannot be used *in vivo*. Also, the specimen is destroyed during the process, and it is a very time consuming process. [35]

Hardness Tests

Surface hardness tests have been used to quantifying dental caries in vitro using a microhardness tester. Indentations are made in the enamel samples with a Knoop diamond, usually with a load of 25 g applied for 10 s. The length of these indentations has been shown to reflect the degree of demineralization.[36] Again the specimen is destroyed during this process, and the method cannot be used in vivo.

Polarizing Light Microscopy

Polarized light microscopy is another method for hard tissue evaluation, especially enamel lesion depth. The specimen is sectioned into 100 µm slices and evaluated under a microscope, using polarized light transmitted onto the specimen. Since the mineral crystals of enamel exhibit an intrinsic birefringence, differences in crystal structures can be observed. This technique again destroys the specimen, and sectioning the specimens requires a great deal of preparation and manipulation.[37]

DIAGNOdent

DIAGNOdent was introduced in 1998, and is a laser fluorescence device, with a wavelength of 655 nm, used for the detection and quantification of both occlusal and smooth surface lesions.[38] The light is absorbed by both organic and inorganic material in the tooth and re-emitted as fluorescence within the infrared region. When decalcification is present, the fluorescence increases, and the change is observed as a higher number displayed on the instrument.[39]

Quantitative Laser/Light Fluorescence

Enamel fluorescence was first described by Benedict, and subsequently used to detect dental caries. [40, 41]Fluorescence results when the wavelength of the incident light rays is changed after it is been reflected from the surface of a material. [42]

In quantitative laser fluorescence (QLF), the tooth is illuminated by blue-green visible light from an argon-ion laser (488 nm), or by blue light from a 50-W xenon microdischarge arc lamp. The fluorescence of enamel occurs in the yellow region (about 540 nm for laser, and 520 for lamp) and is visualized through a yellow high-pass filter to exclude the tooth-scattered blue laser light.[42, 43] The device used a color CCD camera and frame grabber to capture these fluorescent filtered images. A custom-made software is used to collect, store and analyze the data.[42]

The teeth fluoresce naturally. Since areas demonstrating mineral loss fluoresce less, they appear darker in comparison to sound enamel. The analysis program detects these darker areas of the image and uses a reconstruction algorithm to simulate the fluorescence radiance of sound enamel at the lesion site. This is performed by a two-dimensional linear interpolation of sound enamel values adjacent to the lesion. The decrease in fluorescence (ΔF) is determined by calculating the percentage loss between actual and reconstructed fluorescence. The program also calculates the area of the lesion (mm^2) and the ΔQ, which is defined as the fluorescence radiance loss integrated over the lesion area. ΔQ is comparable to the total mineral loss from the lesions measured by transverse microradiography.[44]

Quantitative light fluorescence has been shown to be a valid method of early caries detection. Heinrich-Weltzien et al. completed an in vivo study on 34 adolescents comparing visual examination and quantitative light-fluorescence.[45] They determined that QLF is able to detect a larger number of initial caries, and that QLF is more sensitive at detecting lesions. Aljehani et al. compared QLF and DIAGNOdent readings taken on 41 extracted premolars to TMR and histopathology analyses.[38] A correlation analysis showed that the association between lesion depth and the QLF readings was slightly greater than with the DIAGNOdent, .82 versus .76. They also determined that QLF is a better method for evaluating mineral loss in a carious enamel lesion.

In vivo repeatability and reproducibility of QLF have also been tested. Tranaues et al. determined that the repeatability and reproducibility of QLF is excellent.[46] To investigate the image capturing stage of QLF, three investigators obtained images of 15 teeth with enamel lesions. Two of the investigators were highly experienced, while the third investigator was not. The inter-examiner correlations ranged between .95 and .98. To evaluate the analytical stage, the same three investigators evaluated the images of 15 incipient smooth surface enamel lesions. Intra-examiner reliability were again high, ranging between 0.93 and 0.99. Inter-examiner correlations ranged between 0.95 and 0.99.

Quantitative light fluorescence is the method of choice for evaluating white spot lesions because it allows for the evaluation of early enamel lesions without the destruction of the specimen. It provides a means for detecting and evaluating early

carious lesions prior to the need for restorative treatment, even before the lesions can be seen by the naked eye. Finally, it allows the lesions' activity to be monitored over time.

The Fluorecam® is a quantitative light fluorescence machine manufactured by Daraza Technologies. The Fluorecam® was designed to be a chairside detection instrument, using quantitative light fluorescence technology, which could detect and monitor early caries and monitor the reduction of lesion size and loss of fluorescence. This machine uses a high intensity light source with a filtered wavelength of 405 λ to induce fluorescence of the enamel matrix. A CCD camera captures the image, and then sends the image to a computer. These images are stored for future analysis.

Prevalence of White Spot Lesions in Orthodontic Patients

The incidence of decalcification following orthodontic banding and bonding ranges between 2-96%.[1-4, 6] In a cross-sectional study involving 796 patients, 527 pre-treatment and 269 post-treatment, Mizrahi reported the incidence of white spot lesions, based on clinical observation, in post-orthodontic patients, to be 84%.[3] These patients did not receive fluoride supplementation during treatment. While this study had a large sample size, this reported incidence may be misleading because 72% of pre-treatment patients also had white spot lesions. Øgaard also reported a very high prevalence (96%) of white spot lesions in patients receiving orthodontic treatment, but again, the untreated group also had a high prevalence (85%).[4] There must have been some local environmental factor(s) contributing to the increased prevalence in these two studies.

Gorelick et al. found that white spot lesions occur in 50% of patients undergoing orthodontic treatment and involve 10% of the teeth.[1] At least one white spot lesion was found in 12% of the patients in their control group. This study used clinical evaluations as well as visual examinations of photographs to determine whether white spot lesions were present. No fluoride treatment was provided other than that found in toothpaste. Lucchese and Gherlone evaluated patients in fixed appliances after 6 and 12 months in treatment using Gorelick's visual scale.[47] They found that after 6 months in treatment, 40% of patients had or developed at least one WSL. After 12 months, 43% of the active patients had developed at least one WSL. A control group was evaluated immediately before bracket placement, and 13% of these subjects presented with at least one white spot lesion initially. Chapman et al. utilized pre and post treatment photographs of 332 consecutively treated patients to determine the prevalence of white spot lesions on the maxillary 8 anterior teeth. In their study, 36% of patients developed at least one new white spot lesion during treatment.[32] In a similar study, Julien et al. compared pre and post treatment photographs of the maxillary and mandibular anterior 6 teeth of 885 orthodontic patients. In this study, 9% of the patients had pre-existing white spot lesions and 23% of the patients developed at least one white spot lesion during treatment.[6] Together, these studies indicate that, based on clinical or photographic assessments, approximately 11%-38% of orthodontic patients develop white spot lesions.

When using quantitative light fluorescence to evaluate white spot lesion development, van der Veen et al. found that of the 58 consecutively recruited patients who completed the study, 55 had developed lesions.[48] This study did not have a control

group and did not evaluate pre-treatment records, making it impossible to account for pre-existing white spot lesions. Boersma et al. used quantitative light induced fluorescence to evaluate the presence of white spot lesions in patients at debonding, with 97% of patients having a lesion.[49] Again, this study did not have a control group, so the results may have reported a higher incidence of white spot lesions than actually developed. There is a much higher incidence in white spot lesion development in studies utilizing QLF technology rather than visual/ photographic examination. Even though there is some discrepancy in the reports of white spot lesion development, it is obvious that white spot lesions present a significant problem to clinical orthodontists.

The teeth most likely to develop white spot lesions are the maxillary laterals, and the mandibular molars, canines and premolars.[4] Lucchese and Gherlone evaluated three groups of patients, one group that had been in fixed orthodontic appliances for 6 months, one group that had been in treatment for 12 months and the third group that served as the controls. Based on visual evaluations, the most common site for white spot lesion development was the mandibular first molars (30% of patients), followed closely by the maxillary laterals (29% of patients).[47] More recently, Julien et al. found the maxillary laterals and canines and mandibular canines to be most susceptible to white spot lesions development.[6] Based on QLF, the canines and lateral incisors were most likely to develop white spot lesions in the maxilla, while the canines and first premolars were more likely to develop white spot lesions in the mandible.[49]

White spot lesions have been shown to develop as early as 4 weeks after fixed appliances are bonded.[7, 8] In a study conducted by Øgaard et al., bands were attached to

10 premolars predestined for extraction in 5 patients. Microradiography was utilized for lesion detection. [7] After four weeks, visible lesions were detected on most teeth with the average lesions depth of 101 μm. O'Reilly and Featherstone also found that significant demineralization can occur on teeth with orthodontic appliances after 4 weeks. This study placed brackets on 58 premolars that were scheduled for extraction for orthodontic purposes. After 4 weeks, the premolars were extracted and evaluated with microhardness tests. Teeth in the control group (teeth that did not receive any fluoride treatment) demonstrated 14% mineral loss in the cervical region of the tooth.[8] As mentioned above, Lucchese and Gherlone found lesions in 40% of patients after 6 months in treatment using visual examination.[47]

White Spot Lesion Resolution

Treatment of white spot lesions following orthodontic treatment depends on the severity and location of the lesion as well as on the esthetic goals of the patient. Natural resolution of white spot lesions is contingent on the severity of the lesion, with small lesions often remineralizing with good oral hygiene.[50] Different remineralization and camouflage methods have been utilized in an attempt to reverse the appearance of white spot lesions. More severe and cavitated lesions require restorative care, ranging from composite bonding to porcelain veneers restorations.

Remineralization

As mentioned above, there is a constant flux between demineralization and remineralization in the oral cavity. The minerals necessary for hydroxyapatite formation are present in the saliva. Remineralization of small lesions may occur in the presence of good oral hygiene. In a study completed by Backer-Dirks, 72 WSLs were identified in a group of 9 year olds. [51] These children were followed for six years. Upon re-examination, 37 lesions (50%) had resolved/ remineralized. Remineralization varies among subjects and locations within the mouth. [52] Studies have shown that, on average lesions remineralize 20-30% over 2 weeks (measured as a percentage mineral change). On the other hand in some cases, the amount of remineralization cannot overcome the amount of demineralization which occurred, resulting in a permanent scar. Following the removal of orthodontic appliances, some regression of white spot lesions occurs provided that other etiological factors are favorable.[53] Willmot completed a study evaluating white spot lesions in 9 patients who had undergone orthodontic treatment.[53] The average size of the lesions at debond was 2.72 mm^2 (±1.72), decreasing to 1.30 mm^2 (±3.40) after 26 weeks. In most of the cases, majority of the reduction in size occurred during the first 12 weeks after the removal of appliances, with little further reduction occurring in cases that were followed for more than 26 weeks. Lesion size reduced approximately by a third after 12 weeks, and by a half after 26 weeks. Van der Veen et al. confirmed that white spot lesions will regress after the termination of orthodontic treatment.[48] Their study utilized light-induced fluorescence to visualize white spot lesions, and found that after six weeks after bracket removal,small lesions show rapid

improvements and further improvements were observed after six months. [48] White spot lesions will naturally reduce in size following the cessation of orthodontic treatment with no further intervention.

While lesions may naturally decrease in severity following bracket removal, complete resolution of these lesions does not usually occur. Clinicians have investigated other products, including fluoride and casein phosphopeptide amorphous calcium phosphate (CPP-ACP), to aid in the remineralization process and reverse the development of these lesions.

Studies have been completed evaluating fluoride's ability to reverse the demineralization process and remove white spot lesions following orthodontic treatment. [50] While concentrated fluoride agents have been shown to be effective in arresting the carious process and preventing further lesion progression, Øgaard warned against using highly concentrated fluorides due to the formation of surface hypermineralization. [50] Hypermineralization arrests both the demineralization and remineralization process. When large doses of fluoride are used, the size of the lesion remains unchanged, and the lesion can become stained with organic debris.[10] Hypermineralization results from the blockage of enamel's diffusion pathways by hydroxyapatite crystals. [8] To avoid occluding the surface layer, applications of low-dose fluorides have been recommended to aid in subsurface remineralizaiton.[54, 55] Lagerweij et al. determined that lesions less than 60 µm deep can be remineralized with low dose fluorides (1 ppm or less). [54] Some studies have shown that a 50 ppm fluoride mouth rinse results in greater amounts of remineralization than a control solution, or a regular mouth rinse containing 250 ppm, [54,

[55] but other have concluded that this difference in remineralization is not clinically significant. [53]

Casein phosophopeptide amorphous calcium phosphate (CPP-ACP) has also been explored as a means of remineralization. [18, 56]CPP-ACP aids in the attachment of calcium and phosphate ions to enamel and the formation of calcium phosphate crystals. The free calcium and phosphate ions diffuse from CPP-ACP and into the enamel rods where they reform as apatite crystals. [18] Different methods have been developed to deliver the CPP-ACP; these include: a paste, a water-based mousse, a topical cream, chewing gum and mouth rinses, and sugar-free lozenges. It is present in products under the label "Recaldent" and MI Paste Plus. Bailey et al. found that the use of a cream containing CPP-ACP was effective in reducing the severity of white spot lesions in post-orthodontic patients.[56] Another recent study showed that CPP-ACP in MI Paste Plus was able to prevent white spot lesion formation as well as reduce the number of lesions present. The paste was delivered through prefabricated bleaching trays, once daily at night. [57]

Restoration of White Spot Lesions

A conservative treatment approach should be utilized within the first 6 months after bracket removal when addressing white spot lesions because it has been shown that these lesions can spontaneously improve. Other means of improving the esthetics of the lesion can be utilized if lesions are still present after 6 months.

Microabrasion has been utilized to decrease the severity of white spot lesions appearance.[58, 59] Microabrasion is performed using 18% hydrochloric acid and pumice,[58]

and has been used to remove superficial noncarious enamel defects, including mottling, opacities, pigmentation, and fluorosis.[60-62] Murphy et al. performed a study that quantified the changes in post-orthodontic white spot lesion surfaces areas after microabrasion.[63] Their study showed an 83 percent reduction in white spot lesions. It was determined that microabrasion is effective in improving the esthetics of white spot lesions.

Other methods which have been used to camouflage white spot lesions include external bleaching and resin infiltration. Knosel et al. conducted a study to evaluate the effect of external bleaching on the color and luminosity of inactive white spot lesions, and determined this method is effective in camouflaging white spot lesions.[64] It should be noted that the susceptibility to the formation of carious lesions after bleaching increases, so bleaching should be restricted to patients with excellent oral hygiene.[65] Resin infiltration has recently been developed to mask the appearance of white spot lesions. This method involves penetrating a low-viscosity resin into the enamel to obstruct diffusion of acid and strengthen the enamel. Kim et al. found that this method completely masked the appearance of WSLs in 61% of affected teeth and decreased their appearance in 33%.[66]

While there has been evidence to suggest that the appearance of relatively small white spot lesions can be improved by multiple methods, larger white spot lesions must be addressed by other means. These lesions must be restored by a dentist, which can be expensive and requires many years of upkeep. The best way to decrease white spot lesions is to prevent their development.

Preventive Measures

Patient education, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), sealant application, and fluoride administration have all been introduced as methods to prevent WSL formation.[22, 67]

Patient Education and Oral Hygiene

Geiger et al. showed that professional oral hygiene instruction and regular professional cleanings are effective methods of reducing enamel decalcification.[68] Zimmer and Rottwinkel found that an extended prophylaxis program with a hygienist performing cleanings and patients receiving a chlorhexidine rinse lead to a statistically significant reduction in decalcification frequency in patients with a high caries risk.[69] Obtaining professional cleanings is expensive and is an added cost to orthodontic treatment.

At home oral hygiene programs rely on patient compliance. It has been shown that patients with poor compliance with fixed appliances are at a greater risk for enamel decalcification.[67] Verbal praise and re-education of the patient on the consequences of poor oral hygiene compliance have been found to be effective methods of improving patient cooperation.[69] Lovrov et al., who evaluated patient compliance with monthly exams questionnaires asking them about their oral hygiene habits, showed that a vigorous oral hygiene regimen and weekly use of a prescribed fluoride gel were effective in decreasing WSLs over 12-18 months.[70]

Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP)

Casein phosphopeptide is milk derived protein. CPP-ACP, complexes have been shown to restrict the caries process and limit demineralization by stabilizing the calcium and phosphate ions. Calcium and phosphate salts have a low solubility, so solutions containing calcium and phosphate ions alone have little effect on remineralization.[18, 71] Robertson et al. recruited 60 orthodontic patients to apply MI Paste plus, which contains CPP-ACP, every night after brushing their teeth for 3 months.[57] Sixty patients were randomly divided into a treatment group (MI paste plus) and a placebo group. The paste was delivered in a prefabricated fluoride varnish tray every day for 12 weeks. The patients were asked to apply the paste for 3 to 5 minutes every night after brushing their teeth and were instructed to expectorate and not rinse, eat, or drink anything after applying the paste. The patients were evaluated at the initiation of the study, as well as at 4 weeks, 8 weeks, and 12 weeks. At these time points, photographs of the 8 anterior teeth in both the mandible and maxilla were taken and the teeth were scored according to the enamel decalcification index described by Banks and Richmond.[72] This study showed a 53.5% decrease in enamel decalcification over a 12 week period. Importantly, this study did not differentiate between white spot lesions associated with orthodontic treatment and those that can be caused from developmental reasons and fluorosis. White spot lesions that occur during orthodontic treatment typically occur in the gingival third of the tooth. The greatest change in the demineralization index score in patients receiving MI paste was in the incisal third, which would not be expected for white spot lesions caused by orthodontic treatment.

Sealants

Sealants have been used increasingly to reduce the incidence of WSLs. Benham et al. found that the application of a protective sealant at the gingival region of anterior teeth produced a significant reductions in WSLs during fixed orthodontic treatment.[73] Sixty patients participated in their split mouth study. A highly filled sealant was randomly applied to the anterior teeth on either the right or left sides in both the maxilla and mandible; the contralateral side received no sealant treatment. This study evaluated pre- and post-treatment photographs to determine the presence of white spot lesions. Only 6 of the 60 patients showed visual signs of white spot lesion development. Teeth without sealants had 3.8 times the number of WSLs than teeth that were sealed.

Heinig and Hartmann also reported a significant decrease in decalcification of enamel in orthodontic patients who received full coverage sealants prior to bracket placement.[74] In their study 78 patients participated, 38 received no sealant and 40 received a sealant. The two groups were similar in treatment duration, oral hygiene, age, gender and fluoride application during treatment. Intraoral examinations and photographs were used to assess the development of white spot lesions following the removal of orthodontic appliances. Of the patients who received no sealant treatment, 10% presented with white spot lesions, compared to 5% in the sealed group. Using the grading system adapted from Gorelick and Øgaard, [1, 4] the non-sealed teeth had more severe white spot lesions.

The problem with sealants is that they undergo mechanical wear and erode over time. Another problem with utilizing sealants to prevent white spot lesions is that as a

teeth erupt and extrude over time, enamel that was below the gingiva and not covered with sealant becomes exposed to the oral environment. Such sites could develop white spot lesions unless sealants are re-applied throughout treatment.

Fluoride

Fluoride administration has perhaps been the most thoroughly investigated means of preventing enamel demineralization. When enamel is exposed to ionic fluoride, it may be taken up to form fluorhydroxyapatite or calcium fluoride. Fluorhydoroxyapatite is formed when the fluoride concentration is low (<50 ppm) and an acidic environment is present. It is integrated into the outer layer of enamel. Hydroxyapatite in enamel is broken down when the pH drops below 5.5. If the pH remains above 4.5 and fluoride is available, fluorhydroxyaptite may form in the surface layers of enamel while hydroxyapatite dissolves in the subsurface enamel, reducing demineralization. Once the pH drops below 4.5, the surrounding environment is undersaturated of both hydroxyapatite and fluorapatite and no remineralization will occur.[75] Calcium fluoride is formed when the fluoride concentration is greater than 100 ppm. The greater the fluoride concentration, the more calcium fluoride formed. At a low pH, the solubility of enamel increases, providing calcium for calcium fluoride formation. Therefore, acidulated fluoride gels provide more calcium fluoride to the enamel over a shorter period of time than NaF gels.[11] Saxegaard and Rolla also found that decreasing the pH of the fluoride solution, increasing the fluoride concentration, prolonging exposure times, and etching the enamel surface would lead to an increase in calcium fluoride formation. [76] Larsen and Jensen found that *in vitro,* calcium fluoride is only formed at a fluoride

concentration of at least 300 ppm when the solution is at a neutral pH, but when the pH is decreased to fide calcium fluoride forms at 100 ppm. [77] When fluoride is applied, a calcium fluoride builds up in plaque, on the tooth surface, or in incipient lesions. The cariostatic effect results from absorption of phosphate ions and protein molecules onto the calcium fluoride. This may serve as a pH controlled reservoir of fluoride, available for remineralization or inhibiting demineralization during a carious attack.[50, 78]

Several studies have found incorporating fluorides into the mineral components of the enamel only slightly reduces enamel's solubility .[78-80] Øgaard et al. compared the resistance of shark enamel, which almost completely consists of pure fluorapatite, and human enamel to caries formation. Six patients wore a removable appliance (Hawley retainer) with both human enamel and shark enamel specimens attached for four weeks.[81] Microradiography was used to determine mineral loss. It was shown that human enamel had more mineral loss than shark enamel, but when the human enamel was rinsed daily with .2% sodium fluoride the shark enamel had more mineral loss than human enamel. It was concluded that free fluoride ions in solution around the tooth play a more important role in caries prevention than fluorides incorporated in the enamel crystals themselves.

Fluoride treatments utilized for caries prevention include: water fluoridation; fluoride toothpastes, mouth rinses and gels; fluoride varnishes; and fluoride in orthodontic bonding agents.[67] Caries levels in communities with fluoridated water have been shown to be reduced by approximately 50 percent when compared to communities without water fluoridation.[82] While most communities in the United States have

fluoridated water, white spot lesions continue to be a nuisance to practicing orthodontists.

In populations with and without fluoridated water, topical fluorides used with fluoride toothpastes appear to decrease the incidence of decalcification in orthodontic patients.[83] One form of topical fluoride utilized has been a sodium fluoride rinse. Geiger et al. completed a study evaluating the effectiveness of .05% sodium fluoride rinse in reducing the number of white spot lesions developed during orthodontic treatment.[84] Two hundred and six patients were given sodium fluoride rinse and instructed to rinse daily with 10 ml before retiring and immediately after brushing. Participants were instructed to repeat this protocol for the length of their treatment. Upon debonding, white spot lesions were detected during a clinical evaluation. This study did not account for the white spot lesions that existed prior to orthodontic treatment, making it impossible to determine the lesions that developed as a result of treatment. Only 13% of patients fully complied with the instructions provided, while 42% reported rinsing with the fluoride every other day. All of these patients were categorized as compliant. The remaining 45% of patients reported rinsing less that once every other day, and were identified as non-compliers. The compliant patients had significantly fewer lesions than the non-compliers (21% compared to 49%). This showed that while fluoride rinses can reduce the number of white spot lesions that develop during orthodontics, the majority of orthodontic patients will not comply with the instructions provided.

Historically, stannous fluoride gels and solutions containing 8 or 10% fluoride have been used to prevent caries formation. They have been shown to decrease plaque's

ability to form and produce an acid byproduct due to the stannous ion rather than the fluoride ion. However, stannous fluoride has a poor taste and can result in staining of the teeth, which limits its use.[85]

Another option for professionally applied topical fluoride is a varnish containing 5 percent NaF. This is equivalent to 2.26 percent or 22,600 ppm fluoride ion.[86] Importantly, this method does not require patient compliance. Varnishes have been created to prolong the contact time between fluoride and enamel.[85] Todd et al. demonstrated that fluoride varnish can decrease the amount of demineralization adjacent to orthodontic brackets.[87] In their study, 36 extracted canines and premolars with bonded orthodontic brackets were divided into 3 groups: a control group with no topical fluoride application, a placebo group that received a nonfluoridated placebo varnish, and a group that received a single treatment with fluoride varnish. Each group was presented a carious challenge for 1 hour, 2 times a day, for 37 days, and tooth brush simulation was used to produce the mechanical cleaning. Polarized light microscopy was used to determine the average depth and area of demineralization present on each tooth. The depth and area of the lesions was greatest in the placebo group, followed closely by the control group, and finally the varnish group, which demonstrated the smallest and shallowest lesions. The varnish group demonstrated approximately 50% less demineralization than the control group. Allergic reactions to fluoride varnishes remain a concern; eczema of the hand, contact stomatitis, and edema of the tongue, soft palate, and upper lip have all been reported. [88]

Fluoride releasing bonding agents were developed in an attempt to create a compliance-free topical fluoride, and have been shown to decrease the number of white spot lesions by 16.5%.[67] Marcusson et al. concluded that, with the use of fluoride releasing glass ionomer cement, the number of white spot lesions developed during orthodontic treatment with full fixed appliances was decreased by 16 percent.[29]

Acidulated Phosphate Fluoride

Acidulated phosphate fluoride (APF) was introduced in the early 1960s by Brudevold et al .[89] Prior to that time, it was believed that neutral fluoride was more effective at reducing caries than fluoride solutions with acetic acid. Their study demonstrated that fluoride is readily available for uptake from acidic phosphate solutions. These results were confirmed by Saxegaard and Rolla, who reported that calcium fluoride could be significantly increased by lowering the pH of the fluoride solution.[76] As previously mentioned, the solubility of enamel increases at a low pH, providing calcium for calcium fluoride formation. Therefore, acidulated fluoride gels provide more calcium fluoride in the enamel over a shorter period of time than NaF gels.[11] APF solutions contain 1.23% (12,300 pppm) fluoride ion. The pH is approximately 3.0. At such a low pH, more than 50 percent of the fluoride will be in the form of hydrogen fluoride rather than free fluoride ions.[11] Phosphate is added to the solution to depress calcium fluoride formation and increase fluorapatite formation.[85]

Numerous studies have been performed to determine the most effective method for applying acidulated phosphate fluoride. It has been determined that the efficacy of APF gel on the reduction of caries formation varies according to the risk of the patient,

with high risk patients showing the poorest results.[90] Wiegand et al. determined that acidulated fluoride gel's ability to protect demineralized enamel against subsequent demineralization increased with increasing concentration (up to 1.25%) of the applied gels.[91] One enamel specimen from each tooth was assigned to each of the four experimental groups, and one specimen from each tooth was used as a control to determine the baseline fluoride content. Each of the experimental enamel specimens were covered with 1 mL fluoride gels of different concentrations depending on the experimental group: group A, 1.25% sodium fluoride, Group B, .62% sodium fluoride, group C, .31% sodium fluoride, and group D, .15% sodium fluoride. After 5 minutes, the gels were removed from the enamel surfaces and the teeth were stored in artificial saliva. This study showed that the greater the fluoride concentration of the applied gel, the greater the uptake of fluoride in the enamel surface.

Another study evaluated whether the length of time that fluoride is applied affects its ability to reduce the number of carious lesions. Garcia-Godoy et al. compared the effects of 1 minute APF and 4 minute APF treatment on the development of carious lesions.[92] Ten extracted human molars were quartered and divided between the treatment groups. The distobuccal and distolingual quarters 1 minute of APF treatment, while the mesiobuccal and mesolingual quarters received 4 minute of APF treatment. Paired controls were created by applying an acid-resistant varnish to windows of enamel on the buccal and lingual surfaces. Carious lesions were then simulated on each surface using an acidified gel. Polarized light microscopy was utilized to evaluate lesion depth. No statistical difference was found between 1 and 4 minutes of APF treatments, but

statistical differences were found between the treatment groups versus control. This study concluded that 1 minute APF treatment provided the same degree of caries protection as the 4 minute treatment.

Additional studies have compared acidulated phosphate gel application with foam application. Whitford et al. conducted a study on 46 patients 8-12 years old to compare the fluoride uptake when using acidulated phosphate fluoride gel or foam.[93] Prior to fluoride application, a saliva sample was obtained and an enamel biopsy was completed by placing perchloric acid on the enamel of a central incisor using a fixed volume pipettor. After 15 seconds, the acid was removed by drawing it back into the plastic tip and placed in a beaker contained 50 microliters of Total Ionic Strength Adjustment Buffer. The biopsy solution was analyzed for fluoride and calcium. Fluoride was then administered; half of the patients received the gel treatment first. Another saliva sample was completed immediately following fluoride administration. Fifteen minutes after the fluoride tray was removed from the mouth, another acid etch enamel biopsy was completed. The patients returned to the clinic two weeks after the initial fluoride application. At this appointment an enamel biopsy was completed, and the study was repeated using the opposite fluoride (gel/foam). It was found that significantly less fluoride was retained by the patient with the foam application. The differences in enamel fluoride uptake at 15 minutes and two weeks were not significantly different.

Acidulated phosphate fluoride has also been compared to other fluoride treatments. It has been demonstrated that following 24 hours of fluoride varnish application, extracted teeth will have significantly more fluoride uptake. [94] The same

study showed that when the fluoride was removed after 1 hour (closer to a clinical setting) there was no difference in enamel fluoride concentration between APF and fluoride varnish. Lee et al. evaluated demineralization of 48 bovine enamel samples. These specimens were mounted on a mandibular removable acrylic appliance and worn by six patients (eight specimens per appliance) for a total of four weeks.[95] The enamel samples were divided into four groups: a control group, iontophoresis with 2% sodium fluoride varnish, 1.23% acidulated phosphate fluoride gel, and 5% sodium fluoride varnish. Microhardness of enamel surfaces was used to determine the amount of demineralization present at the end of the study, and the fluoride concentration was analyzed using a fluoride electrode. No significant differences in microhardness were observed between the 3 fluoride regimens. APF showed the greatest fluoride uptake in enamel, but the increase was not significantly different than the fluoride varnish.

Only two clinical studies have been conducted to evaluate the effectiveness of 1.23% APF application in reducing enamel demineralization during orthodontic treatment. O'Reilly and Featherstone completed a study on 20 orthodontic patients scheduled to have premolars extracted to evaluate the amount of demineralization that occurred following fluoride treatment.[8] The patients were randomly divided into 4 groups: a control group (18 teeth) that just bushed with fluoride toothpaste, a group that rinsed with .05% sodium fluoride each night (16 teeth), a group that received weekly 1.2% APF treatment (10 teeth), and a group that received the APF treatment weekly and rinsed with .05% sodium fluoride (14 teeth). All premolars were extracted after 1 month of treatment. Microhardness tests were completed on sections of the extracted teeth. This

study found that teeth treated with 1.2% APF gel once a week for a month (five treatments), even in the presence of orthodontic brackets had a normal enamel profile with hypermineralization in the outer layer of enamel to a depth of 25 micrometers. This indicated rehardening of enamel or inhibition of demineralization. While this study was conducted in a clinical setting, orthodontic patients are not typically seen on a weekly basis. More research is required to evaluate the extent of enamel demineralization with fluoride treatment applied at regular orthodontic appointments.

More recently, Jiang et al. completed a study evaluating acidulated phosphate fluoride foam's ability to decrease the incidence and severity of white spot lesions.[96] In this double blind, randomized controlled trial, 50 patients were in the placebo group, while another 50 patients were in the treatment group. White spot lesion assessments conducted by clinical assessments immediately before brackets were bonded and immediately after debonding. White spot lesions were scored according to the method described by Gorelick. [1] Patients received gel treatments once every two months until debonding. The mean orthodontic treatment time was approximately 18 months. In this study, the incidence of white spot lesion development was significantly different ($p<.001$) between the treatment and control group: 12.5% in the fluoride group and 51.1% in the placebo group. When using Gorelick's white spot lesion assessment, the placebo group also had more severe white spot lesions. This present study will evaluate the effect of 1.23% APF on white spot lesion formation using visualization and QLF readings to determine the incidence and severity of demineralization utilizing a typical orthodontic treatment timeline. The null hypothesis for this study is that there will be no

difference in the incidence or severity of lesions between teeth treated with APF and untreated controls.

CHAPTER II

BACKGROUND

White spot lesions (WSLs)/enamel decalcifications are a persistent problem to clinical orthodontists. These lesions develop in areas surrounding orthodontic appliances and often remain after treatment is completed, forming permanent scars. They compromise orthodontic esthetic results, as well as the health of the dentition. White spot lesions result from the accumulation of plaque and bacteria on the enamel surface, which in turn results in an increase in acidic by-products and demineralization of the enamel. [1] The overall prevalence of WSLs in orthodontic patients has been reported to range between 2-96%, depending on the method of detection.[1-5] In a recent study evaluating 885 orthodontic patients, Julien et al. determined that WSLs developed in 23% of the patients.[6] While enamel lesions have been reported to occur on all teeth; the maxillary laterals and mandibular canines and premolars are most affected by these permanent scars.[4] Øgaard et al. found that white spot lesions can occur as early as four weeks. [7]

Many methods have been utilized to prevent white spot lesions that develop during orthodontic treatment. These include, but are not limited to, filled resin-sealants, glass ionomer cements, fluoride-releasing cements, fluoride varnishes, fluoride rinses, and fluoride gels. [2] Excellent oral hygiene can prevent white spot lesions from developing, but this is complicated by the presence of orthodontic appliances that cause

increased plaque retention. Also, most orthodontic patients are adolescents, who often have poor oral hygiene.

Acidulated phosphate fluoride (APF) was introduced as a prevention agent in the early 1960s.[89] While it was originally believed that neutral fluoride was most effective at reducing caries, Brudevold and coworkers demonstrated that fluoride is more readily available for uptake from acidic phosphate solutions.[89] Saxegaard and Rolla confirmed that calcium fluoride could be significantly increased by lowering the pH of the fluoride solution.[76] The solubility of enamel increases at a low pH, providing calcium for calcium fluoride formation. As such, acidulated fluoride gels provide more calcium fluoride in the enamel over a shorter period of time than NaF gels.[11] APF solutions contain 1.23% (12,300 pppm) fluoride ion. The pH is approximately 3.0. At such a low pH, more than 50 percent of the fluoride will be in the form of hydrogen fluoride rather than free fluoride ions.[11] Phosphate is added to the solution to depress calcium fluoride formation and increase fluorapatite formation.[85]

Only a couple of clinical studies have evaluated the effectiveness of 1.23% APF application in reducing enamel demineralization during orthodontic treatment. O'Reilly and Featherstone, found that 1.2% acidulated phosphate fluoride gel applied once a week for a month decreased demineralization in patients undergoing active orthodontic treatment. [8] While their study was conducted in a clinical setting, orthodontic patients are not typically seen on a weekly basis. More recently, Jiang et al. studied 100 orthodontic patients who received either 1.23% acidulated phosphate foam or a placebo treatment every two months for an average of 18 months.[96] They showed that there was

a 76 percent reduction of WSLs incidence in the 1.23% acidulated phosphate fluoride foam group. More research is required to quantify the extent of enamel demineralization with fluoride treatment applied at regular orthodontic appointments.

The purpose of the present study was to evaluate the effect of 1.23% acidulated phosphate fluoride gel on the incidence and severity of white spot lesion development.

CHAPTER III

MATERIALS AND METHODS

In this double-blind, randomized, placebo controlled trial, 55 patients starting orthodontic treatment, either at Texas A&M University, Baylor College of Dentistry or at a private practice, were selected based on the following criteria: under the age of 18; in the permanent dentition; having a treatment plan that included bonded orthodontic brackets, no significant medical problems; no hypersensitivity to fluoride; no current fluorosis; no active carious lesions. Before treatment, written informed consent to participate in the study was obtained from each patient and their legal guardian; the study was approved by the Institutional Review Board at Texas A&M University, Baylor College of Dentistry (Table 1).

Neither the patient nor doctor was aware of the treatments that were administered. Patients were randomly divided into two groups (A or B) using Excel®(Microsoft, Bellview). The treatment group received an application of Nupro 1.23% APF fluoride gel (Dentsply®), while the placebo group received a gel treatment that was exactly the same composition but lacked fluoride. Each product was packaged the same; only the A/B labels were different.

At the initial bonding appointment, rubber cup prophies were performed prior to the preparation of the teeth for bracket placement. The teeth evaluated included the maxillary lateral incisors, mandibular canines and first premolars, which are the teeth most likely to develop white spot lesions.[4] Prior to the photographs, the teeth were

isolated with cheek retractors and dried for 7 seconds. Standardized photographs of each tooth were taken with the camera held perpendicular to the facial surface, approximately .32 meters from the tooth. Initial Fluorecam® (Daraza, Indianapolis)(quantitative light fluorescence) readings were also captured. The Fluorecam® unit had a positioning bar to ensure that subsequent photos of the same tooth are taken from a similar angle.

Next, the teeth to be bonded were isolated and prepared using 3M/Unitek's self-etching primer on the labial surface, followed by bracket placement. The brackets were bonded by Transbond XT (3M/ Unitek). Once all brackets had been cured and the archwire had been placed, oral hygiene instructions were provided. The patients were instructed to brush their teeth for two minutes at least three times per day. While cleaning their teeth, the patients were instructed to brush each surface of a tooth: the buccal/labial, occlusal/incisal, and lingual. When brushing the buccal/labial surface of the tooth, the patient was instructed to angle the brush at 45 degrees, both below and above, the archwire to ensure that the area beneath the wire was cleaned. Each patient was required to use fluoridated toothpaste throughout the experimental time period.

Following the oral hygiene instruction, the treatment was administered. During gel administration, a Nupro Styrofoam tray was filled with the gel treatment and placed in the patient's mouth for one minute. Slow speed suction was utilized to remove excess saliva. Following the gel application, the patient was allowed to expectorate for 30 seconds and instructed not to eat or drink anything, including water, for a minimum of 30 minutes.

The study included two groups of patients because not all of the patients were able to receive their maxillary and mandibular braces at a single appointment. One group (5 experimental and 12 placebo) either had both the maxillary and mandibular brackets bonded at the initial appointment or had one set of brackets bonded at the initial appointment and their second set of brackets were not bonded within two months of the initial. These patients were followed for six months and received treatments at the initial bonding appointment, two months and four months following bracket placement (Figure 1). After six months, final photographs and Fluorecam readings were obtained. The second group (19 experimental and 19 placebo) had their first set of brackets bonded at the initial records appointment. Their second set of brackets were bonded within two months of the first set. Patients in this group were followed for eight months, and gel treatments were applied at the initial appointment, two months, four months, and six months following bracket placement (Figure 1). At the completion of eight months, final photographs and Fluorecam® readings were obtained of the teeth of interest.

At the final records appointment, the brackets and cement were removed from the research teeth, and final photographs and Fluorecam® readings were obtained. The brackets were replaced and orthodontic treatment continued as dictated by the initial treatment plan.

Analysis

Pre- and post-treatment photographs were evaluated by a single blinded investigator for the presence of white spot lesions. Each photograph was given an Øgaard score (Figure 2).[4] A score of 0 was given if no lesion was present; a score of one was given if a lesion was present and covered less than one-third of the tooth; a score of two was given to lesions that were present and covered more than one-third of the tooth; a score of three was assigned to cavitated lesions. Fifteen patients were randomly selected to be reevaluated a second time for reliability measurements. There were no significant systematic errors, the intraclass correlation between replicate readings was 0.937, and the method error was 1.208.

The amount of demineralization was also assessed based on pre- and post-treatment Fluorecam® readings performed by the same blinded investigator. When the tooth is illuminated by visible light within the blue-green region, the teeth naturally fluoresce. Areas demonstrating mineral loss fluoresce less, and appear darker in comparison to sound enamel (Figure 3). These darker areas are outlined by the Fluorecam® program, and the size (mm^2), intensity (the change in fluorescence between sound and demineralized enamel) and impact (intensity x size) of demineralization were calculated. Reliability was based on replicate readings taken at both the pre-treatment and post-treatment appointments (Table 2).

Statistics

SPSS version 19 (SPSS Inc.; Chicago, IL) was used to analyze the data (at a significance level of $p<0.05$). Chi square tests were used to determine 1) group differences in the initial Øgaard scores, 2) the presence of a significant difference between the treatment and placebo groups in new white spot lesion development per tooth when using both photographic and QLF evaluation, 3) the presence of a significant difference in the number of new white spot lesions developed by patients in the treatment and placebo groups using photographic and QLF evaluation, 4) the presence of a significant change in pre-existing white spot lesions between the treatment and placebo groups using photographic evaluation. Mann-Whitney tests were used to detect a difference in severity of white spot lesions between the treatment and placebo groups.

CHAPTER IV

RESULTS

Photographic Analysis

There were no significant differences in the pretreatment Øgaard scores between the treatment and placebo groups (Table 3). Based on the photographic assessments, 37.5% of patients in the fluoride group and 32.3% of patients in the placebo group developed new white spot lesions (Figure 4), a difference that was not statistically significant (p=.685). There also were no statistically significant differences between the treatment and placebo groups in the number of new white spot lesions that developed on each tooth during treatment (Table 4), or in the number of new white spot lesions that each patient developed (p= .701)(Table 5). The treatment group developed 21 new white spot lesions, while the placebo group developed 17 new white spot lesions. Two patients in the treatment group developed nine new white spot lesions; they accounted for 42.9% of the white spot lesions that developed in the treatment group.

Five patients had pre-existing white spot lesions (1 patient in the treatment group and 4 patients in the placebo group) (Table 2). In the treatment group, none of the pre-existing white spot lesions became more severe (Table 6). In the placebo group, 3.2% of the patients demonstrated worsening of two pre-existing white spot lesions. These differences were not statistically significant (p=.375).

Finally, there were no significant differences in the severity (Øgaard scores) of the white spot lesions between the treatment and placebo groups before or after treatment (Table 7). While the treatment group showed greater increases in the Øgaard scores during treatment, the group differences were small and not statistically significant.

Fluorecam Analysis

Based on the Fluorecam® assessments, 37.5% of patients in the fluoride group developed new white spot lesions, while 58.1% of patients in the placebo group developed new white spot lesions (Figure 5). The difference was not statistically significant (p=.13). Similarly, there were no significant group differences in the development of new white spot lesions on any of the teeth (Table 8). While the patients in the placebo group developed a greater number of white spot lesions than the patients in the treatment group, the difference was not statistically significant (p=.63) (Table 9).

When comparing the size, intensity and impact of demineralization, the only measurement demonstrating a significant difference between the fluoride and placebo group was the intensity of the lower left first premolar (Figure 8). The treatment group showed an improvement (0.38) in the intensity, while the placebo group showed a worsening (-1.45). The size of the lesions in the fluoride group increased less than the placebo group or actually decreased, with the exception being the mandibular right canine (Figure 7). The maxillary right lateral incisor and the mandibular first premolars

showed less of a decrease or an actual improvement in both the intensity and impact (Figures 6 and 8). The maxillary left lateral incisor and the mandibular left canine demonstrated greater worsening of the intensity in the treatment group, but their impacts did not decrease as much as the placebo group (Figure 6 and 7). Finally, the intensity and impact of the lower left canine decreased more in the treatment group than the placebo (Figure 7).

Lesion Detection: Photographic vs. Fluorecam Analysis

The two methods agreed that 40 percent of the teeth did not develop white spot lesions (Table 10). Lesions were detected by both methods in 23.6 percent of the teeth. Photographic analysis alone detected lesions on 10.9 percent of the teeth, while 25.5 percent of the teeth developed lesions that were only visible with the Fluorecam®.

CHAPTER V

DISCUSSION

Multiple studies have shown that approximately one-third of patients receiving orthodontic treatment develop visible white spot lesions. In the present study, 32.25% of patients in the placebo group developed visible white spot lesions. In 2012, Lucchese and Gherlone, using intraoral examinations of each tooth, determined that 40% of patients had at least one visible white spot lesion after 6 months of orthodontic treatment.[47] A recent large scale study reported a prevalence of 23%; white spot lesions were detected by comparing pre- and post-treatment anterior intraoral photographs.[6] Because pre- and post-treatment anterior intraoral photographs were used to detect white spot lesion development rather than close up pre- and post-treatment photographs of each tooth, as in the present study, they might have underestimated the number of white spot lesions.

Importantly, two patients in the treatment group developed more new white spot lesions than any of the other patients in this study. One patient developed five new white spot lesions, and the other developed four. The greatest number of white spot lesions developed by any patient in the placebo group was three. Notably, the two patients who developed the majority of white spot lesions in the treatment group had poor oral hygiene. Improper cleaning techniques lead to increased plaque accumulation, which in turn create an environment that is more susceptible to demineralization.[21] Poor oral hygiene is a major risk factor for developing white spot lesions.[6] Patients with fair or

poor pretreatment oral hygiene are approximately 2.8 times more likely to develop white spot lesions than those patients with good pretreatment oral hygiene.

The maxillary lateral incisors are the teeth most likely to develop white spot lesions. The maxillary laterals developed the most white spot lesions in the present study, followed by the mandibular canines, and then the mandibular first premolars. These teeth have been previously shown to be most likely to develop white spot lesions.[4, 6, 47] Lucchese and Gherlone found that the most common sites for white spot lesion development was the mandibular first molars (30% of patients), followed closely by the maxillary laterals (29% of patients).[47] More recently, Julien et al. found the maxillary laterals were the most susceptible to white spot lesion development, followed by maxillary and mandibular canines.[6] The maxillary lateral incisors could be more susceptible because there is a smaller area between the bracket base and gingival margin on the lateral incisor than on any other tooth, making it more difficult to brush and remove plaque.

No significant differences in white spot lesion development were found between the treatment and placebo groups when using photographic or QLF analysis in the present study. The treatment group had the same percentage of patients who developed white spot lesions based on photographic analyses and QLF (37.50%), while the placebo group had a greater percentage of patients develop white spot lesions based on the QLF (58.06%) than on the photographic analyses (32.25%). Heinrich-Weltzien et al. determined that QLF is able to detect a larger number of initial caries than visual examination, and that QLF is more sensitive at detecting lesions, except for adjacent to

gingival tissues. On that basis, one would expect for the percentage of patients who developed white spot lesions to be greater in both the treatment and placebo group based on QLF analyses. One possible explanation of why the placebo group showed a higher prevalence with QLF analysis, while the treatment group did not, are the sample size differences between the two groups. In the present study there were 24 patients in the treatment group, compared to 31 in the placebo. Another possible explanation is that white spot lesion detection with QLF has been shown to be region dependent; it is not a good method for detecting white spot lesions adjacent to the gingival tissue.[45]

Most importantly, application of 1.23% acidulated phosphate fluoride gel for one minute every two months does not appear to prevent white spot lesion development. This could be due to the fact that the fluoride tray only remained in place for only one minute as recommended by the manufacturer. The length of time that fluoride gel is applied to the tooth may affect white spot lesion development. The present study applied the fluoride gel for one minute using a tray delivery method. Jiang et al. found a 76% reduction of white spot lesion incidence when applying 1.23% acidulated phosphate fluoride foam for four minutes.[96] While some studies have reported no differences in fluoride uptake between 1 and 4 minute acidulated phosphate fluoride groups,[92, 97, 98] others have demonstrated differences.[99-101] However, two of the studies that demonstrated a difference in fluoride uptake between the 1 and 4 minute application tested multiple APF gels.[99, 101] They grouped the gels together in order to determine if there was a significant difference between the 1 and 4 minute application, even though one gel was shown to have a significantly greater amount of fluoride taken up by

enamel. Delbum and Curry found that there was a significant difference in the amount of fluoride uptake by enamel between 1 and 4 minute acidulated phosphate fluoride, but there was no difference in the enamel's resistance to demineralization.[102] More research is needed to determine the appropriate application time using acidulated phosphate fluoride.

The lack of treatment effect could also have been to the length of time between treatments. Acidulated phosphate fluoride gel may need to be applied more often than every 8 weeks. This study applied the acidulated phosphate fluoride gel at approximately 8 week intervals. The ADA recommends applying acidulated phosphate fluoride every three months for patients at high risk (including orthodontic patients).[103] While the anti-cariogenic effect of fluoride has well been established,[104] several studies have demonstrated that a large portion of the fluoride is removed relatively rapidly following application.[105, 106] Dijckman et al. evaluated the fluoride content on the surface of the enamel, as well as the fluoride content incorporated into the enamel, 1 week, 4 weeks, and 12 weeks after acidulated phosphate fluoride gel application.[107] The fluoride on the enamel for the acidulated phosphate fluoride gel was comparable to that of the control 1 week after the application. The fluoride content in enamel was lost within a week and the experiment was stopped after 1 month. Mellberg et al. found that the loss of fluoride from surface enamel of primary teeth was complete within the first week after topical treatment with acidulated phosphate fluoride.[105] Caslavska et al. reported similar results after two weeks for permanent central incisors treated with acidulated phosphate fluoride.[108] O'Reilly and Featherstone, found that orthodontic patients who brushed

nightly with a fluoridated toothpaste and received weekly applications of 1.23% acidulated phosphate fluoride gel for a month had normal enamel profiles, indicating a reduction of demineralization.[8] Because acidulated phosphate fluoride has a high fluoride concentration, calcium fluoride is the main reaction product rather than fluorhydroxyapatite.[109] Calcium fluoride can be washed out by saliva in a relatively short time.[105] These studies suggest that acidulated phosphate fluoride gel may need to be applied weekly in order to prevent white spot lesions from occurring in orthodontic patients.

Even though this study did not find a significant treatment effect it is important because negative research results can have a positive impact in society. Studies have shown that research projects with statistically significant findings are more likely to be published than studies with null results.[110-112] By selectively reporting studies with positive findings, Cleophas and Cleophas believe that both bias and imprecision are introduced into healthcare assessments.[113] By reporting non-significant results, the procedure tested may be altered, or a new method of treatment developed altogether, in future research projects to find a solution to the problem in question.

In the future, more research needs to be conducted to determine which method of fluoride application, under the control of the orthodontist, is most effective in the prevention of white spot lesions. For example, fluoride varnishes have been shown to be effective in carries prevention,[103] and it has been demonstrated that fluoride varnishes are more effective than acidulated phosphate fluoride foam and gel in demineralization protection over the long term.[114] Todd et al. reported that fluoride varnish reduces

demineralization around orthodontic brackets in vitro by 50 percent when compared to a control and placebo group.[87] More recently, Stecksen-Blicks et al. determined that fluoride varnish applied at every orthodontic appointment decreases white spot lesion incidence by 17 percent.[115] Fluoride varnishes adhere to the tooth longer than fluoride gels and foams and therefore allow time for calcium fluoride to be converted into fluorhydroxyapatite.[109] Fluoride varnishes have a higher fluoride concentration than acidulated phosphate fluoride gels (almost twice as much fluoride).[116] Even though there is a greater fluoride concentration in fluoride varnishes, the level of plasma fluoride in young children after the application of a varnish is only one seventh of the peak after application of 1.2% APF gel.[117] Sealants have also been shown to be effective in reducing white spot lesion formation. [73, 74] The problem with sealants is that they undergo mechanical wear over time, and as the teeth erupt and extrude throughout treatment, the enamel that was initially covered by the gingiva becomes exposed to the oral environment. Ideally, research in the future would develop a method of white spot lesion prevention that does not require patient compliance, fits in with a typical orthodontic treatment timeline, is cost effective, and most importantly, is effective at preventing white spot lesions.

CHAPTER VI

CONCLUSIONS

Within the limits of this study, it can be concluded that:

1. Approximately one-third of patients receiving orthodontic treatment develop visible white spot lesions
2. The maxillary laterals are the teeth most likely to develop white spot lesions, followed by the mandibular canines and first premolars
3. Application of 1.23% acidulated phosphate fluoride gel for one minute every eight weeks does not prevent white spot lesion development

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APPENDIX A

FIGURES

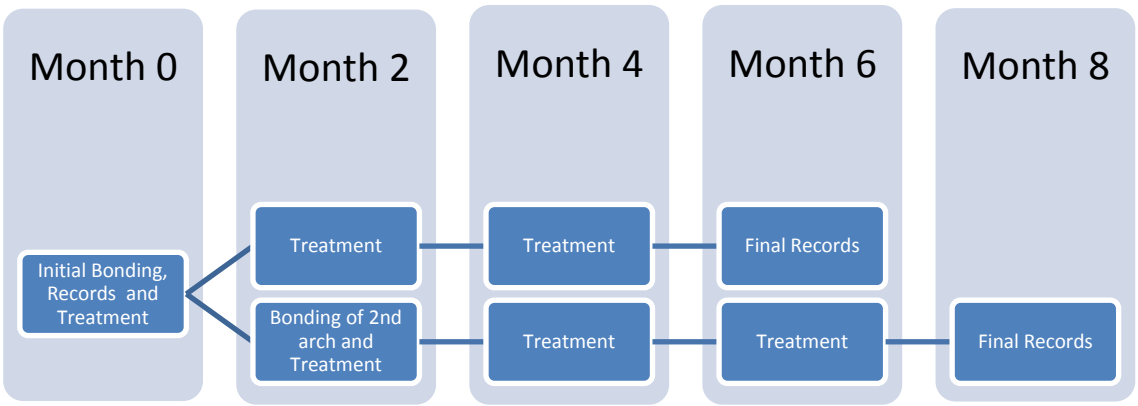

Figure 1: Treatment Timeline.

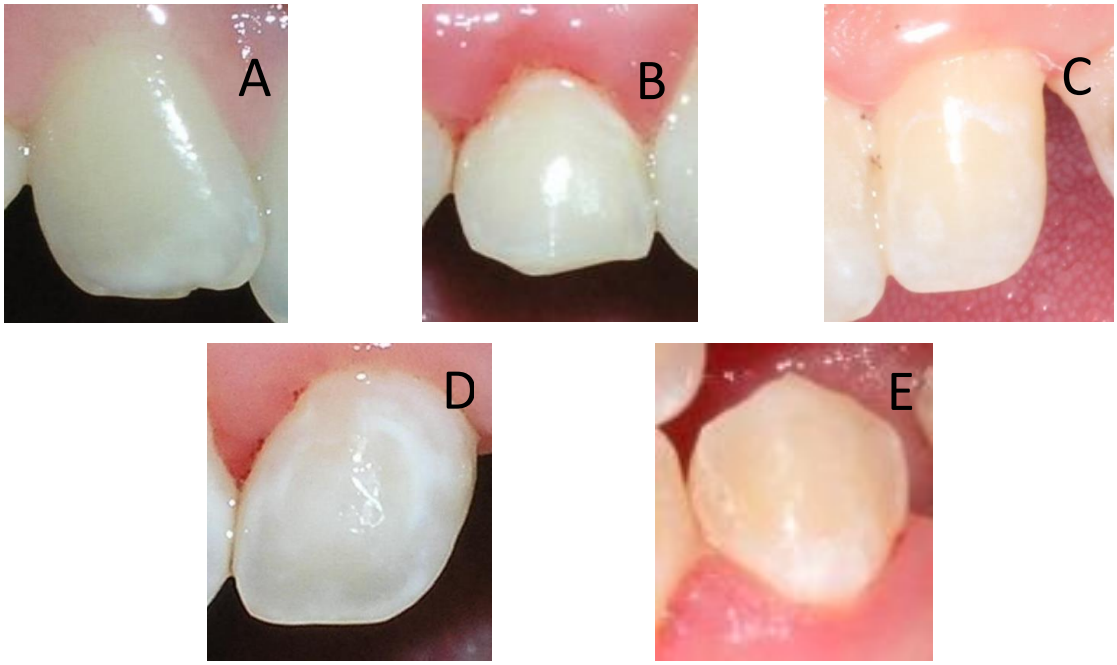

Figure 2: Examples of Øgaard scores. A) Øgaard score of 0 B) Øgaard score of 1 (small). C) Øgaard score of 1 (large) D) Øgaard score of 2. E) Øgaard score of 3.

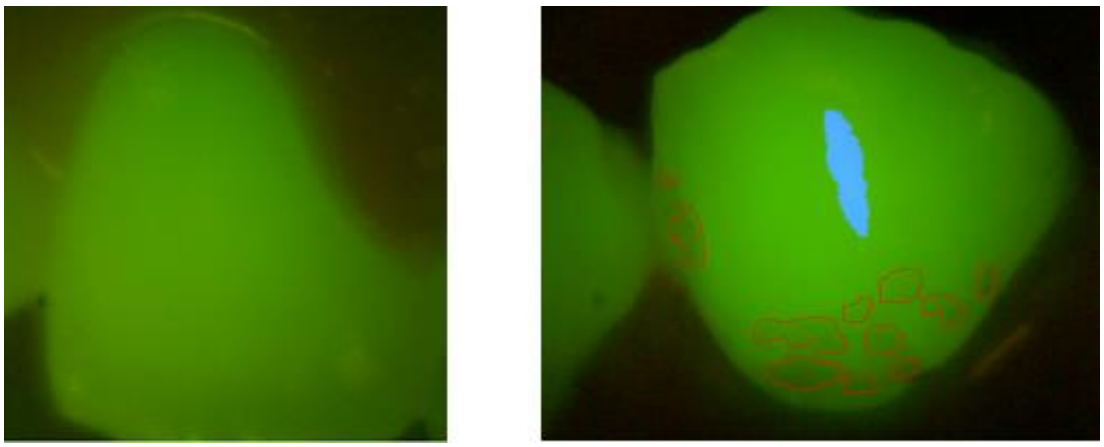

Figure 3: Fluorecam Photos.

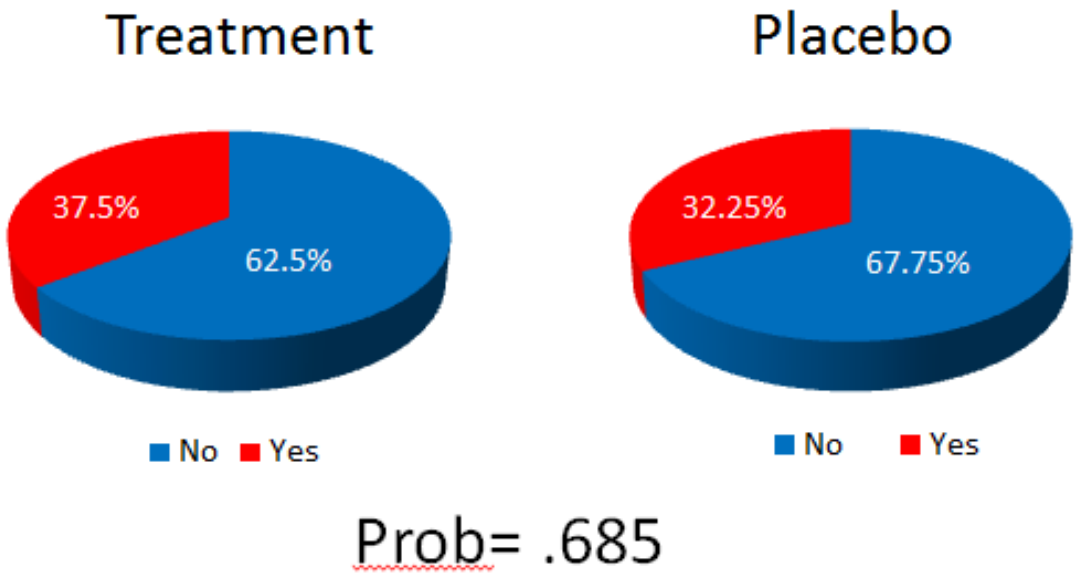

Figure 4: Percentage of patients who developed at least one WSL with photographic analysis.

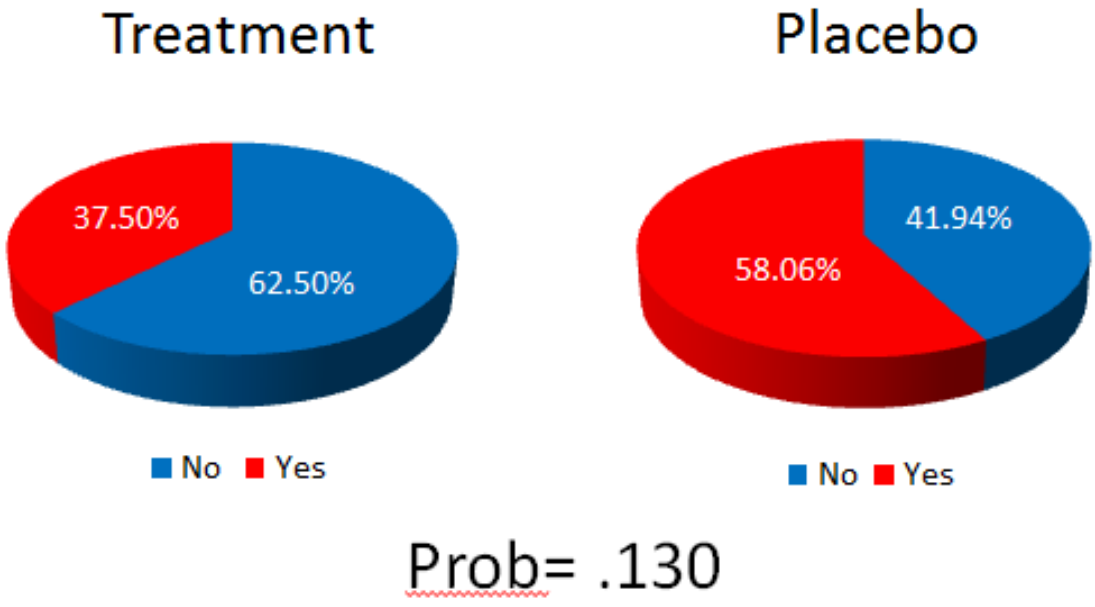

Figure 5: Percentage of patients who developed at least one WSL with QLF analysis.

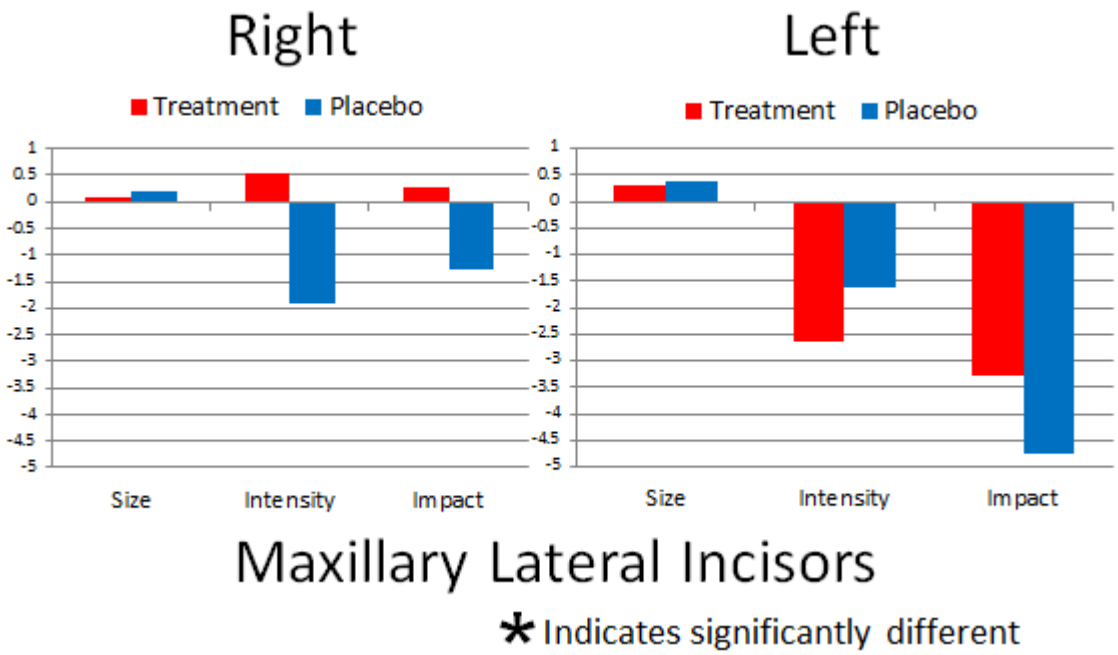

Figure 6: Maxillary lateral incisor QLF measurements: size (mm), intensity, and impact.

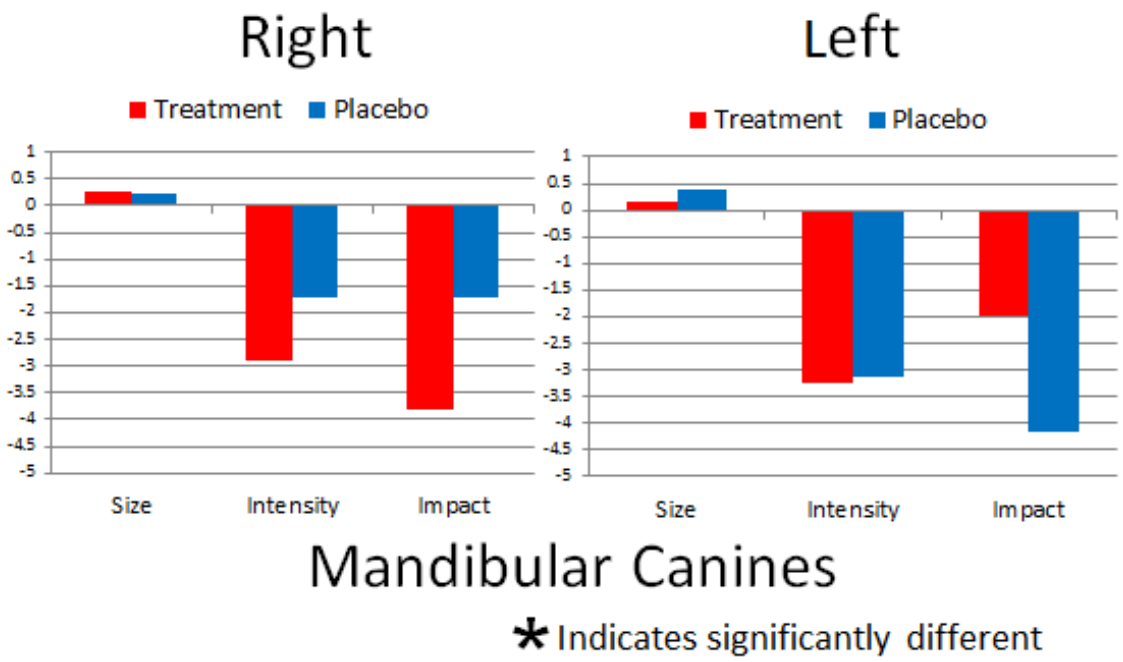

Figure 7: Mandibular canine QLF measurements: size (mm), intensity, and impact.

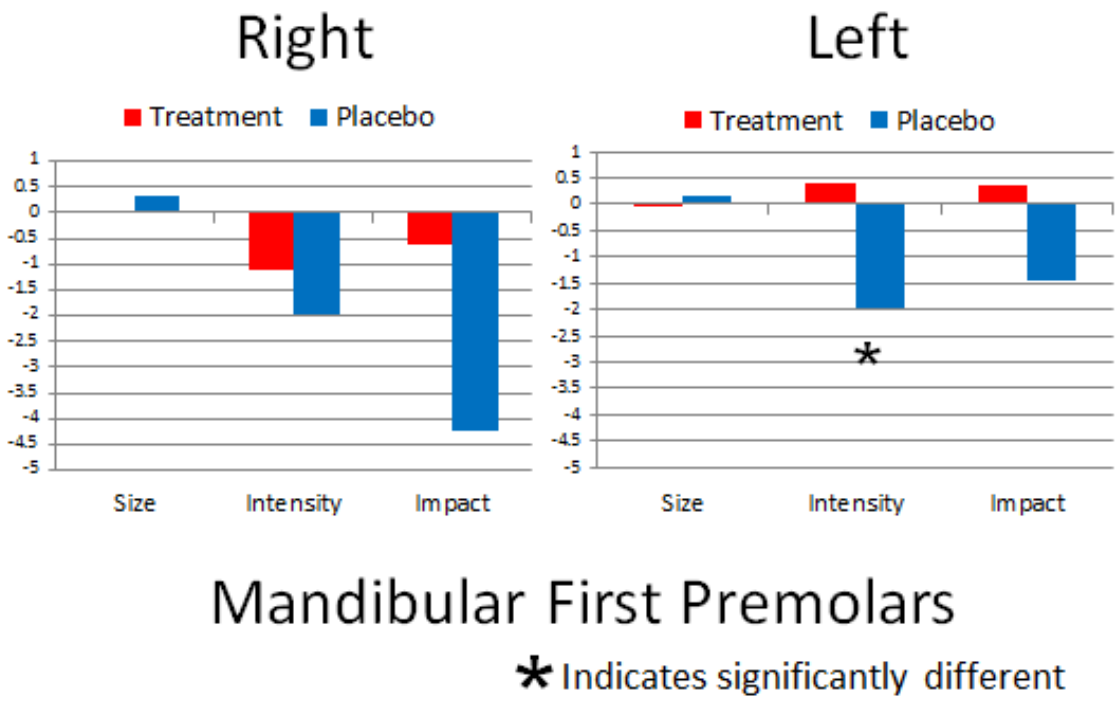

Figure 8: Mandibular first premolar QLF measurements: size (mm), intensity, and impact.

APPENDIX B

TABLES

Group	Sex		Age	
	Male	Female	Pre-tx	Post-Tx
Treatment	15	9	13.9	14.5
Placebo	8	23	13.5	14.0

Table 1. Patient description.

Variable		Systematic Error	Random Error	Interclass Correlation
UR2	Size	-.009	.098	.965
	Intensity	.237	.943	.952
	Impact	.432	2.019	.924
LR3	Size	.007	.059	.987
	Intensity	.061	.798	.964
	Impact	.007	.797	.969
LR4	Size	-.013*	.033	.996
	Intensity	-.068	.442	.987
	Impact	.111	.478	.996
UL2	Size	.005	.152	.971
	Intensity	-.015	.480	.991
	Impact	-.032	2.246	.973
LL3	Size	-.022	.074	.975
	Intensity	.353*	.601	.982
	Impact	.555	1.44	.952
LL4	Size	-.004	.055	.990
	Intensity	-.177	.689	.978
	Impact	-.113	.874	.981

Table 2. Reliability of Fluorecam readings.

Variable	Group	Øgaard Scores			Group Difference (Prob)
		0	1	2	
UR2	Treatment	100.0	0.0	0.0	.369
	Placebo	96.6	3.4	0.0	
LR3	Treatment	100.0	0.0	0.0	1.00
	Placebo	100.0	0.0	0.0	
LR4	Treatment	100.0	0.0	0.0	.118
	Placebo	88.0	12.0	0.0	
UL2	Treatment	100.0	0.0	0.0	1.00
	Placebo	100.0	0.0	0.0	
LL3	Treatment	95.7	4.3	0.0	.257
	Placebo	100.0	0.0	0.0	
LL4	Treatment	100	0.0	0.0	.118
	Placebo	88.0	12.0	0.0	

Table 3. Percentage of patients with pretreatment Øgaard scores from photographic analysis.

Variable	Group	Percentage of Patients	Group Difference Prob.
UR2	Treatment	25.0	.249
	Placebo	14.8	
LR3	Treatment	12.5	.439
	Placebo	6.5	
LR4	Treatment	8.3	.408
	Placebo	3.2	
UL2	Treatment	25.0	.415
	Placebo	16.1	
LL3	Treatment	8.3	.863
	Placebo	9.7	
LL4	Treatment	8.3	.790
	Placebo	6.5	

Table 4. Percentage of patients who developed new white spot lesions from photographic analysis.

Group	Number of new white spot lesions							Group Difference Prob.
	0	1	2	3	4	5	6	
Treatment	62.5	12.5	12.5	4.2	4.2	4.2	0.0	.701
Placebo	67.7	16.1	9.7	6.5	0.0	0.0	0.0	

Table 5. Percentage of patients who developed new white spot lesions from photographic analysis by number of new white spot lesions.

Group	Number of white spot lesions that worsened			Group Difference Prob.
	0	1	2	
Treatment	100.0	0.0	0.0	.375
Placebo	96.8	0.0	3.2	

Table 6. Percentage of patients with worsening of pre-existing white spot lesions from photographic analysis.

Group	Pre-treatment			Post-treatment			Treatment changes		
	25%	50%	75%	25%	50%	75%	25%	50%	75%
Treatment	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	2.0
Placebo	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	1.0
Group Difference	.372			.906			.654		

Table 7. Severity of white spot lesions based on Øgaard scores from photographic analysis.

Variable	Group	Percentage of Patients with New White Spot Lesions	Group Difference Prob.
UR2	Treatment	12.5	.141
	Placebo	29.0	
LR3	Treatment	20.8	.876
	Placebo	22.6	
LR4	Treatment	8.3	.500
	Placebo	12.9	
UL2	Treatment	29.2	.781
	Placebo	25.8	
LL3	Treatment	25.0	.557
	Placebo	32.3	
LL4	Treatment	4.2	.094
	Placebo	19.4	

Table 8. Percentage of patients who developed new white spot lesions based on increase in size measured by QLF.

Group	Number of new white spot lesions					Group Difference Prob.
	0	1	2	3	4	
Treatment	62.5	8.3	8.3	8.3	12.5	.633
Placebo	41.9	16.1	16.1	9.7	16.1	

Table 9. Percentage of patients who developed new white spot lesions with QLF analysis by number of new white spot lesions.

Method of Detection	Photographs		
		No	Yes
Fluorecam®	No	40.0%	10.9%
	Yes	25.5%	23.6%

Table 10. Comparison of photographic and Fluorecam findings.