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Effectiveness of Resin Infiltration and Mi Paste Cpp-Acp in Masking White Spot Lesions

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The Effectiveness of Resin Infiltration and MI Paste CPP-ACP in Masking White Spot Lesions

by

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A Thesis submitted in partial satisfaction of
the requirements for the degree
Master of Science in Orthodontics

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Each person whose signature appears below certifies that this Thesis in his/her opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.

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ABBREVIATIONS

WSLs	White Spot Lesions
RI	Resin Infiltration
CPP-ACP	Casein Phosphopeptide Amorphous Calcium Phosphate
L*	Lightness value on the CIELAB scale
ΔL	Change in lightness value, from T_1 to T_2
T_0	Time at baseline, prior to specimen demineralization
T_1	Time after specimen demineralization (WSLs created)
T_2	Time after specimen remineralization/treatment

ABSTRACT OF THE THESIS

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by

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Graduate Program, Master of Science in Orthodontics and Dentofacial Orthopedics
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The development of decalcification around orthodontic brackets and bands, commonly called white spot lesions (WSLs), is often observed in patients with poor oral hygiene during treatment (Behnan et al., 2010, Rodgers et al., 2010). In many instances, these WSLs continue to be visible after the removal of fixed appliances and after natural remineralization. The purpose of this in vitro study was to compare the effectiveness of two treatment modalities, resin infiltration (RI) and Casein Phosphopeptide Amorphous Calcium Phosphate (CPP-ACP), at improving the light reflectivity and thus the appearance of WSLs using spectrophotometric analysis.

Sixty extracted human third molars were partially demineralized to create artificial WSLs and randomly divided into a control (artificial saliva) and two treatment groups. A spectrophotometer (VITA Easyshade® compact) was used before and after treatment to quantify the amount of light (L*) reflected from the surfaces of each tooth specimen. All three groups showed statistically significant improvements in reflectivity, as indicated by the increase in L* after treatment. However, there were no statistically significant differences among the study groups. In conclusion, both treatment modalities and the control were effective at masking WSLs.

CHAPTER ONE

INTRODUCTION

Patients seek orthodontic treatment to improve their dentofacial esthetics, but occasionally the development of areas of decalcification around orthodontic brackets and bands called white spot lesions (WSLs) can occur as a negative consequence of poor oral hygiene during treatment (Behnan et al., Rogers et al., 2010). Unfortunately, many WSLs are visible after the removal of fixed appliances and natural remineralization. There are numerous published studies on the topic of remineralization of WSLs, but few studies have focused specifically on comparing the performance of various treatments in the masking of WSLs.

Purpose

The purpose of this in vitro study was to compare the effectiveness of two treatment modalities, resin infiltration (RI) and Casein Phosphopeptide Amorphous Calcium Phosphate (CPP-ACP), at improving the light reflectivity and thus the appearance of WSLs using spectrophotometric analysis. The null hypothesis was that there would be no statistical difference in the appearance of WSLs (quantified by the amount of light reflected from the surfaces of each tooth specimen) before and after treatment for the RI and CPP-ACP treatment groups when compared to the control group.

CHAPTER TWO

REVIEW OF LITERATURE

White Spot Lesions

White Spot Lesions (WSLs) associated with poor oral hygiene and plaque accumulation are a relatively common occurrence in fixed orthodontic treatment, with rates reported between 2% and 96% (Mitchell, 1992). WSLs are defined as subsurface enamel porosities caused by imbalances in the demineralization and remineralization process (Beerens et al., 2010). The decrease in the mineral content beneath the intact enamel surface changes the light reflectivity of the normally translucent enamel and the appearance of these lesions can vary from no perceptible change in color to white spots on the enamel (Roger et al., 2010). Thus, although the term white spot lesion is used to describe this phenomenon, the lesions may not actually appear white in all cases.

The translucency of enamel depends on the size of the intercrystalline spaces and whether those spaces are filled with water or air. In the early stages of demineralization, WSL could only be seen with air drying as the water around the enamel prisms are replaced with air. As the demineralization progresses, the intercrystalline spaces become larger and the lesion becomes visible without air drying (Holmen et al., 1985). This phenomenon has to do with the different refractive indices of enamel (1.65), water (1.33) and air (1.00). The porous enamel, with greater intercrystalline air and water space, will scatter more light than sound enamel, resulting in visual enamel opacity (Houwink, 1974).

Before cavitation of the enamel occurs, therapeutic agents can be used to remineralize or reverse the caries process (Featherstone 2008). However, even after remineralization has occurred and the caries process has been arrested, the unsightly white spots often persist. This persistence could be due to the location of remineralization. A number of studies have shown that more remineralization occurs on the surface of the lesion relative to the body of the lesion, thus the body of the lesion does not achieve the same level of remineralization as the surface (ten Cate et al., 1981). Furthermore, optical property experiments have shown that the light scattering intensity depends on the entire mineral volume. As such, even though there may be a decrease in the size of the lesion after remineralization treatment, the lesion may remain clinically visible (Holmen et al., 1985).

Resin Infiltration

The purpose of the resin infiltration (RI) technique is to micro-invasively infiltrate the intercrystalline spaces of enamel with polymerizable low viscous resin to arrest enamel lesions. Before a WSL can be infiltrated, it must be acid etched with hydrochloric acid to remove the highly mineralized pseudointact surface layer of enamel (Kielbassa et al., 2009). Unlike dental sealants, which sit on the surface of the enamel, the RI can penetrate the enamel up to 400 microns (Paris et al., 2007). A study on adhesive penetration showed that just 60 microns of infiltration was enough to prevent further demineralization, despite the breakdown of the surface coating (Davila et al., 1975).

In addition to arresting caries by creating a diffusion barrier for cariogenic acids, RI has the added benefit of masking WSLs because it fills in the intercrystalline spaces within the enamel rods. Because the resin (infiltrant) has a refractive index (1.48) that is similar to enamel (1.65), RI can completely mask the opaque color of less severe inactive WSLs and partially mask the appearance of moderate to severe WSLs (Neuhaus et al., 2010, Paris and Meyer, 2009). An in vivo study of bovine teeth demonstrated that RI was an effective treatment for masking WSLs (Torres et al., 2010).

Casein Phosphopeptide Amorphous Calcium Phosphate

Casein Phosphopeptide Amorphous Calcium Phosphate (CPP-ACP) is a nanocluster that binds calcium and phosphate ions in an amorphous form. When the pH drops in the oral environment, the calcium and phosphate ions are released to produce a supersaturated concentration of ions in the saliva, which precipitates a calcium-phosphate compound onto the exposed tooth surface (Aimutis, 2004). The use of CPP-ACP, in the form of MI Paste (Milk derived phosphopeptide Infiltration), may be a helpful adjunct in preventing or remineralizing WSLs, but there is insufficient and conflicting evidence of its efficacy (Guzman-Armstrong et al., 2010, Tung and Eichmiller, 1999).

An in vitro study using CPP-ACP stabilized calcium phosphate solutions showed subsurface enamel remineralization of human third molars (Reynolds, 1997). Another in vitro study found that the use of CPP-ACP tooth mousse decreased demineralized lesion depth and showed higher remineralizing potential when used in combination with fluoridated toothpaste. Therefore, it was recommended that for children with high caries risk, CPP-ACP should be self-applied after brushing with fluoridated toothpaste (Kumar

et al., 2008). Bailey et al. in 2009 also found that significantly more post-orthodontic WSLs regressed with the CPP-ACP cream compared with a placebo over twelve weeks. Yet another in vitro study found that the application of CPP-ACP paste to teeth surface forms a layer that fills the interprism cavities, preventing dental erosion and acid attack by soft drinks (Poggio et al., 2009).

A recent in vivo study of WSLs after debonding of orthodontic appliances demonstrated a significant reduction in fluorescence and reduced area of lesions after daily application of CPP-ACP paste for a period of four weeks (Brochner et al., 2011). Similarly, Robertson et al., in a prospective randomized controlled trial, found that the use of MI Paste Plus (MI Paste with Fluoride) during orthodontic treatment not only decreased the number of existing WSLs but also prevented the development of new lesions (Robertson et al., 2011).

However, Pulido et al. found that there was no significant difference between the artificial saliva and MI paste group in reducing carious lesion progression (Pulido, 2008). In vitro evaluations of various treatments to prevent demineralization next to orthodontic brackets found that MI paste did not differ statistically from the control group (Behnan et al., 2010). Due to these inconsistent findings, further research should be undertaken to confirm that CPP-ACP has the potential to reverse the visible appearance of white spot lesions.

Artificial White Spot Lesions

Artificially created WSLs of enamel have been successfully used to study the remineralization of enamel in vitro because they are histologically similar to natural

white spot lesions (Itthagarun et al., 1999, 2000). Additionally, artificially created WSLs have proven to be a more reliable experimental model than natural lesions because they can be produced more homogeneously (Silverstone, 1983).

Shade Analysis

Human visual color determination of a patient's tooth color against a shade guide is the most commonly applied method in clinical dentistry. However, due to differences in human perception of color, visual shade assessment lacks standardization. Variation in external light conditions, experience, age, and fatigue of the human eye may lead to inconsistencies. Furthermore, shade guides do not always cover the complete range of natural tooth colors (Joiner, 2004).

The color of a tooth can be measured and quantified using a spectrophotometer on a CIELAB scale. The Commission International de l'Eclariage established in 1931 created the standard CIELAB scale that places the color of an object in a three-dimensional color space. The three axes are L*, a*, and b*. The coordinate L* is a measure of the lightness of an object and ranges from 0 (perfect black) to 100 (white). Therefore, the higher the L* value, the greater the light reflectivity of the object (CIE, 1978). The a* and b* coordinates measure color and represent an object's position between red and green (a*) and yellow and blue (b*). One advantage of the CIE Lab system is that color differences can be quantified in units that can be related to visual perception in the clinical setting (O'Brian et al., 1997).

Spectrophotometer

A study comparing visual and spectrophotometric shade analysis showed that the use of a spectrophotometer is more accurate and more reproducible compared with human shade assessment (Paul et al., 2002). Another study comparing an objective (spectrophotometer) to a subjective method (human evaluation) of tooth shade evaluation confirmed that human evaluation of tooth shade is unreliable and that the spectrophotometer can provide a more predictable and accurate method of evaluating tooth shade *in vitro* (Horn et al., 1998). The use of a spectrophotometer is not without its drawbacks, however, as the equipment is often complicated to set up and it can be difficult to measure tooth color in vivo (Tun et al., 2002).

The repeatability and accuracy of spectrophotometric measurements have been evaluated by several studies. One study comparing four spectrophotometers against a reference system found that all the devices, including the VITA Easyshade® compact, had high intraclass correlation coefficients, between 0.979 and 1.000. Furthermore, the VITA Easyshade® compact had the smallest mean deviation from the reference when measuring the L* value (Lehmann et al., 2010). Another study found that the values obtained from the clinical spectrophotometer (VITA Easyshade®) and the laboratory one (PSD 1000) showed excellent scanning repeatability (Corciolani 2006).

CHAPTER THREE

MATERIALS AND METHODS

Specimen Preparation

Sixty extracted human permanent molars that were free of caries and/or restorations were collected, cleaned, and stored in 0.1% thymol solution prior to the study to prevent dehydration (Torres et al., 2010). The root surface below the cementoenamel junction (CEJ) of each specimen was painted with two coats of an acid resistant varnish (Revlon Red, Revlon Consumer Products Corporation, New York, NY, USA) to prevent undesired root erosion from the demineralization process (Figure 1).

Figure 1: Specimen painted with two coats of acid resistant varnish after demineralization

To increase precision and repeatability of L* measurements, custom jigs were fabricated from vinyl polysiloxane bite registration material (Dentsply® Regisil Rigid

VPS) for each tooth specimen. The teeth were dabbed dry but not allowed to dehydrate. A cylindrical rod that was the same diameter as the tip of the spectrophotometer was placed against the buccal coronal surface of the specimen, and with the rod in place, vinyl polysiloxane was expressed around the rod and the buccal and occlusal surfaces of the tooth, thereby creating a unique jig for each specimen. All the cylindrical rods used were equal in length and diameter. (Figure 2)

Figure 2: Custom jig fabricated for each tooth specimen to increase the precision and repeatability of L* measurements

Shade Measurement

The tooth specimens were randomly divided into three groups (n=20 per group): control, RI, and CPP-ACP. The specimens were measured prior to demineralization (T_0), after demineralization (T_1), and after treatment (T_2), using a spectrophotometer (VITA Easyshade® compact) that was calibrated according to the manufacturer's instructions. The T_0 values were measured prior to demineralization and were recorded to establish the baseline in which subsequent values can be compared to. All spectrophotometric

readings were measured in a full spectrum, color corrected environment with color temperature of 5,500 K with no outside ambient light and neutral-colored surroundings.

The tip of the spectrophotometer was guided by the custom jig to the test area to ensure that all L* values for the same specimen were measured at the same location each time (Figure 3). The jigs were also fabricated with sufficient depth so that the tip of the spectrophotometer can be directed perpendicular to the enamel surface. Each specimen surface was measured three times and the L* value was the average of the three.

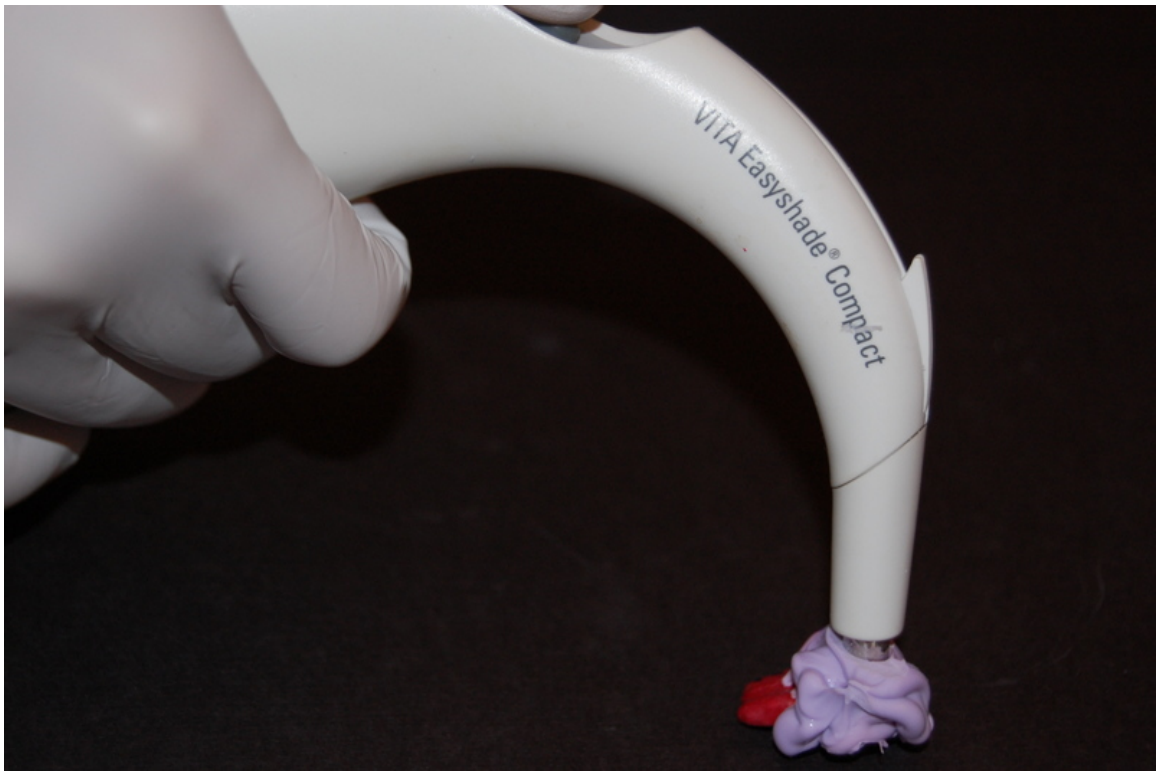


Figure 3: Tip of the spectrophotometer guided by the custom jig to the test area of the tooth specimen

Specimen Demineralization

To create the artificial WSLs, the teeth were placed in one liter of demineralization solution containing 450 mL distilled water, 100 mL lactic acid, 1.5 g calcium phosphate tribes, 200 mL 1% carbopol C907, HCl and NaOH to adjust pH to 5.0 at 37° C for fourteen days (Torrado et al., 2004). During the demineralization period, the pH of the solution was monitored daily, and if necessary, 10% HCl or 10 M NaOH was added to maintain the pH at 5 (Paris and Meyer-Lueckel, 2008).

After demineralization, the teeth were rinsed with water and carefully dried with absorbent paper for five seconds (not desiccated) and the L* value at T_1 was recorded using the spectrophotometer.

Control

Artificial saliva was prepared to serve as the control. The artificial saliva with mucin consisted of 1.45 mM Calcium Chloride, Anhydrous (CaCl2) MW 110.99, 5.4 mM Potassium Phosphate Monobasic (KH2PO4) MW 136.09, 0.1M Tris-HCl MW 156.60, Porcine Gastric Mucin (from Porcine Stomach, Sigma #M1778-10G), and deionized H2O. The final pH was adjusted to 7.0 using HCl or KOH.

The control teeth were submerged in 250 mL of artificial saliva solution and the saliva was changed at the end of each week. At the end of the fourth week, the L* value at T_2 was measured.

Resin Infiltration

The RI group was resin infiltrated (Icon®, DMG, Hamburg, Germany) according to the manufacturer's instructions (Figure 4). The shade, L* value at $T_{2,}$ was measured immediately after the RI treatment.

Step #	Resin Infiltration Protocol
1	Clean the affected tooth and rinse well
	
2	Icon® Etch for 2 minutes
	
3	Rinse with water and air dry for 30 seconds
	
4	Apply Icon®-dry for thirty seconds
	
5	Apply Icon® Infiltrant for three minutes. Remove excess with cotton or with sharp explorer. Light cure for forty seconds
	
6	With a new tip, apply Icon® Infiltrant again for one minute. Remove excess with cotton or with sharp explorer. Light cure for forty seconds
	
7	Measure shade after RI treatment

Figure 4. Protocol for the Resin Infiltration Group

Casein Phosphopeptide Amorphous Calcium Phosphate

(CPP-ACP)

The CPP-ACP group was brushed manually for 5 seconds on the buccal surface using a fluoridated toothpaste (Colgate Total, Colgate-Palmolive, USA) and rinsed with distilled water. MI paste (GC Corp, Tokyo, Japan) was applied topically for three minutes and, without rinsing, the specimens were placed into fresh artificial saliva (Poggio et al., 2009). This procedure was performed daily for a total of four weeks (Figure 5). The shades were measured at the end of each week and the final shade, L* value at T_2, was measured at the end of the fourth week.

Step #	CPP-ACP Protocol
1	Brush manually for 5 seconds using fluoridated toothpaste
	↓
2	Rinse with distilled water
	↓
3	Apply MI Paste® for three minutes
	↓
4	Without rinsing, place specimen into fresh artificial saliva
	↓
5	Repeat daily for four weeks
	↓
6	Measure shade at end of each week

Figure 5. Protocol for the CPP-ACP Group

Step #	Control n=20	RI n=20	CPP-ACP n=20
1	Custom jig fabrication	Custom jig fabrication	Custom jig fabrication
	↓	↓	↓
2	Record pre-demineralization shade T_0	Record pre-demineralization shade T_0	Record pre-demineralization shade T_0
	↓	↓	↓
3	Demineralize for 14 days	Demineralize for 14 days	Demineralize for 14 days
	↓	↓	↓
4	Record initial shade after demineralization T_1	Record initial shade after demineralization T_1	Record initial shade after demineralization T_1
	↓	↓	↓
5	Store in artificial saliva for 4 weeks	Resin infiltration application	Daily application of CPP-ACP for 4 weeks
	↓	↓	↓
6	Record final shade T_2 at the end of the 4th week	Record final shade T_2 immediately after RI	Record final shade T_2 at the end of the 4th week

Figure 6. Outline of steps for each of the three groups: Control, RI, and CPP-ACP

Statistical Analysis

The data was analyzed with both parametric and nonparametric procedures. All tests of hypotheses were two-tailed and conducted at an alpha level of 0.05. Statistical analysis was performed with SAS v9.2 software (SAS Institute, Cary, NC).

There were two values of MI Paste (T_1 and T_2) and one value of RI (T_2 only) that may be considered to be outliers (Figures 7 and 8). To reduce their bias on the statistical analysis, the Kruskal-Wallis and Wilcoxon Signed Ranks Test were used to determine whether there were between-group and within-group differences, respectively, in T_1 or T_2 values. A two factor (1 between, 1 within) mixed model ANOVA was performed on the ranks of the data to control for effects simultaneously.

CHAPTER FOUR

RESULTS

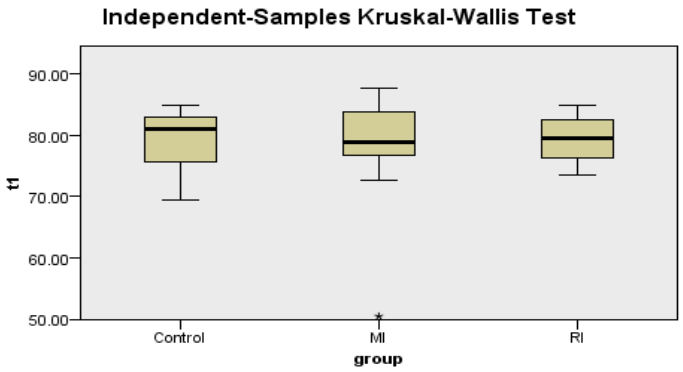
The results of the parametric and nonparametric statistical analysis were similar: there were no statistically significant between-group differences in T_1 or T_2 values. There was, however, a statistically significant T_1- T_2 difference in the L* values (ΔL) seen within each of the groups (Figures 9,10,11). In other words, all three groups (Control, RI, CPP-ACP) showed a significant difference in ΔL after treatment. The T_2 measurements were significantly larger than the T_1 measurements ($p < .001$). This T_1 to T_2 difference in ΔL was statistically the same for all of the groups ($p = .820$). In addition, no statistically meaningful difference in T_1 or T_2 values was detected between the groups ($p = .891$) (See Table 1).

The change in L* value in relation to treatment and time point is illustrated in Figure 12. The L* was the highest before demineralization at T_0 and lowest after demineralization at T_1. The L* increased after treatment at T_2, but not to the level of T_0.

Using the independent-samples Kruskal-Wallis test, the distribution of L* values were similar across the three groups (Control, RI, CPP-ACP) at T_0, T_1 and T_2, with significance at 0.717, 0.967, 0.659, respectively. The mean L* value at T_1 for all three groups was 79.06, with a standard deviation of 5.60. The individual mean T_1 L* values for the control, RI, and CPP-ACP groups were 79.48, 79.41, 78.30, respectively. The mean L* value at T_2 for all three groups was 83.77, with a standard deviation of 3.68. The individual mean T_2 L* values for the control, RI, and CPP-ACP groups were 83.75,

83.53, 84.03, respectively. These values are summarized in Table 2 and Figures 13 and 14.

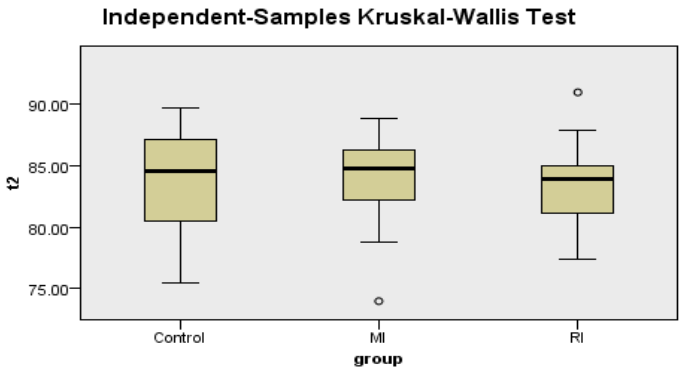
The T_2 L* values for the CPP-ACP group from weeks one to four were 80.00, 83.95, 84.76, 84.03, respectively. There was a rapid increase in L* value up to the end of the second week, with the peak L* value at the end of the third week. The final L* value at the end of the fourth week was similar to that of the second week (Figure 15).



Total N	60
Test Statistic	.067
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.967

1. The test statistic is adjusted for ties.
2. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

Figure 7. Boxplot of T_1 values of the three groups



Total N	60
Test Statistic	.777
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.678

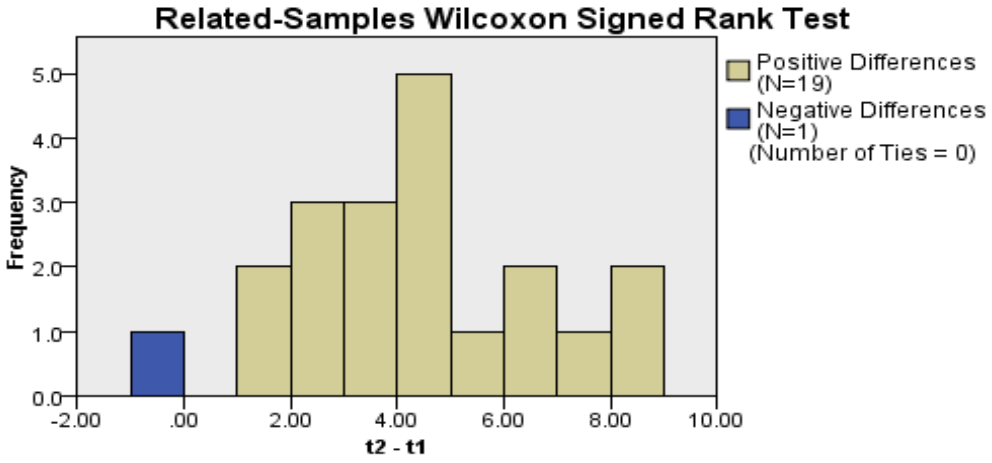
1. The test statistic is adjusted for ties.
2. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

Figure 8. Boxplot of T_2 values of the three groups

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The median of differences between t1 and t2 equals 0.	Related-Samples Wilcoxon Signed Rank Test	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.



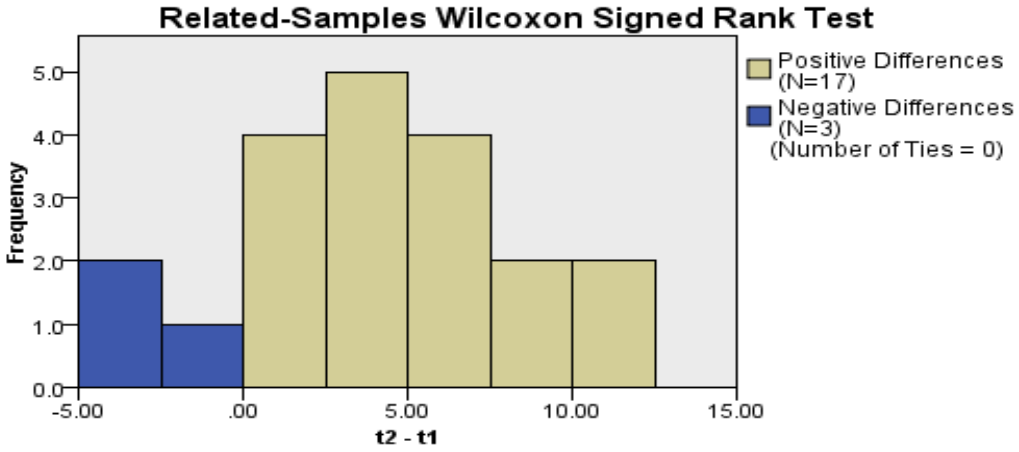
Total N	20
Test Statistic	209.000
Standard Error	26.786
Standardized Test Statistic	3.883
Asymptotic Sig. (2-sided test)	.000

Figure 9: T_1- T_2 difference in the ΔL^* values in Control Group

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The median of differences between t1 and t2 equals 0.	Related-Samples Wilcoxon Signed Rank Test	.001	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.



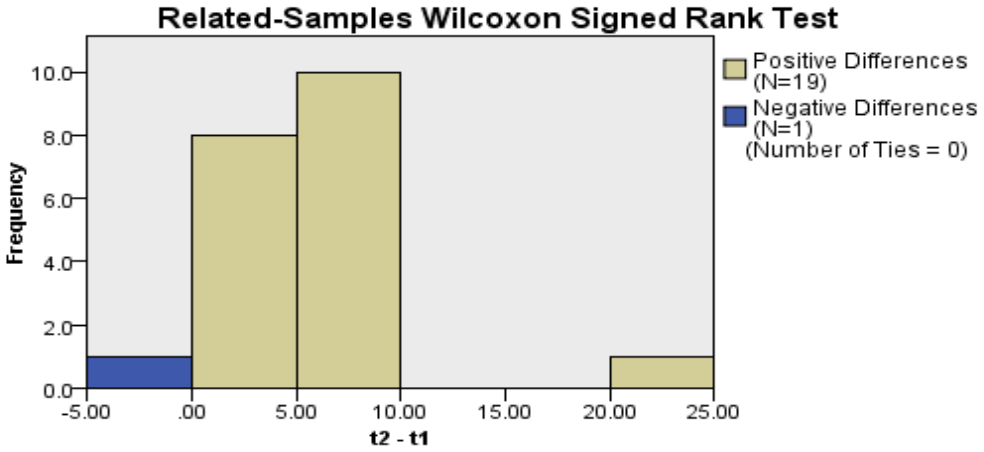
Total N	20
Test Statistic	194.000
Standard Error	26.786
Standardized Test Statistic	3.323
Asymptotic Sig. (2-sided test)	.001

Figure 10: T_1- T_2 difference in the ΔL* values in RI

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The median of differences between t1 and t2 equals 0.	Related-Samples Wilcoxon Signed Rank Test	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.



Total N	20
Test Statistic	206.000
Standard Error	26.784
Standardized Test Statistic	3.771
Asymptotic Sig. (2-sided test)	.000

Figure 11: T_1- T_2 difference in the ΔL* values in CPP-ACP Group

Table 1: Test of within-subject effects, contrasts and tests of between-subject effects

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	34307.008	1	34307.008	92.907	.000
	Greenhouse-Geisser	34307.008	1.000	34307.008	92.907	.000
	Huynh-Feldt	34307.008	1.000	34307.008	92.907	.000
	Lower-bound	34307.008	1.000	34307.008	92.907	.000
time * group	Sphericity Assumed	147.467	2	73.733	.200	.820
	Greenhouse-Geisser	147.467	2.000	73.733	.200	.820
	Huynh-Feldt	147.467	2.000	73.733	.200	.820
	Lower-bound	147.467	2.000	73.733	.200	.820
Error(time)	Sphericity Assumed	21048.025	57	369.264		
	Greenhouse-Geisser	21048.025	57.000	369.264		
	Huynh-Feldt	21048.025	57.000	369.264		
	Lower-bound	21048.025	57.000	369.264		

Tests of Within-Subjects Contrasts

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Linear	34307.008	1	34307.008	92.907	.000
time * group	Linear	147.467	2	73.733	.200	.820
Error(time)	Linear	21048.025	57	369.264		

Tests of Between-Subjects Effects

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	439230.000	1	439230.000	284.110	.000
group	356.850	2	178.425	.115	.891
Error	88121.150	57	1545.985		

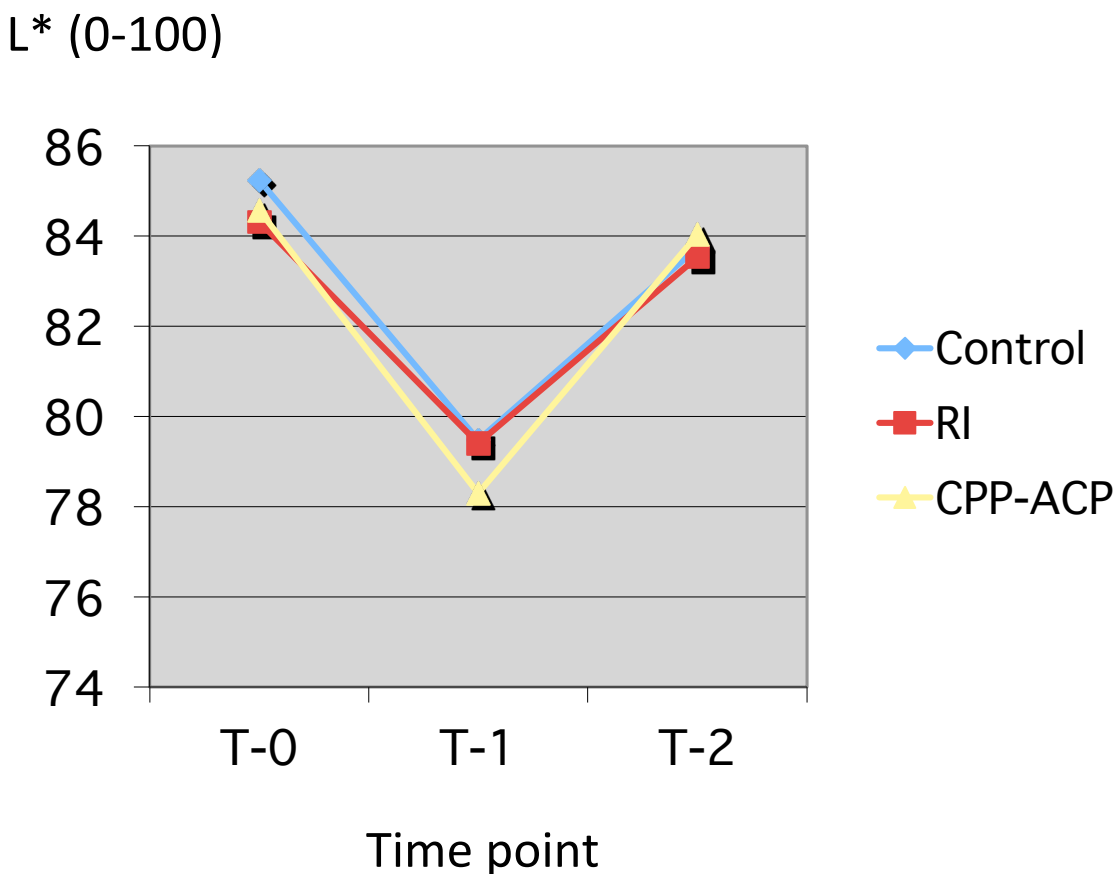


Figure 12: Spectrophotometer readings: L* (from scale of 0-100) at T_0 (before demineralization), T_1 (after demineralization) and T_2 (after treatment)

Table 2: Mean L* Values at T_1 and T_2

	Mean L* Value at T_1	Mean L* Value at T_2
Control (n=20)	79.48	83.75
RI (n=20)	79.41	83.53
CPP-ACP (n=20)	78.30	84.03
Group Average (n=60)	79.48	83.77

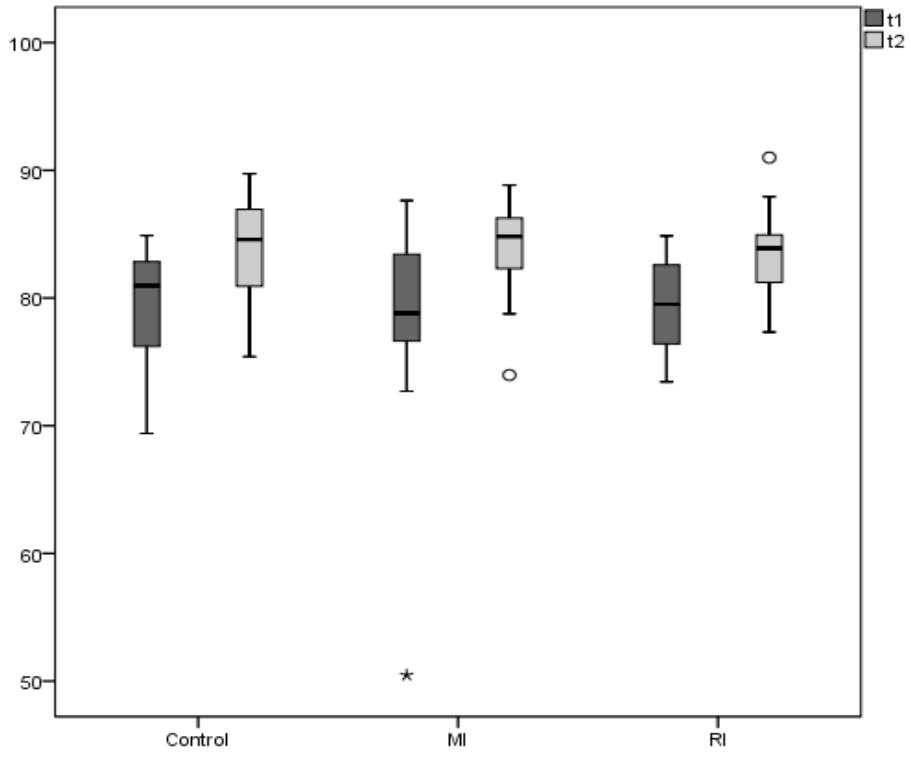
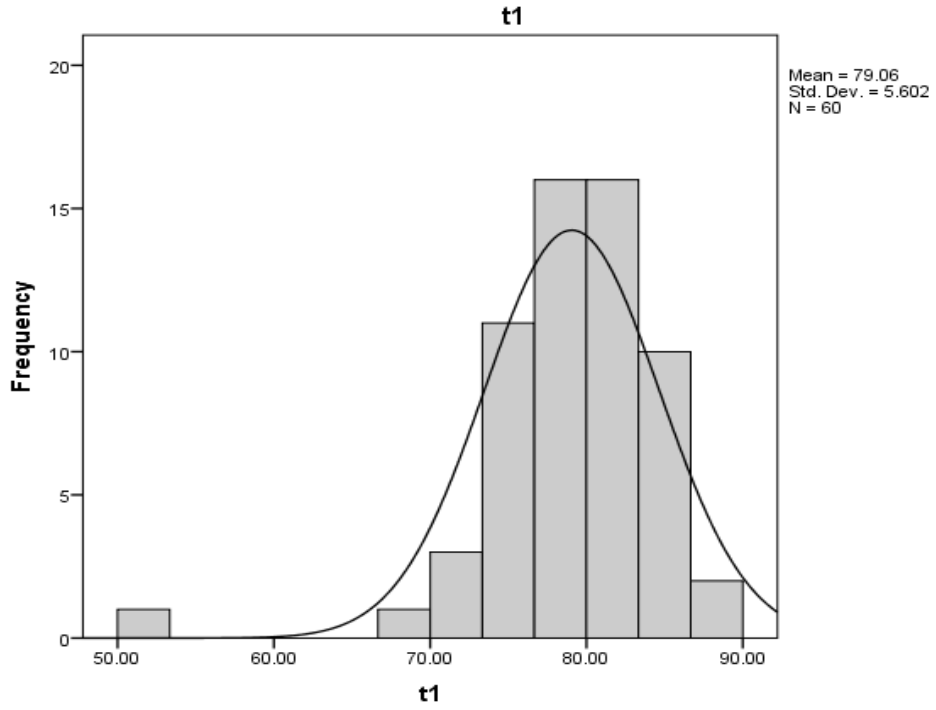


Figure 13: Boxplot of mean L* Values at T_1 and T_2



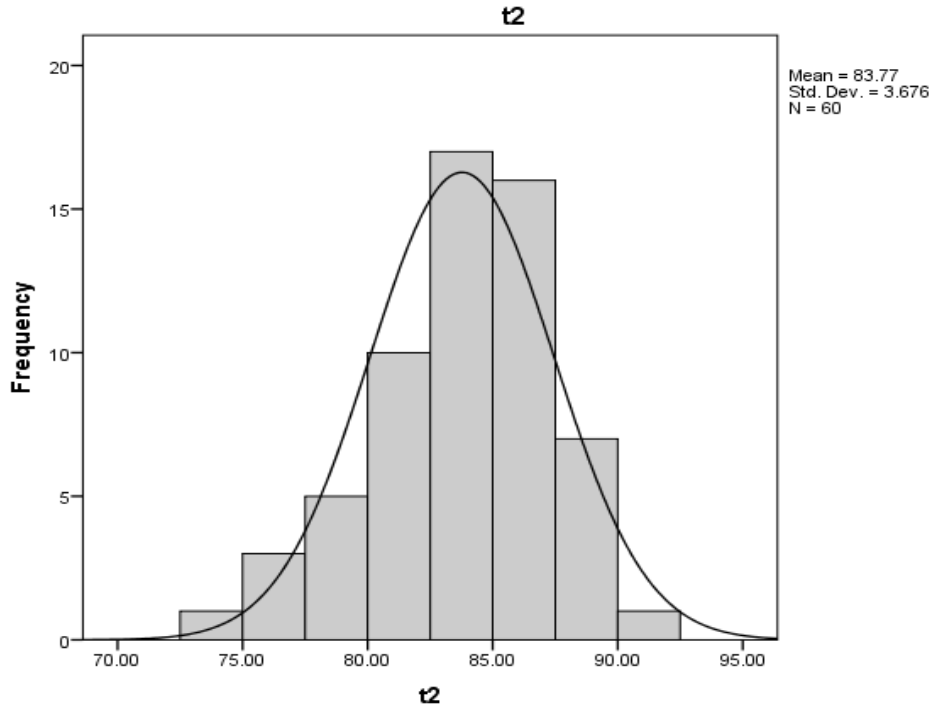


Figure 14: Histogram of mean L* values of the three groups at T_1 and T_2

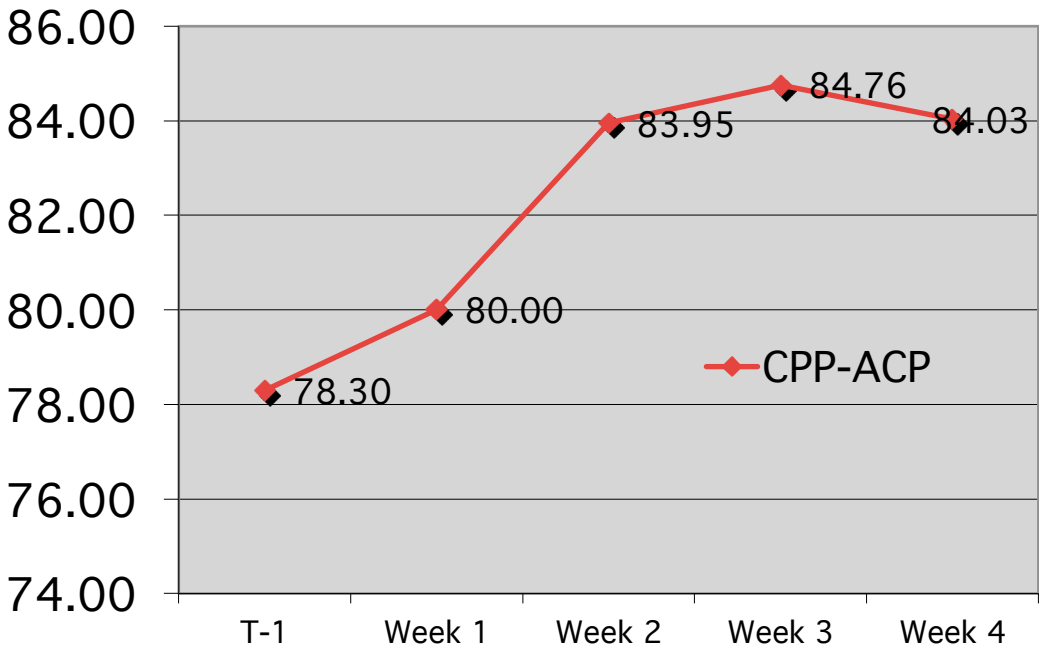


Figure 15: L* values of CPP-ACP by weeks

CHAPTER FIVE

DISCUSSION

Spectrophotometer Reading

Spectrophotometers utilize fiber optic technology to transmit light to an object in order to measure the object's reflected light (Corciolani and Vichi, 2006). The color of an object can then be quantified in a three-dimensional color space (Torres et al., 2011). Because the objective of this study was to evaluate the masking effect of WSLs based on the lightness scale, only the L* value (0-100) was measured.

Objects with lower L* values reflect less light when illuminated by the spectrophotometer than objects with higher L* values. This is because more light is absorbed, scattered elsewhere, or transmitted through in lower L* value objects (Fondriest, 2003). Darling et al. studied the light scattering properties of natural and artificially demineralized dental enamel and found that with increasing mineral loss, the scattering coefficient increased exponentially, by more than two orders of magnitude. Ko et al., also measured the light scattering of enamel blocks as a result of mineral loss and found that demineralization of enamel increased the optical scattering coefficient by a factor of three.

Partial dissolution of individual mineral crystals in demineralized teeth creates micropores within the body of the lesion. These micropores act as scattering centers, strongly scattering visible light (Darling et al., 2006). Thus, teeth with demineralization have lower L* values because they scatter more light and reflect less light than healthy

teeth. The results of this study showed that the average L* values at T_1 (after demineralization) were in fact significantly lower than the average T_0 values (baseline before demineralization).

Demineralization

The demineralization protocol used in this study (extracted human third molars, demineralized for fourteen days, using the demineralization solution formula provided by the LLU CDR) was developed from the results of our pilot study. We determined that fourteen days of demineralization produced visible WSLs that were free of surface cavitation of the outer surface of the enamel. Tooth specimens that were demineralized for more than fourteen days exhibited cavitated WSLs, which were not acceptable for the purposes of this study. The decrease in L* value combined with the chalky white visual appearance of the specimen were the two pre-defined criteria for successful demineralization in this study.

Remineralization

There was a statistically significant increase in L* values at T_2 compared to T_1 for all three groups, including the control. This indicates that all three treatments, including the artificial saliva control, were effective in masking the WSLs. Many in vitro and in vivo studies have found that natural remineralization alone is sufficient to stop the carious lesion progression and repair of the subsurface of the WSL, without the intervention of restorative dentistry (Featherstone, 2000, 2008). The natural remineralization repair process in non-cavitated lesions relies on calcium, phosphate, and

fluoride ions to rebuild the existing crystal remnants in the subsurface of the WSL (Featherstone, 2008).

A non-cavitated WSL can continue to be visible clinically and radiographically, even after remineralization because of the increased radiolucency and physical property changes. In this study, the T_2 values approached but did not reach the T_0 values. The larger the body of the lesion, the lower the potential masking effect of remineralization. This is due to a difference in remineralization between the surface of the WSL and the body of the lesion (Gonzalez-Cabezas, 2010, ten Cate et al., 1998).

Comparisons between RI, CPP-ACP, and Control

Although all three groups showed a statistical improvement in L* values from T_1 to T_2, the difference in L* values between the groups was not statistically significant. In this study, RI, CPP-ACP, and artificial saliva proved to be equally effective in improving the esthetic appearance of WSLs by increasing light reflectivity. This finding differed from that of Torres et al., which concluded that treatment with RI was superior to artificial saliva (Torres et al., 2010).

One possible reason for this difference in results may be related to the size of the WSLs created by the artificial demineralization process. Neuhaus et al. in 2010 observed that smaller WSLs were completely masked when treated with RI. Moderate to large WSLs, on the other hand, had esthetic improvements after RI, but were still visible after treatment. The WSLs created in this study may have been smaller than the WSLs used in Torres' study. A WSL with a smaller body cavity will likely have a higher degree of remineralization when compared to a WSL with a larger body cavity, regardless of the

treatment modality used. Since the T_2 L* values for all groups came close to T_0 values, there may be insufficient remineralization capacity remaining for RI and CPP-ACP to outperform artificial saliva.

Specimen Type

Other factors that may account for the difference in the findings of the Torres study and this study include the type of tooth specimens used and the demineralization protocol. Torres et. al used bovine teeth as their test specimens while our study used extracted human third molars. Bovine enamel is more porous than human enamel because it has larger crystalline structures (Arends and Jongebloed, 1978). The larger crystals of bovine enamel may explain why bovine enamel demineralization progresses about three times faster than human enamel demineralization (Featherstone and Mellberg, 1981). Furthermore, after exposure to erosion and erosion-abrasion, the losses of enamel in human teeth are significantly less than that of bovine teeth, possibly due to the higher calcium and phosphorus contents of human enamel (Attin et al., 2007 and Wang et al., 2012).

A review of published research showed that a variety of specimens and demineralization solutions have been used to study WSLs, including bovine teeth (Paris et al., 2008, Torres et al., 2010) and extracted human premolars (Behnan et al., 2010). The bovine teeth were demineralized for fifty days in Paris' study and only sixteen hours in Torres' study. By contrast, the human premolars in Behnan's study were demineralized for fifteen days and the human third molars in this study were demineralized for fourteen days.

The differences in the physical properties of bovine teeth and human teeth, as well as the differences in the demineralization process (including the formulation of the demineralization solution and duration of demineralization) make it problematic to compare the results of these studies, and may explain why the L* value after demineralization increased in the Torres study but decreased in this study.

Because extracted human third molars were used in this study, natural variations inevitably exist between the specimens. Some specimens demineralized more readily than others, creating possible outliers. However, the sixty specimens were randomly divided into the three groups and the average L* values among the three groups were statistically similar at T_1. This indicates that the degree of demineralization of the specimens was similar at T_1 and that these natural variations did not compromise the significance of the results.

Resin Infiltration

The enamel surface of a WSL has a pseudo-intact highly mineralized layer that may inhibit remineralization (Flaitz and Hicks, 1994). Therefore, acid etching, in the form of 15% hydrochloric acid gel for 90-120 seconds, is used in RI to increase the surface porosity and maximize remineralization (Kielbassa et al., 2009, Flaitz and Hicks 1994, Hicks and Silverstone 1985). After the surface layer is removed, the infiltrant, a low viscosity resin that mimics the refractive index of enamel, fills the intercrystalline spaces of the body of the WSL (Kielbassa et al., 2009).

This study, along with several other in vitro and in vivo studies (Torres et al., 2010, Paris et al., 2009, ten Cate 1981), have found RI to be effective at masking small to

moderately sized WSLs. Unlike Torres' study, however, in this study RI was not found to be superior to artificial saliva in masking WSLs. This difference in results may be related to the size of the artificial WSLs and the type of specimen used, as discussed above.

CPP-ACP

The efficacy of CPP-ACP in the remineralization of WSLs is conflicting, as described in the review of literature. This study showed that while CPP-ACP significantly enhanced the esthetic appearance of WSLs, its improvement, as measured by ΔL, did not surpass that of artificial saliva. This finding is consistent with the Behnan et al., 2010 in vitro study, which showed that MI paste and artificial saliva were not different from each other in terms of preventing demineralization of extracted human premolars. Pulido's study on the inhibitory effect of MI paste on the progression of artificial caries-like lesions in 2008 presented similar results.

An interesting finding, not related to the main objective of this study, was that CPP-ACP achieved 99% of its remineralization potential (L^*_{T2} divided by L^*_{T0}), after two weeks of application. Thus, additional benefits with continued use of CPP-ACP may not be achievable, since the remineralization capacity was already at 99%.

Limitation of Study

Under normal clinical situations, the teeth that are most likely to develop WSLs are maxillary lateral incisors and canines (Gorelick et al., 1982, Geiger et al., 1988). Ideally, these teeth would have been the test specimens for this study. However, extracted human

maxillary centrals and canines were difficult to obtain in numbers large enough to support this study. As a result, extracted human third molars were used. Furthermore, because the third molars used in this study were obtained from oral surgeons, it can be assumed that many of these third molars were impacted and never exposed to the oral environment. It is possible that different results may have been observed if maxillary laterals and canines were used in this study, and this may limit the validity of the study results. Nevertheless, the extracted human third molars used in this study may still be more clinically relevant than the bovine teeth used in other studies.

Furthermore, it would have been ideal if it were possible to only demineralize an isolated section of each tooth specimen rather than the entire coronal surface. That way, each tooth specimen could serve as its own control for spectrophotometric and visual comparison. The creation of the "demineralization window" was attempted during the initial pilot study, but was unsuccessful because it was difficult to isolate the small area of each tooth specimen for demineralization for fourteen days while maintaining the pH and temperature of the solution. During the pilot testing, unique acrylic jigs were fabricated with "demineralization wells" to hold the demineralization solution. However, the acrylic wells did not seal tightly enough against the tooth surface to prevent the demineralization solution from spreading to the surrounding area. Another attempt to demineralize only a section of the tooth surface involved painting the entire tooth specimen with two coats of red varnish except for the "demineralization window" to protect the other surfaces from demineralization. When the red varnish was removed with acetone, however, traces of the red color were still visible on the tooth surface, preventing an accurate spectrophotometric comparison. Clear varnish was also tested in

an attempt to avoid the problem of color contamination. The clear varnish, however, was not strong enough to withstand the demineralization solution. Since attempts at demineralizing a small section of the tooth surface were unsuccessful, measuring the L* value of each tooth specimen prior to demineralization proved to be the best method of comparing the effectiveness of each treatment modality at restoring the teeth to their original state.

Future Study

It is pertinent to note that after natural remineralization with diet and oral hygiene instruction, WSLs may continue to be visible. There are a variety of procedures, including RI, CPP-ACP, microabrasion, fluoride, whitening, and composite restoration, that can be added as adjunctive treatment to enhance the final esthetic result. A future study may include some or all of the aforementioned techniques. To enhance the validity of this study, a large scale in vivo study involving human subjects should be considered.

CHAPTER SIX

CONCLUSIONS

The objective of this in vitro spectrophotometric study was to evaluate the effectiveness of RI and CPP-ACP in improving the appearance of WSL, as quantified by increased optical reflectivity. This study demonstrated that RI, CPP-ACP, and artificial saliva were all effective at masking WSLs, as indicated by the statistically significant improvement in L* values from T_1 to T_2. However, there was no statistical difference in the effectiveness of the experimental groups (RI and CPP-ACP), when compared to the control (artificial saliva).

Under the conditions of this study, the result suggests that one group is not significantly better at masking WSLs when compared to another group. However, the advantages of RI and CPP-ACP may lie outside the confines of visual improvement. CPP-ACP may offer a protective remineralization benefit that is not matched by that of artificial saliva. RI, a promising minimally invasive procedure, may surpass the masking ability of artificial saliva in larger sized WSLs.

The second noteworthy finding in this study revealed that daily usage of CPP-ACP for two weeks versus four weeks resulted in similar improvements in L* values. Nevertheless, longer treatment time with CPP-ACP may yield additional benefits (remineralization, reduction of lesions) that extend beyond merely the esthetic improvement in light reflectivity.

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APPENDIX

RAW DATA

RI Group Values at T_0, T_1, and T_2

Specimen #	**Average L* @ T_0**	**Average L* @ T_1**	**Average L* @ T_2**
1	81.50	83.03	80.30
2	84.47	77.40	81.33
3	87.17	75.73	79.07
4	85.80	84.67	85.57
5	78.73	74.03	85.07
6	86.10	84.87	84.70
7	81.10	80.73	77.33
8	88.70	82.67	87.37
9	85.33	77.87	81.47
10	84.23	82.40	83.23
11	88.00	80.80	87.93
12	81.57	78.27	81.10
13	85.30	83.93	84.37
14	87.27	82.50	83.60
15	84.73	81.43	91.00
16	84.10	76.70	84.83
17	87.60	76.10	82.93
18	86.93	77.90	84.20
19	79.17	73.67	80.47
20	78.57	73.43	84.77
Average L* Value	**84.32**	**79.41**	**83.53**

CPP-ACP Group Values at T_0, T_1, and T_2

Specimen #	**Average L* @ T_0**	**Average L* @ T_1**	**Average L* @ T_2**
1	85.60	75.27	82.47
2	81.57	79.13	81.63
3	81.80	76.63	86.17
4	86.60	84.73	88.83
5	86.83	78.67	79.37
6	88.83	85.03	83.47
7	81.37	76.87	83.10
8	87.13	76.63	85.07
9	79.87	87.63	88.57
10	84.73	77.23	83.50
11	83.70	87.07	87.77
12	84.57	74.10	82.13
13	86.10	82.83	87.37
14	85.10	84.00	86.03
15	85.97	81.27	85.73
16	81.73	50.47	73.97
17	84.07	72.70	78.77
18	86.57	79.93	86.37
19	84.03	76.77	84.57
20	85.43	78.93	85.80
Average L* Value	**84.58**	**78.30**	**84.03**

Control Group Values at $T_{0,}$ T_1, and T_2

Specimen #	**Average L* @ T_0**	**Average L* @ T_1**	**Average L* @ T_2**
1	89.43	84.60	89.23
2	80.40	69.40	75.40
3	85.60	82.80	84.27
4	84.30	82.07	81.70
5	89.40	84.90	89.73
6	87.57	81.27	89.30
7	87.47	82.77	86.03
8	88.83	84.27	87.30
9	83.57	77.23	82.63
10	83.53	77.73	80.13
11	88.03	82.77	86.60
12	79.47	72.97	77.80
13	84.63	80.67	84.90
14	82.77	78.83	83.60
15	81.17	75.20	76.50
16	87.13	74.70	82.90
17	88.40	82.90	85.77
18	84.20	72.63	78.77
19	83.70	77.23	85.07
20	85.13	84.60	87.33
Average L* Value	**85.24**	**79.48**	**83.75**