EFFECTS OF MICROABRASION—WITH OR WITHOUT MI PASTE PLUS—ON THE TREATMENT OF WHITE SPOT LESIONS

A Thesis

by

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ABSTRACT

Purpose: To determine whether microabrasion treatment, with or without the addition of MI Paste Plus, improves the esthetics and mineral density of white spot lesions (WSLs).

Materials and Methods: This in vitro prospective longitudinal study included 60 halved premolar specimens, randomly allocated to two groups. Artificial white spot lesions were created in all teeth following the Quieroz protocol. Control specimens were subjected to microabrasion treatment alone, while experimental specimens received daily MI Paste Plus® application after microabrasion. Both groups were subjected to a 28-day pH cycling protocol to test the longevity of treatment. The groups were evaluated at T1(intact enamel), T2 (WSL formation), T3 (after microabrasion), and T4 (post pH cycling). Visual appearance was assessed using photographs, which were shown using an online survey administered to dental students (36.6%) and recent dental graduates (63.4%). Enamel lightness was quantitatively assessed using spectrophotometer analysis, and mineral density was analyzed using micro CT analysis.

Results: Dentists and dental students detected a greater esthetic improvement in WSLs treated with MI Paste Plus. Analysis of covariance controlling for initial differences in lightness between groups showed a statistically significant between-group difference in lightness at T4 ($p<0.001$), with the experimental teeth being 3.7 units lighter than controls. There were statistically significant between-group differences for the lightness changes that occurred from T3-T4 ($p<0.001$), as well as differences in lightness from T1-T4,($p<0.001$). Apparent and material densities were significant higher ($p<0.05$), 104.62 and 29.79 units respectively, in the experimental than control group. Material density increased significantly (31.4 units) more in the experimental than control group ($p<0.05$) from T2-T4. Apparent density between T1-T4 decreased 17.02 units more in the control than the experimental group ($p<0.05$).

Conclusions: Mineral density and esthetics of specimens with white spot lesions were greatly improved by treatment with microabrasion and MI Paste Plus. This minimally invasive treatment is a viable option for patients requiring treatment of white spot lesions after orthodontic treatment.

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NOMENCLATURE

WSL(s)	White spot lesions(s)
CPP-ACP	Calcium phosphopeptide amorphous calcium phosphate
L*	Lightness
μCT	Microcomputed tomography
VAS	Visual analogue scale

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

White spot lesions (WSLs) are major problems that occur during orthodontic treatment with bonded appliances. It has been reported that 23.4% of patients will develop at least one white spot lesion (WSL) during their treatment.[1] The prevalence of these lesions has been reported to be as high as 96%.[1-4] Bonded orthodontic appliances alter the oral environment, hinder hygiene, serve as retention sites for plaque accumulation, and serve as a barriers to the normal movement of food and cleaning mechanisms.[2, 5, 6] By blocking oral musculature and preventing salivary access, the resultant accumulation of acid producing plaque creates a physical barrier that prevents diffusion of acid away from the enamel surface. This barrier prevents the normal remineralization that typically takes place when saliva delivers calcium and phosphate ions to the enamel surface.[2] All of these factors contribute to demineralization of enamel around bracket bases, and the development of WSLs.

While WSLs are preventable with good oral hygiene, most adolescent orthodontic patients demonstrate underdeveloped hygiene practices and poor compliance[3]. As a result, the orthodontic community has implemented various preventive measures, including oral hygiene instructions, fluoride mouth rinses, fluoride toothpaste, fluoride varnishes, and labial sealant application prior to bonding[2, 7]. Thus far, complete prevention of WSLs has not been shown to be possible.[8]

Since white spots do not disappear completely after treatment, especially the more advanced lesions, they remain an esthetic problem for patients.[2, 9] Treatment options for

WSLs remaining after treatment include non-invasive remineralization,[10, 11] and invasive restorative options.[12, 13] Minimally invasive options are also available, however, they have not been adequately researched and supported by literature.[14, 15] Two recent systematic reviews conducted by Abdullah et al in 2016 and Paula et al in 2017 evaluated minimally invasive treatments for white spot lesions. Both reviews concluded that there was a lack of reliable evidence to support the efficacy of these treatment modalities in general for post-orthodontic white spot lesions, and both stressed the need for more high-quality studies with controlled methodology. Additionally, neither of these reviews even recognized microabrasion as a treatment option even though it is still being used.

Microabrasion is considered to be a minimally invasive treatment technique that has been recommended for use on white spot lesions.[16-18] Current protocols and associated treatments for its use on WSLs are highly variable. Products vary with respect to the types and concentrations of acids, abrasive materials and particle sizes, as well as application specifics.[5, 17, 19, 20] Post microabrasion protocols also vary, with some studies advocating no additional products[21, 22] and others recommending bleaching[23] or application of topical fluoride.[24] There is a paucity of evidence supporting this technique. The available literature is either of low quality, has flaws in methodology, or relies solely on visual assessment.[16, 25, 26] Reviews have called for additional high quality studies,[14, 15] as few have evaluated the effects of additional products after microabrasion.[22] Only one study has assessed the combination of treatments similar to that of the present study. Pliska et al in 2012 was the first to evaluate the effects of microabrasion plus CPP-ACP Paste on white spot remineralization. They concluded that application of this paste after microabrasion did not provide any additional benefit in increasing mineral content, but they also recommended further quantitative assessments on the effectiveness of combining these two treatment options. Problems with this study include it being underpowered, which makes their conclusion unfounded, but more important is the flaw in their methodology. The study did not incorporate pH-cycling, and an acid pH is necessary for the effects of the paste to be evident.[27] However, this literature lays the foundation for the present study.

The purpose of this study was to determine whether microabrasion treatment, with or without the addition of MI Paste Plus, improved the esthetics and mineral density of WSLs. MI Paste Plus was chosen because it contains CPP-ACP in addition to 900 ppm fluoride. This is the lowest concentration of fluoride available for topical use, and is desirable for this study due to the necessity of avoiding creation of a hypermineralized surface layer. If adding MI Paste Plus to the microabrasion procedure offers a benefit in remineralization and improvement of esthetics, this will allow practitioners to achieve the esthetic result they desire without abrading the enamel surface repeatedly, which is the often the result of using microabrasion alone.

Literature Review

Definition of White Spot Lesion

White spot lesions are the earliest visible manifestation of the carious process,[5, 28] and are characterized by an intact surface with subsurface demineralization.[28, 29] These lesions present an esthetic concern since they most commonly affect the gingival margin of the facial/buccal surfaces of teeth.[30] While they can occur on any enamel surface, the incidence is highest in the maxillary anterior due to the site's high exposure to carbohydrates and low exposure to saliva.[1, 2] The affected enamel appears dull, chalky, and white due to increasing porosity that affects light refraction[2, 5, 28, 31] and ultimately color perception. Cavitation is not usually evident, but the surface may be rougher than intact enamel. In order to understand the development and prevention of WSLs, the process of caries development must be understood.

Etiology of Demineralization

Dental tissues are continuously covered by a pellicle that allows bacterial cells to attach within 24 hours. Bacteria begin to colonize the surface, generating extracellular glucans and matrix that increase bacterial colonization of the plaque. By the end of 1 week, an organized community of bacteria exists. This makes up the mature biofilm that can become increasingly cariogenic. This metabolically active biofilm is rich with bacteria like mutans streptococci and lactobacilli, which produce acid as a byproduct and increase the pH lowering ability of plaque. [2, 28, 29, 32] Acid demineralizes enamel by reacting with hydroxyapatite and forming soluble calcium and phosphate ions that are lost from the mineral matrix. [29, 33] Demineralization and remineralization are dynamic processes that normally are in equilibrium. One process follows the other as dietary substrates such as food and drinks are introduced, broken down, and then cleared from the oral cavity. The pH increases towards neutral as saliva removes and buffers the acid. Calcium and phosphate ions are delivered to the tooth surface via saliva to promote remineralization. This natural process occurs multiple times a day throughout life. Lesions only occur when the balance is disturbed, and the frequency and magnitude of acid production overcome the repair process.[2, 13, 28, 29]

Risk Factors for Demineralization

Development of WSLs is multifactorial, and involves many different patient risk factors including salivary flow rate, pH and buffer capacity, oral hygiene, diet, and fixed appliances.

Saliva is considered one of the most important factors in the demineralization and remineralization process. The factors listed above (flow rate, pH, buffer capacity) can influence the degree of demineralization following an acid attack. Saliva regulates exposure of the tooth to carbohydrates and plaque, and affects the microbial composition of that plaque. It delivers fluoride ions to the enamel-plaque interface, to tip the scales toward remineralization. Increased flow rate aids in physical cleansing, increases buffering and anti-bacterial potential, and increases clearance of substrates from the mouth. Conversely, a decreased flow rate results in a lower pH following carbohydrate exposure, and a slower recovery to neutral pH, providing ideal conditions for demineralization. The pH of the saliva is directly related to its buffering capacity and ability to neutralize the acid produced by plaque. Saliva's buffering ability is derived from its carbonic acid-bicarbonate system, which maintains the oral pH between 6 and 8.

There is a negative correlation between the buffering capacity and development of caries.[2]

Oral hygiene is necessary for the physical removal of plaque that has accumulated throughout the day. Orthodontic attachments and appliances make cleaning more difficult and lead to an accumulation of plaque. As carbohydrates from the diet are introduced into the system, dental plaque is able to metabolize them and create a prolonged acid challenge. This plaque not only produces acid, but also acts as a physical barrier preventing remineralization. Diets that are high in carbohydrate consumption provide a plethora of fermentable substrate leading to acid production. Frequency of intake also plays a large role as consecutive episodes of acid attack on the enamel prevent substantial repair time via salivary buffering. This will ultimately result in a net loss of minerals—demineralization.[2]

White Spot Lesions in Orthodontics

Orthodontic wires, brackets, and bands provide an increase in the number of plaque retention sites, and create an obstacle to adequate oral hygiene in adolescent patients with already underdeveloped hygiene practices and poor compliance.[3] Plaque retention is also increased at sites of excess resin at the bracket/tooth interface.[34-38] This results in areas of stagnation where plaque remains undisturbed. The acid produced causes a decrease in pH that starts the demineralization process leading to caries.[2, 5, 6, 28, 39] Several studies have shown that patients with fixed appliances have an increased level of strep mutans and lactobacilli, and therefore an increase in acid production.[28, 40, 41 42] The process of demineralization is multifactorial, but orthodontic treatment and appliances provide additional factors that hinder oral hygiene and perpetuate the process.

For this reason, white spot lesions are an extremely common problem following orthodontic treatment. Clinically visible lesions have been reported to occur in approximately 23% of university and 28% of private practice orthodontic patients, according to Julien and Brown.[1, 43] Other studies have reported the prevalence to be as high as 96%[1, 2, 30], indicating a significant problem for both the patient and the orthodontist. Patients undergo orthodontic treatment in pursuit of an improvement in esthetics, but the formation of WSLs combats this treatment goal. Patients that are unhappy with the final result, and who will potentially need invasive treatment and restoration, often blame the orthodontist. This can result in time consuming and costly lawsuits, as well as negative publicity. As a result, there has been much research and many protocols implemented for prevention of white spots.[2] Even with preventive practices in place, however, the prevalence of white spots remains high. For this reason, there is a need for developing a minimally invasive technique for treating white spots even after they have formed.

White Spot Lesion Prevention

Oral Hygiene

Mechanical plaque removal via tooth brushing is a crucial step in preventing WSLs.[44, 45] Poor compliance with oral hygiene practices has been shown to be significantly associated with the development of WSLs. For this reason, professional instruction and regular dental cleanings are of great importance in reducing decalcification. [46] Motivating adolescent patients to change their behavior, however, can be a difficult task.[47] Some have recommended praising and re-educating patients as a means of improving their cooperation. Brushing twice a day is the standard recommendation by dental professionals.[46] Manual and electric toothbrushes are available to suit different patients' needs. For those with poor oral hygiene, the active heads of electric toothbrushes have made it easier to achieve better hygiene results. The rotation and oscillation action can remove plaque more effectively than manual brushes for some patients.[45, 46, 48, 49]

Fluoride

Fluoride has historically been used as the first line of prevention against demineralization and caries. Its preventive properties are based on its ability to form calcium fluoride as well as integrate into the crystalline lattice of enamel to form fluorapatite, a structure that is more resistant to dissolution.[46] The critical pH of fluorapatite and hydroxyapatite is 4.5 and 5.5, respectively. This lower critical pH of fluorapatite provides a protective mechanism against demineralization, so long as the oral pH does not drop below it. During acidic conditions with low fluoride present, fluorapatite forms and is integrated into the outer layer of enamel. Low fluoride concentrations in saliva ensure a supersaturation with respect to fluorapatite. Under these conditions, there is dissolution of hydroxyapatite from the enamel surface with a simultaneous formation of fluorapatite. The result is a reduction in the overall amount of demineralization, and a slow increase in enamel fluoride concentration. However, if the pH drops below 4.5, the cariostatic effects of fluorapatite are lost. The plaque becomes undersaturated with respect to both hydroxyapatite and fluorapatite, meaning that no remineralization will occur. [2, 11, 34, 50]

Calcium fluoride is another important factor that plays a significant role in the cariostatic effect of fluoride. It is the major reaction product when topical fluoride is applied to enamel.[46] It is present as globules of varying sizes depending on the pH and concentration of fluoride in the environment, and can persist within plaque for many weeks after topical application. CaF_2 provides a reservoir of fluoride ions that can be released and subsequently incorporated into the crystal lattice as fluorapatite when the oral pH drops during one of the many daily acid attacks. The released fluoride can diffuse into enamel and promote reformation of apatite. It also adsorbs to the surface of enamel to inhibit further dissolution. Phosphate ions and protein molecules adsorb to the surface, and limit the rate of solubility of CaF_2 in saliva. Furthermore, CaF_2 also has the potential to transform into fluorapatite. This process as a whole provides a cariostatic effect by liberating fluoride ions into the environment, promoting remineralization.[11, 34, 46] Fluoride can be administered in the form of toothpastes, mouth rinses, gels, pastes, or varnishes.

Sealants

Orthodontists have attempted to prevent white spot lesion formation using methods that do not require patient compliance. One such method that has been employed is the use of sealants as a barrier to protect the labial surface of enamel. General dentists have historically used sealants for occluding pits and fissures on teeth. Numerous studies have been published supporting their effectiveness in preventing occlusal decay.[51] It stands to reason that this same concept could be applied to protecting labial enamel from decay, and thus, has led to orthodontists utilizing this technique in an attempt to prevent demineralization and decay around brackets. A study by Benham et al. evaluated enamel sealant along the gingival margins of anterior teeth using a split mouth design. The study showed a significant reduction in WSLs during orthodontic treatment. Teeth without sealants had 3.8 times more WSLs than those that did not.[8]

Sealants are only effective as long as they remain on the tooth. Unfortunately, retention is a major issue. Over time, sealants are eroded via mechanical abrasion from tooth brushing and mastication.[8] The duration of retention and protection is dependent on sealant thickness, particularly when discussing wear. Unfilled sealants display low abrasion resistance and wear rapidly in areas adjacent to brackets. Therefore, they should not be expected to provide long-standing protection against demineralization.[52] Adding fillers to resin sealants can increase their ability to withstand mechanical abrasion and chemical erosion.[8, 53, 54] Sealant loss and continued eruption result in unprotected areas of enamel that are susceptible to demineralization and ultimately WSL formation or cavitation.

White Spot Lesion Treatment Options

Prevention should be the first line of defense against demineralization.[12] As stated previously, some patients will still develop WSLs despite preventive practices. Once a white spot lesion has formed, the clinician is faced with a number of options for improving the appearance. Treatment options include noninvasive techniques such as remineralization, minimally to moderately invasive such as microabrasion or resin infiltration, and invasive restorative treatment. Generally, treatment should start with the most conservative approach. Aggressive treatment options may be used if conservative techniques do not resolve the issue, and the patient seeks further treatment.[12, 55]

Remineralization

Remineralization is a non-invasive technique for the treatment of WSLs, and is usually the first step in treatment.[12, 55] It has been shown that lesion depth and mineral loss are significantly reduced following removal of orthodontic appliances. Some lesions will recover naturally through remineralization from saliva once treatment ceases, appliances are removed, and oral hygiene improves.[10, 35, 44, 45] However, removal of plaque and exposure to saliva is not enough to completely repair WSLs, and some will persist for years after treatment. Furthermore, the remineralizing potential of saliva has little effect on the esthetics and structure of deeper lesions. These require treatment with additional remineralizing agents.[10]

Remineralization is achieved via repeated application of fluoride to the enamel surface. Fluoride can be delivered to the enamel surface by various methods including mouthwash, paste, foam, gel and varnish. It has been shown to arrest the development and progression of the lesions.[10] Application of high concentrations of fluoride, however, can have undesirable esthetic effects and should therefore NOT be used. A high concentration causes the complete arrest of the carious process and remineralization of the most superficial layer of enamel. This leaves the deeper enamel unaffected and does not allow slow remineralization and regression. Surface hypermineralization creates a barrier to diffusion pathways and maintenance of the white appearance of the lesion. These arrested lesions will eventually stain with organic debris, which further perpetuates the esthetic concern.[10, 22, 35, 44, 55] In order to avoid arresting the lesion and forming the hypermineralized surface layer, it is recommended to use low fluoride preparations for small lesions. The repeated application of low doses of fluoride allows slower penetration by calcium and fluoride, enhanced subsurface remineralization, and lesion regression.[12, 35, 56, 57]

Casein Phosphopeptide Amorphous Calcium Phosphate

Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) is a milk-derived product that has been shown to promote remineralization of initial enamel lesions as well as decrease demineralization. The casein phosphopeptides (CPP) have the ability to stabilize calcium phosphate in solution through binding amorphous calcium phosphate (ACP) to their phosphoserine residues, forming small CPP-ACP clusters. Stabilization prevents the calcium and phosphate ions from transforming into crystalline phases. This provides a localized reservoir with high concentrations of calcium-phosphate in a bioavailable form. The result is a maintained state of supersaturation with respect to enamel minerals, and the potential for depression of enamel demineralization, and enhanced remineralization.[58-61]

Multiple studies have demonstrated that the use of CPP-ACP on post-orthodontic WSLs results in regression of the lesion.[58-60] By stabilizing calcium phosphate at the enamel surface, a large concentration gradient is generated into the enamel subsurface lesion.[59] A study by Reynolds et al. in 2008 demonstrated that CPP-ACP promoted remineralization throughout the body of the lesion rather than at the surface alone, which is the case with fluoride.[62] This technology has a synergistic effect when combined with topical fluoride. When used together, the results seem promising for non-invasive management of early lesions.[60, 62]

While some white spots will remineralize to a more normal appearance, others may persist. Lesions with an ICDAS score of 2 or higher have not been shown to remineralize to the depth of the lesion, and require more invasive techniques for treatment.[45] This brings us back to the issue of an unacceptable esthetic issue that requires restorative treatment.[46]

Restorative Treatment

Dentists should chose restorative treatments only as a last resort to addressing the patient's esthetic concerns. Restorative options include composite restorations, veneers, or even crowns. These procedures are considered maximally invasive and destructive as they involve the removal of sound tooth structure. Not only are they much more invasive, but are also generally more expensive.[12, 13]

Erosion Infiltration

The erosion-infiltration technique is relatively new in dentistry and was originally introduced for use in arresting proximal carious lesions. It is considered to be microinvasive, and centers around the idea that noncavitated enamel lesions have the potential to be arrested or remineralized.[13, 63] The treatment sequence begins by preparing the enamel surface with HCl etching. It has been shown that the surface of a white spot lesion is more resistant to phosphoric acid etching than sound enamel, thereby requiring a stronger acid (HCl) to achieve the increased permeability necessary to allow resin penetration into the underlying porous structure.[13, 64] Etching is followed by infiltration of the lesion with a low viscosity triethylene glycol dimethacrylate-based light-cured resin. This goal of resin infiltration is to occlude the porosities of incipient lesions and create a diffusion barrier to acid entry. The result is inhibition of lesion progression and possibly even complete arrest of the process. Secondarily, penetration of the resin into the porous structure offers some mechanical support to the weakened hard tissue.[13, 45, 55, 65]

Treatment with resin infiltration has been shown to restore the color of WSLs.

The light refraction index of the resin used is similar to sound enamel, thereby improving the appearance of the lesion. [13, 55 65] A study by Knösel et. al in 2013 investigated the durability of esthetic improvement following Icon resin infiltration of white spot lesions over 6 months. They determined that white spot color compared to the surrounding enamel after infiltration was stable with no significant changes over 6 months.[66] Another study by Paris et. al in 2013 evaluated the ability of resin infiltration to camouflage WSLs immediately after infiltration as well as after a staining period. They concluded that infiltrated lesions showed a better color match with sound enamel than untreated controls, and that staining was significantly reduced for the infiltrated and polished lesions compared to untreated lesions.[65] The results of these studies seem promising, but the technique still requires the use of restorative materials. Time will tell if this procedure subjects these teeth to the same cycle of "replacement dentistry".

Microabrasion

Enamel microabrasion, introduced in the 1900s for the treatment of fluorotic enamel, involves the use of acidic and abrasive agents applied to the enamel surface via mechanical pressure from a rubber cup coupled to a rotary mandrel of a low-rotation micromotor.[67] The technique of using 18% HCl acid with fine pumice, referenced in many articles[16, 26, 68], was described by Croll in 1986.[25] The results from multiple studies have shown that the technique produces significant esthetic changes, and is considered to be a safe and effective treatment of fluorosis [17, 25, 69-71]that should be considered prior to restorative options.[17, 25, 69-71] While some papers have advocated the technique for the removal of post-orthodontic decalcification,[16-18] it is still not a widely accepted treatment option, and has not been adequately researched. A recent systematic review in 2016 evaluating minimally invasive treatments for white spot lesions did not even recognize microabrasion as a treatment modality. This review, in addition to one published in 2017, concluded that there was a lack of reliable evidence to support the efficacy of minimally invasive treatment modalities for post-orthodontic WSLs, and that more studies are necessary in order to reach a conclusion.[14, 15] In addition, a study by Pliska et al recommended that a quantitative assessment of the effectiveness of combining microabrasion with the application of fluoride and calcium ions be performed, since the majority of available literature is clinical case reports.[39]

As indicated, previous studies concerning microabrasion have focused primarily on treatment of fluorosis.[69, 72-74] Fluorosis is an enamel defect caused by excessive fluoride present during tooth development. This affects the enamel development by interfering with the process of protein removal and mineral acquisition during enamel maturation.[50] Enamel maturation requires that proteins like amelogenins and water be removed at the same time that crystal growth is occurring in order to allow enamel to become fully mineralized.[75] The result is teeth with a well-mineralized surface layer, but subsurface enamel that is increasingly porous and hypomineralized.[50] The classic presentation of fluorosis is white spots on teeth after eruption, but in severe cases, fluorosis can present as brown stains, pitting, and mottling of the enamel. These defects are a great esthetic concern for patients and often cause them to seek restorative treatment, which is how the first microabrasion techniques came about.

While the available literature focuses on microabrasion as a treatment for fluorosis, multiple studies have suggested that it may also be useful for the treatment of post-orthodontic WSLs.[16, 18, 76] Fluorosis is a developmental defect that is completed prior to eruption, but the white spot lesion is a post-eruption problem. It occurs through the repeated loss of minerals from the enamel surface, followed by reconstitution with subsurface minerals. WSLs are the first visible stage of the demineralization process. They are unesthetic, irreversible, and can eventually result in frank cavitation if remineralization periods are too short to reconstitute the surface. The resulting white spot is an opaque, chalky white area on the enamel that is due to a subsurface demineralization and an increase in porosity which alters the refractive index of enamel[2, 28]. While fluorosis and white spot lesions are etiologically different, they do share some similarities.

Both fluorotic enamel and post-eruption white spot lesions exhibit an intact surface zone, with an underlying area of demineralization.[2, 77] Additionally, both types of tooth scars are esthetic concerns for patients and often cause them to seek restorative care. Considering that the final presentation of both fluorotic and demineralized enamel is similar, it stands to reason that a treatment that is effective for fluorosis may also prove effective for white spot lesions. However, there is inadequate literature on the use of microabrasion for treating white spot lesions. [21]

There are a number of articles that simply describe the proposed technique for the treatment of WSLs,[69, 78] as well as a handful of case reports that show promising results.[70, 76, 79] However, most of these papers have only assessed the visual changes of the lesion.[68] In 2012, Pliska et al performed the first study comparing the effects of microabrasion and the application of CPP-ACP (casein phosphopeptide amorphous calcium phosphate) on white spot remineralization.[39] CCP-ACP is a milk derivative complex that functions as a reservoir for calcium and phosphate. It creates a supersaturated mineral state that can be used to deliver high concentration of calcium and phosphate ions to the tooth to promote remineralization.[39, 61] It has been suggested that mechanical abrasion removes the hypermineralized surface enamel, which then allows the applied product and ions to access the deeper hypomineralized layers.[39] The study measured changes in enamel fluorescence over the first two weeks after treatment using quantitative light-induced fluorescence (QLF). They found statistically significant gains in fluorescence (indicative of increase in mineral content) associated with microabrasion alone, as well as with microabrasion and CCP-ACP treatment. They concluded that microabrasion resulted in significant reduction in the size of white spot lesions, regardless of whether it was performed with or without CCP-ACP paste.[39]

While the study by Pliska et al lays the foundation for the proposed study, there were some methodological problems.[39] First, the study did not specify how they determined when a white spot lesion was formed, or quantify the severity of the lesion. This is important because it is necessary to determine the extent of the lesion prior to treatment in order to assess if the amount of reduction is significant. The chemical etch and the mechanical abrasion were performed separately, rather than using the currently advocated technique of a combined acid and abrasion system. The 2-week duration of the study was also not sufficient in order for the effect of the applied fluoride to be evident.[80] The study's conclusion that microabrasion was successful with or without the paste was unfounded. A power analysis based on a sample size of 16 gave a power estimate of 0.11, indicating that the study was underpowered. Most importantly, a visual assessment of the lesions was not included in the study. Increasing mineral content is a great goal for the dentist. The patient, however, seeks an improvement in esthetics. For this reason, it is necessary to investigate and find a treatment that accomplishes both goals: restoration of mineral density, and improvement of esthetics. Finally, the study did not incorporate a pH cycle, which is necessary for the effects of the paste to be evident.[27] Without subjecting specimens to an acidic pH, the CPP ACP complex does not break apart.

The proposed study will quantify the extent of WSL created, and assess visual and mineral changes after treatment with the currently advocated technique of a combined acid/pumice paste for microabrasion[25, 71 81] along with CPP-ACP and fluoride application.

Product Selection

There have been various types and combinations of pastes used for microabrasion treatment in the literature. In the Pliska study, the teeth were etched with 35% phosphoric acid for 2 minutes, then rinsed and pumiced separately with a prep and polish paste for 20-seconds.[39] In other studies, 18% HCl has been used to achieve the enamel etch.[17, 71] A study by Meireles et al in 2009 used SEM (scanning electron microscope) analysis to show that HCl demonstrated non-selective enamel etching, demineralized the enamel surface equally, and resulted in lower surface roughness than enamel treated with phosphoric acid. [19]

Croll suggested that the ideal system should include a lower acid concentration with abrasive particles suspended in a water-soluble mixture.[17, 71] He thought treatment could be made safer by combining a less-concentrated acid with a more abrasive particle, which would prevent the acid from flowing uncontrollably. The combination would provide pressure induced abrasion, allowing for an increase in the erosive effects of the acid.[71] Since Croll's recommendation of combining the acid and abrasive, multiple products have become commercially available. In 2007, Loguercio et. al used a split mouth design to test the effectiveness of two of these combination materials, PREMA and Opalustre, which contain HCl and a silica mixture. Their study demonstrated that Opalustre, which contains only 6.6% HCl, was more effective than Prema, 10% HCl, at removing enamel stains. While Opalustre had a lower HCl concentration, the superior results were attributed to the larger size of the silica particles (20-160microns) compared to those in PREMA (30-60microns). The ease of lesion removal is dependent upon the type of acid and abrasive, as well as the application cycles and pressure.[73]

The ideal technique should result in insignificant enamel loss, no damage to the pulp or periodontal tissues, and satisfactory and permanent results in a short clinical time without patient discomfort.[17] While hydrochloric acid is more erosive than phosphoric acid, its reduced concentration of 6.6% makes it an equivalent to phosphoric acid, which is a weak acid at high concentrations.[21] Opalustre fulfills the recommendations made by Croll: a lower acid concentration and increased particle size combined into one product for ease of use and patient safety. There is currently no published material that has quantified the effects of microabrasion with this material, and most studies have assessed only visual changes.[73] It is important to test the effects of this treatment on both the esthetics and mineral content of WSLs in order to know its limitations and create a guideline for the use of this technique clinically.

Because poor compliance during orthodontic treatment often results in WSLs, no matter the intervention, there is a need for a minimally invasive and effective method of ameliorating white spots after they have occurred. Chemical and mechanical microabrasion with or without MI Paste Plus application could become a safe and minimally invasive procedure for removing WSLs following orthodontic treatment in patients for whom prevention was unsuccessful. The gap in the literature warrants further investigation to determine whether microabrasion with 6.6% HCl and large abrasive particles combined with MI Paste Plus application is an effective treatment option.

CHAPTER II

MATERIALS AND METHODS

This in vitro prospective longitudinal study was approved by the Institutional Review Board and faculty advisors at Texas A&M University College of Dentistry. It was determined that this study did not constitute human subjecs research. Since the utilized teeth were extracted solely for therapeutic reasons and were de-identified, this study was determined to be IRB exempt.

Specimen Preparation

30 extracted human premolar teeth were collected from dental clinics in central and north Texas. The teeth were rinsed and stored in a solution of 1:10 NaHCl in water overnight for disinfection. Following this, the teeth were transferred to a 0.1% solution of thymol for storage. During the experiment, teeth were stored in distilled water.

A diamond disc was used to section teeth mesio-distally, producing a total of 60 specimens. The enamel surface was minimally polished with an 800-grit polishing disc using a Buehler Ecomet 3 Digital variable speed grinder-polisher. Teeth were polished under running water for 20 seconds at 50 rpm. This procedure resulted in a renewed enamel surface that was essentially free of background fluoride.[82] A modified technique of minimal polishing was employed following this rationale. After drying, an adhesive strip 2X3 mm in size was placed in the center of the polished surface, and the remaining enamel surface was coated with two coats of an acid-resistant nail polish (O.P.I. Clear Top Coat) leaving a 6.0 mm^2 surface area of enamel exposed for treatment (Figure 1).

Additionally, a groove was cut in the enamel outlining the treatment window and used as a marker during µCT analysis (Figure 2).[83]

Creation of White Spot Lesions

Artificial white spot lesions were created in the 2X3 mm window of exposed enamel following the Queiroz protocol. Specimens were suspended in Falcon® Centrifuge Tubes using soft wax and orthodontic wire (Figure 3A). Each tube was filled with 12 ml of demineralizing solution. The demineralizing solution[80, 84, 85] (Queiroz's solution) was 0.05M acetate buffer containing 1.28 mmol/L Ca, 0.74 mmol/L Pi and 0.03 µg F/mL (pH 5), prepared from $Ca(NO_3)_2.4H_2O$, KH_2PO_4 and NaF, respectively.[80] The proportion of demineralizing solution per area of exposed enamel was 2 mL/mm^2.[84] The tubes were placed in a water shaker bath set to 37°C with constant agitation (Figure 3B). The demineralizing solution was replaced every 3 days until visual white spot lesions were formed (14 days). Following lesion creation, the teeth were removed from the wax, washed with distilled water and the nail polish was removed.

Teeth were randomly assigned, using excel number randomization, into two groups. The control group received microabrasion alone and the experimental group received microabrasion followed by daily MI Paste Plus application.

Treatment

Microabrasion and MI Paste Plus

Both the control and experimental groups received microabrasion following procedures described by the manufacturer. One investigator performed the microabrasion. A thin layer of Opalsutre (Ultradent Products Inc.), approximately 1 mm thick, was applied to the facial surface over the WSLs. The product was applied to the treatment windows for 60 seconds using a rubber cup in a slow speed hand piece with medium to heavy pressure. The surface was then rinsed with distilled water to remove all remaining compound.

After microabrasion, group 3 had MI Paste Plus applied over the 2X3 mm window following microabrasion treatment (Figure 3C). In accordance with manufacturer guidelines, the paste was applied for 5 minutes and then rinsed with distilled water. Application of MI Paste Plus occurred once a day, for 28 days, between the remineralization and demineralization cycles described below.

pH Cycling

Both groups were subjected to a 28-day pH-cycling protocol previously described.[7, 80] Each specimen was suspended by orthodontic wire and wax in a 50 mL Falcon tube. Two sets of Falcon tubes were prepared: one set with 37.5 mL demineralizing solution, and one with 18.5 mL remineralizing solution. Specimens were alternately immersed in demineralizing solution for 4 hours, then transferred to remineralizing solution for 20 hours over a 28-day period. On the 28th day, the specimens were stored in remineralizing solution for 24 hours. Specimens were rinsed with distilled water and blotted dry between cycles to avoid cross contamination and dilution of the solutions. Both solutions were replaced by fresh solution every 4 days. The tubes were kept in a shaker bath set at 37°C and under constant agitation (Figure 3B).[7, 84]

Data Collection

Quantitative Color Assessment with Spectrophotometer

Lesion color was objectively assessed at four time points with a spectrophotometer, using the system of the Commission Internationale de l'Eclairage.[86] Spectrophotometers are considered to be one of the most accurate instruments to use for color assessment.[87] This study utilized the Vita Easyshade Compact spectrophotometer to record and compare lightness (L*) values for the samples in order to quantitatively measure the color changes with treatment.

The CIELAB system is the most frequently used system to analyze color space in dentistry. Objective determination of perceived color difference can be achieved by calculating the colorimetric distance between two samples (ΔE).[31] The CIELAB system is a nonlinear system for describing color using a three-dimensional color space. It is comprised of a* and b* axes, which form a plane that is orthogonal to the L* axis. The system incorporates difference signals that are similar to the processes that take place in the human visual system. CIELAB represents color similarly with one achromatic lightness value (L*) and two chromatic values a* and b*, representing the red-green and yellow-blue signals respectively. This system allows color to be represented by 3 components: lightness, chroma, and hue. The nonlinear nature of the CIELAB system allows the colorimetric distance (ΔE) between two points in space to better predict the visual color difference between them. It is calculated using the following formula[88]:

$$\Delta E=[L_1^*-L_2^*)^2 + (a_1^*-a_2^*)^2 + (b_1^*-b_2^*)^2]^{1/2}$$

The Vita Easyshade Compact is a spectrophotometer containing 19 fiberoptic bundles that run to a 5 mm diameter probe tip. A halogen light inside the machine illuminates the bundles in the periphery of the probe so that light is directed toward the tooth. Bundles in the interior of the probe tip receive light returning from the tooth and deliver it to the filters and photodiode arrays within the unit. This allows spectral reflectance of the scattered light to be measured in bandwidths of 25 nm.[87] According to the manufacturer (Vita Zahnfabrik, Germany), the fiber optics of VITA Easyshade illuminate a spot of 5mm diameter in the tooth surface, but measures an area of approximately 1 mm diameter using the central fiber optic bundle.

Molds using vinyl polysiloxane impression material (Exaflex® Putty, GC America, Inc.) were made for each specimen to ensure reproducibility of the readings (Figure 4A).[84] A round mold of putty was created in a metal ring with the tooth inserted in the center, ensuring the test window was centered, and two orientation grooves were added. The spectrophotometer tip was then placed against the enamel surface covering the treatment window, and impression material was molded around the tip, being sure to fill the orientation grooves and leaving a reference indentation for the reading tip. This allowed for the same positioning of the device during each reading (Figure 4B).

The unit was operated as described in the user manual. The base was plugged into a power source, and automatic calibration was performed by placing the unit into the holder with the probe tip pushed against the calibration block. Lightness (L*) values were recorded using the single tooth mode. Using the positioning molds, the probe tip was placed flush against the enamel surface in the center of the treatment window, and a reading was taken. Each reading was taken twice to ensure accuracy, and to allow for an average measurement. This procedure was repeated for all specimens at each time point. All readings were taken in the same room to ensure consistency of lighting, even though the VITA Easy shade technology claims to be unaffected by ambient conditions.

Initial L* values were recorded for the untreated enamel surface in order to obtain a quantitative record of baseline enamel lightness. A second reading was taken following white spot lesion creation. The third reading was taken following microabrasion, and a final reading was taken following treatment and pH cycling. Lightness change (ΔL) was analyzed for the changes from pre treatment to post treatment. This allowed the investigator to determine the ability of this treatment to return the enamel to its initial untreated color.

To test reliability and validity, 10 randomly selected specimens were remeasured at each time point.

Imaging for Visual Assessment

Images of the enamel surfaces were captured at 4 time points: intact enamel, WSL creation, after microabrasion, and post treatment (Figure 5). Images were captured with a Canon Rebel T4i camera using a 60 mm macro lens and ring flash, under standardized conditions with the teeth still hydrated since a hydrated surface could mask the WSL. Settings for the camera were 1/200 shutter speed, F32 aperture, and ISO100.

Photographs were presented to a population of blinded dental students and recent dental school graduates using an online survey prepared with Qualtrics® research software. Survey respondents were asked to select their level of education as part of the survey. Paired images of either WSL versus post treatment, or initial versus post treatment of the same specimen were randomly presented. The survey population was asked to rate the amount of difference between enamel treatment windows from one specimen to the next using a 100mm visual analog scale (Figure 6). A set of 6 teeth with WSLs was presented to the population to choose their preferred treatment (Figure 7). Finally, a spectrum of teeth with varying severities of WSLs was presented for respondent's to choose the severity at which treatment would be necessary (Figure 8).

MicroCT Protocol

The samples were evaluated using microcomputed tomography. The ability of μCT to represent three-dimensional structures while being non-destructive makes it extremely useful for evaluating white spot lesions and remineralization treatment.[89, 90] Scans were conducted at 3 time points: intact enamel, WSL creation, and post treatment. Samples were stacked vertically in 12.3 mm diameter viewing tubes, with an incisal-gingival orientation, and filled with distilled water for each scan. Scans were acquired using the μCT 35 Desktop MicroCT Scanner (Scanco Medical, Switzerland) using the following parameters as per manufacturer recommendations for dental tissue samples: energy/intensity of 70kVp, 114μA, 8W; medium resolution, field of view/diameter of 12.3mm, and voxelsize of 6.0mm.

Scans were post-processed using the μCT 35 software by defining a region of interest within the treatment window bordered by the enamel surface, the previously made enamel orientation grooves, and to the depth of the lesion, for 50 layers (Figure 2). The analysis for mineral density (BV/Density) was completed and produced three-dimensional reconstructed images and mineral density values for the regions of interest (Figure 9).[89] Replicate analysis of 15 scans to assess operator reliability found no systematic errors. Method errors for apparent and material density measurements were 41.8 and 10.0, respectively.

To ensure standardization of the procedures, one investigator performed all of the enamel treatments and testing. The data were coded and entered into SPSS Software Version 25 (IBM SPSS Statistics Inc., Chicago, IL) for statistical testing. Significance level was set at 0.05.

Statistical Analysis

All spectrophotometer variables were assessed for normality. Variables were normally distributed, with no statistically significant skewness or kurtosis. Variables were evaluated using T-tests, and were described using means and standard deviations. Analysis of covariance was used to control for initial differences in lightness between control and experimental groups. Variables derived from the survey were not normally distributed, with significant skew and kurtosis. They were described using medians and interquartile ranges. Variables describing mineral density were normally distributed, with no significant skewness or kurtosis. Variables were described with means and standard deviations. Operator reliability was assessed based on 15 randomly reanalyzed scans.

CHAPTER III

RESULTS

All 60 samples had WSLs created. The WSLs were 135 μm and 100 μm deep in the control group and experimental group, respectively. The between-group difference was not statistically significant (Table 1).

Spectrophotometer Analysis: L Values (Lightness)

Statistically significant differences ($p<0.05$) were found between the control and experimental groups at T1 (initial lightness), T2 (after WSL formation), and T3 (after microabrasion). Initial lightness values were significantly higher (2.46 units) in the control than the experimental group (Table 2). The same trend was evident at T2 and T3, with controls being 2.03 and 1.93 units lighter, respectively. No statistically significant between-group difference was found at T4 (post pH cycle).

Analysis of covariance (ANCOVAR) controlling for initial differences in lightness between control and experimental groups showed there was a statistically significant ($p<0.001$) between-group difference in lightness at T4 (Table 3), with the control teeth being 3.7 units less than the experimental teeth. No significant between-group differences were evident at T2 or T3.

There were statistically significant differences ($p<0.001$) between groups from T3-T4 and T1-T4 (Table 4). After controlling for initial lightness differences with ANCOVAR, statistically significant differences were evident for the change that occurred between T3-T4 and T1-T4 (Table 5). Lightness value decreased 3.2 units in the control group, and increased slightly (0.10) in the experimental group from T3-T4.

Overall (T1-T4), values decreased 5.19 units in the control teeth and 1.49 units in the experimental teeth.

Photographic Analysis: Survey

Level of education varied among survey respondents (Figure 10); 36.5% were 1-year post dental school, 26.9% were 2 years post dental school, 23.1% were 3 years post dental school, and 13.5% were fourth year dental students.

When asked to choose the type of treatment for WSLs (Figure 7), 51.9% chose non-invasive treatment, 44.2% chose restorative treatment, and only 3.8% chose no treatment (Figure 11). When shown the spectrum of WSL severity (Figure 11), the majority of respondents (73.1%) chose to treat the two teeth with the most severe lesions (Figure 12). More specifically, 42.3% chose #5, 30.8% chose #4, and 11.5% chose to treat tooth #3.

There were statistically significant ($p<0.001$) between-group differences in the perceived changes in visual appearance of enamel for control and experimental teeth (Table 6). The median change between initial WSL formation and post pH cycling (T2-T4) was 81.3 (IQR 51.1-108.9) for the control group and 99.6 (IQR 70.4, 122.1) for the experimental group. The median change from T1-T4 was 58.5 (IQR 46.4, 69.6) for the control group, and 30.9 (IQR 15.4, 43.9) for the experimental group, with the experimental group scoring 27.9 units less than the control group.

Micro CT Analysis: Mineral Density

No significant differences were found in mineral densities between groups at T1 or T2. There were statistically significant differences between the control and experimental groups in apparent ($p<0.05$) and material ($p<0.05$) density at T4 (Table 7). Apparent and material densities were 104.62 and 29.79 units higher, respectively, in the experimental than control group. Apparent and material densities decreased between T1-T2 and increased for T2-T4, resulting in net overall (T1-T4) decreases.

Statistically significant between-group differences were evident for the changes from T2-T4 and the overall change from T1-T4 (Table 8). Material density increased 31.4 units more in the experimental than control group ($p<0.05$) between T2-T4. Apparent density between T1-T4 decreased 17.02 units more in the control than the experimental group ($p<0.05$).

CHAPTER IV

DISCUSSION

Current microabrasion protocols and associated treatments for white spot lesions have not been standardized and thus are highly variable. Products currently in use vary with respect to the types and concentrations of acids, abrasive materials and particle sizes, as well as number and timing of applications.[5, 17, 19, 20] Post microabrasion protocols also vary, with some studies utilizing no additional products[21, 22] and others recommending bleaching[23] or application of topical fluoride.[24] Overall, the procedures are usually left up to dentist preference, which makes for great variability from practitioner to practitioner. Multiple reviews have called for additional high quality studies[14, 15] to validate this treatment method and support it with evidence. The fault does not lie solely with the practicing dentists, however, as the available evidence is either lacking or of low quality. A majority of the available literature includes descriptive reports and case reports that lack control groups and rely on visual assessment.[16, 25, 26] The studies that use controls demonstrate a methodology flaw, and use qualitative assessments of lesion improvement.[18, 39, 68] Few studies have evaluated the effects of additional fluoride or bleaching regimens after microabrasion.[22] In addition, only one other study assessed the combination of treatments used in the present study.[39] However, their specimens did not undergo pH cycling, which is necessary to determine the effects of CPP-ACP paste and fluoride.[80, 89] They did not detect any additional benefit from the application of the paste, which might be expected if a demineralization solution was used to activate release of ions in the applied paste.[14, 39, 61, 62] The present randomized study was designed to collect quantitative data in order to evaluate the effects of combining MI Paste Plus with microabrasion treatment. Microabrasion was limited to 1 application in order to be as minimally invasive as possible while using a newer product for remineralization. MI Paste Plus was selected for use because it contains not only CPP-ACP, but also has fluoride. Finally, the present study involved a 28-day pH cycle, which is sufficient time for the effects of the paste to become evident.[89] The pH cycle is equivalent to, or even harsher than the oral environment as demineralization and remineralization are much faster than what is expected to occur in *in vivo* conditions.[91]

The present study showed that MI Paste Plus application after microabrasion significantly increased the mineral density of treated white spot lesions. The teeth that received MI Paste Plus showed greater increases in apparent and material densities. MI Paste Plus utilizes CPP-ACP technology to prevent the rapid transformation of ions into the calcium phosphate phase. Stabilization of these ions in a bioavailable form creates a localized reservoir at the tooth surface.[58-61, 92] Increased concentration of available ions at the surface creates a concentration gradient that drives diffusion of ions deep into the subsurface lesion. This results in higher ion activity in the subsurface lesion fluid, ultimately resulting in higher levels of remineralization and fluoride incorporation into the mineral phase.[12, 62, 93, 94] The desirable effects of MI Paste Plus application was probably increased when used in combination with microabrasion. It has been shown that a one-time acid etch of a WSL results in a significant decrease in lesions depth as well as increase in mineral content. However, it is not able to achieve full remineralization as these changes are mainly seen in the surface zone and the superficial portion of the body of the lesion.[95, 96] The remineralization potential of CPP ACP has also been validated in the literature. Its use has proved successful in increasing the rate of remineralization of subsurface lesions and decreasing demineralization. However, the remineralization is often incomplete.[22, 97, 98] Studies have shown that microabrasion treatment removes the hypermineralized layer of surface enamel, effectively eliminating the barrier to diffusion. Secondarily, the acidic gel formulation creates porous enamel that is more easily infiltrated by ions. [73, 99] The product selected for the present study, Opalustre, contains hydrochloric acid, which has been shown to be more effective than phosphoric acid at removing hypermineralized surface enamel and eroding to a depth that is well below the threshold of clinical significance.[5] In combination, microabrasion and MI Paste Plus synergistically increased mineral density of white spot lesions.

Decreases in apparent mineral densities were roughly 4 times greater than changes in material mineral densities. This was to be expected, given how the densities are measured.[100] Since apparent density included voids present within the material selected, it should show greater changes after lesion creation and treatment. The creation of white spot lesions created pores within the enamel, and the absence of minerals in those pores accounted for the decreases in apparent density observed but not in the material density values.

MI Paste Plus application also significantly improved enamel translucency. Teeth treated with the paste were significantly lighter than teeth that were not treated. An increased translucency of enamel indicates that the opacity caused by the presence of the white spot lesions was substantially decreased and even eliminated in some cases. The opacity associated with white spots causes less light to be reflected, resulting in more scatter when illuminated by a spectrophotometer. The light scattering properties of demineralized enamel are different from those of healthy enamel. Since individual mineral crystals are partially dissolved during demineralization, micropores that act as scattering centers are formed. As mineral loss increases, the scattering coefficient of light increases exponentially, by a factor of two to three.[101, 102] MI Paste Plus created a state of supersaturation with respect to enamel minerals and allowed for remineralization of the white spot lesions.[58-61] Remineralization of the lesions resulted in a decreased amount of light scattering and therefore, an increase in translucency. Survey results were also consistent with this finding. Dentists and dental students were able to detect almost 20% greater esthetic improvement in teeth treated with MI Paste Plus.

Lightness of teeth treated with MI Paste Plus can be almost fully restored. Post treatment enamel lightness, and therefore translucency, returned to within approximately 1 unit of the pretreatment values. In contrast, teeth that did not receive MI Paste Plus showed a significant overall decrease in lightness and were therefore not as translucent as intact enamel. Two previous in vitro studies have attempted to quantify color changes resulting from treatment of white spots,[31, 103] but neither compared the differences between intact and post treatment enamel. Existing case reports and studies analyzed visual improvement in the size and appearance of the lesions, compared to the initial WSL present.[23, 25, 26, 70, 76, 78, 79, 99, 104] The present study took the evaluation one step further by assessing the ability of treatment to return treated teeth to their initial pre white spot enamel lightness. Survey results were consistent with the lightness results.

Dentists and dental students were able to detect only a very subtle difference in pre-white spot and post-treatment enamel of teeth that received MI Paste Plus. The difference was twice as noticeable in teeth that did not receive Paste. It appears that the addition of MI Paste Plus restores enamel lightness, almost to its initial value.

It also appears that teeth treated with MI Paste Plus are able to maintain their lightness over time. Immediately after microabrasion (T3), the control and experimental lightness values were similar. However, after undergoing pH challenges that allowed time for the effects of fluoride to become evident,[89] teeth treated with MI Paste Plus maintained their lightness, while the untreated teeth did not. In fact, the untreated teeth showed significant decreases in lightness.

pH cycling is essential for evaluating the effect of fluoride on inhibition of enamel-dentin demineralization and enhancement of remineralization, because it mimicks caries development *in vivo*.[92] The pH cycling models have been validated for their ability to evaluate the anti-caries potential of fluoride preparations.[92 80] There are only a few case reports and no controlled studies that have quantitatively evaluated the longevity of microabrasion treatment for white spot lesions. The present results, however, are consistent with previous in vitro studies showing that acid challenge results in substantially more mineral loss for untreated teeth than teeth treated with CPP-ACP and fluoride.[62] The increase in remineralization can be related to the previous finding that greater mineral content reduced light scattering. Therefore, teeth that received MI Paste Plus during pH cycling continued to have fluoride incorporated into the enamel, ultimately increasing mineral content, which was reflected in their superior translucency.

Dentists and dental students in the present study were almost equally divided in terms of their preferred treatment for white spot lesions. 51.9% preferred a non-invasive treatment method, while 44.2% preferred restorative treatment. This suggests that half of the respondents preferred a non-invasive approach, making the treatment evaluated in the present study a viable option for them. For the other half of respondents who preferred restorations, the results of the present study should make them reconsider that decision. Survey respondents were also able to detect a significant esthetic improvement when shown photographs of treated teeth (Figure 6 & Table 6), which may further convince them that minimally invasive techniques are able to deliver a more than acceptable esthetic result.

A transition from invasive restorative treatment to a minimally invasive treatment is important from a longevity standpoint for patients. Clinically, the result that patients and doctors seek is an improvement in the appearance of the white spot lesion. If this can be achieved with a minimally invasive approach, then more destructive treatments are not necessary. Since there is no restorative material that can adequately replace natural tooth structure forever, preservation of that natural structure is extremely important.[13, 105] Avoiding invasive treatments prevents teeth from entering the cycle of replacement dentistry, which is the continual enlargement of restorations and increased damage to hard tissue as restorations are replaced over the lifetime of the patient.[13, 105] Since it is now possible to remineralize and heal demineralized tooth structure, there is no reason to remove excessive tooth structure simply because calcium and phosphate ions have been lost from the matrix.[105] This combination treatment evaluated in the present study takes advantage of the effects of microabrasion to enhance the effects of the fluoride, calcium, and phosphate supplied by MI Paste Plus. While microabrasion does remove some enamel, it is very minimal. It has been shown to only remove about 25 microns per application, which is not clinically significant and has been described to be clinically unrecognizable.[17, 106] Removal of this minimal amount of enamel not only eliminates the hypermineralized surface layer allowing easier ion penetration, but also immediately changes the optical characteristics of enamel. The simultaneous abrasion and acid erosion leaves a lustrous and shiny surface able to camouflage the remaining subsurface stains while the process of remineralization continues to restore mineral content long after the microabrasion is complete.[17, 106] Patients will notice this immediate esthetic improvement, which is the result they are seeking.

The type of tooth selected may have limited the present study. The most likely teeth to develop WSLs are maxillary lateral incisors and canines.[1, 3, 47] It would have been ideal to utilize a sample of laterals and canines as specimens, but these teeth are difficult to obtain in large numbers. As a result, a sample of extracted premolar teeth was used. It is possible that results may have varied slightly if maxillary laterals and canines were used. Another possible limitation is the sample size. Some results of the present study did not attain significance, but were very close. An increase in sample size may have made it possible to realize a significant difference in some areas. The greatest limitation of the study is its in vitro design. *In vitro* studies are unable to completely simulate the complex oral conditions that lead to caries development.[107] It is difficult to replicate the development of bacterial biofilms or the saliva/plaque fluid composition found *in vitro*,[108] and rates of demineralization and remineralization are much faster than those occurring in *in vivo* conditions.[91] Finally, when evaluating the effects of remineralizing agents, it is necessary to note that an *in vitro* design may not adequately simulate topical use and clearance of products from the oral cavity.[108] These limitations must be considered when evaluating results of *in vitro* studies.

A clinical protocol for this treatment is necessary for implementing its use on patients. It is important to note that the general dentist should be the dental professional overseeing this treatment, and that the role of the orthodontist is to refer and communicate effectively on treatment needs for patients with WSLs after orthodontic treatment. The general dentist should perform this procedure using a rubber dam with as few applications as possible, in order to preserve enamel. The patient should be instructed to apply MI Paste Plus to the enamel surface for 5 minutes once a day. Options for paste application could include using the orthodontic retainer, depending on the type of material, or custom trays fabricated by the general dentist to maintain the paste for the specified amount of time. The length of time for MI Paste Plus application *in vivo* should be less than the one month time frame used in this study, since WSLs are not always as severe as those in the present study, and pH challenges *in vivo* are less intense. It is in the best interest of the patient for orthodontists to collaborate with their general dental colleagues to promote the use of minimally invasive techniques for treatment of white spot lesions.

CHAPTER V

CONCLUSIONS

1. Mineral density increased more in teeth whose white spot lesions were treated with MI Paste Plus.
2. The desirable effects of MI Paste Plus application were increased when used in combination with microabrasion.
3. Microabrasion treatment restores enamel lightness.
4. MI Paste Plus application maintains enamel lightness.
5. Lightness/translucency of enamel was greater in teeth treated with MI Paste Plus.
6. Survey respondents noticed a significantly greater esthetic improvement in white spots treated with MI Paste Plus after microabrasion.
7. Survey respondents felt that intact and post-treatment enamel appeared most similar in teeth treated with MI Paste Plus.

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APPENDIX A

FIGURES

Figure 1. Preparation of treatment window with 2x3 mm adhesive tape centered on enamel surface, and remaining enamel coated with two cots of acid resistant nail polish

Figure 2. Grooves in enamel (red arrows) used as reference markers during µCT analysis of white spot lesions (green outline)

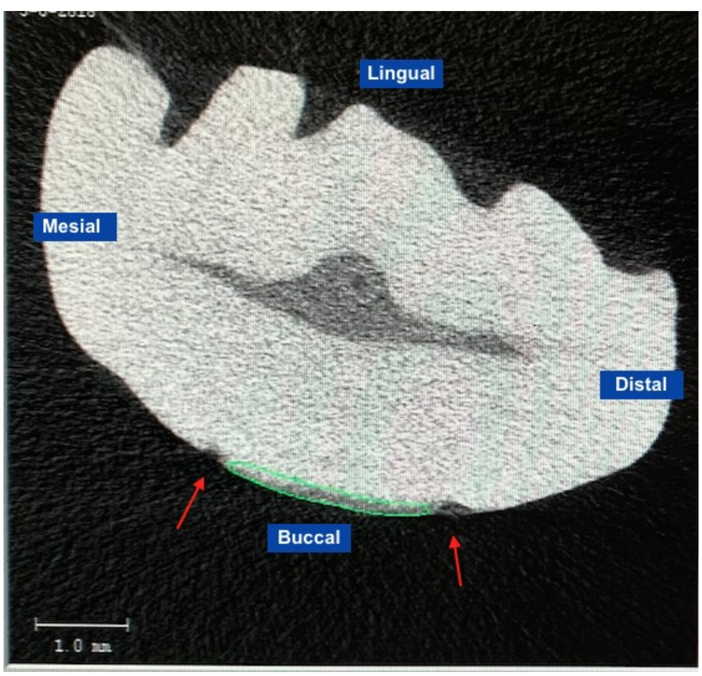
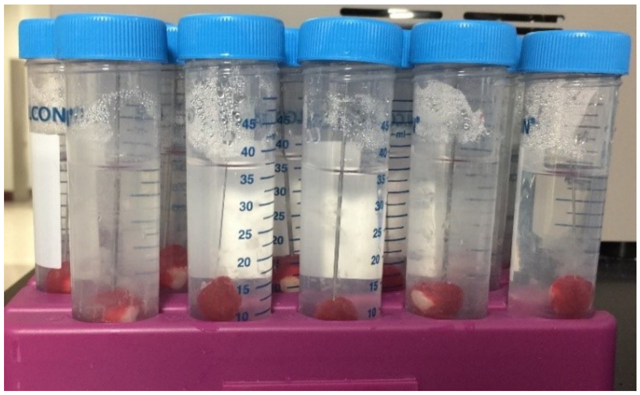
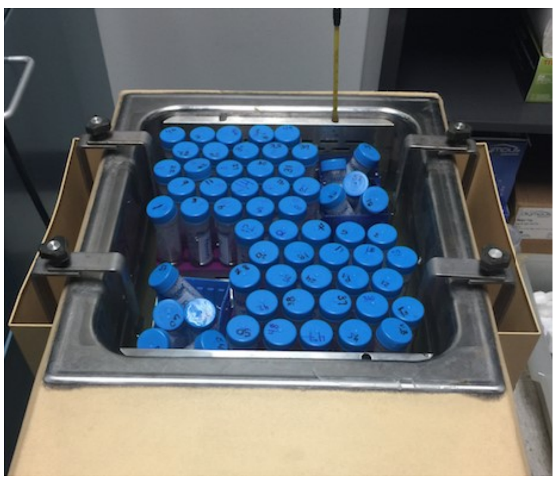


Figure 3. Samples suspended in Falcon centrifuge tubes (A), and stored in a shaker bat at 37°C (B). MI Paste Plus applied over exposed treatment window between remineralizing and demineralizing cycles (C).

A



B



C

Figure 4. Vinyl polysiloxane molds (A) for accurate repeated positioning for spectrophotometer readings (B)

A

B

Figure 5. Images of the enamel surfaces captured at 4 time points: T1 (intact enamel), T2 (WSL creation), T3 (after microabrasion), and T4 (post treatment)

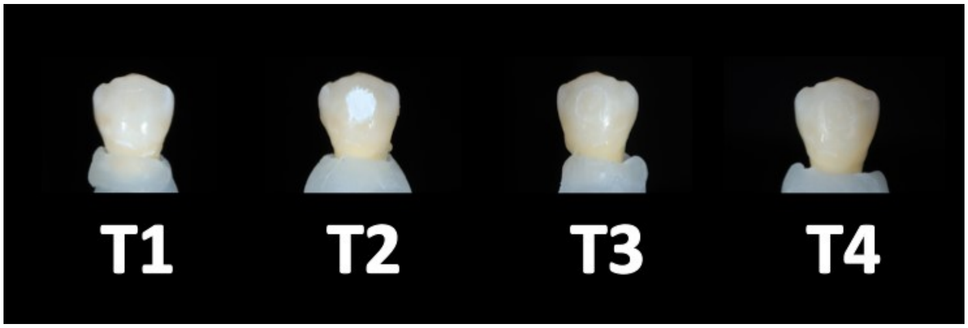


Figure 6. Sample question surveying perceived change using paired images of specimens at different time points on a 100 mm VAS

Figure 7. Photograph used in survey to determine respondents' preference for type of treatment necessary on this severity of WSL

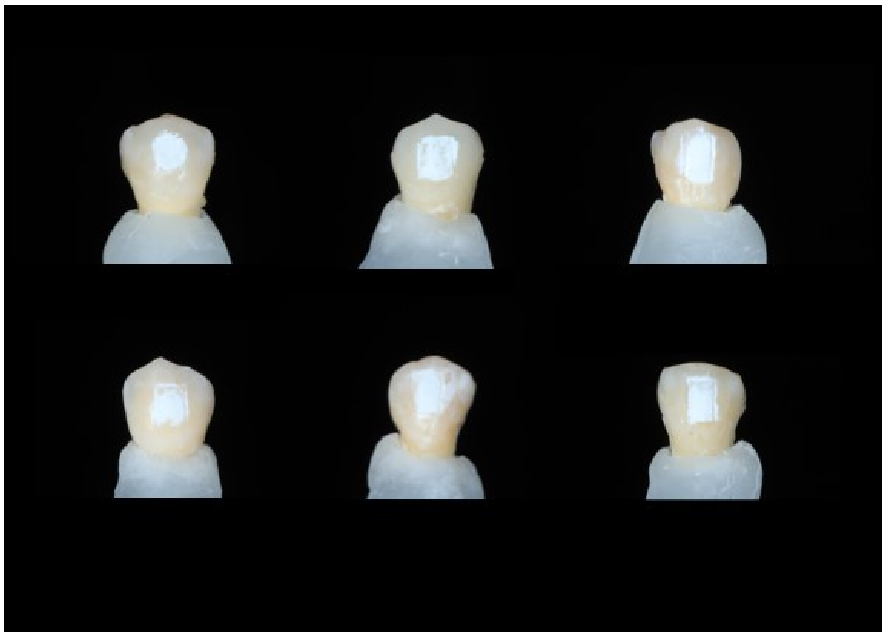


Figure 8. Photograph used to survey the severity of WSL at which respondents felt treatment would be necessary

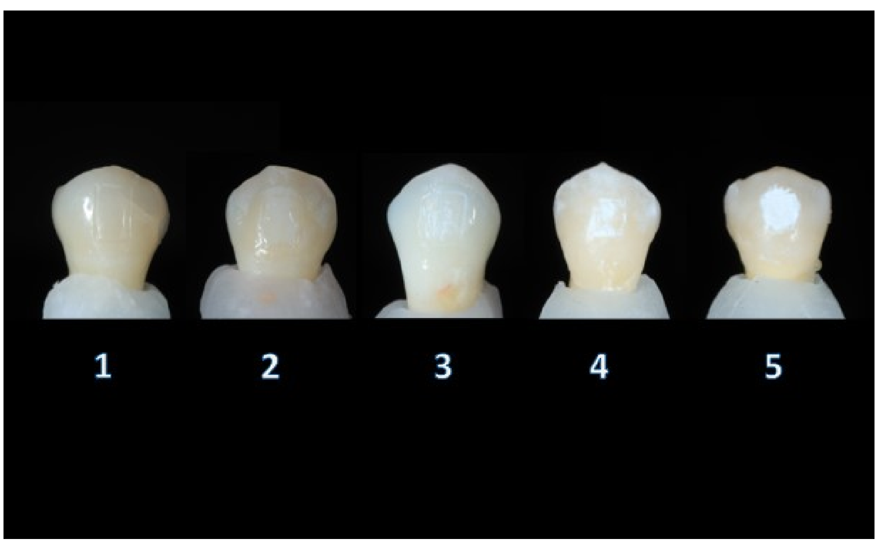


Figure 9. Three-dimensional reconstructed image of a WSL from µCT density analysis

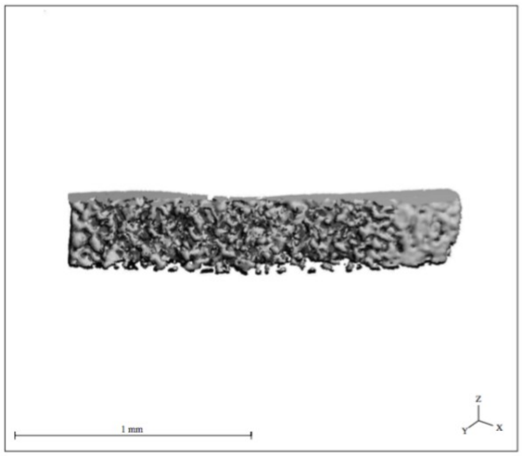


Figure 10. Survey respondents' level of education

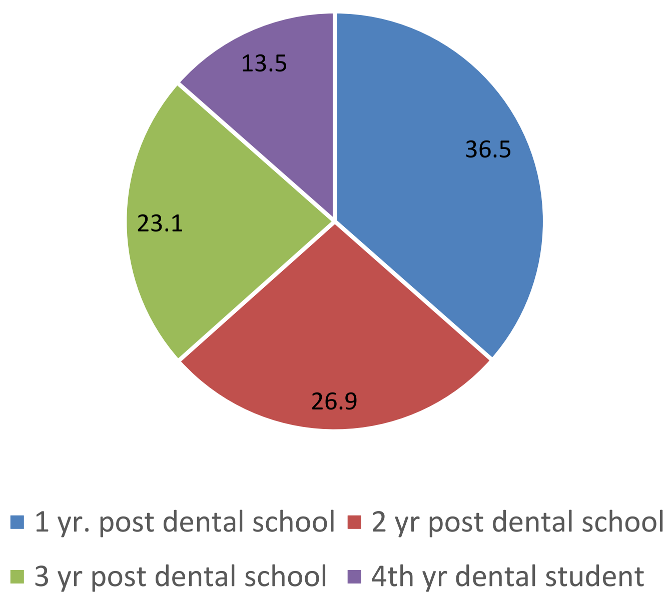


Figure 11. Survey respondents' preferred treatment method for white spot lesions

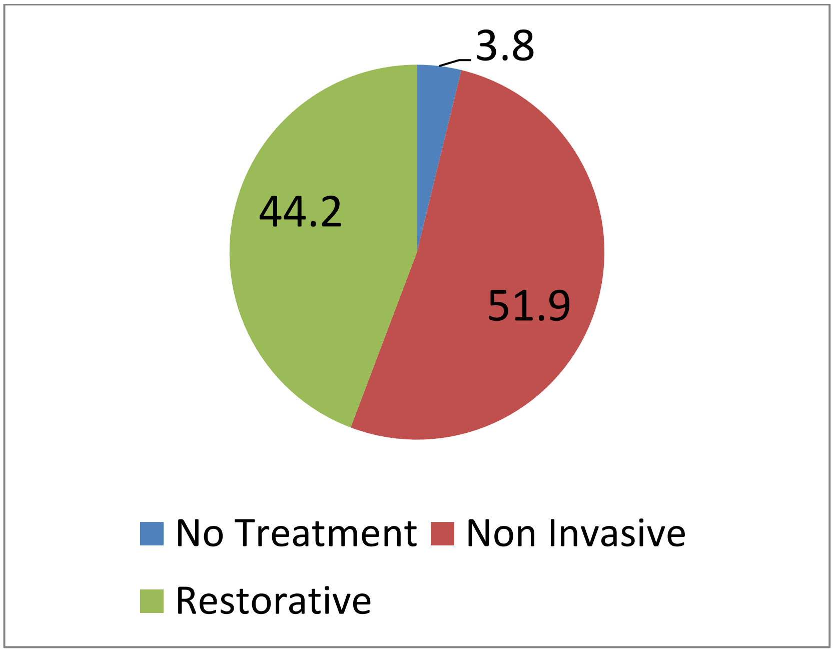
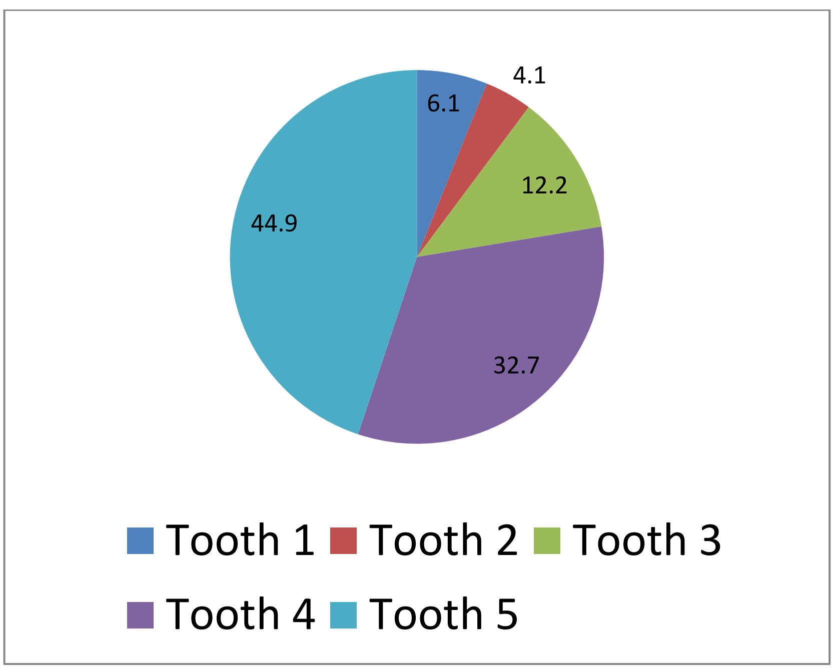


Figure 12. Percentage of respondents who chose the severity of WSL at which a restoration was necessary



APPENDIX B

TABLES

Table 1. White spot lesion depth measured with μμCT 35 Desktop Software

	Depth		Difference		
Group	**Mean**	**SD**	**Mean**	**SE**	**Probability**
Control	0.135	0.162	0.04	0.03	0.24
Experimental	0.1	0.012			

Table 2. Spectrophotometer lightness values for control and experimental groups at 4 time points: T1 (intact enamel), T2 (WSL formation), T3 (after microabrasion), T4 (post pH cycling)

	Control		Experimental		Differences	
Time Pt	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**Probability**
T1	83.18	3.02	80.73	3.81	2.46	0.008
T2	75.38	2.89	73.35	4.33	2.03	0.037
T3	81.17	3.05	79.25	3.90	1.93	0.037
T4	77.84	2.96	79.39	3.70	-1.56	0.078

Table 3. ANCOVAR for spectrophotometer lightness values at 3 time points: T2 (WSL creation), T3 (after microabrasion), and T4 (post pH cycling) after controlling for T1 (intact enamel)

	Control		Experimental		Group Difference
Time Point	**Mean**	**SE**	**Mean**	**SE**	**Probability**
T2	74.30	0.40	74.43	0.40	0.824
T3	79.97	0.17	80.46	.017	0.058
T4	76.76	0.28	80.47	0.28	<0.001

Table 4. Changes in spectrophotometer lightness values between time points: T1 (intact enamel), T2 (WSL creation), T3 (after microabrasion), T4 (post pH cycling)

	Control		Experimental		Differences	
Time Pt	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**Probability**
T1-T2	-7.81	2.18	-7.38	2.11	-0.43	0.44
T2-T3	5.80	2.12	5.90	2.05	-0.10	0.853
T3-T4	-3.34	1.77	**0.145**	1.20	-3.48	<0.001
T1-T4	-5.35	1.99	-1.34	0.93	-4.01	0.001

Table 5. ANCOVAR for chagnes in spectrophotometer lightness values between time points: T1 (initial enamel), T2 (WSL creation), T3 (after microabrasion), T4 (post pH cycling)

	Control		Experimental		Probabilities	
Time Pt	**Mean**	**SE**	**Mean**	**SE**	**Initial**	**Group Difference**
T1-T2	-7.66	0.40	-7.53	0.4	0.137	0.824
T2-T3	5.67	0.39	6.03	0.39	0.187	0.530
T3-T4	-3.20	0.28	0.10	0.28	0.056	<0.001
T1-T4	-5.19	0.28	-1.49	0.28	0.032	<0.001

Table 6. Perceived change between time points as measured by a visual analog scale: T1 (intact enamel), T2 (WSL creation), and T4 (post pH cycling)

	Control		Experimental		Group Difference
Time Point	**Median**	**IQR**	**Median**	**IQR**	**Probability**
T2-T4	81.3	51.1, 108.9	99.6	70.4, 122.1	<0.001
T1-T4	58.5	46.4, 69.6	30.9	15.4, 43.9	<0.001

Table 7. µCT mineral density for control and experimental teeth at three time points: T1 (intact enamel), T2 (WSL creation), T4 (post pH cycling)

		Control		Experimental		Difference		
Time Pt.	**Density**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SE**	**Prob.**
T1	**Apparent**	1967.03	88.97	1959.64	72.93	7.39	21.36	0.731
	Material	2175.07	68.41	2179.4	71.88	-5.33	18.58	0.775
T2	**Apparent**	1273.44	125.19	1314.62	157.9	-41.18	36.79	0.268
	Material	1999.15	38.74	1997.13	37.09	2.02	9.79	0.838
T4	**Apparent**	1604.88	137.14	1709.49	133.96	-104.62	35.29	0.004
	Material	2068.03	36.77	2097.82	41.71	-29.79	10.25	0.005

Table 8. Changes in mineral density between time points: T1 (intact enamel), T2 (WSL creation), and T4 (post pH cycling)

		Control		Experimental		Difference		
Time Pt.	**Density**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SE**	**Probability**
T1-T2	**Apparent**	-695.38	162.71	-651.74	185.69	-43.64	45.85	0.345
	Material	-173.63	80.47	-179.11	81.63	5.45	21.47	0.800
T2-T4	**Apparent**	335.37	193.26	394.87	193.05	-59.5	50.3	0.242
	Material	69.29	48.22	100.69	49.95	-31.4	12.79	0.017
T1-T4	**Apparent**	-368.58	162.74	-251.1	182.71	-117.48	45.89	0.013
	Material	-98.68	68.51	-81.66	87.22	-17.02	20.96	0.42