White spot lesion regression using casein phosphopeptide amorphous calcium phosphate complexes alone or combined with microabrasion.

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

Gary Andrew Warner

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF Science in Dentistry

Dr. Daranee Versluis-Tantbirojn and Dr Brent Larson

June 2010

Acknowledgements

A special thank you to Dr. Daranee Versluis for her generosity in letting me work in her laboratory and utilize her expertise, equipment, time, and materials at no cost. Thank you to Dr. Benjamin Pliska for guiding me through this process and helping me to find this opportunity to conduct this research. Thank you to David Augustson for all of his help with the materials used in this study. Thank you to the biostatistical team, Andrew Wey and Philippe Gaillard, for their help in analyzing the data produced in this study.

Dedication

This thesis is dedicated to the loving memory of my grandmother Joye Warner who always told me that the heights of great men met and kept were not attained by sudden flight, but they while their companions slept were toiling upwards through the night.

Abstract

Introduction: Current literature suggests that the appearance of white spot lesions (WSLs) can be improved by treatment with casein phosphopeptides (CPP) and amorphous calcium phosphate (ACP). The aim of this study was to test the null hypothesis that the treatment of WSLs with CPP-ACP complexes produces no difference in the outcome of WSL regression when compared to controls regardless of whether or not microabrasion is used. **Method:** WSLs were artificially induced on 4 tooth sections obtained from each of 16 bovine central incisors, and then each section was randomly assigned to one of four groups. The three treatment groups were: CPP-ACP only group; a microabrasion only group, and a microabrasion and CPP-ACP group. The control group was neither CPP-ACP nor microabrasion. Quantitative light-induced fluorescence (QLF) was used to measure mineral content changes in WSLs immediately before (T1) and 2 weeks after treatment (T2). A two-within subject factor ANOVA was used to analyze the significance of any change from T1 to T2 in fluorescence, indicating mineral gain or loss. **Results:** There was a statistically significant gain in mineral content ($p=0.0058$) associated with the microabrasion technique, but no such gain for CPP-ACP ($p=0.40$). **Conclusions:** The null hypothesis was accepted. CPP-ACP complexes do not significantly improve the mineral content of bovine white spot lesions in vitro. Within the limitations of this in vitro study, the gains in mineral content seen with CPP-ACP were not statistically significant, and the gains in mineral content found when CPP-ACP was preceded by microabrasion were due to microabrasion, but not to the CPP-ACP complexes.

Table of Contents

List of Tables

List of Figures

Introduction

It has been estimated that 36-97% of patients receiving orthodontic treatment develop white spot lesions on their enamel.[1-3] Over the last decade, remineralization based on the milk derivative casein phosphopeptide (CPP) and amorphous calcium phosphate (ACP) has been suggested by researchers at the University of Melbourne.[4] It has been proposed that CPP can not only stabilize calcium phosphate nanoclusters of ACP on the tooth surface, but also in the subsurface part of a demineralized lesion.[4] Claims are that the nanoclusters of ACP are small enough to access the demineralized areas underneath an already remineralized surface zone.[5] A corollary of this is that CPP-ACP complexes would support remineralization of a WSL in both the surface and subsurface zones to create a deep regression in the appearance of the white spot lesion producing a more favorable esthetic previously unattainable by conventional techniques with fluoride.

At this time, CPP-ACP complexes are available in a variety of gels, creams and mousses and may also be incorporated into chewing gums. For this study, MI Paste (Tooth Mousse RECALDENT™ (CPP-ACP), GC America Inc., Tokyo, Japan 070118M) was used to provide the CPP-ACP complexes in the form of a cream. A review of the literature shows a number of promising *in situ* studies from the University of Melbourne Australia[4-8] on the effectiveness of CPP-ACP on the regression of WSLs. However, a comparison of the effectiveness of MI Paste application alone versus in conjunction with microabrasion has yet to be studied, and quantified.

The purpose of this study was to compare the outcomes of daily CPP-ACP regimens with and without a prior microabrasion regimen for efficacy in inducing

regression of early enamel lesions *in vitro*. One of the challenges for a study such as this is quantifying the enamel alterations in an objective way. The enamel mineral content fluctuations present in the demineralization and remineralization process causes changes in the optical properties of the enamel such as its fluorescence. As such, this study uses quantitative light-induced fluorescence, measured by Inspektor Pro version 2.0.0.38 software, to objectively measure the changes in fluorescence produced as a result of the enamel mineral content changes produced by the treatments.

Review of the Literature

Relationship between orthodontic treatment and white spot lesions (WSLs)

An unfortunate, but prevalent iatrogenic effect of orthodontic therapy is the decalcification of the enamel surface adjacent to fixed orthodontic appliances. The increased number of plaque retention sites created by orthodontic appliances makes optimal oral hygiene a challenge. Patients with poor dietary and oral hygiene practices can develop white spot lesions within 4 weeks after the beginning of treatment in the absence of fluoride supplementation.[9]

Gwinnett and Ceen showed that fixed orthodontic appliances induce a rapid increase in the volume of dental plaque.[10] Chatterjee and Kleinberg furthermore showed that plaque in orthodontic patients had a resting pH lower than that in nonorthodontic subjects.[11] Scheie and coworkers observed significantly elevated plaque and salivary levels of *S. mutans* after insertion of orthodontic appliances.[12]

In the majority of instances, lesions are small and restricted to thin bands surrounding the bracket bases or areas between the brackets and the gingival margin. However, in some patients the lesion development may be extensive, and result in early termination of treatment unless oral hygiene and fluoride regimens are followed accurately. Complicated appliance designs with loops, auxiliary archwires, springs, coils, and some Class II correctors create areas that are almost impossible to clean with normal skills and equipment.[13] Additionally, excess bonding material around the bracket base creates sites where bacteria can multiply.[13] Steel ligatures or self-ligating brackets are preferable to elastic ligatures for less plaque retention; however, they do not eliminate the risk of WSLs.[14]

Review of Results of Prevalence Studies on WSLs following Orthodontic Treatment

In a prospective study conducted in Norway,[1] roughly 50% of the patients receiving orthodontic treatment developed one or more WSLs during treatment with 5.7% of the teeth affected. This result was compared to a matched group of nonorthodontic patients in whom 11% developed WSLs on the labial surfaces in the same period of time with 0.4% of the teeth affected.[1]

Using more advanced detection techniques like quantitative light-induced fluorescence (QLF) Boersma and coworkers[2] observed that 97% of all subjects and on average 30% of buccal surfaces in a person post orthodontic treatment were affected. On average 40% of the surfaces in males, and 22% of the surfaces in females showed white spot lesions in this study.

Mizrahi[15] found no significant gender difference in the prevalence of white spot lesions, but did find that prior WSLs in males got more opaque following orthodontic treatment, than did prior WSLs in females after orthodontic treatment.

In a case controlled study done by Willmot,[3] the mean prevalence of WSLs post orthodontic treatment was 36% of his 657 consecutively debonded subjects, with 7.3% of tooth surfaces involved over 5 years. Willmot's study found that the prevalence of orthodontically related lesions was significantly greater in treated orthodontic arches ($p<0.01$) when compared with untreated control arches.

It has been reported in the literature that the first molars, upper lateral incisors and lower canines are the teeth most affected by WSL.[13] It has also been found that larger

lesions occur in gingival quadrants of a tooth, and particularly in upper central and lateral incisors.[16]

Etiology of white spot lesions (WSLs)

Sound enamel is a low light scattering material, whereas a demineralized lesion in enamel is white due to an increase in the backscatter of light. When a light photon enters sound enamel, it travels an average distance of 0.1 mm before being scattered.[17] Typically a large portion of light penetrates the enamel, which is about 1 mm thick, and is backscattered by dentin. This is why the color of dentin is often more clinically apparent than the color of enamel. When mineral is lost from enamel it becomes more porous, and the mineral is partly replaced by water, leading to an increased difference in refractive index between sound and demineralized enamel. A light photon travels a much shorter distance in carious enamel before being backscattered.[13] Most photons are scattered within the lesion, rather than penetrating to dentin, and the backscatter is greater resulting in the clinical appearance of a white spot. When the lesion is dried, the water is replaced by air and the average refractive index declines even more, leading to an even whiter lesion.[18]

Furthermore, tooth enamel that is demineralized is porous and may take up stain from food and beverages. Therefore the prevention, diagnosis and treatment of WSLs are important not only to prevent tooth decay but also to minimize the sequelae of tooth discoloration that could compromise the esthetics of the smile.

Small caries lesions in enamel have subsurface demineralization deep to a well-mineralized surface zone. Using polarized light microscopy, naturally occurring white

spot lesions have been classified into four zones: surface zone, body of lesion, dark zone and the translucent zone on the advancing front of the lesion. The lesion typically has a triangular shape pointing towards dentin as mineral loss follows the grain of the enamel rods. The translucent zone is the first visible sign of caries. This zone appears translucent because the spaces, or pores, are located at prism boundaries and other junctional sites of enamel such as the striae of Retzius. A slight remineralization of enamel takes place in the dark zone because caries is an active process of demineralization and remineralization. The dark zone is like a molecular sieve excluding large molecules from the micropores filled with air or vapor. The body of the lesion is where the largest area of demineralization is present resulting in this zone being visible radiographically. The surface zone remains relatively mineralized and is present until loss of tooth structure creates a cavitation. The range of sizes for all 4 zones is variable (Fig 1).

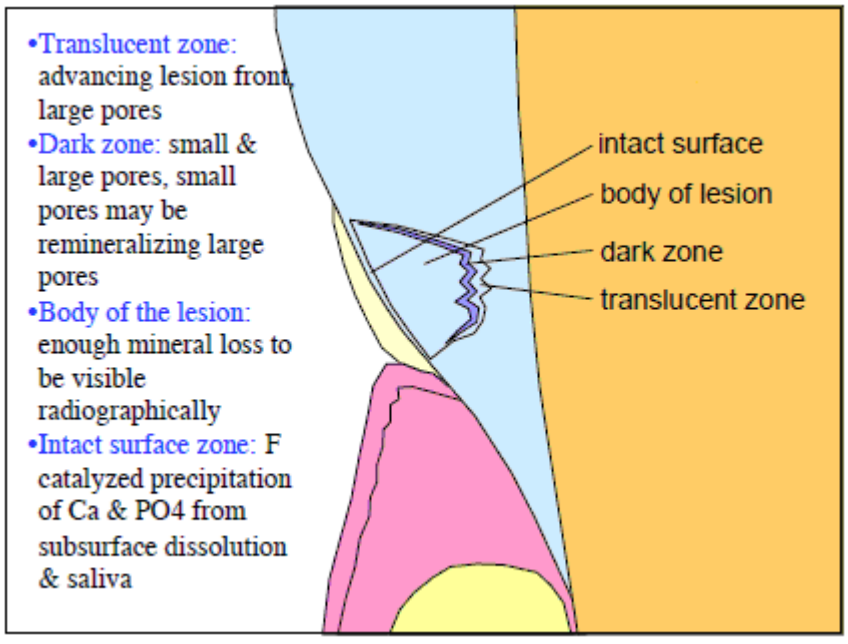

Image shown with permission of Dr. Gary Hildebrandt

Figure 1- The 4 zones of a white spot lesion.

What happens when white spot lesions are left untreated?

A study where 40 individuals who had participated in a randomized, controlled clinical study on the effect of a caries preventive program 6 years after debonding showed that left untreated 75% of small WSLs had regressed during that period, with 25% of the most severe lesions remaining visible on the surfaces.[19]

Backer-Dirks found on average remineralization of 20% to 30% (measured as percent mineral change) over 2 weeks in a group of 9-year olds with 72 carious WSLs and observed a disappearance of 50% of these lesions when examined 6 years later.[20]

Al-Khateeb and coworkers[21] followed 7 patients with WSLs developed during orthodontic treatment for 1 year post debonding using quantitative laser fluorescence and found that during this period the fluorescence radiance in the lesions increased and the area of the lesions decreased indicating remineralization. However, complete regain of lost minerals was not achieved since the minerals are not necessarily deposited in the same way as sound enamel, and so therefore light scattering from the partly remineralized lesion may not be identical to that of sound enamel. Another study using quantitative light-induced fluorescence of enamel surfaces 2 years post treatment showed this same lack of complete remineralization of WSLs with time.[22]

Using polarized light photography on 9 subjects with WSLs post orthodontic treatment, Willmot[23] found an exponential reduction in WSLs with time with most of the size change occurring 12 weeks after appliance removal (1/3 reduction in size) and 1/2 original size after 26 weeks and little further reduction after that.

A more recent study using light-induced fluorescence has shown that small lesions show rapid improvement 6 weeks after debonding ($P< 0.01$) and a further improvement after 6 months ($P<0.01$) confirming the work of Willmot.[13]

It is clear, therefore, from these studies that WSLs left untreated after removal of fixed orthodontic appliances will naturally reduce in size with no intervention. Tooth paste abrasion abrades demineralized bovine enamel moreso than sound bovine enamel, and may be a mechanism for the regression of untreated WSLs.[24] However, remineralization varies considerably from subject to subject and from site to site in the mouth.[25] A highly remineralized surface layer of the lesion may hinder the diffusion of ions that can remineralize the subsurface area. Chemical and/or mechanical modifications of the lesion surface have been suggested to facilitate lesion regression.

Effect of etching white spot lesions

When 37% phosphoric acid etches enamel, it dissolves about 5 to 10 μm of the enamel surface and creates a zone of etched enamel rods 15 to 25 μm deep.[26] It has been suggested that acid etching of fluoride-treated lesions could facilitate remineralization of the lesion by oral fluids.[27] Acid etching of WSLs increases surface porosity and this has been proposed to thereby enhance remineralization. Al-Khateeb and coworkers[21] induced WSLs in enamel in vitro and investigated longitudinally the rate of remineralization in etched and non-etched lesions in the presence and absence of fluoride. The rate of remineralization varied considerably between the groups during the first few weeks of the experiment. Etched enamel exhibited more pronounced lesion reduction than non-etched enamel, especially in the absence of fluoride. Later, the remineralization process slowed

down for all groups and at the end of the experiment no significant differences were found for any of the treatments. The etched lesions retained a porous structure of their surface layer even after a long period of remineralization in vitro.

Treatment options for white spot lesions suggested by Bishara[28]

Fluoride must not be used in high concentration, as it arrests the remineralization as well as the demineralization and can lead to unsightly staining.[16] In patients recently debonded from braces, the effect of applying a high fluoride concentration to their WSLs may immediately remineralize the most superficial layer of enamel but leave the deeper enamel crystals relatively unaffected. Therefore, if white spot lesions are present immediately following orthodontic treatment it is advisable to first allow slower calcium and fluoride ion penetration of the white spot lesion (WSL) from saliva or through the application of lower concentrations of fluorides. Such a treatment regimen may remineralize the mild WSL from the deeper parts of the lesion to the outer surface layers of the enamel, thus increasing the chances for a successful and more esthetic treatment result. If time and fluoride do not improve or correct the esthetic concerns of the patient and clinician, tooth whitening should be considered as the next step. If whitening the teeth is unsuccessful the clinician may consider the use of microabrasion on the enamel surface in an effort to eliminate localized WSLs. The last resort to meet the esthetic objective of the patient and clinician is having composite restorations or porcelain veneers.[28]

Proposed MI Paste Regimens in the Literature and Product Brochures

Conventional treatments such as enamel microabrasion (etching followed by gentle abrasion with pumice) only affect the surface, and will improve the surface, but not the subsurface. Regeneration of the subsurface, using MI Paste or MI Paste Plus immediately after microabrasion has been proposed to address the underlying opacity and maximize the esthetic benefit of treatment.

Walsh asserts that in cases of incipient carious lesions, the subsurface water can be converted back into enamel because the neutral ion species move by diffusion through the porous surface, and when they react with the water, the hydroxyapatite formed will regenerate in the subsurface space.[29] Once 80%-85% regeneration has occurred, the enamel will appear optically normal meaning the appearance of the white spot lesion also disappears.[29]

For existing active white spot lesions, Walsh proposes that there is no need to etch prior to applying MI Paste, whereas in arrested lesions, the clinician should etch for 15 seconds with phosphoric acid to make the surface permeable. Walsh asserts that it is important to maximize the microscopic porosity of the enamel surface overlying the defect, by etching combined with gentle microabrasion when treating arrested lesions. Well demarcated areas will respond relatively poorly to treatment, however, diffuse poorly demarcated areas are shallower and will respond better.[29]

Ardu et al[30] in a case report recommend using an abrasive paste containing silicon carbide microparticles in a water soluble paste and 6.6% hydrochloric acid (Opalustre, Ultradent) to microabrade with slight pressure for 60 to 120 seconds, followed by application of CPP-ACP undisturbed for 15 minutes, then aspirated but not rinsed with

water. Ardu et al then recommend having the patient apply a moderate amount of CPP-ACP after brushing and flossing twice a day, after breakfast and just before bedtime for several months eventually supplemented with home bleaching to achieve a more uniform tooth color. The photographic results of one successful case were shown by Ardu et al for a patient following 3 months on this regimen.

Casein Phosphopeptide Amorphous Calcium Phosphate (CPP-ACP) Mechanism of Action.

In the 1980s, Reynolds drew attention to the fact that casein phosphopeptide amorphous calcium phosphate, which is a product derived from milk casein, was capable of absorbing through the enamel surface and could affect the carious process.[31] In the process where fluoride facilitates remineralization, for every 2 fluoride ions, 10 calcium ions and 6 phosphate ions are required to form 1 unit cell of fluorapatite ($Ca_{10}(PO_4)_6F_2$). Hence on topical application of fluoride ions, the availability of calcium and phosphate ions can be the limiting factor for net enamel remineralization to occur. The clinical use of calcium and phosphate ions for remineralization has not been successful in the past due to the low solubility of calcium phosphates, particularly in the presence of fluoride ions. Calcium phosphates are not easily applied, do not localize effectively at the tooth surface and require acid for solubility to produce ions capable of diffusing into the enamel subsurface lesions. If calcium and phosphate ions are used, it can only be done at very low concentrations due to the intrinsic insolubility of the calcium phosphates, in particular the calcium fluoride phosphates. Furthermore, soluble calcium and phosphate ions do not substantially incorporate into dental plaque or localize at the tooth surface to

produce effective concentration gradients to drive diffusion into the subsurface enamel.[32] CPP-ACP is a delivering system that allows freely available calcium and phosphate ions to enter enamel and reform into calcium phosphate crystals. The free calcium and phosphate ions move out of the CPP-ACP and into the enamel rods and freeform as apatite crystals.[33] According to Cross et al, CPP contains a cluster of phosphoseryl residues in the motif-Ser(P)-Ser(P)-Ser(P)-Glu-Glu- which markedly increases the apparent solubility of calcium phosphate by stabilizing amorphous calcium phosphate (ACP) under both neutral and alkaline conditions. This prevents transformation to the insoluble phase, forming metastable solutions that are supersaturated with respect to calcium phosphates in plaque.[34] CPP-ACP then acts as a calcium reservoir, buffering the activities of free calcium phosphate ions in the plaque fluid helping to maintain a state of super saturation with respect to the enamel mineral, thereby depressing the enamel demineralization and enhancing remineralization.[35]

A randomized, controlled caries clinical trial showed that CPP-ACP containing chewing gum in 2720 school children produced an 18 percent reduction in caries progression after 24 months at the subject level and a 53 percent greater regression (remineralization) of baseline lesions when compared with the control gum.[36] A number of different media have been produced to deliver the CPP-ACP, including a water based mousse, a topical cream, chewing gum, mouth rinses, and sugar free lozenges. The material is marketed under the trade name "Recaldent." Examples include sugar free chewing gum (Recaldent™; GC Corp., Japan and Trident White®; Cadbury Adams USA, Parsippany, New Jersey, USA), mints (Recaldent Mints™; Cadbury Japan Ltd., Japan), and topical gels (Tooth Mousse™; GC Corp., Japan).

Microabrasion

Microabrasion is the application of an acidic and abrasive compound to the surface of enamel. Research indicates that 1 minute applications of commercially available microabrasion compounds remove 12 μm of surface enamel on the first application and 26 μm on subsequent applications.[37] The first application removes less enamel than subsequent applications due to fluoride rich enamel that is at the enamel surface. The microabrasion process removes small amounts of surface enamel, but also leaves a highly polished enamel surface. The highly polished surface does not have the typical enamel surface appearance because the microabraded enamel has no interprismatic spaces.[38] The microabrasion process abrades surface enamel while compacting calcium and phosphate ions into the interprismatic spaces. This polished surface reflects light differently than natural enamel. Therefore, a portion of the whitened enamel is removed and a portion is camouflaged by the highly polished surface. Usually, 5 to 10 applications of the microabrasion compound indicates whether the technique will be successful in adequately eliminating the undesirable discoloration. Research has shown that although microabrasion removes small amounts of enamel surface, the newly polished surface is less susceptible to bacterial colonization and demineralization than the natural non-abraded enamel.[39,40]

Quantitative Light-Induced Fluoroscopy (QLF)

In the eighties, when lasers were first being used for caries excavation, the Swedish dentist Dr. SundStröm from the cariology group in Stockholm started experiments with a blue argon laser. Although he was unsuccessful in using the laser for

excavation, he noticed that the blue light caused the teeth to fluoresce green and that early carious lesions, or white spots, showed up as dark grey spots. The Dutch Physicist Elbert de Josselin de Jong met with Dr. Sundström and with Prof. Birgit Angmar-Månsson in 1989 and was asked to help develop the technology for in vivo caries detection. Dr. de Jong immediately recognized the enormous potential and within Inspektor Dental Care he worked for 15 years to develop the technology for practical in vivo application. These efforts led to the Inspektor Pro technology that was used in this study. Inspektor Pro is the first FDA approved instrument that is powered by QLF technology (Figure 2).

The property of fluorescence is a function of light absorption.[17] A material that absorbs light will be more fluorescent than a material that reflects light. Demineralization leads to more backscatter of light, hence less absorption and a lower intensity of fluorescence. Carious enamel will therefore show up as a dark area with fluorescent techniques (Figure 3). When a tooth becomes carious, the fluorescence radiance at the location of the caries lesion decreases. The fluorescence image of enamel with incipient lesions can be digitized and the fluorescence loss in the lesion can then be quantified in relation to the fluorescence radiance level of sound enamel.[41,42] The amount of fluorescence radiance loss has been validated by use of transverse and longitudinal micro-radiography and is very closely correlated ($r = 0.97$) with the mineral loss in the lesion.[43-45] QLF has been cross-validated with transverse micro-radiography (TMR), which is the gold standard for measuring mineral loss, and strong correlations between the two techniques have been found.[46-48]

The QLF device used in this study (Inspektor Research Systems, BV, Amsterdam, The Netherlands) has been shown to have validity and reliability and good inter- and

intra- examiner reproducibility even despite the subjectivity involved in determining sound or unsound enamel and the presence or absence of borders and morphology of any detected lesion.[49] This device can calculate the difference in fluorescence between the demineralized area and the surrounding sound enamel and thereby quantify mineral loss and lesion size (Figure 3). A percent fluorescence change of 10% equals a mineral content change of 0.15 kgm^{-2} when using QLF to study natural carious lesions, but the relationship is less linear for artificially induced carious lesions.[50]

In light induced fluorescence, an arc lamp with a liquid light guide emits light that is passed through a blue filter with a peak intensity of 370 nm. The detecting camera has a yellow high pass filter (<540 nm) to exclude scattered blue light and the combination is optimized so that there are no reflections. The images are stored, processed, and analyzed using custom software. The software is designed to ensure that reproducible images of the tooth are taken over a period of time. The technique is an effective way to measure changes in mineral loss without employing destructive methods such as microhardness testing, polarized microscopy and microradiography. In this study, the Inspektor Pro QLF device was used to follow lesion regression or mineral gain as indicated by the changes in fluorescence intensity.

Aim

To determine if CPP-ACP containing MI Paste is a good therapy for white spot lesion regression, and to determine if its efficacy is enhanced by microabrasion.

Objectives

1) Quantify the change in mineral content of white spot lesions treated with MI Paste.
2) Quantify the change in mineral content of white spot lesions treated with microabrasion.
3) Quantify the change in mineral content of white spot lesions when microabrasion precedes MI Paste application.
4) Determine if any of the changes in the mineral content observed with MI Paste application, microabrasion or both are significantly different from the controls and/or each other.

Null hypothesis

The treatment of WSLs with CPP-ACP complexes produces no difference in the outcome of WSL regression when compared to controls regardless of whether microabrasion is used or not.

Materials and Methods

Based on the variability in observations found in a pilot study (data not shown) of five bovine incisor samples that were divided into four treatment groups, a sample size of sixteen freshly extracted bovine incisors was chosen in order to produce less variable data. Bovine incisors without cracks or erosions were cleaned and stored in physiological saline. These teeth were then sectioned mesiodistally and along the long axis into 4 sections using a diamond disc mounted on a straight slow-speed handpiece. Sections from the same tooth were placed in distinct treatment groups in this study to reduce the variability between teeth and their effect on the mean percentage of change for the groups. However, it should be noted that there is variability even between areas of the same tooth.

The baseline light-induced fluorescent readings were recorded using Inspektor Pro. QLF images were captured using an intraoral fluorescence camera (Inspektor Research Systems, BV, Amsterdam, The Netherlands) on a personal computer using the image capturing software (Inspektor Pro version 2.0.0.38) delivered with the system (Figure 2). To ensure that the images of the tooth surfaces are always captured with the same camera position and from the same angle, the software uses video-repositioning techniques. The video-repositioning technique displays the baseline live image simultaneously with the new image and computes their correlation based on similar geometry of the fluorescence intensities.[51] Images are stored in a list when the correlation is higher than 0.90 and the system automatically stops "grabbing" when the correlation reaches 0.98. In this way, the images captured at different time points in the study show the tooth surface from the same angle and at the same magnification. To measure the

WSLs, a patch was drawn surrounding the lesion site with its borders on sound enamel. Inside this patch, the fluorescence levels of sound tissue were reconstructed using the fluorescence radiance of the sound enamel (Figure 3). Subsequently the percentage difference between the reconstructed and original fluorescence levels was calculated. Pixels inside the patch were considered part of the lesion when the fluorescence loss exceeded the 5 per cent threshold.[21]

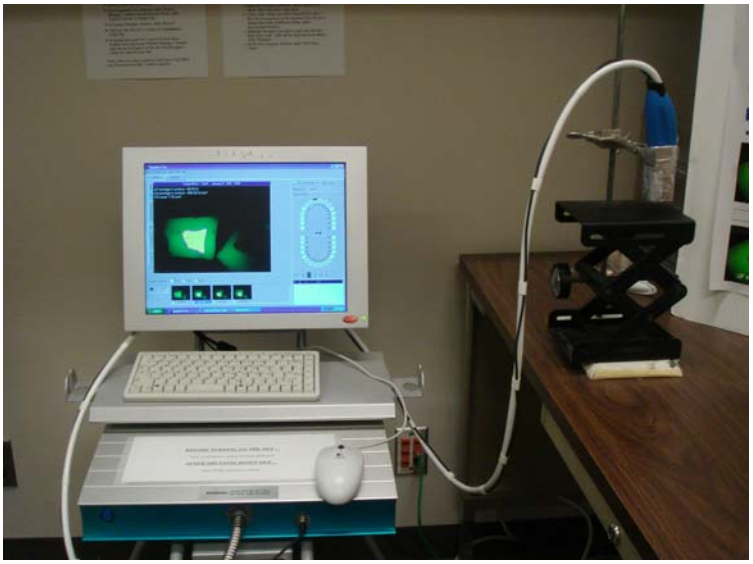

Figure 2 - QLF machine showing the Inspektor Pro software for WSL analysis on the monitor, and the QLF light source where teeth are analyzed on the table.

The sections were then painted with an acid resistant nail varnish exposing a window of 2 x 2 mm on the center of the buccal surface. The varnish was allowed to dry and then the sixty four sections of teeth were soaked for 2 weeks at room temperature in demineralizing solution to create a WSL. The demineralizing solution consisted of 1.5 mM calcium chloride ($CaCl_2$), 0.9 mM potassium phosphate (KH_2PO_4), and 50 mM acetic acid, adjusted to pH 5.0 with 1 M potassium hydroxide (KOH). QLF values were again recorded though not needed for this study.

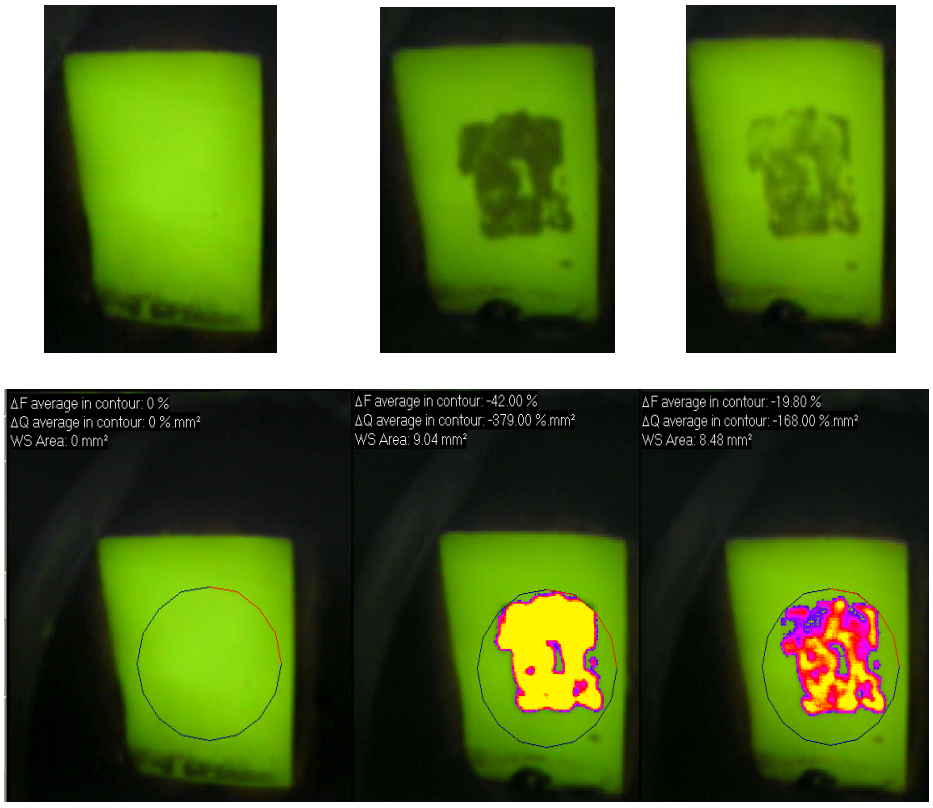

Figure 3- QLF analysis for one tooth section, P3, at baseline, T1 and T2. From Upper left to right- tooth section before demineralization, tooth section after demineralization, tooth section at the end of 2 weeks of MI Paste and microabrasion treatment. From lower left to lower right- QLF analysis of tooth section before demineralization, tooth section after demineralization, tooth section after 2 weeks of MI Paste and microabrasion treatment.

In order to produce a remineralized surface zone to simulate the anatomy of the WSLs induced by orthodontic treatment, the teeth were then placed in remineralizing solution for 1 week. This remineralizing solution consisted of 1.5 mM calcium chloride ($CaCl_2$), 0.9 mM potassium phosphate (KH_2PO_4), 130 mM potassium chloride (KCl), and 20 mM HEPES, adjusted to pH 7.0 with 1 M potassium hydroxide (KOH). At the end of this week, the QLF values were again recorded (T1), and these values were recorded as $\Delta F_{initial}$ and used as the baseline value for the starting mineral content of the WSL pre-treatment. ΔF is a value that

measures the percent difference in fluorescence between the sound and demineralized enamel of the tooth.

Using online randomizing software (http://www.random.org), each of the 4 sections per tooth was divided into 4 groups: the control, the MI Paste only group, the microabrasion only group and the MI Paste and microabrasion group. For the two treatment groups receiving microabrasion at the beginning of treatment, the technique involved a 2 minute 35% phosphoric acid etch with Gel-Etch Semi Gel in a Syringe (Temrex Corp., Freeport, NY, USA 6580) and rinse, followed by a 20 second pumice with Topex® Prep & Polish™ Paste (Sultan Dental Products Inc., Englewood, NJ, USA 020810B) with a rubber cup, attached to a slow speed hand piece, in a clockwise direction followed by a rinse. Over the next two weeks, using a q-tip, the control group and the "abrasion only" group were rubbed with deionized water for 20 seconds twice daily before being returned to a freshly replenished container of remineralizing solution. The MI Paste and microabrasion and the MI Paste only group were similarly rubbed with 1:1 diluted MI Paste and deionized water for 20 seconds twice daily before being returned to a freshly replenished container of remineralizing solution. This remineralizing solution in all 4 groups consisted of 1.5 mM calcium chloride ($CaCl_2$), 0.9 mM potassium phosphate (KH_2PO_4), 130 mM potassium chloride (KCl), 20 mM HEPES, adjusted to pH 7.0 with 1 M potassium hydroxide (KOH). The 1:1 dilution of the MI Paste was done as an approximation for the dilution that occurs on paste application to teeth *in situ*. QLF values were measured at the end of two weeks (T2), and recorded as ΔF_{final}. The difference between ΔF_{final} and $\Delta F_{initial,}$ ΔF, was calculated to measure the change in fluorescence/mineral content for each subject. The overall experimental design is shown in Figure 4.

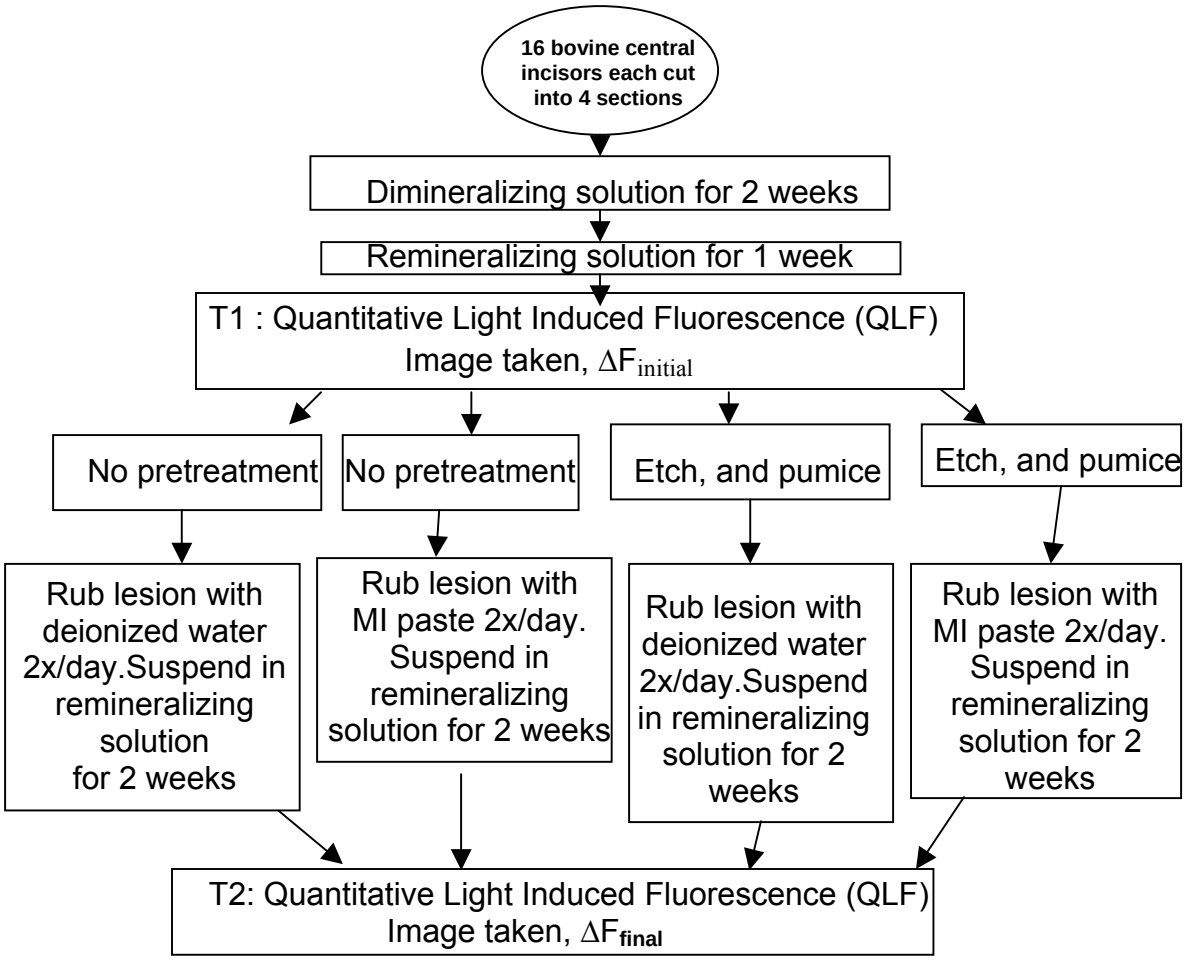

Figure 4 – Flowchart showing the study design used to test the null hypothesis of this investigation.

Statistical Analysis

The gain in fluorescence for each tooth section was analyzed with a two within-subject factor ANOVA for comparison of treatment outcomes at $\alpha = 0.05$. The fluorescence values among treatment groups at T1 were analyzed with ANOVA to determine any differences among the mineral content of the WSLs between groups at the start of treatment.

Results

From the initial 64 tooth sections used in this study three were rejected. Two were lost during treatment, and one was rejected owing to a WSL that was too minimal to distinguish from sound enamel ($\Delta F < 8\%$). Of the remaining sections, the control group and MI Paste group showed the smallest gain in mineralization from T1 to T2 at 2.9 % and 3.1 % respectively. The microabrasion group and the MI Paste and microabrasion group showed the largest gains in mineralization at 6.8 % and 8.2 % respectively. The results are shown graphically in Figure 5 and can be found in Table 1.

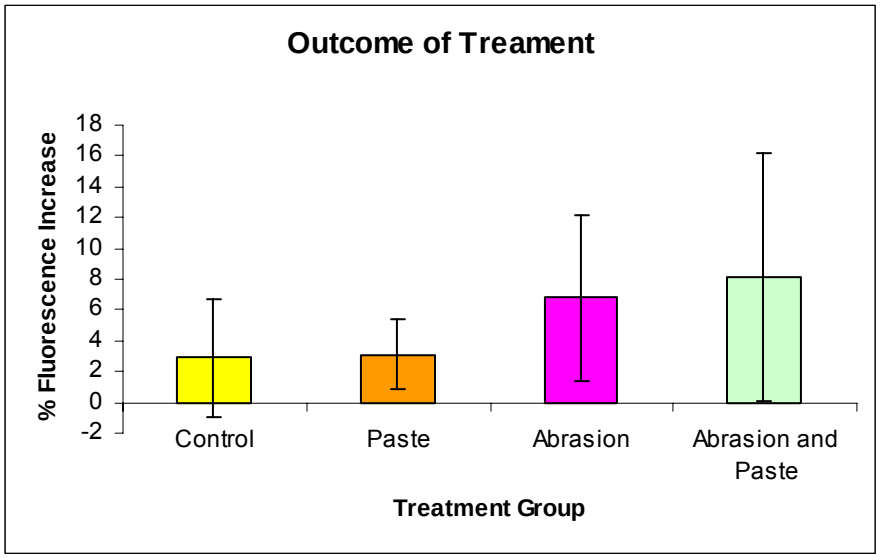

Figure 5- Mean percent fluorescence gain for each group**.** There was an increase in fluorescence (gain in mineral content) for each group. The mineral content gains for the microabrasion and microabrasion and paste treatment groups were significant at $p < 0.05$.

The mean values of $\Delta F_{initial}$ for each group at T1 were compiled in table 2, and compared for differences using ANOVA. It was determined that there was no significant difference between the mean $\Delta F_{initial}$ among all 4 groups.

Two-within subject factor ANOVA was used to analyze the changes in fluorescence from T1 to T2 for each group. The results of the two-within subject factor ANOVA indicated the following. First, the interaction between MI paste and microabrasion was not statistically significant. This indicates that the effect of MI paste does not depend on the levels of microabrasion, and that the effect of microabrasion does not depend on the levels of MI Paste. Second, MI paste did not have a statistically significant effect on fluorescence gain ($p = .40$). Third, microabrasion lead to statistically significant gains in fluorescence ($p = 0.0058$). These results indicate that microabrasion was the only factor to significantly contribute to gains in fluorescence. The use of microabrasion is associated with a higher average gain in fluorescence.

Control				MI Paste				Etch and Pumice				Etch, Pumice and MI Paste			
	ΔF initial	ΔF final	ΔF final - ΔF initial		ΔF initial	ΔF final	ΔF final - ΔF initial		ΔF initial	ΔF final	ΔF final - ΔF initial		ΔF initial	ΔF final	ΔF final - ΔF initial
A3	-15.4	-18.2	-2.8	A4	-26.0	-23.2	2.8	A2	-26.9	-14.9	12.0	A1	-8.31	-6.93	1.38
B4	-30.9	-27.2	3.7	B2	-14.2	-13.1	1.1	B3	-10.6	-7.91	2.69	B1	-28.4	-14.7	13.7
C3	-11.4	-9.7	1.7	C2	-22.5	-20.9	1.6	C4	-32.5	-14.5	18.0	C1	-27.0	-22.7	4.3
D2	-32.3	-27.6	4.7	D1	-9.17	-12.3	-3.13	D3	-21.5	-8.87	12.63	D4	-14.8	-14.5	0.3
E2	-18.5	-14.7	3.8	E4	-23.6	-22.2	1.4	E3	-14.3	-17.0	-2.7	E1	-9.0	-9.76	-0.76
F3	-8.79	-10.0	-1.21	F4	-18.0	-16.1	1.9	F1	-14.9	-8.99	5.91	F2	-8.43	-7.08	1.35
G3	-24.1	-22.4	1.7	G4	-24.0	-22.9	1.1	G1	-18.6	-9.66	8.94	G2	-16.9	-9.99	6.91
H4	-8.35	-8.87	-0.52	H3	-26.0	-20.1	5.9	H1	-13.6	-10.3	3.3	H2	-21.5	-10.7	10.8
I3	-11.3	-9.76	1.54	I1	-16.8	-17.2	-0.4	I2	-23.1	-10.9	12.2	I4	-19.8	-18.4	1.4
J2	-20.5	-16.9	3.6	J4	-21.3	-18.8	2.5	J3	-19.0	-11.2	7.8	J1	-24.7	-13.6	11.1
K2	-31.0	-25.0	6.0	K3	-29.0			K1	-15.8	-10.9	4.9	K4	-34.6	-14.2	20.4
L3	-24.9	-21.4	3.5	L1	-40.3	-35.7	4.6	L2	-16.4	-14.4	2.0	L4	-29.0	-21.7	7.3
M1	-18	-17.7	0.3	M2	-26.8	-24.2	2.6	M4	-10.5	-8.1	2.4	M3	-12.9	-11.8	1.1
N2	-7.37	-6.7	0.67	N1	-33	-29.3	3.7	N4	-22.2	-12.9	9.3	N3	-12.3		
O2	-14.7	-10.9	3.8	O1	-29.8	-23.3	6.5	O3	-17.0	-7.88	9.12	O4	-35.8	-12.9	22.9
P1	-30.7	-17.2	13.5	P4	-44.7	-30.2	14.5	P2	-14.1	-13.5	0.6	P3	-40.2	-19.8	20.4
		Mean	2.7[a]			**Mean**	3.1[a]			**Mean**	6.8[b]			**Mean**	8.2[b]
		SD	3.7			**SD**	3.9			**SD**	5.4			**SD**	8.1

Table 1 - Percent change in fluorescence from T1 to T2 for each of 4 randomly assigned tooth sections from each of the 16 teeth labeled A through P. Means labeled with the same letter are not significantly different from each other at $\alpha = 0.05$.

	Control	MI Paste	Etch and Pumice	Etch, Pumice and MI Paste
	-15.4	-26	-26.9	-8.31
	-30.9	-14.2	-10.6	-28.4
	-11.4	-22.5	-32.5	-27
	-32.3	-9.17	-21.5	-14.8
	-18.5	-23.6	-14.3	-9
	-8.79	-18	-14.9	-8.43
	-24.1	-24	-18.6	-16.9
	-8.35	-26	-13.6	-21.5
	-11.3	-16.8	-23.1	-19.8
	-20.5	-21.3	-19	-24.7
	-31	-29	-15.8	-34.6
	-24.9	-40.3	-16.4	-29
	-18	-26.8	-10.5	-12.9
	-7.37	-33	-22.2	-12.3
	-14.7	-29.8	-17	-35.8
	-30.7	-44.7	-14.1	-40.2
Mean	-19.3[a]	-25.3 [a]	-18.2 [a]	-21.5 [a]
SD	8.8	9.1	5.9	10.3

Table 2- Table providing a comparison between the means of $\Delta F_{initial}$ among all 4 groups. Means labeled with the same letter are not significantly different at $\alpha = 0.05$.

Discussion

Enamel decalcifications affect many orthodontic patients. White spot lesions are caused by inadequate oral hygiene leading to plaque accumulation around orthodontic appliances. Other factors that predispose a patient to white spot lesions are appliance design, cement lute failure, poor salivary flow and composition, enamel susceptibility, and dietary practices.[36] At this time there are no predictable non-restorative approaches to white spot lesion regression. The advent of CPP-ACP complexes for WSL regression has made it imperative to conduct quantitative studies such as this one to evaluate not only its efficacy, but also to determine how best to use CPP-ACP complexes for an optimum esthetic result. Anecdotal reports from Ardu et al,[30] and Walsh[29] have suggested that prior to CPP-ACP regimens, chairside microabrasion of the WSL to make the lesion more porous allows for better subsurface remineralization. This is the first quantitative study to attempt to evaluate this recommendation.

The results of this study, however, do not show a remineralizing effect for MI Paste, but it does show a significant effect for microabrasion. Microabrasion studies have shown that chairside chemomechanical microabrasion of WSLs can cause significant reduction in visible enamel demineralization by as much as an 83% reduction in the appearance of the lesion.[52] Even abrasion from tooth brushing over time may be a source of WSL regression. As mentioned earlier in this study, one investigation suggested that for bovine enamel, tooth brushing abrades the demineralized lesion more so than the sound enamel.[24]

Even without microabrasion, data shows that initial surface-softened lesions appear to remineralize rapidly in saliva even without fluoride.[53] The remineralizing solutions

used in this study have calcium and phosphate content similar to saliva. This explains the average gain in mineral content seen in the controls for this study which were soaked in remineralizing solution for 2 weeks (Figure 5 and Table 1).

The role of CPP-ACP has been described as localization of ACP at both the tooth surface and the subsurface of the lesion. The CPP-ACP is said to buffer free calcium and phosphate ion activities, helping to maintain a state of supersaturation with respect to enamel depressing demineralization and enhancing remineralization.[35] The presence of CPP-ACP might permit a rapid return to resting calcium concentrations and may allow more immediate remineralization of enamel substrate.

For this study, there was a statistically insignificant increase in remineralization from applying MI Paste to the lesion twice a day for two weeks. Microabrasion produced a statistically significant increase in remineralization of the WSLs for both the microabrasion only, and the microabrasion and MI Paste group ($p < 0.05$). Adding MI paste to the microabrasion treatment did not increase this effect significantly (Figure 5).

Contact Time of CPP-ACP with the lesion in this study

A 1:1 diluted mixture of MI Paste was applied for 20 seconds twice a day to the lesions. The dilution was done to simulate the dilution that would occur on applying MI Paste in the oral environment. The short contact time was chosen because the MI paste was not rinsed off of the teeth after each application. The paste was left as an undisturbed film on the teeth that persisted between the twice daily MI Paste applications. This persistent film of MI Paste was achieved because the tooth sections sat mounted in the base of the remineralizing solution container in a stable orientation so that the film of

paste on the surface of the lesion remained undisturbed between treatments. It has been demonstrated that CPP could still be detected on tooth surfaces 3 hours after consuming xylitol gum containing CPP-ACP.[4] Other studies have demonstrated that CPP-ACP in a mouthwash significantly increased the level of calcium and inorganic phosphate ions in supragingival plaque with the CPP bound to the salivary pellicle, and to the surface of bacteria in the plaque biofilm.[4] Because of this known affinity of CPP for biofilm, it was thought that rinsing off the MI Paste after each treatment application would not simulate the actual clearance rates in situ where contact time is increased by binding of CPP to biofilms on the lesions. In vitro studies placing undiluted MI Paste for 3 minutes on the lesion before suspending the lesion in artificial saliva are simulating a scenario that is not practical to home care MI Paste regimens where salivary clearance does not allow for such prolonged super saturation with the full concentration of the MI Paste product. Also, studies where all remnants of the MI Paste were rinsed off of the tooth completely before immersion in artificial saliva are not simulating the effect of biofilms in vivo.

Contact Time of CPP-ACP with the lesion in other studies

Table 2 shows contact times used in other studies.[54] The Pai et al[55] study left the undiluted paste on the lesion for 3 minutes before immersing the samples in artificial saliva. Similar to this study, the Pai et al study was done over the course of 2 weeks, but they observed a significant gain in mineral content from the GC Tooth mousse (GC Corporation, Japan) when measured both by laser fluorescence and scanning electron microscopy. Pulido et al[56] left the paste on the lesion for 2 minutes before rinsing all remnants of the paste off completely, and performed treatment two times a day for 6

days. The Pulido et al study, however, did not find any enhanced remineralization from CPP-ACP, and the authors suggested that the negative findings may have been because the 2 minute treatment period may have been too short.

In situ demin model	
Buccal flange appliance Reynolds (1987)[31]	Six 20-minute sugar exposures/day
Plaque Retention	
Reynolds et al. (2003)[4]	Mouthrinse 15 mL/30 sec/3x/day/5 days
Palatal Appliance	
Shen et al (2001)[6]	Chewing gum 20 min + 20 min intra-oral exposure/4x/day/14 days
Reynolds et al (2003)[4]	Chewing gum 5 min chewing/4x/day/14 days
Cai et al (2003)[7]	Lozenge 8 min 4x/day/14 days
Iijima at al (2004)[5]	Chewing gum 20 min
Walker et al (2006)[8]	Milk with 2-5 grams CPP-ACP sipped over 1 min + 40 min intraoral exposure/1x/day on weekdays/ 3 weeks
Cai et al (2007)[57]	Chewing gum 20 min + 40 min intra-oral exposure
Lingual appliance	
Itthagarun et al (2005)[58]	Chewing gum 20 min chewing/5x/day/21 days continuous intra-oral exposure
Buccal appliance	
Schirrmeister et al (2007)[59]	Chewing gum 20 min chewing + 20 min intra-oral exposure
Morgan et al (2008)[36]	Chewing gum 10 min chewing/3x/day/
Andersson et al (2007)[60]	Dental cream daily use for 3 mos/ followed by 3 mos of daily F dentifrice

Table 3 - Contact times between CPP-ACP complexes and white spot lesions found in treatment groups of past studies reporting positive findings for CPP-ACP on WSL regression. Table adapted from Zero, D.T. Recaldent™ Evidence for Clinical Activity. 2009 Adv. Dent. Res. 21: 30-34

The contact time of CPP-ACP complexes with WSLs are high for chewing gums and mints. The consumption of chewing gums and mints has been demonstrated to result

in increased production of stimulated saliva. Stimulated saliva has been shown to contain increased calcium and phosphate ionic concentrations when compared with non-stimulated saliva.[61] One study, however, did not support the finding that CPP-ACP containing gum was any better at remineralizing the dentition than non-CPP-ACP containing gums tested.[59]

Possible Treatment Effect from Using Bovine Enamel versus Human Enamel

There is general agreement that the use of human teeth is more relevant for conducting *in vitro* studies. However, the advantage of using bovine teeth instead of human teeth is that they are easy to obtain in large quantities in good condition, and have less variability in composition than human teeth.[62] Bovine teeth have large flat surfaces and would not have had prior caries challenges that might affect the test results. Mineral distribution in the carious lesions in bovine teeth is reported to be similar to that found in human teeth, and structural changes in human and bovine teeth are similar.[62] Positive *in vitro* findings for the efficacy of CPP-ACP paste on bovine enamel have been found in several studies.[31,55,63,64,65]

Possible Treatment Effect from Not Introducing Acid Challenges

There were no acid challenges in this study. Reynolds and Walsh suggested that CPP-ACP molecules need an acid challenge to be activated to separate the ACP from the casein.[66] However, Shen[6] and others had study designs without acid challenges and found positive outcomes for the efficacy of CPP-ACP in aiding remineralization, and so the absence of acid challenges should not affect efficacy.

Possible Treatment effect from physical differences between White Spot Lesions.

The physical differences between the teeth caused variability in the sizes of WSLs produced. Past *in vitro* thesis research projects conducted by the University of Iowa have shown that the upper limit on the depth of artificially induced WSLs that still had an intact enamel surface layer is about 150 μm.[67] The depth of the WSLs created by the demineralizing technique used in this study was tested prior to the start of this study using microhardness measures. It was found that with the technique used in this study, the WSLs produced showed variability but were generally on the order of 100-200 μm. The two weeks of demineralization used in this study was followed by one week of remineralization to ensure that the WSLs created were subsurface lesions with intact surface zones as opposed to being simply surface demineralizations. In remineralizing studies with positive findings for CPP-ACP efficacy, such as Shen et al,[6] lesion depth was around 110 ± 9μm with the demineralizing technique employed.

Possible Effect of Sample Size

One of the WSLs created in this study was barely over the Inspektor Pro software's 5% threshold for being a WSL (ΔF_{intial} = 8%) and so this was eliminated from the study, while two other tooth sections were lost during the remineralization period leaving a total of 61 tooth sections available for analysis from the original 64. For this experiment, treatment time was 14 days. It should be noted that *in vitro* remineralization is known to proceed more rapidly than *in vivo* remineralization.[13] The data collected from this study showed that the remineralizing process was sufficiently progressed at 14 days to detect fluorescence changes in all 4 groups. The Pai et al study showed positive in vitro findings

for CPP-ACP using 14 days of treatment. The mean ΔF in the MI paste group is relatively small when compared with the variability of the observations, and therefore, it would probably take an unreasonably large sample size to achieve a reasonable level of power (~80%) for this study. Because of this, the sample size used (n=16) was considered sufficient for accepting the null hypothesis.

Photographic Evaluation of White Spot Lesions

Photographs were taken at T1 and T2 for this study. However, these photographs were not analyzed or shown for this study since cameras can record the details differently from the naked eye. Photographs tend to overestimate the incidence of opacities, partly due to reflection of the flash from the tooth surface.[18] The problems of extraneous light can be reduced by slanting slightly or by filtering out the flash using cross-polarizing filters. In this study it was done by slanting. Using polarized light, and filters it is possible to obtain photographic images of WSLs without the light reflections which make image processing difficult. However, standardization of the procedure is difficult, particularly with respect to the wetness of the tooth, and lighting conditions might differ. For these reasons, the data obtained from the photographs were not analyzed in this study.

Summary and Future Study

The null hypothesis tested in this study was that the treatment of WSLs with CPP-ACP complexes produces no difference in the outcome of WSL regression when compared to controls regardless of whether microabrasion is used or not. The outcome measure, ΔF, was the percent change in the value of the difference in fluorescence

between the demineralized area, and the surrounding sound enamel immediately before (ΔF_{intial}) and after (ΔF_{final}) the 14 days of treatment. After 14 days of treatment, analysis of variance indicated that significant differences ($p < 0.05$) existed only for the two treatment groups employing microabrasion at the start of the treatment regimen. Two within-subject factor ANOVA showed that it was the microabrasion ($p = 0.0058$), and not the MI Paste ($p = 0.40$) that caused significant gains in remineralization.

However, clinical trials to accurately assess the efficacy of treatment regimens in post orthodontic patients are required. The duration of this *in vitro* study was only 2 weeks, and the results are not comparable to longitudinal *in vivo* studies. A clinical trial is needed to make reliable recommendations about the role of MI Paste in treating WSLs. Nevertheless, an *in vitro* study can provide some useful information as a quantification study. This study showed that two weeks of MI Paste use does not provide any advantage in the regression of WSLs *in vitro* compared to the regression observed with artificial saliva alone, nor does it confer any advantage to the efficacy of the microabrasion regimen for WSL regression. This study provides support for the use of microabrasion in remineralizing white spot lesions *in vitro*.

Conclusion

The following conclusions can be drawn:

1) MI Paste confers no advantage to the regression of WSLs *in vitro* for this study.
2) Adding MI Paste to a microabrasion regimen confers no advantage to the regression of WSLs *in vitro* for this study.
3) Microabrasion results in significant regression of WSLs

A comprehensive metanalysis of all the available literature conducted by Azarpazhooh[68] has led to the conclusion that there is insufficient clinical trial evidence (in quantity, quality or both) to make a recommendation regarding long term effectiveness of casein derivatives, specifically CPP-ACP in preventing caries in vivo and in treating dentin hypersensitivity or dry mouth. CPP-ACP use for white spot lesion regression is still purely anecdotal, and further research is required before it can be advocated to orthodontic patients for white spot lesion treatment.

References

1. Sandvik K, Hadler-Olsen S, El-Agroudi M, Øgaard B. Caries and white spot lesions in orthodontically treated adolescents. Eur J Orthod 2006;28(6):e258.

2. Boersma JG, va der Veen MH, Lagerweij MD, et al. Caries prevalence measured with QLF after treatment with fixed orthodontic appliances: Influencing factors. Caries Res 2005;39:41-7.

3. Willmot DR, Brook AH. Enamel demineralization following orthodontic treatment. J Dent Res 1999;78:1049.

4. Reynolds EC. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. Journal of Dental Research 2003;82:206-11.

5. Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC. Acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. Caries Res 2004;38:551-6.

6. Shen P, Cai F, Nowicki A, Vincent J, Reynolds EC. Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. Journal of Dental Research 2001;80:2066-70.

7. Cai F, Shen P, Morgan MV, Reynolds EC. Remineralization of enamel subsurface lesions *in situ* by sugar-free lozenges containing casein phosphopeptide-amorphous calcium phosphate. Aust Dent J 2003;48:240-3.

8. Walker G, Cai F, Shen P, Reynolds C, Ward B, Fone C, Koganei M, Oda M, Reynolds E. Increased enamel mineralization of tooth enamel by milk containing casein phosphopeptide-amorphous calcium phosphate. J Dairy Res 2006;73:74-8.

9. Øgaard B, Rølla G, Arends J. Orthodontic appliances and enamel demineralization. Part 1- Lesion development. Am J Orthod Dentofacial Orthop 1988;94:68-73.

10. Gwinnett AJ, Ceen RF. Plaque distribution on bonded brackets: A scanning electron microscope study. Am J Orthod Dentofacial Orthop 1979;75:67-77.

11. Chatterjee R, Kleinberg I. Effect of orthodontic band placement on the chemical composition of human incisor tooth plaque. Arch Oral Biol 1979;24:97-100.

12. Scheie AA, Arneberg P, Krogstad O. Effect of orthodontic treatment on prevalence of *streptococcus mutans* in plaque and saliva. Scand J Dent Res 1984;92:211-7.

13. Øgaard B. White spot lesions during orthodontic treatment: Mechanisms and fluoride preventive aspects. Seminars in Orthodontics 2008;14(3):183-93.

14. Sukontapatipark W, el-Agroudi MA, Sellisth NJ. Bacterial colonization associated with fixed orthodontic appliances. A scanning electron microscopy study. Eur J Orthod 2001;23:475-84.

15. Mizrahi E. Enamel demineralization following orthodontic treatment. Am J Orthod Dentofacial Orthop 1982;82:62-7.

16. Willmot DR. White spot lesions after orthodontic treatment. Seminars in Orthodontics 2008;14(3):209-19.

17. Angmar-Mansson B, ten Bosch JJ. Optical methods for the detection and quantification of caries. Adv Dent Res 1987;1:14-20.

18. Benson P. Evaluation of white spot lesions on teeth with orthodontic brackets. Semin Orthod 2008;14(3):200-8.

19. Øgaard B, Larsson E, Henriksson T. Effects of combined application of antimicrobial and a fluoride varnishes in orthodontic patients. Am J Orthod Dentofacial Orthop 2001;120:28-35.

20. Backer-Dirks O. Post eruptive changes in dental enamel. J Dent Res 1966;45:503-22.

21. Al-Khateeb S, Forsberg CM, de Josselin de Jong, E. A longitudinal laser fluorescence study of white spot lesions in orthodontic patients. Am J Orthod Dentofacial Orthop 1998;113:595-602.

22. Al-Khateeb S, Exterkate RAM, Angmar-Månsoon B. Effect of acid-etching on remineralization of enamel white spot lesions. Acta Odontol Scand 2000;58:31-6.

23. Willmot DR. White lesions after orthodontic treatment: Does low fluoride make a difference? J Orthod 2004;31:233-40.

24. Kielbassa AM, Gillmann L, Zantner C, Meyer-Lueckel H, Hellwig E, Schulte-Mönting J. Profilometric and microradiographic studies on the effects of toothpaste and acidic gel abrasivity on sound and demineralized bovine dental enamel. Caries Res 2005;39(5):380-6.

25. Mellberg JR, Chomicki WG, Mallon DE. Remineralization in vivo of artificial caries lesions by monofluorophosphate dentifrice. Caries Res 1985;19(2):126-35.

26. Powers JM, Messersmith ML. Enamel etching and bond strength. In; Brantley WA, Eliades T, eds: Orthodontic Materials, Scientific and Clinical aspects. Stuttgart, Germany: Stuttgart-Thieme;2001:105-22.

27. Hicks MJ, Silverstone LM, Flaitz CM. A scanning electron microscopic and polarized light study of acid etching of caries like lesions in human enamel treated with sodium fluoride in vitro. Arch Oral Biol 1984;29:765-72.

28. Bishara SE, Ostby AW. White spot lesions: Formation, prevention, and treatment. Seminars in Orthodontics 2008;14(3):174-82.

29. Walsh L. White spots: Unlock the mystery, identify the problem, find the appropriate treatment solution. Available at: http://www.gceurope.com/pid/126/leaflet/en_White_Spot_Lesions.pdf. Accessed on March 27, 2010.

30. Ardu S, Castioni NV, Benbachir N, Krejci I. Minimally invasive treatment of white spot lesions. Quintessence International 2007;38(8):633-6.

31. Reynolds EC. The prevention of subsurface demineralization of bovine enamel and change in plaque composition by casein in an intra-oral model. J Dent Res 1987;66:1120-7.

32. Reynolds EC, Cai F, Cochrane NJ, Shen P, Walker GD, Morgan MV, Reynolds C. Fluoride and casein phosphopeptide-amorphous calcium phosphate. J Dent Res 2008;87(4):344-8.

33. Reynolds. E.C. Remineralization of enamel subsurface lesions by casein phosphopeptide stabilized calcium phosphate solutions. J Dent Res 1997;76:1587-95.

34. Cross KJ, Huq NL, Palamara JE, Perich JW, Reynolds EC. Physicochemical characterization of casein phosphopeptide-amorphous calcium phosphate nanocomplexes. J Biol Chem 2005;280(15):15362-9.

35. Reynolds EC. Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: A review. Spec Care Dentist 1998;18:8-16.

36. Morgan MV, Adams GG, Bailey DL, Tsao CE, Fischman SL, Reynolds EC. The anticariogenic effect of sugar free gum containing CPP-ACP nanocomplexes on aproximal caries determined using digital bitewing radiography. Caries Res 2008;42:171-84.

37. Waggoner WF, Johnston WM, Schumann S. Microabrasion of human enamel in vitro using hydrochloric acid and pumice. Pediatr Dent 1989;11:319-23.

38. Donly KJ, O'Neil M, Croll TP. Enamel microabrasion: A microscopic evaluation of the "abrasion effect." Quintessence Int 1992;23:175-9.

39. Segura A, Donly KJ, Wefel JS. Effect of enamel microabrasion on bacterial colonization. Am J Dent 1997;10(272):274.

40. Segura A, Donly KJ, Wefel JS. The effects of microabrasion on demineralization inhibition of enamel surfaces. Quintessence Int 1997;28:463-6.

41. Bjelkhagen H, Sundström F, Angmar-Månsson B, Ryden H. Early detection of enamel caries by the luminescence excited by visible laser light. Swedish Dental Journal 1982;6:1-7.

42. de Josselin de Jong, E., Sundström F, Westerling H, Tranaeus S, ten Bosch JJ, Angmar-Mansson B. A new method for *in vivo* quantification of changes in enamel caries with laser fluorescence. Caries Res 1995;29:2-7.

43. Hafström-Björkman U, Sundström F, de Josselin de Jong, E., Oliveby A, Angmar-Mansoon B. Comparison of laser fluorescence and longitudinal microradiography for quantitative assessment of *in vitro* enamel caries. Caries Res 1992;26(4):241-7.

44. Emami Z, Al-Khateeb S, de Josselin de Jong, E., Sundström F, Trollas K, Angmar-Mansson B. Mineral loss in incipient caries lesions quantified with laser fluorescence and longitudinal microradiography. A methodologic study. Acta Odontologica Scandinavica 1996;54:8-13.

45. Ando M, van der Veen MH, Schemehorn BR, Stookey GK. Comparative study to quantify demineralized enamel in deciduous and permanent teeth using laser- and light-induced fluorescence techniques. 2001;35:464-70.

46. Lagerweij M, van der Veen M, Ando M, Lukantsova L, Stookey G. The validity and repeatability of three light-induced fluorescence systems: An *in vitro* study. Caries Res 1999;33:220-6.

47. al-Khateeb S, ten Cate JM, Angmar-Mansson B. Quantification of formation and remineralization of artificial enamel lesions with a new portable fluorescence device. Adv Dent Res 1997;11:502-6.

48. Ando M, Hall AF, Eckert GJ, Schemehorn BR, Analoui M, Stookey GK. Relative ability of laser fluorescence techniques to quantitate early mineral loss *in vitro.* . Caries Res 1997;31:125-31.

49. Pretty IA, Hall AF, Smith PW, Edgar WM, Higham SM. The intra- and inter-examiner reliability of quantitative light-induced fluorescence (QLF) analyses. British Dental Journal 2002;193(2):105-9.

50. Angmar-Månsoon B, Bosch JJ. Quantitative light-induced fluorescence (QLF): A method for assessment of incipient caries lesions. Dentomaxillofacial Radiology 2001;30:298-307.

51. Buchalla W, de Josselin de Jong, E., Ando M, Eggertsson H, Lennon A, Stookey GK. Video repositioning-increased repeatability for QLF caries monitoring method. Journal of Dental Research 2005;80:115.

52. Murphy TC, Willmot DR, Rodd HD. Management of postorthodontic demineralized white lesions with microabrasion: A quantitative assessment. Am J Orthod Dentofacial Orthop 2007;131(1):27-33.

53. Ogaard B, Ten Bosch JJ. Regression of white spot enamel lesions. A new optical method for quantitative longitudinal evaluation in vivo. Am J Orthod Dentofacial Orthop 1994;106(3):238-42.

54. Zero DT. Recaldent™ evidence for clinical activity. Adv Dent Res 2009;21:30-4.

55. Pai D, Bhat SS, Taranath A, Sargod S, Pai VM. Use of laser fluorescence and scanning electron microscope to evaluate remineralization of incipient enamel lesions remineralized by topical application of casein phosphopeptide amorphous calcium phosphate (CPP-ACP) containing cream. J Clin Pediatr Dent 2008;32(3):201-6.

56. Pulido MT, Wefel JS, Hernandez MM, Denehy GE, Guzman-Armstrong S, Chalmers JM, Qian F. The inhibitory effect of MI paste, fluoride and a combination of both on the progression of artificial caries-like lesions in enamel. Operative Dentistry 2008;33(5):550-5.

57. Cai F, Manton DJ, Shen P, Walker GD, Cross KJ, Yuan Y, Reynolds C, Reynolds EC. Effect of addition of citric acid and casein phosphopeptide-amorphous calcium phosphate to a sugar-free chewing gum on enamel remineralization in situ. Caries Res 2007;41(5):377-83.

58. Itthagarun A, King NM, Yiu C, Dawes C. The effect of chewing gums containing calcium phosphates on the remineralization of artificial caries-like lesions in situ. Caries Res 2005;39(3):251-4.

59. Schirrmeister JF, Seger RK, Altenburger MJ, Lussi A, Hellwig E. Effects of various forms of calcium added to chewing gum on initial enamel carious lesions in situ. Caries Res 2007;41(2):108-14.

60. Andersson A, Skold-Larsson K, Hallgren A, Petersson LG, Twetman S. Effect of a dental cream containing amorphous cream phosphate complexes on white spot lesion regression assessed by laser fluorescence. Oral Health Prev Dent 2007;5(3):229-33.

61. Dawes C, Macpherson LM. Effects of nine different chewing-gums and lozenges on salivary flow rate and pH. Caries Res 1992;26(3):176-82.

62. Edmunds DH, Whittaker DK, Green RM. Suitability of human, bovine, equine, and ovine tooth enamel for studies of artificial bacterial carious lesions. Caries Res 1988;22(6):327.

63. Yamaguchi K, Miyazaki M, Takamizawa T, Inage H, Moore K. Effect of CPP-ACP paste on mechanical properties of bovine enamel as determined by an ultrasonic device. Journal of Dentistry 2006;34:230-6.

64. Oshiro M, Yamaguchi K, Takamizawa T, Inage H, Watanabe T, Irokawa A, Ando S, Miyazaki M. Effect of CPP-ACP paste on tooth mineralization: An FE-SEM study. J Oral Sci 2007;49(2):115-20.

65. Tantbirojn D, Huang A, Ericson MD, Poolthong S. Change in surface hardness of enamel by a cola drink and a CPP-ACP paste. J Dent 2008;36(1):74-9.

66. Reynolds EC, Walsh LJ. Additional aids to remineralization of tooth structure. In: Mount GJ. Hume WR, editors. Preservation and restoration of tooth structure. 2nd ed. Brisbane Australia: Knowledge Books and Software; 2005:111-8.

67. Staley RN. Effect of fluoride varnish on demineralization around orthodontic brackets. Seminars in Orthodontics 2008;14(3):194-9.

68. Azarpazhooh A, Limeback H. Clinical efficacy of casein derivatives: A systematic review of the literature. J Am Dent Assoc 2008;139(7):915-24; quiz 994-5.