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In vitro comparison of microabrasion, CPP-ACP, CPP-ACFP and combination therapies on the remineralization of white spot lesions

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IN VITRO COMPARISON OF MICROABRASION, CPP-ACP, CPP- ACFP AND COMBINATION THERAPIES ON THE REMINERALIZATION OF WHITE SPOT LESIONS

TETYANA PARSONS, D.M.D.

Submitted to the College of Dental Medicine of Nova Southeastern University

in Partial Fulfillment of the Requirements for the Degree of

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By

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I certify that I am the sole author of this thesis, and that any assistance I received in its preparation has been fully acknowledged and disclosed in the thesis. I have cited any sources from which I used ideas, data, or words, and labeled as quotations any directly quoted phrases or passages, as well as providing proper documentation and citations. This thesis was prepared by me, specifically for the M.Sc.D degree and for this assignment.

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Dedication

To my loving husband, my wonderful son, my amazing mom and my sister who have supported me throughout my education and my life. Without your generous help, encouragement and support I would not be where I am today. Thank you for helping me to reach my goals!

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Abstract

IN VITRO COMPARISON OF MICROABRASION, CPP-ACP, CPP-ACFP AND COMBINATION THERAPIES ON THE REMINERALIZATION OF WHITE SPOT LESIONS

DEGREE DATE: DECEMBER 2014

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Objectives: To determine whether treatment of demineralized enamel white spot lesions with CPP-ACP paste, CPP-ACFP paste or microabrasion technique decreases lesion depth in vitro. Additionally, to determine whether treatment of demineralized enamel white spot lesions with microabrasion technique in combination with CPP-ACP paste or CPP-ACFP paste decreases lesion depth greater than any of the three techniques alone. **Background:** White spot lesions (WSLs) after the removal of orthodontic appliances remain a problem for clinicians and patients. Previous studies suggest that application of casein phosphopeptide amorphous calcium phosphate

(CPP-ACP) and casein phosphopeptide amorphous calcium fluoride phosphate (CPP-ACFP) may promote enamel remineralization. [1-5] Recently, microabrasion of enamel was proposed as another treatment modality of white spot lesions.[6] A review of literature showed that there was no comprehensive in vitro study that combined microabrasion, casein phosphopeptide amorphous calcium phosphate and casein phosphopeptide amorphous calcium fluoride phosphate for treatment of WSLs. **Methods:** A total of one hundred and twelve bovine incisor teeth were randomly assigned to seven treatment groups:

1. Control 1 (demineralization control)
2. Control 2 (remineralization solution control)
3. CPP-ACP paste
4. CPP-ACFP paste
5. Microabrasion
6. Microabrasion with CPP- ACP paste
7. Microabrasion with CPP-ACFP paste

Teeth in all groups were placed in demineralizing solution for 96 hours to produce artificial caries-like lesions. At the end of the 96 hr period, teeth in Control 1 group were sectioned to establish adequate amount of demineralization. The rest of samples were treated with assigned regimen once a day for 10 days and stored in remineralization solution. At the end of ten days, teeth were sectioned with a hard tissue microtome and observed under polarized microscopy to analyze enamel lesion depth. One-way ANOVA at $\alpha=0.05$ was performed to assess difference in lesion depth between groups followed with *post hoc* Tukey's test. **Results:** Statistical analysis showed a significant difference between groups ($p<0.05$). The greatest decrease in the lesion depth was achieved in CPP-ACFP paste alone and combination of microabrasion with CPP-ACFP. There was no statistically significant difference in mean lesion depth between these two groups. The second largest decrease in lesion depth was achieved with CPP-ACP paste alone and combination of CPP-ACP paste with microabrasion. Again, there was no statistically significant difference in mean lesion depth whether CPP-ACP was used alone or in combination with microabrasion. Microabrasion alone, while statistically significantly superior to the control groups, was the least effective of the above therapeutic options. **Conclusions:** Based on the results from this study, we can conclude that: (1) treatment of WSLs with CPP-ACP paste, CPP-ACFP paste or microabrasion decreases lesion depth in vitro; (2) microabrasion in combination with CPP-ACP paste or CPP-ACFP paste did not decrease lesion depth greater than that observed with either paste technique alone; (3) both CPP-ACP and CPP-ACFP pastes in combination with microabrasion treatments showed greater decrease in lesion depth than microabrasion alone.

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Chapter 1: Introduction

1.1 Enamel Structure

Enamel provides shape and a hard, durable surface for teeth and a protective cap for the dentin and pulp.[2] The enamel thickness varies from 0.1 mm cervically to about 2.0 mm occlusally.[7] Enamel is translucent and varies in color from light yellow to gray-white.[8] Fully formed enamel is composed of approximately 96 % mineral and 4% organic material and water.[8] The inorganic portion of enamel is crystalline and consists mainly of calcium hydroxyapatite, whose stoichiometric formula is $Ca_{10}(PO_4)_6(OH)_2$.[9,10] The apatite in the enamel may also have variations in its structure such as missing calcium ions and hydroxyl groups, extraneous ions such as carbonate, fluoride, sodium and magnesium.[9] Enamel is built from closely packed apatite crystals that are 60-70 nm in width and 25-30 nm in thickness.[8] These crystals are grouped together in bundles of approximately 1000 crystals as enamel rods. The rod's crystals run along the longitudinal axis of the rod.[8] The interrod enamel surrounds rods and its crystals are oriented almost perpendicular to the rod crystals.[7] The crystals in the enamel rods and interrod enamel differ only in their orientation.[8] Enamel also contains a small volume of organic matrix and water, which is contained in the intercrystalline spaces and in a network of micropores opening to the external surface.[8] The intercrystalline spaces are approximately 1-2 nm wide.[7] These intercrystalline spaces and micropores provide communication between oral cavity into dentinal tubules and pulpal tissue.[11] Various ions, fluids and low molecular weight substances can diffuse through semipermeable enamel.[11] The organic matrix of enamel is made from non-collagenous enamel proteins and enzymes.[8] Of the enamel proteins, 90% are a heterogeneous group of proteins known as amelogenins and the remaining 10% consist of nonamelogenins such as enamelin and ameloblastin.[8] Members of the nonamelogenins family are believed to promote and guide the formation of enamel crystals, whereas amelogenins regulate growth in width and thickness of crystals.[12]

1.2 Caries Lesion

Dental caries is a dynamic and multi-factorial disease that involves the interaction between diet, dental plaque containing bacteria, and host factors, such as tooth surface, saliva, and the acquired pellicle.[13, 14] High levels of cariogenic bacteria such as *Streptococcus mutans* and *Lactobacilli* are present in bacterial plaque.[15] When these bacteria metabolize fermentable carbohydrates, they produce organic acids.[13] As result, when dietary carbohydrates become available to acidogenic bacteria, the pH rapidly decreases from a physiologic pH of 7.0 to a pH of less than 5.5, leading to a more than 100-fold increase in hydrogen ion concentration.[14] Hydrogen ions diffuse into the enamel surface micropores and intercrystalline spaces, dissolve enamel minerals, freeing calcium and phosphate ions to move toward the enamel surface and eventually to diffuse out of the tooth.[13] As previously mentioned, the apatite in the enamel may also have variations in its structure. The defects and substitutions in apatite structure have a great effect on solubility of apatite at low pH. Fluoride ions may fill hydroxyl vacancies or displace hydroxyl ions in hydroxyapatite, decreasing its solubility.[9] Carbonate may replace either hydroxyl or phosphate groups while magnesium can replace calcium inside the hydroxyapatite structure.[9] Both carbonate and magnesium increase hydroxyapatite solubility.[9] When enamel is exposed to acids, the most accessible and most soluble material is removed from the periphery of enamel rods, increasing enamel surface porosity permitting further diffusion of acids into the tissue and mineral ion loss from it.[9] This is the demineralization process. Enamel caries are first observed clinically as a "white spot lesion".[13] The lesion appears white because the loss of mineral changes the refractive index of the affected enamel. Frequent and prolonged acid attacks result in further mineral loss from deeper layers and the formation of a subsurface body of the lesion with decreased mineral content, pores of which enlarge, producing mechanical destruction of tissue, or cavitation.[9] The dissolution of calcium phosphates within hydroxyapatite is dependent on whether the supernatant solution is supersaturated or under-saturated with respect to the dissolving mineral.[16]

Enamel carious lesions are not uniform. Using polarized microscopy, Darling described four porosity-related zones in enamel caries lesions.[17] The translucent zone lies at the inner periphery of the lesion and corresponds to 1-2 % of mineral loss.[9, 17] Directly above the translucent zone is the dark zone which corresponds to a 5-10% mineral loss.[9, 17] The body of the lesion lies between the dark and surface zones.[17] The body of the lesion has the largest mineral loss (approximately 25-50%).[9] The surface zone has relatively intact enamel porosity with mineral content similar to that of sound enamel.[9] The relatively intact mineral content of this layer is due to redeposition of mineral dissolved from deeper layers with some contribution of mineral ions from plaque and saliva.[9] As a result, during demineralization, some remineralization occurs at the surface layer of the enamel.[9]

The natural repair response to demineralization is remineralization. Remineralization is defined as the process where calcium and phosphate ions are supplied from a source external to the tooth to promote ion deposition into crystal voids of demineralized enamel to produce net mineral gain.[1] Under normal conditions the tooth is continually bathed in saliva. Human saliva composition varies from person to person.[7] Saliva contains organic components such as proteins, carbohydrates, lipids, enzymes and organic acids as well as inorganic ions such as calcium, phosphorus, sodium, potassium, magnesium and carbonate.[7] Saliva plays several important roles in enamel remineralization.[13] First, bicarbonate, phosphate and peptides in saliva buffer and neutralize acids produced by bacterial fermentation of carbohydrates causing the pH to rise towards neutral.[13] Secondly, saliva can provide minerals that were dissolved during the demineralization challenge. Saliva is usually supersaturated with calcium and phosphate ions and is capable of remineralizing the very early stages of lesion formation, particularly when the fluoride ion is present.[13]

Featherstone described caries progression versus reversal as a delicate balance.[13] When pathologic factors such as large number of bacteria, salivary dysfunction, tooth defects, poor dietary habits, and inadequate oral hygiene outweigh the protective factors that include normal salivary flow, use of antimicrobial and remineralizing agents, effective diet and good oral hygiene, the caries lesion will form and progress.[13, 14] Conversely, if the protective factors outweigh the pathologic factors, the caries process will arrest.[13, 14] This balance may tip either way several times a day.

1.3 White Spot lesions in Orthodontic Patients

Fixed orthodontic treatment can offer many benefits to patients such as improved occlusal function, esthetics, and self-confidence. Unfortunately, the esthetic result of orthodontic treatment is frequently compromised by enamel demineralization around fixed orthodontic appliances, especially on the facial surface of anterior teeth.

Orthodontic appliances make routine oral hygiene more difficult by increasing the number of plaque retention sites and hampering access to tooth surfaces.[18] These changes in oral environment favor an increase in the number of acidogenic bacteria. The level of S. Mutans can increase up to fivefold during orthodontic treatment.[19] With thick plaque around fixed orthodontic appliances, enamel demineralization can be a very rapid process. Visible white spot lesions (WSLs) may form within 4 weeks.[20, 21]As a result, often upon removal of appliances, a patient may present with white, opaque areas of demineralization on the facial surfaces of the teeth. According to a recent study, the prevalence of WSLs is 38% for patients who are 6 months into treatment and 46% for patients who are 12 months into orthodontic treatment. [22] Another study by Boersma *et al* [23] found that 97% of individuals who had orthodontic treatment had one or more decalcified lesions. Depending on the oral environment, these spots can develop into cavities, remain stable for long periods, or heal to a certain extent. Mattousch *et al* conducted follow-up of 370 white spot lesions in 49 post-orthodontic patients and found that after two years of observation, 40% of lesions improved, 15 % worsened, and 45% remained stable.[24] They noted that the magnitude of remineralization was greatest during the first six months following removal of the fixed orthodontic appliances. They concluded that these lesions have limited ability to improve on their own after removal of braces and that additional treatment measures are necessary to enhance their improvement.[24]

An incipient caries lesion, if not treated successfully, may progress to a cavitated lesion that requires conventional surgical restorative procedures. Restorations have a limited lifespan and every time a restoration is replaced, the preparation is enlarged and more tooth structure is removed.

The goal of WSL treatment is to attempt to remineralize the enamel surface, prevent disease progression, and improve esthetics. Current conservative treatment options that attempt to supply ions needed for remineralization include fluoride application, use of casein phosphopeptide amorphous calcium phosphate (CPP-ACP) or casein phosphopeptide amorphous calcium fluoride phosphate (CPP-ACFP) pastes, or microabrasion.

1.4 Fluoride Treatment

The application of fluoride containing dentifrices and mouthrinses has been demonstrated to decrease caries occurrence.[25] Fluoride has several mechanisms of action that aid in preventing dental decay. Fluoride in plaque interferes with bacterial metabolism of carbohydrates by inhibiting enolase, an enzyme necessary for fermentation.[26] As result, oral bacteria have a decreased-acid production.[26] Fluoride ions in plaque can also promote the formation of fluorapatite in the presence of calcium and phosphate ions. This is believed to be the major mechanism of fluoride action in preventing enamel demineralization.[27] In addition, fluoride ions can promote remineralization of previously demineralized enamel if salivary or plaque calcium and phosphate ions are available. [28] For every 2 fluoride ions, 10 calcium and 6 phosphate ions are required to form one unit of fluorapatite.[28] With topical application of fluoride, the availability of calcium and phosphate ions can be a limiting factor in enamel remineralization.[28] The protective mechanisms of fluoride on erupted teeth require frequent exposures to low levels of fluoride in order to achieve and maintain adequate concentrations of the ion in dental plaque and enamel.

Although low levels of topical fluoride show many beneficial properties, excessive amounts may be associated with rapid mineral precipitation on the enamel surface, subsequent obliteration of surface enamel pores, and formation of a hypemineralized surface layer that prevents further subsurface remineralization of the enamel lesion.[14, 20] This process results in an arrestment of the lesion without significantly improving subsurface mineral content and esthetic appearance.[4, 7, 14, 28, 29] High amounts of topical fluoride also have the potential to be ingested, increasing the amount of systemic fluoride intake. Systemic fluoride can be incorporated into developing tooth enamel crystallites and bone.[30] The main adverse effects associated with systemic, excess fluoride intake are enamel and skeletal fluorosis, especially in children under eight years of age.[30] Based on a lowest-observed-adverse-effect level of 0.10 mg/kg/day for enamel fluorosis, a Tolerable Upper Intake Level of 0.10 mg/kg/day was established for infants, toddlers, and children through 8 years of age.[30]

1.5 CPP-ACP and CPP-ACFP Treatment

Casein is the major protein found in milk.[31] Casein has the ability to stabilize calcium and phosphate ions and prevent their precipitation.[31] Stabilization of calcium and phosphate ions ensures that these ions remain bioavailable, allowing remineralization of the enamel lesion, while preventing surface deposition in the form of calculus. This benefit of casein protein resides in a protein sequence that can be released as small casein phosphopeptides.[32] These casein phosphopeptides (CPP) contain a cluster of phosphoseryl residues in the motif –Ser(P)-Ser(P)-Ser(P)-Glu-Glu-, which increase the solubility of calcium phosphate.[32] The casein phosphopeptide phosphoseryl residues, within CPP-ACP and CPP-ACFP complexes stabilize calcium, phosphate and fluoride ions preventing transformation into crystalline phases and maintaining a high concentration of these ions, thereby generating large concentration gradients that favor diffusion of these ions down the concentration gradient into enamel porosities.[33] The CPP-ACP and CCP-ACFP clusters attach themselves to dental plaque and tooth surfaces.[34] Under acidic conditions, the attached CPP-ACP and CCP-ACFP complexes release calcium and phosphate ions, thus maintaining a supersaturated mineral environment, which inhibits enamel demineralization and enhances remineralization.[34] Once CPP-ACP or CPP-ACFP present inside of the lesion, they release weakly bound calcium and phosphate or calcium, phosphate and fluoride ions, respectively, which then deposit into crystal voids and form hydroxyapatite or fluorhydroxyapatite.[3] This finding led to development of the remineralization technology based on casein phosphopeptide amorphous calcium phosphate (CPP-ACP and CPP-ACFP).

The capacity of CPP-ACP in promoting enamel remineralization was first demonstrated in animal caries models in 1995 by Reynolds *et al* [32]. Since then the remineralization of enamel decalcified lesions with CPP-ACP complexes has been extensively studied with conflicting results. The *in situ* remineralization study by Reynolds *et al.*[2] found that a dentifrice containing 2% CPP-ACP achieved superior remineralization to a dentifrice containing 1100 ppm fluoride, and produced a level of remineralization similar to that achieved with a 2800-ppm fluoride dentifrice. The greatest amount of remineralization was achieved with a dentifrice containing 2% CPP-ACP plus 1100 ppm fluoride.[2]

The clinical trial of Andersen *et al.* [35] compared a CPP-ACP regimen with a conventional fluoride program for efficacy of inducing regression of early enamel lesions. They found the CPP-ACP regimen produced better esthetic regression of WSLs compared to a fluoride program when assessed visually.[35] However, when treatment outcomes were assessed with the laser fluorescence, DIAGNOdent, no differences between the treatment regimes were found.[35] Both regimens promoted regression of WSLs.[35]

In another *in situ* randomized clinical trial by Chi *et al.*[36] subjects were randomly assigned to four treatment groups: a lozenge containing 56.4 mg of CPP-ACP, a lozenge containing 18.8 mg of CPP-ACP, a lozenge without CPP-ACP, and a no lozenge control group. Subjects in each group wore removable palatal appliances that contained human enamel slab inserts with subsurface lesions.[36] Lozenges were consumed four times per day for 14 days. After 14 days, the enamel slabs were removed and microradiography was used to analyze enamel remineralization. They found that lozenges containing 18.8mg CPP-ACP and 56.4mg CPP-ACP significantly increased enamel subsurface lesion remineralization by 78 and 176 percent, respectively, relative to the controls.[36]

Morgan *et al.*[37] investigated the progression and regression of caries lesions using digital radiography and visual-tactile examination in adolescent subjects who chewed a sugar-free gum containing CPP-ACP, compared to subjects who chewed the identical gum without CPP-ACP. The CPP-ACP sugar-free gum significantly slowed progression and enhanced regression of caries lesions relative to the control sugar-free gum in this 24-month trial.[37]

In another clinical trial, Bailey *et al* [4] investigated regression of WSLs in post-orthodontic patients with and without a CPP-ACP regimen. The severity of white-spot lesions was quantified according to the International Caries Detection and Assessment System II (ICDAS II) criteria.[4] They found that over a twelve-week period, significantly more post-orthodontic WSLs regressed with the remineralizing cream compared with placebo.[4]

An in vitro remineralization study by Ogata *et al.*[5] investigated enamel remineralization with a fluoride regimen, a CPP-ACP regimen, or a combination of CPP-ACP with fluoride. As assessed by polarized light microscopy and microradiographs, neither the CPP-ACP nor the fluoride regimen alone were successful in remineralizing WSLs.[5] However, combined treatment resulted in significant remineralization of artificially induced WSLs.[5]

In a prospective, randomized clinical study, Beerens *et al.*[38] compared a fluoride-containing CPP-ACP paste with a control past and found no advantage for the fluoridated CPP-ACP paste over regular oral hygiene in WSL regression as measured by Quantitative light-induced fluorescence (QLF).[38]

Similarly, Brochner *et al.,*[39] in a prospective clinical trial using non-fluoridated CPP-ACP paste found, after a four week treatment measured with QLF, that WSLs regression was comparable to that observed with traditional toothpaste.

The conflicting results of these studies may be related to the different methods of assessing WSLs regression.

In the U.S., the Food and Drug Administration (FDA) has approved GC Corporation products MI Paste and MI Paste Plus (GC Corporation, Tokyo, Japan), based on CPP-ACP and CPP-ACFP technology, respectively, for the following indications: cleaning and polishing procedures as part of a professionally administered prophylaxis treatment, management of tooth sensitivity, and dental hypersensitivity.[40] Both MI Paste and MI Paste Plus contain approximately 10% CPP-ACP molecule by weight. In addition, MI Paste Plus contains 900 parts per million (ppm) of sodium fluoride (0.2%). This level of fluoride is similar to the amount of fluoride in over-the-counter toothpaste. Currently, these products are not FDA approved for the treatment of WSLs and are a subject for further extensive dental research.

1.6 Microabrasion Treatment

Recently, microabrasion of enamel was proposed as another treatment modality for white spot lesions.[6] It was reported that acid-pumice abrasion can successfully remove white enamel opacities, multicolored defects, and many brown, orange, or yellow enamel spots and streaks if the stain is limited to a thin layer of the tooth enamel surface no more than 100 μm deep.[41, 42] The original microabrasion technique included an application of acidic and abrasive material to the enamel surface, followed by subsequent enamel polishing.[41] The chief mechanism of microabrasion requires limited enamel abrasion, rather than enamel dissolution by acid. [41] Each application is estimated to cause a total loss of enamel surface of approximately 10 μm.[42] The procedure can be repeated up to a maximum of ten applications. If the stain or defect is present after ten applications, the stain or defect is too deep for the procedure.[42] Microabrasion creates a smooth, polished surface layer through deposition and compaction of calcium and phosphate breakdown products.[43] In an in vitro study, Segura *et al.*[43] investigated the effect of microabrasion on enamel demineralization and found that enamel surfaces that were microabraded and treated with 1% neutral sodium fluoride were more resistant to demineralization than enamel surfaces that were treated with fluoride or microabrasion alone.

Murphy *et al.*[44] first proposed the microabrasion technique for treatment of WSLs. They examined the visible change in the WSL surface area following microabrasion of WSLs in eight postorthodontic patients and noted that the microabrasion technique significantly reduced visible enamel WSLs with a mean reduction in lesion size of 83%.

Ardu et al.[6] recently described a modified technique in which microabrasion was combined with the application of a CPP-ACP cream for the treatment of WSLs in postorthodontic patients. They suggested that microabrasion removes the hypermineralized superficial layer of enamel, and when followed by daily home application of CPP-ACP, could help to remineralize and eliminate WSLs without invasive restorative procedures.[6] In this modified technique, microabrasion reactivates superficial enamel by mechanical and chemical abrasion, increasing porosity of the enamel lesion allowing better penetration of ions required for remineralization.[1]

Pliska *et al.*[18] examined the effects of applying microabrasion and CPP-ACP paste on the remineralization of WSLs using quantitative light-induced fluorescence and found that microabrasion treatment, with or without CPP-ACP, improved the fluorescence and thus reduced WSLs.

1.7 Purpose

The high prevalence of post-orthodontic WSLs is a significant clinical problem. Theses incipient caries lesions, if not treated successfully, may progress to cavitated lesions that require conventional surgical restorative procedures.

A review of the literature showed that there was no comprehensive, in vitro study that combined microabrasion, CPP-ACP, and CPP-ACFP for the treatment of WSLs. Previous in vitro and in vivo studies used a combination of microabrasion with casein phosphopeptide amorphous calcium phosphate only.[6, 18] The purpose of this in vitro study is to compare microabrasion, CPP-ACP, CPP- ACFP and combination therapies on the remineralization of WSLs as evidenced by a decrease or increase in lesion depth, as analyzed by polarized light microscopy.

Most of the previous remineralization studies used light-induced fluorescence, digital photography or clinical examination for assessment of treatment outcomes. Conflicting results from these studies may be related to the methods of assessment. Light-induced fluorescence is reported to have greater sensitivity to detect surface changes and lower sensitivity to detect subsurface changes in mineral content.[45] Both digital photography and light-induced fluorescence can be affected by imaging geometry, tooth hydration, enamel thickness and the surrounding lighting.[45] Clinical visual examination is highly subjective and varies from one examiner to another. Polarized light microscopy (PLM) will be used for quantitative examination of the carious lesion depth in this study. PLM has been widely used for histologic examination of early enamel lesions.[17, 46] In fact, most of the descriptive information on caries enamel has arisen from studies utilizing polarized light.[46] When lesion depth is used as the main measurement of demineralization and remineralization, PLM is a highly sensitive method and can give an accurate measurement of lesion depth even when it is difficult to determine the outer surface of the lesion under Transverse Microradiography (TMR).[47]

The results of this study may provide important information about the remineralization potential of investigated treatment modalities and help to improve the treatment protocol for WSLs. Successful remineralization of incipient lesions will prevent further progression of the disease, restore enamel hardness and smoothness, and improve a patient's oral health and esthetics.

1.6 Specific Aims and Hypotheses

1.6.1 Specific Aims

I. To determine whether treatment of demineralized enamel white spot lesions with CPP-ACP paste, CPP-ACFP paste or microabrasion technique decreases lesion depth in vitro.

II. To determine whether treatment of demineralized enamel white spot lesions with a microabrasion technique in combination with CPP-ACP paste or CPP-ACFP paste decreases lesion depth greater than any of the three techniques alone.

1.6.2 Hypotheses

I. Ho: The null hypothesis is that there will be no difference in lesion depth between these groups and the control group.

II. Ho: The null hypothesis is that there will be no difference in lesion depth when microabrasion is combined with CPP-ACP or CPP-ACFP pastes or when these three techniques are used alone.

1.7 Location of Study

The design, preparation, data collection, and analyses for this study took place at:

Bioscience Research Center

Nova Southeastern University

College of Dental Medicine

3200 South University Drive

Fort Lauderdale, FL 33328

Chapter 2: Materials and Methods

2.1 Study

Based on a power analysis, one hundred twelve bovine incisor teeth were collected for this in vitro study.

2.1.1 Ethical Issues

No potential ethical issues could be identified as part of this research study.

2.1.2 Grant

This study was awarded a grant by the Health Professions Division at Nova Southeastern University.

2.2 Sample

In the present study, bovine teeth were used as a substitute for human teeth. Using human teeth in the study has several disadvantages. They often have defects from natural caries challenges or other causes, and they usually are of a variable age and source, which gives them a variable composition leading to larger variations in treatment or test response. Also, they usually do not have a large flat surface that is desirable for experimental manipulation. Bovine incisor enamel has a composition that is less variable than that of human enamel, and is thought to result in a more consistent experimental response. [48] Featherstone *et al* [49] demonstrated that even though the rate of progression of an artificial carious enamel lesion is faster in bovine than human teeth, the subsurface lesion characteristics in bovine enamel are similar to natural caries lesions in human teeth. Edmunds *et al* [50] used both bacterial cultures and acid gel systems to study artificial caries formation in bovine enamel. The mineral distribution in the lesions of bovine teeth was similar to that in human teeth, and structural changes in human and bovine enamel were also similar.[50] The overall difference in response to cariogenic challenges and anti-caries agents between bovine and human enamel appears to be mainly one of degree with slightly faster lesion formation in bovine teeth.[48, 49]

2.3 Sample Size

Sample size calculations were based on previous studies similar to the proposed study.[18, 51] The in vitro study of Pliska *et al*[18] investigated the effects of an application of CPP-ACP paste and microabrasion treatment on the regression of WSLs. The treatment protocol had four treatment groups with sixteen bovine enamel slabs per group. A total of 64 enamel slabs were used. There was a statistically significant ($P < 0.05$) gain in mineral content associated with the microabrasion only, as well as with microabrasion and CPP-ACP.[18]

In the study by Akin *et al*[51] the power analysis was established by G*Power version 3.0.10 software. Based on the 1:1 ratio between groups, a sample size of 20 participants per group gave more than an 80% power to detect significant differences with a 0.40 effect size and at the α .05 significance level.[15]

The results of that study are presented in the table bellow. Based on the data provided, we used one-way ANOVA post hoc test to calculate the achieved power.

Table 4. Descriptive Statistics and Statistical Comparison of Success Rate (%)

Group	Age, Year	n	Mean	SD	Range	Post Hoc Tukey*
Control	14.4 ± 2.1	20 (208 teeth)	0.45	0.10	0.31–0.58	A
Fluoride	14.5 ± 2.1	20 (282 teeth)	0.48	0.07	0.38–0.60	A
CPP-ACP	14.4 ± 1.7	20 (212 teeth)	0.58	0.11	0.40–0.80	B
Microabrasion	14.6 ± 2.0	20 (264 teeth)	0.97	0.02	0.88–1.00	C

* $P < .05$.

Based on the means provided, the number of groups (4), the teeth per group (20) and the SD, the effect size was calculated. Effect size = 1.9783. The post hoc analysis of achieved power =1.00000 or 100%.

As in the above study we used 80% power to detect significant differences with a 0.40 effect size at the α= 0.05 significance level. Our study has seven experimental groups. Using one-way ANOVA, A piori test we calculated the total sample size needed to be 105 teeth, or 15 teeth per group. We used one hundred twelve bovine teeth, or 16 teeth per treatment group, to accommodate for possible loss of teeth during the study due to damage.

2.4 Sampling Method

A total of one hundred twelve (112) freshly extracted, caries free bovine incisor teeth without cracks or erosions were collected. Teeth were cleaned and stored in deionized water until ready for use. A systematic sampling method was used to assign teeth (n=16) to seven treatment groups as follows:

1. Control 1 (demineralization control)
2. Control 2 (remineralization solution control)
3. CPP-ACP paste (MI Paste, GC Corporation, Tokyo, Japan)
4. CPP-ACFP paste (MI Paste Plus, GC Corporation, Tokyo, Japan)
5. Microabrasion
6. Microabrasion with CPP- ACP paste (MI Paste, GC Corporation, Tokyo, Japan)
7. Microabrasion with CPP-ACFP paste (MI Paste Plus, GC Corporation, Tokyo, Japan)

Systematic sampling involves a random start and then proceeds with the selection of every n^{th} element from then onwards. Teeth from each animal were arranged in individual rows. Numbers 1,2,3,4,5,6 and 7 corresponded to each individual treatment group. At the random starting point on the line of arranged teeth, we counted from 1 to 7 until all teeth from one animal were assigned to seven different treatment groups. The same process was repeated for all other teeth from different animals. Teeth from the same animal were randomly placed into distinct treatment groups to reduce variability between groups. As result each treatment group had 16 bovine teeth (Figure 1).

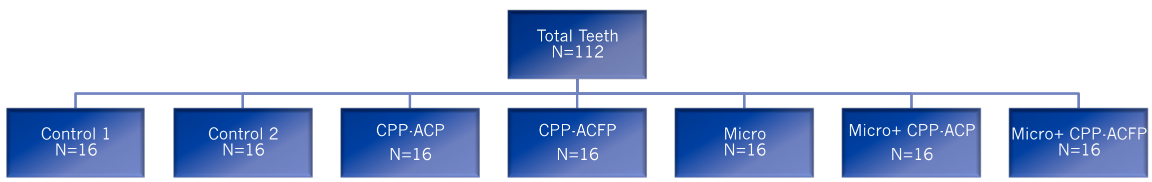

Figure 1. Flow chart of the division of the total sample into treatment groups by random assortment.

2.5 Sample Inclusion Criteria

A total of one hundred twelve (112) freshly extracted, caries free bovine incisor teeth without cracks or erosions were collected.

2.6 Sample Exclusion Criteria

Teeth with caries, attrition, hypomineralized lesions, external and internal staining, microcracks, or extraction damage were excluded from the study. Teeth that became damaged during the study were also excluded.

2.7 Sample Preparation

The buccal surfaces of all selected teeth were cleaned with a non-fluoridated pumice and water paste (Whip Mix, Louisville, KY, USA), followed by a thorough rinse with distilled water. Each tooth was then coated with a thin layer of acid-resistant varnish (Revlon Red, New York, USA) to isolate 2.0 x 4.0 mm window of exposed enamel in approximately the center of the buccal surface (Figure 2).

Figure 2 Teeth painted with acid-resistant varnish to produce a 2x4 mm window of exposed enamel.

The varnish was allowed to dry, and then, in order to create a WSL, teeth in each treatment group were soaked for 96 hours at 37°C in a demineralizing solution[52], which contained 2.2 mM $CaCl_2$, 2.2 mM KH_2PO_4, 0.05 M acetic acid and had the pH adjusted to 4.4 with 1 M KOH. A low fluoride concentration of 0.5 parts per million was added to the demineralization solution to prevent an erosive defect or direct cavitation.[27, 53] The low fluoride concentration helps to preserve the enamel surface layer in artificially induced enamel subsurface lesions.[27, 53]

2.8 Experiment

After the 96 hour demineralizing period, sixteen teeth in Control 1 group were sectioned prior to beginning the ten-day treatment study in order to establish if an adequate amount of demineralization had occurred. This group was a positive control to verify WSL formation. An average of 75 microns WSL depth was obtained. The remaining ninety-six teeth in next six treatment groups were subjected to treatment regimens that were previously determined based on random assignment. Treatment groups were as follows:

1. Control 2 group (remineralization solution control). 16 teeth with artificial WSLs were rubbed with a cotton swab and deionized water for 20 seconds twice a day for 10 days.
2. CPP-ACP group. Direct application of CPP-ACP paste (MI Paste) with microbrush for 20 seconds daily for 10 days (Figure 3).
3. CPP-ACFP group. Direct application of CPP-ACFP paste (MI Paste Plus) with microbrush for 20 seconds daily for 10 days (Figure 3).
4. Microabrasion group. At the start of treatment period, a 2-minute 37% phosphoric acid etch (Vista Dental, Racine, WI.), followed by water rinse and 20 seconds pumice rub with a microbrush. After this initial treatment, deionized water was rubbed with a microbrush for 20 seconds daily for 10 days (Figure 4).
5. Microabrasion + CPP-ACP group. At the start of the treatment period, a 2-minute 37% phosphoric acid etch (Vista Dental, Racine, WI.), followed by water rinse and 20 seconds pumice rub with a microbrush. After this initial treatment direct application of CPP-ACP paste (MI Paste) with microbrush for 20 seconds daily for 10 days (Figure 5).
6. Microabrasion + CPP- ACFP group. At the start of the treatment period, a 2-minute 37% phosphoric acid etch (Vista Dental, Racine, WI.), followed by water rinse and 20 seconds pumice rub with a microbrush. After this initial treatment

direct application of CPP-ACFP paste (MI Paste Plus) with microbrush for 20 seconds daily for 10 days (Figure 5).

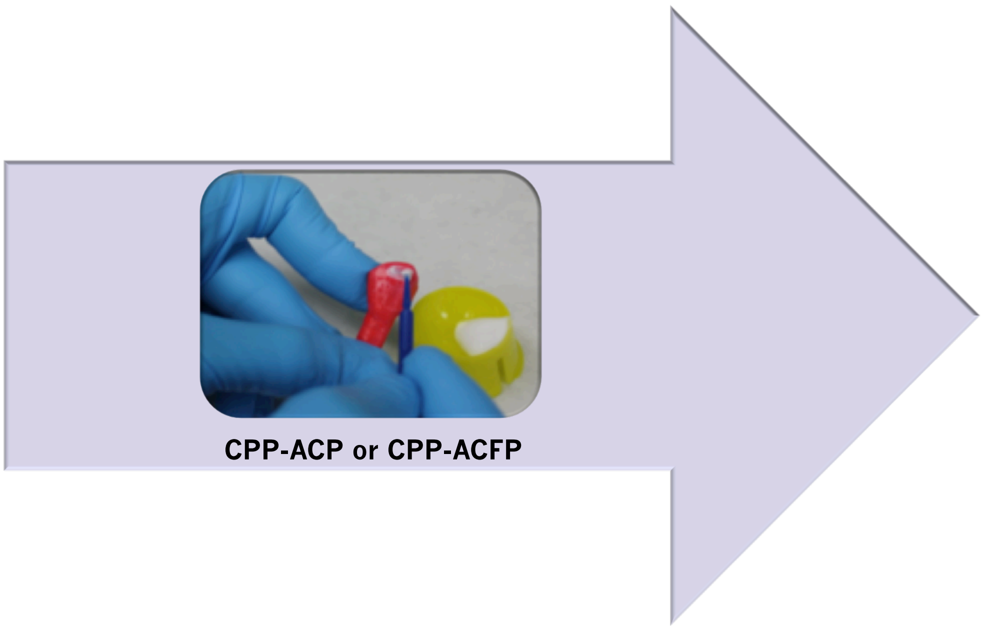

Figure 3. CPP-ACP or CPP-ACFP Treatment

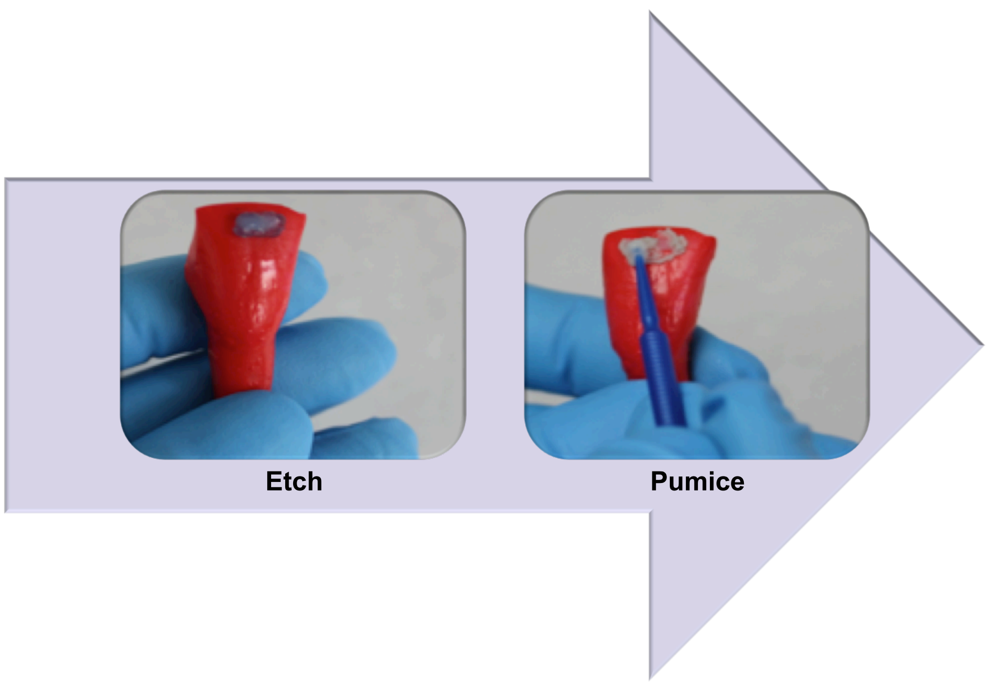

Figure 4. Microabrasion Treatment

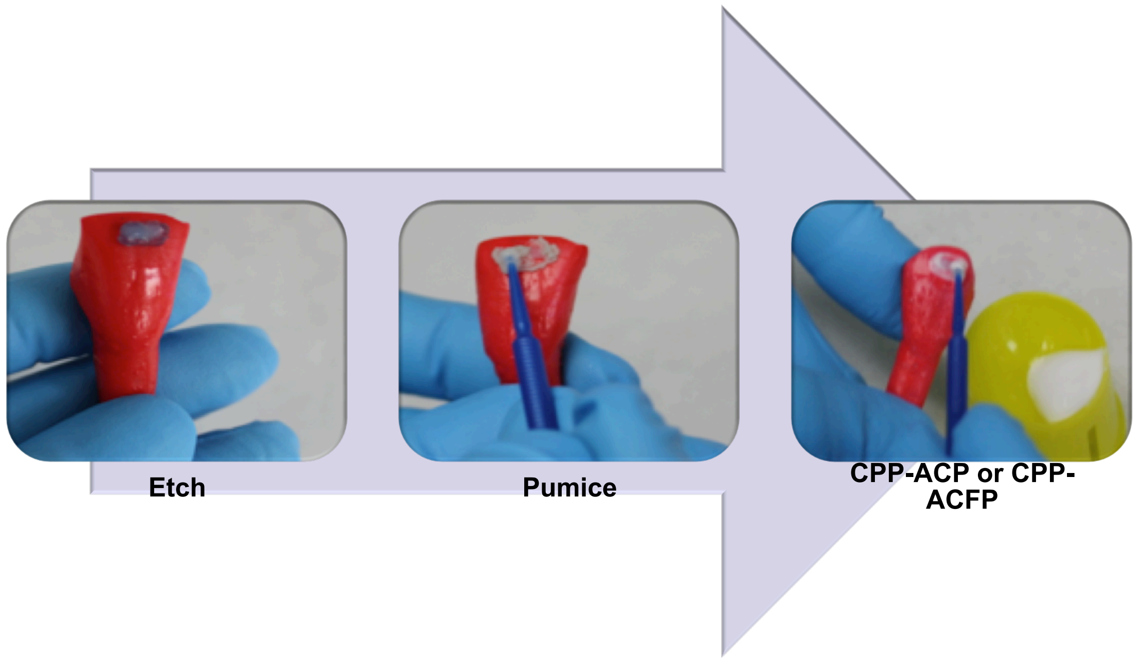

Figure 5 Microabrasion and CPP-ACP or CPP-ACFP Treatment

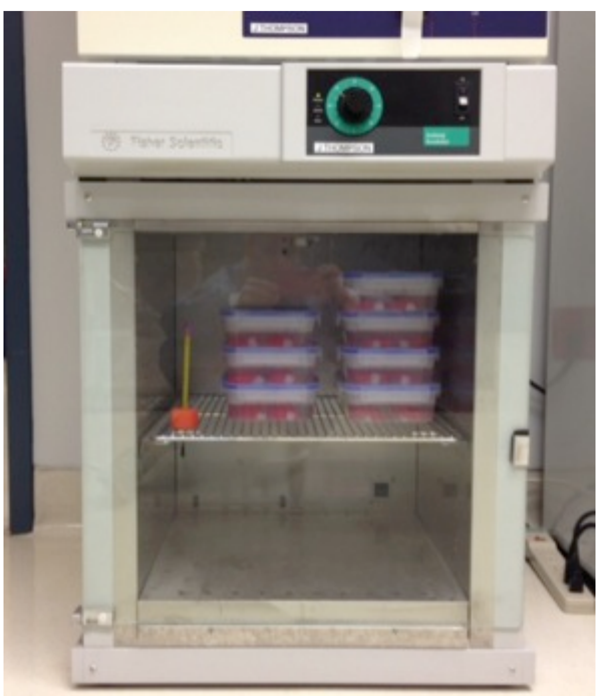

Figure 6. Teeth stored at 37^{o}C.

Between treatments, teeth were stored at 37°C in a remineralizing solution containing 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 0.15 M KCL, at a pH of 7.0 (Figure 6). This solution approximates the saturation of minerals found in saliva and was similar to that utilized by ten Cate and Duijsters.[54]

At the end of the treatment period (10 days), each tooth was cut and fitted into a metal mandrel in such a way that once the tooth was sectioned and analyzed under polarized microscopy, we would be able to observe the WSL window with intact enamel incisal and gingival to the lesion. Dental sticky wax was used to mount and secure the tooth inside the mandrel (Figure 7). The mandrel was attached to the hard tissue microtome (Series 1000 Deluxe Silverstone-Taylor hard-tissue microtome, Scientific Fabrications, Littleton, CO, USA) (Figure 8). The microtome was used to section all enamel slabs bucco-lingually perpendicular to the tooth surface to obtain sections approximately 100-140um in width (Figure 9).

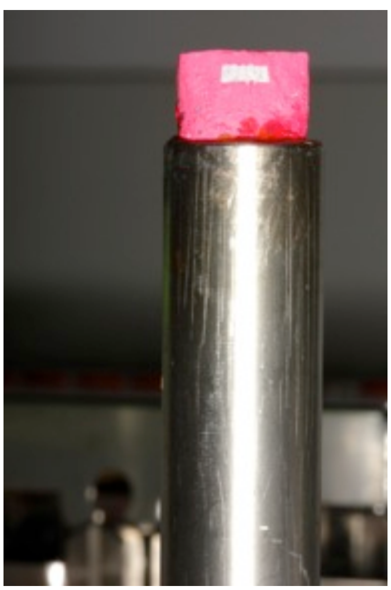

Figure 7. Tooth mounted into a specially designed metal mandrel

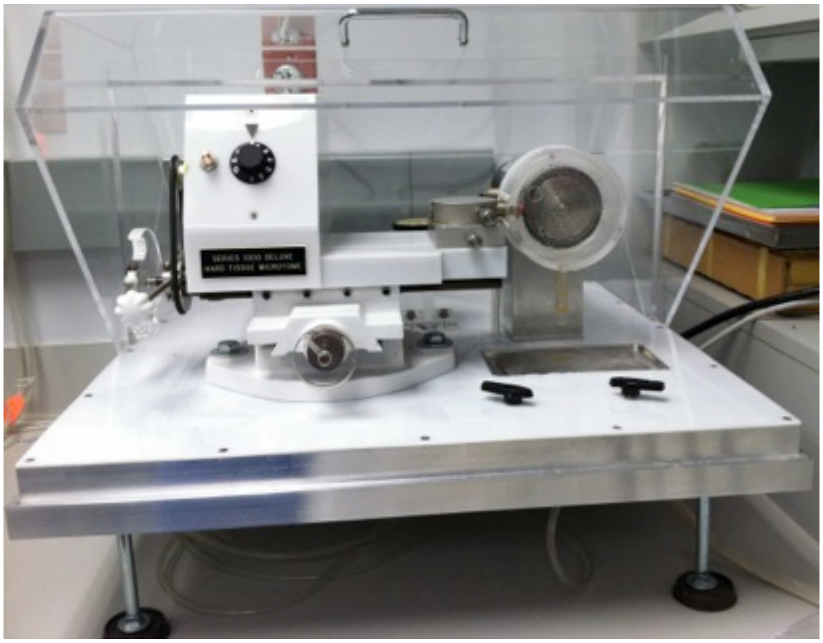

Figure 8 Hard tissue microtome used to obtain longitudinal sections bucco-lingually of all teeth

Figure 9. Tooth sectioned bucco-lingually perpendicular to the tooth surface

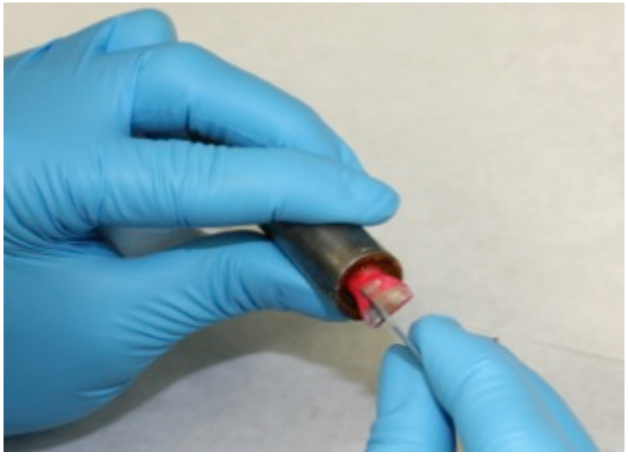

Figure 10. Sections separated for viewing under polarized light microscope.

The sections were separated and washed with deionized water and oriented on glass slides with a cover slip (Figure 10). They were then, imbibed with deionized water (refractive index = 1.33) and viewed under polarized light microscopy (Olympus, Pittsburgh, PA, USA) to analyze the enamel lesions depth. Three sections of each tooth were evaluated for lesions and images were captured with Olympus MicroSuite™ Basic imaging software (Olympus, Melville, NY, USA and Soft Imaging System Corp., Lakewood, CO, USA). Five measurements (D_1, D_2, D_3, D_4, and D_5) for each section from the surface to the bottom of the lesion were measured (Figure11). The measurements that were taken were two at the borders, one in the middle, and two between the borders and the middle. For each tooth, fifteen measurements of lesion depth were recorded. The reliability of the measurements was verified by remeasuring lesions' depth in 10% of the sample.

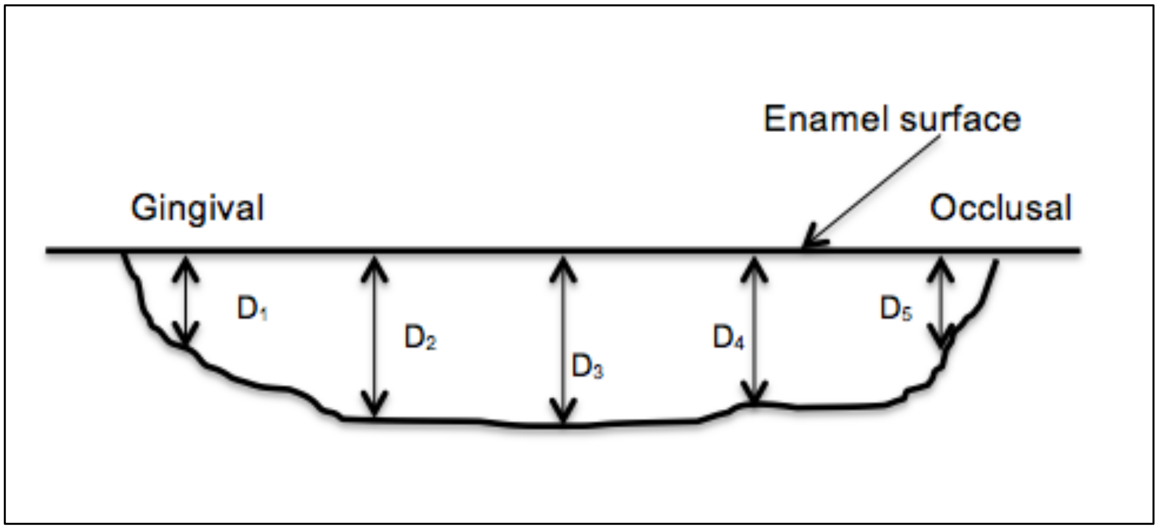

Figure11. Diagram of longitudinal section of demineralized lesion and the five depth measurement lines

2.9 Data Storage

The de-identified data was entered and stored on excel spreadsheets on a password protected computer.

2.10 Statistical Analyses

Descriptive statistics, means, and standard deviations were calculated for all seven groups' lesion depth measurements. The mean difference in lesion depth between untreated demineralized controls, remineralization solution controls, and treatment groups was measured with one-way analysis of variance (ANOVA) at $\alpha=0.05$. Tukey's test was used to find means that are significantly different from each other. The results of the proposed study were organized into tables and supplemented with bar graphs for visualization.

Chapter 3: Results

Descriptive statistics, means, and standard deviations were calculated for all seven groups lesion depth measurements and summarized in Table 1.

Group	N	Mean (μm)	SD	Min (μm)	Max (μm)
Control 1	240	75.86	13.05	32.26	123.37
Control 2	240	70.09	14.34	35.11	115.04
CPP-ACFP	240	55.56	13.40	16.51	87.06
CPP-ACP	240	60.00	14.33	6.31	98.09
Micro	240	63.85	12.98	24.76	96.78
Micro+ CPP-ACP	240	59.31	13.49	3.63	95.29
Micro+CPP-ACFP	240	54.25	15.27	5.41	88.16

Table 1. Descriptive Statistics

Control 1 group (demineralization control) established baseline mean lesion depth of artificially created WSLs. The mean lesion depth created was 75.86 μm. Teeth in all treatment groups were stored in remineralization solution between treatments. This solution approximated the saturation of minerals found in saliva. Since saliva is usually supersaturated with calcium and phosphate ions, it contributes to the remineralization of early enamel carious lesions. Control 2 group (remineralization solution control) was used to determine mean lesion depth of teeth stored in remineralization solution without treatment. The mean lesion depth of Control 2 group was 70.09 μm.

Statistical analysis showed a significant difference between all groups ($p<0.05$) when compared to Control 2 group. There was a statistically significant difference in lesion depth between Control 1 and Control 2 groups. Control 1 group had 5.78 μm deeper lesion than Control 2 group, indicating that a small amount of lesion remineralization occurred in remineralization solution while teeth were stored between treatments. In addition, teeth in all treatment groups had mean lesion depth that were statistically significantly smaller than in Control 2 group (Table 2, Figure 12, Figure 13).

			Diff	Lower 95% CI	Upper 95% CI	P-Value
CPP-ACFP	vs.	Control 2	-14.53	-17.01	-12.05	0.000
CPP-ACP	vs.	Control 2	-10.09	-12.57	-7.61	0.000
Control 1	vs.	Control 2	5.78	3.29	8.26	0.000
Micro	vs.	Control 2	-6.24	-8.72	-3.76	0.000
Micro+ CPP-ACP	vs.	Control 2	-10.78	-13.26	-8.30	0.000
Micro+CPP-ACFP	vs.	Control 2	-15.84	-18.32	-13.36	0.000

Table 2. Tukey HSD Contrasts against Control Group Two

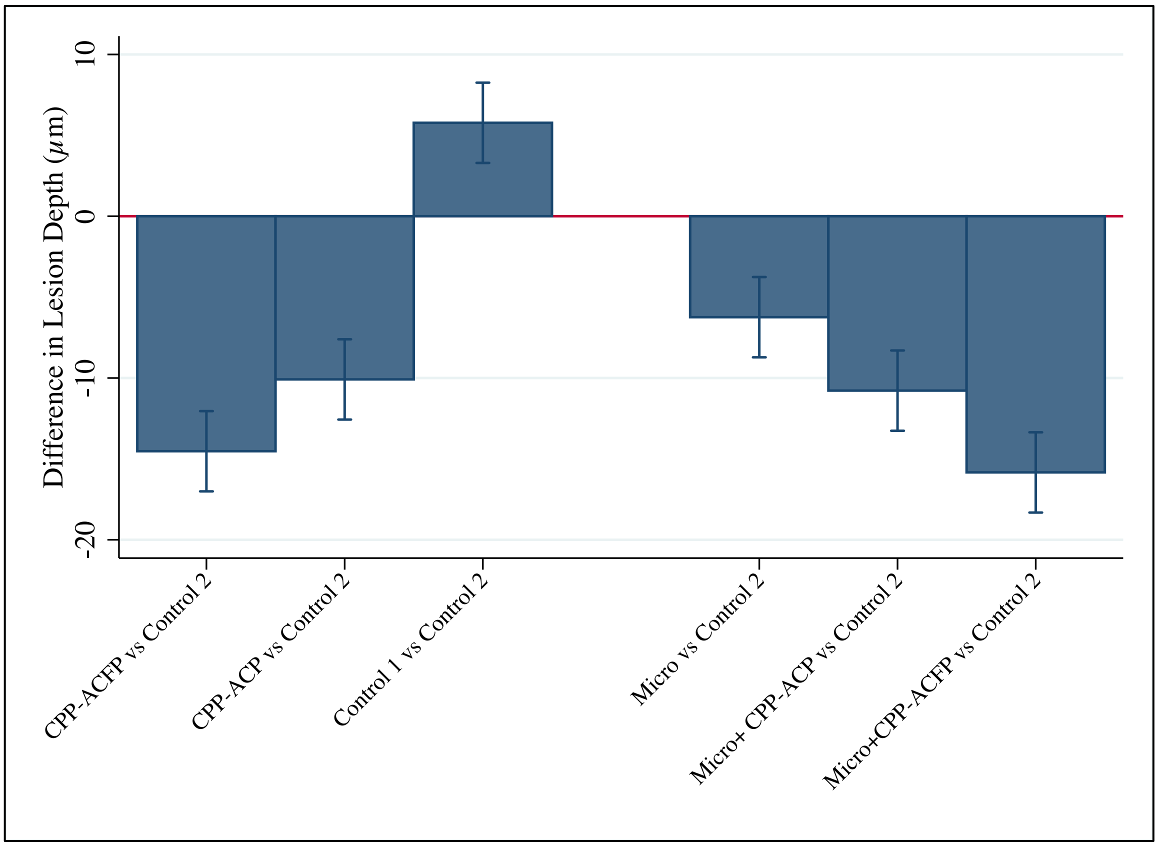

Figure 12. Linear Contrasts vs. Control Group 2.

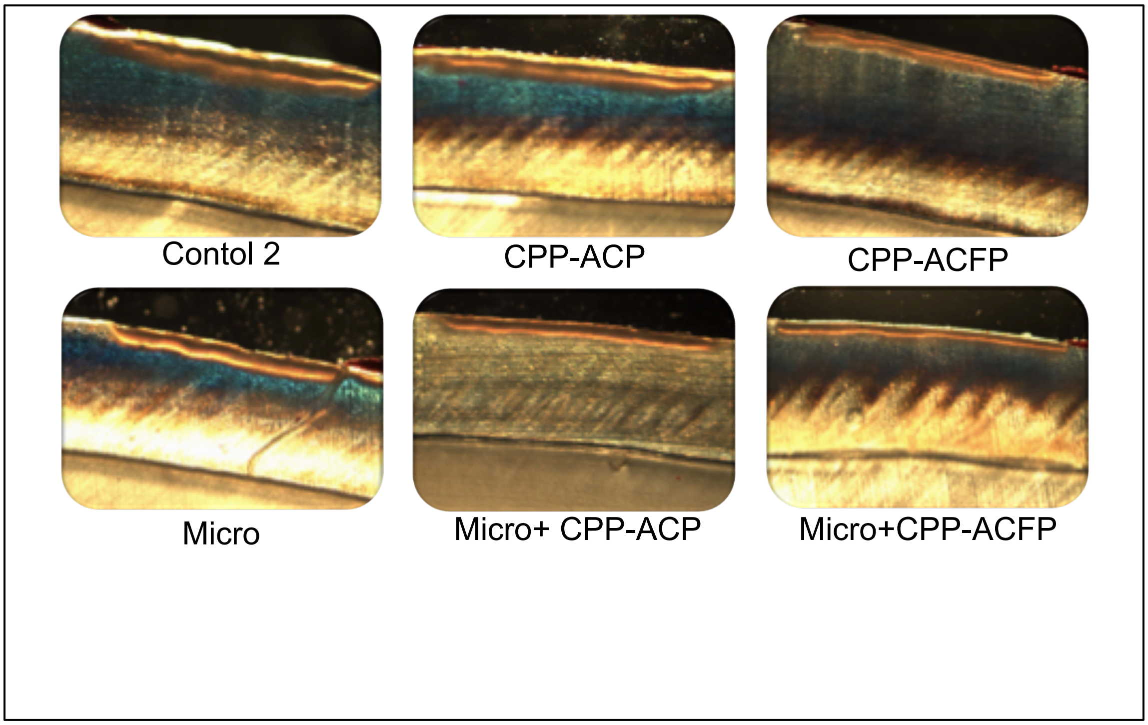

Figure 13. Composite of polarized light microscopy images of representative lesions from all treatment groups.

The data summarized in Table 3 and Figure 14 demonstrates that the greatest decrease in lesion depth was achieved in with CPP-ACFP paste alone or the combination of microabrasion with CPP-ACFP. There was no statistically significant difference in mean lesion depth between these two groups. The second largest decrease in lesion depth was achieved with CPP-ACP paste alone or the combination of CPP-ACP paste with microabrasion. Again, there was no statistically significant difference in mean lesion depth whether CPP-ACP was used alone or in combination with microabrasion. Microabrasion alone, while statistically significantly superior to the control groups, was the least effective of the above therapeutic options.

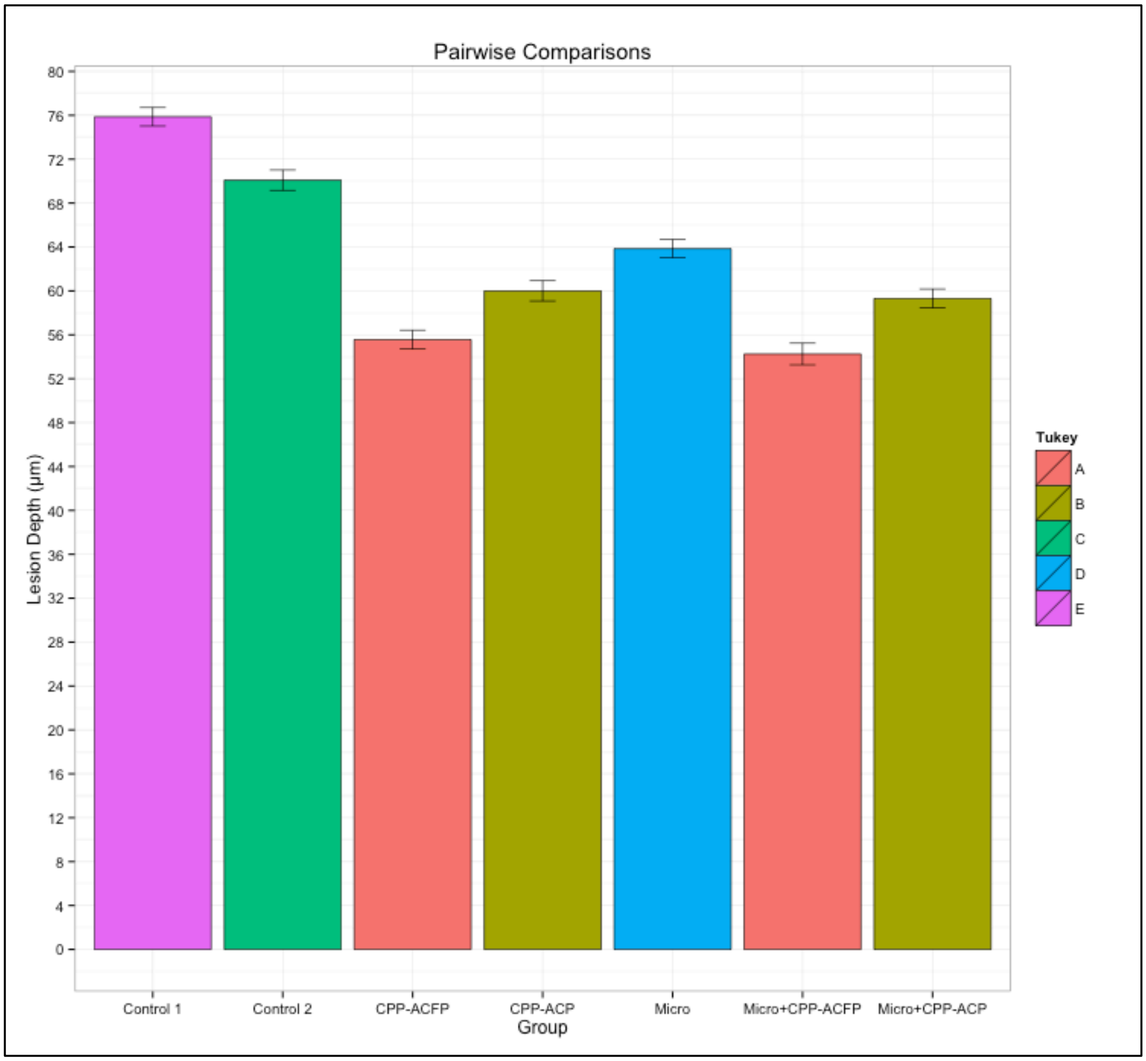

Figure 14. Pairwise Comparisons. Groups not connected by the same letter are significantly different.

			Diff	Lower 95% CI	Upper 95% CI	P-Value
CPP-ACP	vs.	CPP-ACFP	4.44	0.59	8.29	0.010
Control 1	vs.	CPP-ACFP	20.30	16.45	24.15	0.000
Control 2	vs.	CPP-ACFP	14.53	10.68	18.38	0.000
Micro	vs.	CPP-ACFP	8.29	4.44	12.14	0.000
Micro+ CPP-ACP	vs.	CPP-ACFP	3.75	-0.10	7.60	0.065
Micro+CPP-ACFP	vs.	CPP-ACFP	-1.31	-5.16	2.54	1.000
Control 1	vs.	CPP-ACP	15.86	12.01	19.71	0.000
Control 2	vs.	CPP-ACP	10.09	6.24	13.94	0.000
Micro	vs.	CPP-ACP	3.85	0.00	7.70	0.050
Micro+ CPP-ACP	vs.	CPP-ACP	-0.69	-4.54	3.16	1.000
Micro+CPP-ACFP	vs.	CPP-ACP	-5.75	-9.60	-1.90	0.000
Control 2	vs.	Control 1	-5.78	-9.62	-1.93	0.000
Micro	vs.	Control 1	-12.02	-15.86	-8.17	0.000
Micro+ CPP-ACP	vs.	Control 1	-16.56	-20.40	-12.71	0.000
Micro+CPP-ACFP	vs.	Control 1	-21.61	-25.46	-17.76	0.000
Micro	vs.	Control 2	-6.24	-10.09	-2.39	0.000
Micro+ CPP-ACP	vs.	Control 2	-10.78	-14.63	-6.93	0.000
Micro+CPP-ACFP	vs.	Control 2	-15.84	-19.69	-11.99	0.000
Micro+ CPP-ACP	vs.	Micro	-4.54	-8.39	-0.69	0.007
Micro+CPP-ACFP	vs.	Micro	-9.60	-13.45	-5.75	0.000
Micro+CPP-ACFP	vs.	Micro+ CPP-ACP	-5.06	-8.91	-1.21	0.001

Table 3. Tukey HSD Pairwise Comparisons

Reliability was measured using an intra-class correlation (ICC). The ICC was 96% [95% CI: 96% to 97%] indicating consistent intra-rate reliability.

Chapter 4: Discussion

The purpose of this *in vitro* study was to compare microabrasion, CPP-ACP, CPP- ACFP and combination therapies on the remineralization of WSLs as evidenced by a decrease or increase in lesion depth as analyzed by polarized light microscopy. Hypothesis tested:

I. Ho_1: The null hypothesis is that there will be no difference in lesion depth between experimental groups and the control group.

II. Ho_2: The null hypothesis is that there will be no difference in lesion depth when microabrasion is combined with CPP-ACP or CPP-ACFP pastes or when these three techniques are used alone.

Based on the results of this study the null hypothesis Ho_1 was rejected. We found that treatment of WSLs with CPP-ACP paste, CPP-ACFP paste, microabrasion or combination of microabrasion with either of the pastes decreases lesion depth in vitro. Teeth in all treatment groups had mean lesion depths that were statistically significantly smaller than teeth in Control group.

The null hypothesis Ho_2 was not rejected. The results indicate that microabrasion in combination with CPP-ACP paste or CPP-ACFP paste did not decrease lesion depth greater than that observed with either paste technique alone. There was no statistically significant difference in mean lesion depth between CPP-ACFP paste alone and the combination of microabrasion with CPP-ACFP paste. There was also no statistically significant difference in mean lesion depth between CPP-ACP paste alone and the combination of microabrasion with CPP-ACP paste.

Murphy *et al.*[44] first proposed the microabrasion technique for treatment of WSLs. He and his colleagues examined the visible change in WSL surface area following microabrasion of WSLs in eight postorthodontic patients. For each patient, two demineralized lesions affecting either incisors or canines were treated with ten cycles of 10 seconds applications of acid and pumice mixture. They used standardized intraoral images for quantitative assessment of pre and post-treatment lesion surface area. They found that the microabrasion technique significantly reduced visible enamel WSLs with a mean reduction in lesion size of 83%. Each microabrasion application is estimated to remove approximately 10 μm of enamel surface.[42] This procedure can be repeated up to a maximum of ten applications. With multiple applications, the entire white spot lesion surface may be removed if lesion depth is within 100 -120 μm. It is likely that with the ten microabrasion applications used in this study, the entire lesion was removed and treatment outcome was limited to the visible cosmetic improvement.

The combination treatment of microabrasion and CPP-ACP for the successful treatment of WSLs has been advocated by Ardu et al.[6] In the clinical case report, they suggested that microabrasion reactivates superficial enamel by mechanical and chemical abrasion, increasing porosity of the enamel lesion and, when followed by daily home application of CPP-ACP paste, could help remineralize and eliminate WSLs without invasive restorative procedures.[6] Teeth with WSLs were etched for 2-3 minutes and followed by several applications of microabrasion. Subsequently, the CPP-ACP paste was applied for 15 minutes. At home, patients were instructed to continue twice a day use of CPP-ACP paste for a duration of up to several months. Eventually, treatment could have been supplemented with at home bleaching to achieve more uniform color.

Visual non-quantitative assessment of WSLs was used to evaluate the outcome of treatment. The clinical outcome could have been influenced by a variable number of microabrasion applications. With multiple applications, instead of activating superficial enamel and increasing enamel porosity for greater ion penetration, the entire white spot lesion could have been removed and not remineralized. The outcome could have been also affected by at home bleaching, which would mask the visual appearance of WSLs.

Pliska *et al.*[18] examined the effects of application of CPP-ACP paste, microabrasion and combination treatment on the remineralization of artificially induced WSLs in bovine teeth using quantitative light-induced fluorescence. In the CPP-ACP group, teeth were treated with twice-daily applications of 1:1 diluted MI Paste and deionized water for 20 seconds. Teeth in microabrasion only and combination of CPP-ACP with microabrasion groups were treated once at the beginning of the treatment with 2 minutes etch followed by one application of 20 seconds microabrasion with rubber cup that was attached to a slow-speed handpiece. Subsequent to microabrasion treatment, teeth in the combination group were treated with twice-daily applications of 1:1 diluted MI Paste and deionized water for 20 seconds and teeth in the microabrasion group received deionized water treatments for 20 seconds twice a day. The duration of the treatment was 2 weeks. They found that microabrasion treatment with or without CPP-ACP improved the fluorescence and thus reduced WSLs. They also determined that the application of CPP-ACP paste alone did not result in any significant remineralization of WSLs. In contrast, the results of our study showed that microabrasion in combination with CPP-ACP paste or CPP-ACFP paste did not decrease lesion depth greater than that observed with either paste technique alone. In addition, microabrasion treatment alone, while statistically significantly superior to the control groups, was the least effective of the treatment modalities. The difference in the two studies' treatment outcomes can be related to the different methods of assessing WSL regression. Light-induced fluorescence is reported to have greater sensitivity to detect surface changes and lower sensitivity to detect subsurface changes in mineral content.[45] Light-induced fluorescence can also be affected by imaging geometry, tooth hydration, enamel thickness and surrounding lighting.[45] When lesion depth is used as the main measurement of demineralization and remineralization, PLM is a highly sensitive method and can give an accurate measurement of lesion depth even when it is difficult to determine the outer surface of the lesion under Transverse Microradiography (TMR).[47] In addition, variations in the microabrasion technique could have contributed to difference in results of two studies. In the study by Pliska *et al* the microabrasion was performed with a rubber cup that was attached to a slow-speed handpiece. In our study WSLs were rubbed with a microbrush. The goal was to activate the surface lesion and minimize removal of the enamel surface. When the affected enamel layer is removed by the microabrasion technique, it creates a smooth, polished surface layer through deposition and compaction of calcium and phosphate breakdown products.[43] This newly highly polished surface layer may prevent penetration of calcium and phosphate ions in deeper layers and subsequently prevent subsurface lesion remineralization. There was also a difference in application of CPP-ACP and CPP-ACFP pastes to the surface of WSLs. In our study paste was not diluted with deionized water, but rather was applied directly. The manufacturer of MI Paste and MI Paste Plus recommends applying a generous layer of paste to the tooth surfaces, with 1-2 min of direct paste contact. Lastly, product instructions advise patients to not eat or drink for 30 minutes following paste application.

The in vitro results presented in this study are consistent with recent in situ and clinical trial results showing remineralization of enamel WSLs by use of CPP-ACP or CPP-ACFP products. The *in situ* randomized, double-blind remineralization study by Reynolds *et al.*[2], investigated remineralization potential of 4 dentifrice slurries: 1100 ppm fluoride as NaF, 2800 ppm fluoride as NaF, 2% CPP-ACP, and 2% CPP-ACP plus 1100 ppm fluoride as NaF. Participants wore an appliance housing enamel slabs with subsurface lesions and were instructed to perform mouth rinses with assigned slurries for 60 sec, 4 times per day for 14 days. At the end of each treatment period, the enamel slabs were analyzed with microradiography. They found that the dentifrice containing 2% CPP-ACP achieved superior remineralization to the dentifrice containing 1100 ppm fluoride, and produced a level of remineralization similar to that achieved with the 2800 ppm fluoride dentifrice. The greatest amount of remineralization was achieved with a dentifrice containing 2% CPP-ACP plus 1100 ppm fluoride.[2] Reynolds suggested that this was probably due to the ability of CPP-ACP to interact with fluoride ions to produce an additive anticariogenic effect through the formation of a stabilized amorphous calcium fluoride phosphate phase. In the presence of CPP, which prevents rapid transformation of the calcium phosphate phases, calcium, phosphate and fluoride ions are stabilized and maintained in a form that drives these ions down the concentration gradient into the enamel subsurface lesion instead of rapid precipitation at the surface. In our study the greatest decrease in lesion depth was achieved in the two groups that contained CPP-ACFP paste. The second largest decrease in lesion depth was achieved in groups with CPP-ACP paste.

Chi *et al.*[36] also demonstrated the anticariogenic potential of CPP-ACP paste. In this *in situ* randomized clinical trial subjects were randomly assigned to four treatment groups: a lozenge containing 56.4 mg of CPP-ACP, a lozenge containing 18.8 mg of CPP-ACP, a lozenge without CPP-ACP, and a no-lozenge group. Subjects in each group wore removable palatal appliances that contained human enamel slab inserts with subsurface lesions.[36] Lozenges were consumed four times per day for 14 days. After the treatment period, the enamel slabs were removed and microradiography was used to analyze enamel remineralization. They found that lozenges containing 18.8mg CPP-ACP and 56.4mg CPP-ACP significantly increased enamel subsurface lesion remineralization by 78 and 176 percent respectively relative to the controls.[36]

Similarly, Morgan *et al.*[37] investigated the progression and regression of caries lesions in adolescent subjects chewing sugar-free gum containing CPP-ACP relative to the identical gum without CPP-ACP. 2700 subjects from 29 schools participated in this randomized clinical trial. Standardized digital bitewing radiographs were taken at the baseline and at the end of 24-month. The CPP-ACP sugar-free gum significantly slowed progression and enhanced regression of caries lesions relative to the control sugar-free gum.[37]

In another clinical trial, Bailey *et al.* [4] investigated regression of WSLs in post-orthodontic patients with and without the CPP-ACP regimen. They compared the effect of a 12-week home application of a remineralizing cream containing CPP-ACP against that of a placebo cream in a fluoridated environment. The severity of WSLs was quantified according to the International Caries Detection and Assessment System II (ICDAS II) criteria.[4] They found that significantly more post-orthodontic WSLs regressed with the remineralizing cream compared with placebo over 12 weeks.[4]

Yengopal *et al*.[34] conducted a systematic review with meta-analysis of published randomized clinical trials that investigated caries-preventive effects of CPP-ACP. In this study, data from 5 in situ trials were considered clinically and methodologically homogenous and were used in the meta-analyses. They concluded that within the limitations of the meta-analysis, the results of the in situ clinical trials support the short-term remineralization effect of CPP-ACP. Additionally, the in vivo randomized clinical trials provide promising results for the long-term use of CPP-ACP for caries prevention.

In contrast to the previously mentioned studies, other studies report no benefit on remineralization of enamel subsurface lesions with CPP-ACP paste. In a prospective, randomized clinical study, Beerens *et al.*[38] compared a fluoride-containing CPP-ACP paste with a control paste. They found no advantage for use of the fluoridated CPP-ACP paste over regular oral hygiene in WSL regression as measured by Quantitative light-induced fluorescence (QLF).[38] Similarly, Brochner *et al.,*[39] in a prospective clinical trial using non-fluoridated CPP-ACP paste found WSL regression to be comparable with traditional toothpaste after a four week treatment measured with QLF. The conflicting results of these studies may be related to the method of assessment of WSL regression. As previously discussed, light-induced fluorescence can be affected by imaging geometry, tooth hydration, enamel thickness and surrounding lighting.[45]

Further studies are required to determine the most effective way to treat post-orthodontic WSLs. Results of the current study demonstrated greatest decrease in lesion depth in groups that contained CPP-ACFP or CPP-ACP pastes. The group that was treated with microabrasion only, while providing significantly decreased lesion depth over control, was the least effective of the above therapeutic options.

4.1 Limitations of the Study

The results reported in this study are based upon an *in vitro* model, which has several innate limitations. Prior to the experiment, all teeth were cleaned with a non-fluoridated pumice and water paste to remove microbial biofilm and calculus. However, within *in vivo* conditions dental pellicle and plaque are always present on the teeth. One of the important mechanisms of action of the MI or MI Paste Plus is the ability of CPP-ACP and CCP-ACFP clusters to attach to dental plaque and tooth surfaces.[34] This adhesion has the ability to act as a reservoir of calcium and phosphate ions and prolong the duration of their action.[34]

The environment in which teeth were stored during the experiment was created to maximize remineralization time. Once lesions were created they were not subjected to multiple daily acidic challenges that would normally occur in the oral environment. In the oral environment during acidic challenges, the CPP-ACP and CCP-ACFP that is attached to dental plaque releases calcium and phosphate ions, thus maintaining a supersaturated mineral environment, which inhibits enamel demineralization and enhances remineralization.[34]

Another limitation of this study is that bovine teeth were used as a substitute for human teeth. Even though research shows that bovine teeth are an acceptable substitution for human teeth[48-50], ideally, human teeth from the same donor should be used to decrease variability between groups. Teeth from variable sources may lead to large variations in treatment or test response. In this study teeth were collected from 16 animals. Teeth from the same animal were randomly placed in all seven treatment groups. As result each group had similar composition.

Lastly, the data collection was not performed blind. The principal investigator gathered the data.

4.2 Clinical Application

A goal of contemporary dentistry is the non-invasive management of non-cavitated caries lesions using remineralization systems to repair the enamel with fluorapatite or fluorhydroxyapatite. In orthodontic patients who are at increased caries risk, preventive procedures such as fluoride varnishes, fluoride mouthrinses, antibacterial and antiplaque agents, MI or MI Plus Paste, and sealants should be instituted to avert the onset of disease. However in those patients in whom disease is already present, the incipient lesions should be treated non-invasively by remineralization with bioavailable calcium, phosphate, and fluoride ions to restore the strength and aesthetic appearance of the lesion and to increase resistance to future caries challenge.

Based on the results of this study and previous studies, we can infer that both MI Paste and MI Plus Paste can be recommended for treatment of active WSLs in post-orthodontic patients. While microabrasion treatment was better than no treatment, it did not add to the remineralization benefit of MI Paste or MI Plus Paste. As result, we would not recommend the additional step of microabrasion prior to either paste treatment.

4.3 Future Research

Additional in vivo randomized clinical trials on the outcome of remineralization of enamel white spot lesions by use of CPP-ACP or CPP-ACFP products are warranted. Further research is needed to improve remineralization treatment of WSL's. Future studies may investigate reactivation of the lesion's surface by acid etching, bleaching or deproteination.

Chapter 5: Conclusion

The null hypothesis Ho_1 was rejected: treatment of WSLs with CPP-ACP paste, CPP-ACFP paste, microabrasion or combination of microabrasion with either of pastes decreases lesion depth in vitro.

The null hypothesis Ho_2 could not be rejected: microabrasion in combination with CPP-ACP paste or CPP-ACFP paste did not decrease lesion depth greater than that observed with either paste technique alone.

Based on the results from this study, we can conclude that: (1) treatment of WSLs with CPP-ACP paste, CPP-ACFP paste or microabrasion decreases lesion depth in vitro; (2) microabrasion in combination with CPP-ACP paste or CPP-ACFP paste did not decrease lesion depth greater than that observed with either paste technique alone; (3) both CPP-ACP and CPP-ACFP pastes in combination with microabrasion treatments showed greater decrease in lesion depth than microabrasion alone.

While microabrasion treatment was better than no treatment, it did not add to the remineralization benefit of MI Paste or MI Paste Plus.

Appendix A: Raw Data

Tooth	Group	Section	Measurement
1	1	1	61.7
1	1	1	63.77
1	1	1	75.32
1	1	1	71.45
1	1	1	81.4
1	1	2	65.39
1	1	2	58.97
1	1	2	51.87
1	1	2	65.01
1	1	2	79.01
1	1	3	61.01
1	1	3	70.19
1	1	3	68.35
1	1	3	64.13
1	1	3	68.2
2	1	1	60.62
2	1	1	59.54
2	1	1	61.84
2	1	1	62.81
2	1	1	63.79
2	1	2	78.79
2	1	2	69.82
2	1	2	60.85
2	1	2	68.7
2	1	2	74.09
2	1	3	59.82
2	1	3	70.8
2	1	3	69.16
2	1	3	68.86
2	1	3	79.89
3	1	1	78.79
3	1	1	77.32
3	1	1	78.63
3	1	1	74.79
3	1	1	91.08
3	1	2	86.29
3	1	2	68.4
3	1	2	84.7

3	1	2	77.2
3	1	2	76
3	1	3	78.4
3	1	3	75.4
3	1	3	69.4
3	1	3	69.5
3	1	3	82.7
4	1	1	80.27
4	1	1	77.39
4	1	1	72.14
4	1	1	76.31
4	1	1	66.65
4	1	2	63.99
4	1	2	65.9
4	1	2	64.9
4	1	2	66.8
4	1	2	68.67
4	1	3	70.96
4	1	3	73.08
4	1	3	75.27
4	1	3	74.52
4	1	3	68.79
5	1	1	57.78
5	1	1	70.96
5	1	1	53.26
5	1	1	69.49
5	1	1	94.64
5	1	2	58.53
5	1	2	58.53
5	1	2	57.07
5	1	2	63.15
5	1	2	63.15
5	1	3	66.54
5	1	3	63.53
5	1	3	62.11
5	1	3	63.67
5	1	3	54.67
6	1	1	71.15
6	1	1	77.16
6	1	1	69.49
6	1	1	71.45

6	1	1	72.92
6	1	2	73.51
6	1	2	80.1
6	1	2	66.14
6	1	2	70.69
6	1	2	62.84
6	1	3	75
6	1	3	72.12
6	1	3	65.96
6	1	3	73.75
6	1	3	71.71
7	1	1	73.34
7	1	1	84.03
7	1	1	86.04
7	1	1	82.03
7	1	1	78.83
7	1	2	68.09
7	1	2	84.25
7	1	2	84.19
7	1	2	73.91
7	1	2	72.39
7	1	3	66.49
7	1	3	71.04
7	1	3	66.73
7	1	3	62.48
7	1	3	66.9
8	1	1	88.29
8	1	1	91.56
8	1	1	123.37
8	1	1	122.06
8	1	1	105.25
8	1	2	49.52
8	1	2	58.43
8	1	2	62.13
8	1	2	67.77
8	1	2	50.93
8	1	3	36.21
8	1	3	38.35
8	1	3	32.26
8	1	3	42.04
8	1	3	49.1

9	1	1	98.2
9	1	1	93.42
9	1	1	96.45
9	1	1	86.7
9	1	1	95.29
9	1	2	98.6
9	1	2	98.6
9	1	2	89.85
9	1	2	89.57
9	1	2	90.68
9	1	3	109
9	1	3	96.39
9	1	3	93.89
9	1	3	88.87
9	1	3	98.2
10	1	1	75.46
10	1	1	79.78
10	1	1	69.57
10	1	1	78.3
10	1	1	75.39
10	1	2	75.22
10	1	2	77.54
10	1	2	79.89
10	1	2	69.49
10	1	2	75.72
10	1	3	75.33
10	1	3	76.82
10	1	3	78.34
10	1	3	82.73
10	1	3	75.33
11	1	1	80.81
11	1	1	79.17
11	1	1	80.27
11	1	1	80.27
11	1	1	69.94
11	1	2	69.99
11	1	2	72.75
11	1	2	83.78
11	1	2	77.71
11	1	2	75.04
11	1	3	76.31

11	1	3	74.52
11	1	3	77.74
11	1	3	79.17
11	1	3	86.7
12	1	1	72.44
12	1	1	75.69
12	1	1	79.94
12	1	1	84.21
12	1	1	74.28
12	1	2	72.14
12	1	2	70.98
12	1	2	72.42
12	1	2	75.04
12	1	2	72.74
12	1	3	72.74
12	1	3	80.86
12	1	3	77.74
12	1	3	67.37
12	1	3	82.04
13	1	1	78.3
13	1	1	79.82
13	1	1	81.25
13	1	1	67.96
13	1	1	69.49
13	1	2	67.77
13	1	2	79.41
13	1	2	82.78
13	1	2	71.3
13	1	2	60.56
13	1	3	65.41
13	1	3	62.2
13	1	3	59.08
13	1	3	68.52
13	1	3	53.5
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14	1	1	86.45
14	1	1	87.83
14	1	1	91.56
14	1	1	95.29
14	1	2	79.89
14	1	2	81.47

14	1	2	105.13
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14	1	2	93.16
14	1	3	86.13
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14	1	3	91.69
14	1	3	93.07
14	1	3	92.16
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15	1	1	79.94
15	1	1	79.34
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15	1	1	81.35
15	1	2	86.96
15	1	2	78.01
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15	1	3	86.29
15	1	3	75.69
15	1	3	87.2
16	1	1	87.8
16	1	1	83.83
16	1	1	86.03
16	1	1	82.78
16	1	1	84.21
16	1	2	89.28
16	1	2	99.63
16	1	2	97.63
16	1	2	95.36
16	1	2	92.79
16	1	3	75.33
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16	1	3	85.67
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16	1	3	85.68
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2	2	1	75.27
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6	2	1	59.08
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6	2	3	67.27
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7	2	1	42.63
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7	2	2	46.64
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7	2	2	42.3
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7	2	3	52.04
7	2	3	44.41
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7	2	3	52.04
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10	2	1	66.75
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11	2	2	83.36
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11	2	3	75.35
11	2	3	72.38
11	2	3	67.96
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12	2	1	95.02

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12	2	1	96.77
12	2	1	82.85
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12	2	3	84.21
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14	2	2	90.3
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14	2	2	95.29
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14	2	3	90.1

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15	2	3	59.18
15	2	3	64.27
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16	2	1	92.13
16	2	1	80.27
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16	2	2	89.92
16	2	2	94.97
16	2	2	97.1
16	2	2	84.61
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16	2	3	95.66
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16	2	3	100.31
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1	3	1	38.52
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1	3	2	39.88
1	3	2	36.96
1	3	2	32.63

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1	3	3	37.19
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2	3	1	60.85
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2	3	1	59.08
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2	3	2	43.06
2	3	3	48.09
2	3	3	46.38
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3	3	1	44.26
3	3	1	56.84
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4	3	1	53.51
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4	3	2	55.6
4	3	2	65.88
4	3	2	57.02
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4	3	3	68.35
4	3	3	57.36
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4	3	3	64.89
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5	3	1	31.89
5	3	1	30.45
5	3	1	31.89
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5	3	3	71.39
5	3	3	63.99
6	3	1	78.63
6	3	1	79.98
6	3	1	78.79
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6	3	1	80.26
6	3	2	82.5
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6	3	3	79.01
6	3	3	82.29
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7	3	1	57.68
7	3	1	77.95
7	3	1	73.59
7	3	2	81.72
7	3	2	79.82
7	3	2	74.39
7	3	2	69.67
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7	3	3	71.65
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7	3	3	77.32
7	3	3	80.63
7	3	3	85.02
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8	3	1	72.75
8	3	1	79.98
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8	3	2	65.21
8	3	2	72.42
8	3	2	58.53
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8	3	3	60.27
8	3	3	61.28
8	3	3	56.21
8	3	3	49.98
8	3	3	64.52
9	3	1	56.61
9	3	1	57.68
9	3	1	61.7
9	3	1	69.24
9	3	1	73.08
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9	3	2	53.69
9	3	2	64.13
9	3	2	65.41
9	3	2	80.43
9	3	3	75.69
9	3	3	79.41

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9	3	3	70.07
9	3	3	71.47
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10	3	1	46.38
10	3	2	51.95
10	3	2	51.95
10	3	2	50.16
10	3	2	42.3
10	3	2	44.07
10	3	3	55.9
10	3	3	54.8
10	3	3	47.68
10	3	3	52.72
10	3	3	40.18
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11	3	1	60.27
11	3	1	60.56
11	3	1	63.77
11	3	1	62.7
11	3	2	60.85
11	3	2	56.44
11	3	2	65.26
11	3	2	54.73
11	3	2	59.25
11	3	3	51.7
11	3	3	53.18
11	3	3	62.04
11	3	3	64.99
11	3	3	67.96
12	3	1	81.26
12	3	1	72.87
12	3	1	79.54
12	3	1	66.63
12	3	1	81.29
12	3	2	70.96
12	3	2	72.62
12	3	2	65.41

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12	3	2	59.75
12	3	3	79.17
12	3	3	81.4
12	3	3	6.31
12	3	3	84.61
12	3	3	70.07
13	3	1	59.75
13	3	1	54.47
13	3	1	68.09
13	3	1	53.03
13	3	1	57.07
13	3	2	57.07
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16	7	3	53.36

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