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Clinical Assessment of Early Demineralization using PS-OCT

Author
Louie, Tiffany Michelle

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Clinical Assessment of Early Demineralization using PS-OCT

by

Tiffany M. Louie, DDS

THESIS

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I would like to dedicate this thesis to my parents. They have encouraged and supported me in everything I've done. I know that I am where I am today because of their continued guidance. To my sister Natalie, who is the best sister and best friend to me. Thank you for being my role model, and helping to proof read my essays over the many years. And finally to my husband, Barron, who has been my rock these past four years. Thank you for putting up with me during my stressful moments, and for just loving me.

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Clinical Assessment of Early Demineralization using PS-OCT
Tiffany Louie

ABSTRACT

Aims: The aims of this study were to test the hypothesis that PS-OCT can be used to nondestructively measure and quantify the severity of the early demineralization of buccal and occlusal enamel surfaces and assess the effect of fluoride varnish in inhibiting demineralization *in vivo*.

Material & Methods: Twenty subjects requiring bilateral premolar extractions were used. A cariogenic challenge was introduced by cementing orthodontic bands with a buccal window to these premolars and by making incisions on their occlusal surface. Bands were removed after 30 days and PS-OCT scans were acquired *in vivo* of occlusal and buccal areas. A split mouth design was used to test the effects of fluoride varnish. Teeth were extracted, serial sectioned and analyzed using Polarized Light Microscopy and Microradiography for comparison with the PS-OCT images.

Results: High quality PS-OCT images were acquired *in vivo*, with excellent optical penetration through the sound enamel to the dentin. Both occlusal and buccal surfaces showed a significant difference between the "sound" and "carious" enamel groups. The "sound" group had an average $\Delta R = 67.72$ dB * μm, and the "carious" group had an average $\Delta R = 206.64$ dB * μm. Both PLM and PS-OCT lesion depth measurements showed a significant difference between the "sound" and "carious" enamel groups for the occlusal surface, but not for the buccal surface, when calculated with PS-OCT. There was not a significant difference between the fluoride varnish and control groups in both TMR and PS-OCT tests.

Conclusion: This is the first clinical study to show the great potential of PS-OCT as a clinical tool, to nondestructively measure the depth and severity of early enamel demineralization including the high risk areas of occlusal surfaces and around orthodontic appliances, and to assess the efficacy of chemical intervention. With further studies, PS-OCT can prove to be valuable in the clinical setting due to its: ease of use, high contrast of early detection compared to traditional x rays or visual evaluation, and value of early detection in taking preventive action.

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INTRODUCTION

I. Background

During orthodontic treatment, patients are often at an elevated risk of developing areas of enamel decalcification, and subsequent dental caries. These areas of decalcification are commonly referred to as white spot lesions, are due to demineralization of the enamel by organic acids produced by cariogenic bacteria.[1] These unaesthetic areas of surface and subsurface demineralization around orthodontic appliances that form are often due to the inherent plaque retaining abilities of the brackets[2] and the additional obstacles for patients to maintain their oral hygiene around them. In addition to increasing the volume of dental plaque, studies have shown that orthodontic appliances physically alter the microbial environment so that proliferation of the acidogenic bacteria, such as *S. mutans*, occurs.[3]

Previous studies by Gorelick et al., have demonstrated that 50% of orthodontic patients will exhibit an increase in clinically visible white spot lesions,[4] and that even after more than five years post orthodontic treatment, orthodontic patients have a significantly higher amount of white spots than untreated subjects.[5] Equal susceptibility to white spot formation has been reported whether teeth are banded or bonded.[4] Demineralization underneath ill-fitting orthodontic bands is a very rapid process and has been shown to occur after only one month.[2,6]

In this study, the surfaces examined for demineralization are both the buccal surface, where the majority of lesions are found during orthodontic treatment[7,8], and the pits and fissures of occlusal surface where 80% of new decay is often found. Detection

of carious lesions is often obscured by the convoluted topography of the pits and fissures, or is limited by debris that frequently accumulate in those regions of the posterior teeth.

Early demineralized lesions are initially confined to only the enamel, however, once these lesions pass through the dental-enamel junction (DEJ) where there is less mineral content, demineralization occurs at a more rapid rate. Once a lesion has reached the point of the DEJ, surgical intervention is necessary.[9] Fortunately, before white spot lesions reach the DEJ or begin to cavitate, they can be arrested and undergo remineralization.[10] Hence, early detection of carious lesions could help assess its severity and help avoid the possibility of further breakdown of the tooth's structures and the need for invasive restorative treatment. However, conventional methods for diagnostic detection, such as visual/tactile sensation and radiography, do not have sufficient sensitivity to detect carious lesions in the early stages, particularly in occlusal lesions.[11]

II. Preventative Measures

The apparent degree of iatrogenic damage during orthodontic treatment suggests the need for preventive programs. There have been various preventative techniques introduced to decrease demineralization around orthodontic appliances. Some of these methods require the compliance of the patient, which is often difficult to monitor and enforce. Other methods that do not require the cooperation of the patient have, therefore, proven to be more effective.

The self-administered methods requiring compliance that are currently available to patients with white spot lesions are to improve their oral hygiene, change their diet, or apply topical fluoride treatments. O'Reilly and Featherstone in 1987, provided strong

evidence that the combination of daily brushing with a fluoridated dentifrice, coupled with daily rinsing with a fluoride (0.05% sodium fluoride) mouth rinse, will allow for complete protection for the orthodontic patient by inhibiting demineralization and promoting remineralization.[2] Unfortunately, the patients who are experiencing the most demineralization, are the ones who are failing to comply with the maintenance of their oral hygiene or the use of topical fluoride treatments. It was shown that a compliance rate of only 13% was obtained from patients asked to decrease their caries risk with a daily fluoride mouth rinse.[12]

To avoid the problems of compliance, some advocate using in-office topical applications of acidulated phosphate fluoride immediately after bonding. However, Geiger et al. has shown that this provides little benefit in reducing the incidence of white spots during orthodontic treatment.[13] Over the last few years, studies have focused on the incorporation of fluoride into orthodontic bonding cements to help reduce decay around the brackets. Gorton and Featherstone provided evidence that these fluoride-releasing glass ionomer cements are successful in releasing the incorporated fluoride and in inhibiting caries *in vivo*.[14] However, it was shown that these cements do not provide complete caries protection under loose bands or in areas where cement is missing.[15]

The additional use of fluoride varnish in orthodontic treatment, over the buccal surfaces before cementation with glass ionomer cements was advocated by Ogaard et al.[16-18] It was demonstrated that the application of fluoride varnish on sound enamel reduced lesion development by 48% to 50%[19] compared to non-treated enamel and prevented further lesion progression. Schmidt *et al.* also showed fluoride varnish to be beneficial in reducing the depth of the demineralized lesion by 35% and demonstrated a

greater efficacy when using resin-modified glass ionomers, with a 50% depth reduction of the demineralized area compared to conventional composite resins.[20]

Recently in the past 30 years, several studies have introduced the use of lasers to thermally modify the chemical composition of dental enamel to render it more resistant to acid dissolution and potentially more resistant to dental caries.[21,22] Hsu et al. showed that low intensity irradiation with CO_2 laser to the dental enamel results in significant reduction of the effective solubility of enamel mineral. Hsu successfully measured this inhibition with OCT. He also demonstrated that with regard to this effect, there is a significant synergism between laser irradiation and solution fluoride.[23]

III. Diagnostic Methods

In clinical studies, the ability to detect and quantify the changes in the enamel around orthodontic brackets can aid in comparing the efficacy of various preventive methods at reducing demineralization. In addition, early detection can help avoid the possibility of further breakdown, and the need for invasive restorative treatment. A range of diagnostic techniques have been employed, and in a recent study by Benson, he outlines the advantages and disadvantages of many techniques.[24] Some of the more widely used methods are: conducting a clinical exam, photographic examination, microradiograpy, polarized light microscopy, laser fluorescence, microhardness, and electron microscopy have been used to explore the characteristics of demineralized enamel. Unfortunately, many of these widely used detection techniques have the disadvantages in that: the validity is difficult to clinically evaluate, they require a

destructive analysis, they do not provide a quantitative analysis, or they are very technique sensitive.

Transverse microradiography (TMR) of thin sections is the principal method for determining mineral changes in carious lesions. It has become the "gold standard", by which other newly-developed methods for clinical diagnosis of caries, are compared and validated.[25] TMR requires the preparation of transverse thin sections from calcified tissue, and calculates the average mineral loss (ΔZ) in vol % and the lesion depth (μm) in enamel or dentin.[26]

Teeth naturally fluoresce upon irradiation with UV and visible light. Laser light-induced fluorescence was introduced in 1982 for the detection of enamel caries at an early stage because the fluorescence radiance of enamel, at the site of carious lesions, is decreased.[27] It has been shown, that changes in mineral contents of enamel lesions, which occur upon demineralization or remineralization, may be accurately recorded with the Quantitative Laser Fluorescence (QLF) method *in vivo* for orthodontic patients.[28] However, this is a very technique sensitive method, and demands a standardized procedure. Benson et al. in 2003 showed that QLF missed obvious areas of demineralization taken in the occlusal region.[29] Overall, QLF performs well on carefully produced shallow uniform lesions, on smooth surfaces, however high performance cannot be expected on highly convoluted occlusal surfaces.

Optical transillumination was used extensively before the discovery of x-rays for the detection of dental caries. Several studies with near-infrared (Near-IR) imaging have demonstrated that the high transparency of enamel in this region (1310 nm) can be exploited to image occlusal and interproximal lesions via transillumination of the tooth.[30]

There is an increase in the light scattering corresponding to increasing mineral loss in enamel caries. Near-IR can provide valuable information relating to the depth and the severity of developmental defects and dental caries.[31]

Other methods to detect demineralization of the dental enamel around orthodontic brackets *in vitro* include longitudinal sectioning with polarized light microscopy (PLM) assessment which will detect the depth of the demineralized lesion.[20] *In vivo,* cross-sectional microhardness tests were found to have a good correlation between enamel microhardness and % of mineral loss in caries lesions.[32] However, both of these widely used diagnostic paradigms have the disadvantage of requiring the tooth to be sectioned in order to quantify the amount of demineralization. Polarization sensitive optical coherence tomography (PS-OCT) is a novel technique of assessing caries lesions, in that it non-invasively detects early demineralization and remineralization.[33]

IV. Polarized Sensitive-Optical Coherence Tomography

Conventional optical coherence tomography (OCT) is a noninvasive technique for creating cross-sectional images of internal biological structures.[34] Colston et al. in 1998 was the first to image the soft and hard tissue structure of the oral cavity with OCT.[35] Polarization-sensitive OCT (PS-OCT) at 1310 nm has been shown to be more effective in imaging dental caries and early lesions. Areas of demineralization can rapidly depolarize incident polarized light and provide an improved contrast of carious lesions.[36] In addition, the confounding influence of the strong surface reflectance of the tooth surface that is found with OCT, is reduced in the PS-OCT system. Baumgartner in 1999, was the first to present the polarization resolved images of dental caries.[37]

PS-OCT directly measures the light reflected from each layer of the carious lesion, enabling the measurement of surface and sub-surface demineralization in the dental enamel. One approach to quantifying the severity of the lesion is by integrating the reflectivity of the perpendicular polarization ($\perp$).[36] In the $\perp$-axis image, the intensity of the strong reflection from the tooth surface is reduced, and hence the lesions appear with higher contrast to the surrounding sound enamel. Whereas, the parallel ($\parallel$)-axis image represents the linearly polarized light reflected in the original polarization incident on the tooth, showing more of the tooth surface and hence less contrast with respect to the lesion. PS-OCT can quantify the severity of demineralization by correlating the reflectivity (ΔR) of the $\perp$-axis PS-OCT images with the decrease in mineral content (ΔZ) of the dental enamel calculated from the microradiography.[38] PS-OCT was used in this study to quantify *in vivo* the depth-resolved changes of light scattering of dental hard issues that occurred upon demineralization.

PS-OCT has been used in numerous studies and has proven to be beneficial in many aspects of dentistry. It can be used for the clinical assessment of caries inhibition after CO_2 laser treatments,[21] and after fluoride treatments.[39] It has been proven successful in assessing the severity and extent of enamel developmental defects,[31] and it has been shown to be able to quantify and detect early enamel occlusal caries.[38] In addition, the imaging depth of PS-OCT through composite is sufficient to resolve early demineralization under sealants or restoration. A study in 2004 was able to detect occlusal secondary caries formation under sealants, with PS-OCT images.[40] PS-OCT can even be used to assess the state of the carious lesion and determine whether it is active and progressing or whether it is arrested and been remineralized. Jones *et al.* in 2006,

showed that PS-OCT images of a remineralized lesion will result in decreased reflectivity.[33] These previous *in vitro* studies have successfully correlated the ability to detect early carious lesion between PS-OCT and transverse microradiography. A strong correlation between the lesion depth calculated from both imaging modalities, and their respective ΔR and ΔZ values exist.

Clinical validation of PS-OCT in the early detection of surface and subsurface demineralization will enable *in vivo* monitoring of early caries formation and its subsequent prevention. With this in mind, *the objective of this research is to demonstrate that polarization sensitive-optical coherence tomography (PS-OCT) can be used in vivo as a non-invasive optical device for the accurate and early clinical assessment of tooth demineralization and remineralization.*

V. Aims

The specific aims of our study were 3-fold: to test the hypothesis that PS-OCT can be used for the early detection of demineralization *in vivo*; to test the hypothesis that the severity of early enamel demineralization under orthodontic bands and on occlusal surfaces with PS-OCT can be quantified *in vivo;* and to test the hypothesis that the inhibition of fluoride can be measured with PS-OCT *in vivo.*

MATERIALS AND METHODS

I. Subjects

This study was carried out on 20 patients in a randomized, controlled, clinical trial with human subjects' approval Grant # R01-17869. Patients were recruited from the University of California at, San Francisco, Orthodontic Clinic who were scheduled to have at least two premolars extracted for orthodontic reasons. All patients sign an informed consent and received a reimbursement of $300.00 for participation in this study. Eleven participants (55%) were male, and 9 (45%) were female. The age range was 10 to 33 years (mean 19.12 years; SD 6.71). (Table 1) In addition, all patients received oral hygiene instructions and were advised to use a fluoridated toothpaste. Since control and test teeth are present in the same oral environment, differences caused by various oral hygiene habits were not considered important.

The inclusion criteria for our subjects were that they must be adolescents between the ages of 10-18 years old or adults; be able to give informed consent in either English or Spanish; reside in locales with community fluoridation (to eliminate water fluoridation as a confounding variable); be ready for orthodontic fixed treatment; be treatment planned for bilateral premolar extractions in the same arch; and to have all restorative needs met and teeth cleaned prior to bonding. The exclusion criteria for our subjects were patients suffering from systemic diseases or conditions that can effect their oral health (ie. diabetes, HIV, heart conditions requiring antibiotic prophylaxis); patients taking medications that effect oral flora or saliva (ie. antibiotics taken in the last 3

months); patients with a drug or alcohol addiction; and patients who have taken in-office fluoride treatment within the past 3 months.

II. Clinical Methods

Our study design was divided into two portions: the clinical study and the laboratory portion. The study used 20 pairs of premolars treatment planned to be extracted for orthodontic reasons. During the bonding appointment (T_1) of these premolars, a cariogenic challenge was introduced to both the sound buccal enamel surfaces, and the occlusal pits of these premolars. For the buccal surfaces, we used modified orthodontic bands that were developed by Ogaard.[6] Two metal posts (0.8mm thick) were welded to the inner surface of the buccal part of the bands to mimic a space for plaque accumulation, which can occur with ill-fitting bands. (figure 1) For the occusal surface, conservative superficial preparations of 2-mm in length, 600-μm in width, and 500-μm in depth were made with a FG-329 carbide bur in the central fissure of each premolar. These small lesions served as an adequate site for plaque retention and subsequent acid dissolution.

This study was designed in split-mouth technique. Before cementation of the bands, one premolar in each patient was randomly selected to be pretreated with a fluoride varnish containing 5% NaF in a natural colophonium resin (Cavity Shield®; 3M Espe Omni Preventive Care, Inc., Bentonville, AR) on their buccal surface. The control contralateral premolar did not receive any additional fluoride treatment. The specially designed orthodontic bands were then cemented with glass ionomer cement (Ultra Band-Lok; Reliance Orthodontic Products, Itasca, IL) to the mesial, distal and lingual surfaces

of both the premolars treatment planned for extractions. At this initial banding appointment (T_1), only the occlusal surfaces of the premolars were imaged with PS-OCT.

After an average time of 34 days (range 27-43 days; SD 4.8 days), allowing sufficient time for demineralization to take place, the teeth were imaged a second time (T_2) with the PS-OCT on both the buccal and occlusal surfaces. The teeth were then extracted the same day as the second scan for 18 of the 20 patients. Of the two patients who received their extractions on another day, one waited 3 days, and the other waited 24 days. (Table 1) The extracted teeth were collected from the oral surgeon, labeled, and stored in a thymol solution.

Figure 1. Picture of an extracted premolar cemented with the specially designed band, which would create a buccal window where plaque retention and subsequent demineralization would form.

III. Laboratory Methods

During the laboratory portion of the study, the extracted teeth were first sterilized with gamma irradiation, and then mounted in acrylic cubes to facilitate the handling of the samples. (figure 2) Each of the 40 premolar samples (20 pairs), were analyzed with digital photographs taken at a magnification of 12X (figure 3), and with a Near-IR laser to help visualize the demineralized areas with a polarized filter (figure 4). Light from a single-mode fiber-pigtail coupled to a 1310-nm superluminescent diode (SLD) with an output power of 15-mW and a 70-nm bandwidth, Model SLED1300D20A (Optospeed,

Zurich, Switzerland), was used as the illuminating source. An InGaAs focal plane array (FPA) (318X252 pixels) the Alpha NIR (Indigo Systems, Goleta, California) with a Infinimite video lens (Infinity, Boulder, Colorado) was used to acquire all images. The acquired 12-bit digital images were analyzed using IR Vita software (Indigo Systems, Goleta, California). Two modes of imaging were used for imaging demineralization on occlusal and buccal surfaces.

Once all of this was completed, the teeth were cut into sections of approximately five 200-µm thick slices, using an Isomet 5000 saw (Buehler, Lake Bluff, I), for polarized light microscopy. PLM was carried out using a Meiji Techno RZT microscope (Meiji Techno Co., Ltd., Saitama, Japan) with an integrated digital camera, Canon EOS Digital Rebel XT (Canon Inc., Tokyo, Japan). The sample sections were imbibed in water and examined in the bright field mode with crossed polarizers and a red I plate with 500-nm retardation. (figure 5)

	Mean (S.D.)	Range
Age	19y1m (6y7m)	10y5m - 33y3m
Treatment time (T1-T2)	34 days (4.9 days)	27 - 43 days
Extraction time (T2-ext)	1.4 days (5.4 days)	0 - 24 days

Table 1. Descriptive Statistic of the study group showing the mean, standard deviation and range for the age of our patients and the treatment time from T1-T2 and from T2 -extractions.

Figure 2. Picture of the samples mounted in acrylic blocks and labeled.

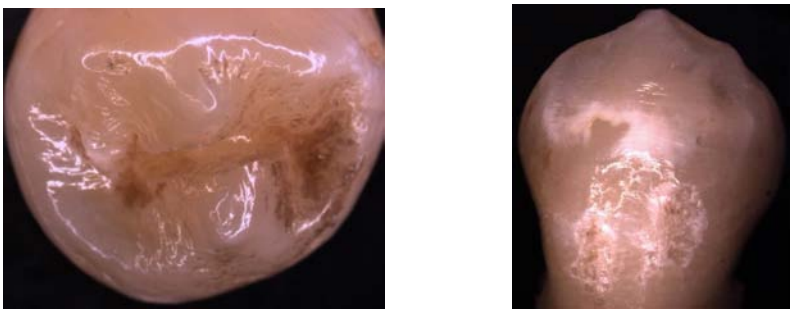

Figure 3. Photo of both the occlusal and buccal surfaces taken at high magnification of 12X.

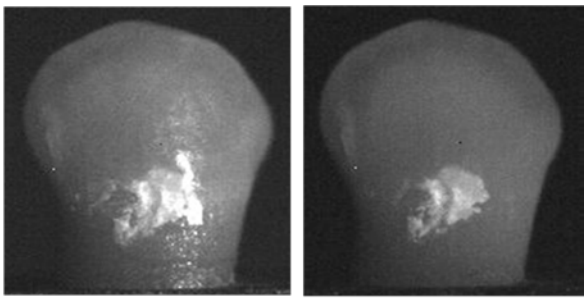

Figure 4. Near-IR images of the buccal surfaces with demineralization. The image without glare was more accurate in the depiction of demineralization due to the polarized filter.

Figure 5. PLM image of a premolar section taken at 10x magnification.

IV. Digital Microradiography

A custom built digital microradiography (TMR) system was used to measure the volume percent mineral content in the areas of demineralization on the tooth sections. High-resolution microradiographs were taken using Cu Ka radiation from a Philips 3100 x-ray generator and a Photonics Science FDI x-ray digital imager (Microphotonics,

Allentown, Pennsylvania). The x-ray digital imager consists of a 1392 X 1040 pixel interline CCD directly bonded to a coherent micro fiber-optic coupler that transfers the light from an optimized gadolinium oxysulphide scintillator to the CCD sensor. The pixel resolution is 2.1 μm, and the images can be acquired in real time at a frame rate of 10fps. A high-speed motion control system with Newport UTM150 and 850G stages and an ESP 300 controller coupled to a video microscopy and laser targeting system was used for precise positioning of the tooth sample in the field of view of the imaging system. (figure 6)

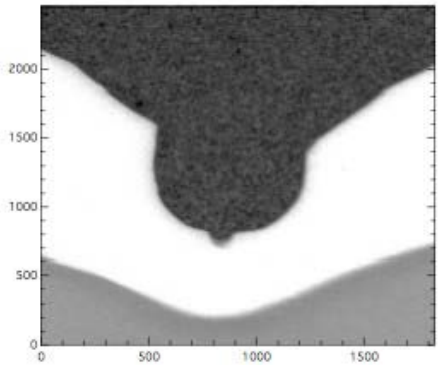

Figure 6. TMR image of a premolar section showing the occlusal lesion prepared at T1, and a small occlusal lesion in the center.

V. Polarization-Sensitive Optical Coherence Tomography

A coherence domain reflectometry (OCDR) system with pm-fiber, high-efficiency piezoelectric fiber-stretchers, and an InGaAs receiver that was fabricated by Optiphase, Inc. (Van Nuys, California), was used to acquire images of occlusal and smooth surface tomography of our premolar samples. This OCDR system was coupled with a broadband high-power superluminescent diode (SLD) from Denselight (Jessup, Maryland) with an output power of 10 mW and a bandwidth of 83 nm for a spatial resolution of 12 μm in air and 7.5 μm in enamel. The probe was designed to provide a

spot diameter of 20 μm. The rate of the scans was 150 scan/sec. The PS-OCT system was controlled using Labview software from National Instruments (Austin, Texas).

The PS-OCT was used to scan the teeth, *in vivo*, at 2 time points: T_1 was the initial banding appointment, and T_2 was the debanding appointment, which was approximately 30 days from the initial banding appointment. (Table 1) At T_1, only the occlusal surfaces of the sample premolars were scanned. However at T_2, both the occlusal surfaces and buccal surfaces of the dental enamel were scanned.

The PS-OCT images were analyzed to see if it could accurately detect and measure the formation of early demineralization by comparing these images with both polarized light microscopy and the gold standard, transverse microradiography. All three imaging modalities of each sample, the PS-OCT *in vivo*, and the PLM and TMR *in vitro*, were best-matched according to their slices. A line profile was drawn through the same area of demineralization in all three images, and calculations were taken at these points. (figure 7) The lesion depth of the demineralized areas was measured in both the PLM and PS-OCT images, and were compared. The intensity of backscattered light (ΔR) was also measured in the PS-OCT images, as a function of depth within the tissue. Two-dimensional (2-D) OCT intensity plots were obtained by laterally scanning the beam across the tooth. The PS-OCT integrated light reflectivity (ΔR) was compared with the structural changes and relative mineral loss (ΔZ) measured in the TMR images.

The PS-OCT images taken *in vivo* at T_2, were also compared and assessed as to whether they could measure significant differences in lesion severity between the premolars pretreated with fluoride varnish and the contralateral control premolars.

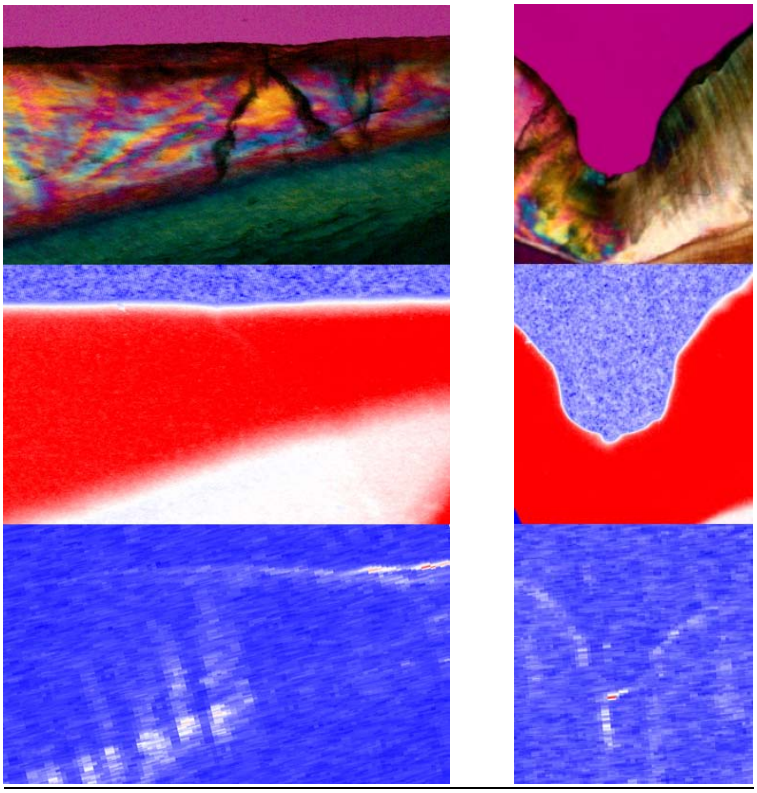

Figure 7. PLM, TMR and PS-OCT images of both the buccal and occlusal surfaces of each sample, were matched according to their slices. A line profile was drawn through an area of demineralization represented in all three slices, and their respective measurements were taken.

VI. Statistical Analysis

The sensitivity of PS-OCT to detect a lesion was analyzed by a unpaired t-test, at $p < 0.05$ to test if there was a significant different in ΔR for, sound and carious enamel groups that were defined by TMR ΔZ values. Unpaired t-tests were also used to test if there was a significant difference, at $p < 0.05$, in the lesion depth between sound and carious enamel groups calculated with both PLM and PS-OCT. A kappa test statistic was conducted to detect the amount of agreement between the PS-OCT ΔR and TMR ΔZ values. Differences in mineral loss values or lesion depths between the test group (fluoride-releasing varnish) and the control teeth were analyzed by a unpaired t-test using the statistical package Graph Pad InStat (version 3.0); the level of significance was set at $p < 0.05$.

RESULTS

I. Reflectivity (ΔR): Sound versus Carious areas

To calculate whether PS-OCT could accurately detect demineralization compared to our gold standard, TMR, we first calculated the ΔZ values for the buccal and occlusal surfaces of each sample. Structural changes and relative mineral loss (ΔZ) were calculated in the TMR images by comparing the volume percent mineral content in an area of sound enamel to an area of demineralized enamel. (figure 8) The ΔZ values were used to divide all our samples into two resultant groups: the sound enamel and the carious enamel groups. The sound enamel group was defined as $\Delta Z < 100$ vol % min. * μm, whereas the carious enamel group was defined as $\Delta Z > 100$ vol % min. * μm. The sound enamel groups had an average ΔZ value of 3.00 vol % min. * μm. The carious enamel group had an average ΔZ value of 1224.73 vol % min. * μm, and was assumed to have developed early enamel demineralization within our 30 day test period (T1-T2). (figure 9)

We then calculated the PS-OCT ΔR value (reflectivity) for each of these two groups, and performed an unpaired t-test to see if there was a significant difference between these groups. (figure 10) We found that there was a statistically significant difference ($p < 0.05$) between the sound enamel and carious enamel groups. The sound enamel group had an average ΔR value of 67.72 dB * μm, and the carious enamel group had an average ΔR value of 206.64 dB * μm.

We then divided the occlusal and buccal surfaces to evaluate if we could detect a significant difference between both the sound and carious enamel groups in each surface.

We calculated each surfaces' respective TMR ΔZ and PS-OCT ΔR values to determine the amount of demineralization in both of these enamel groups. We found that for both the TMR and PS-OCT analysis, there was a statistically significant difference between the sound and carious enamel groups in both the occlusal and buccal surfaces, $p < 0.05$. (figure 11 & 12) In addition we performed a Kappa Statistic to detect the amount of agreement between the PS-OCT ΔR and TMR ΔZ values. (table 2) The Kappa statistic revealed a value of 0.324, indicating that there is a fair agreement between PS-OCT and TMR.

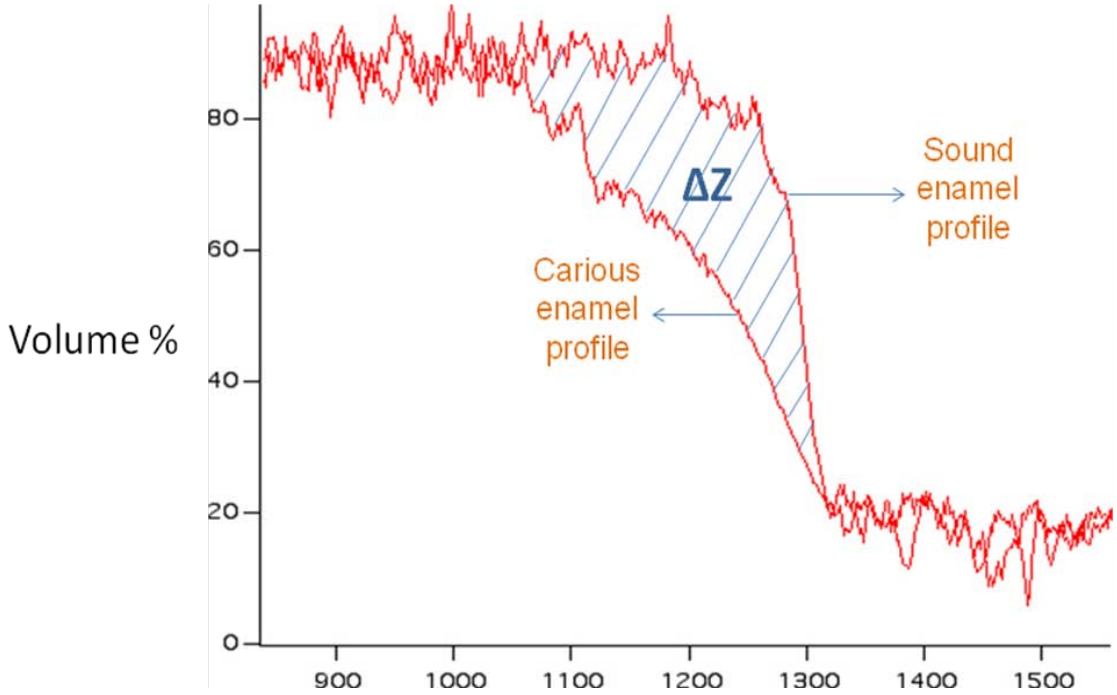

Figure 8. Line profiles of both a sound enamel area and a demineralized area. The difference in & mineral volume between these two line profiles is ΔZ, change in volume of mineral.

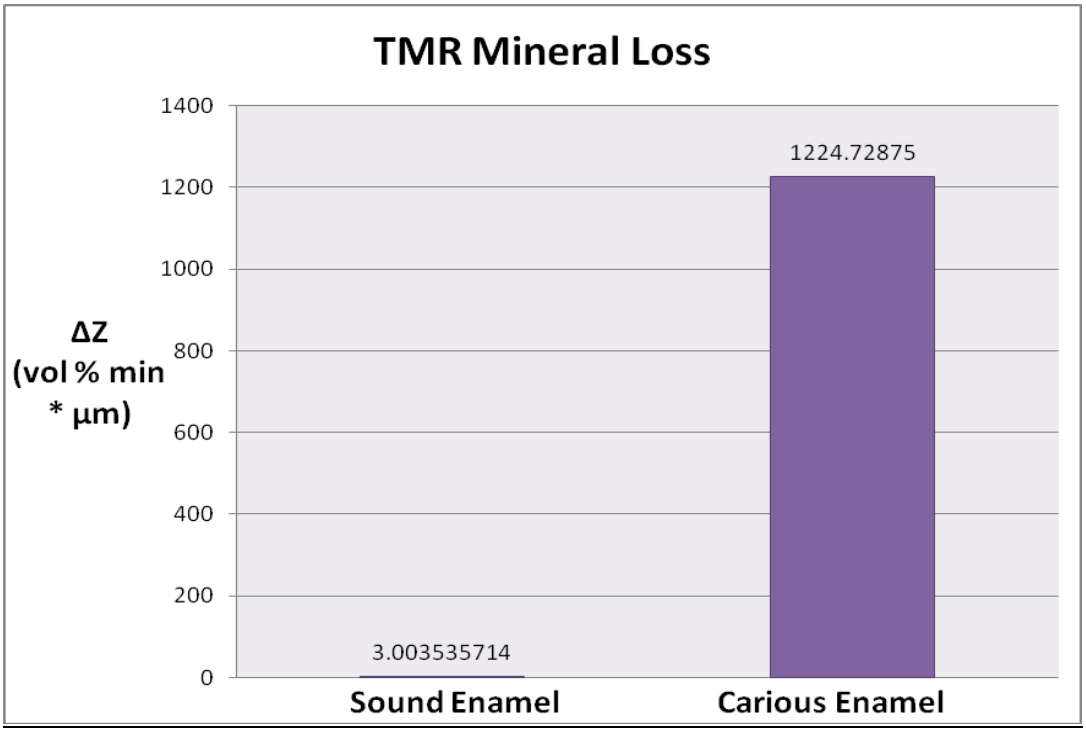

Figure 9. The samples were divided into 2 groups, based on their ΔZ values. They groups are labeled as "sound" enamel and "carious" enamel.

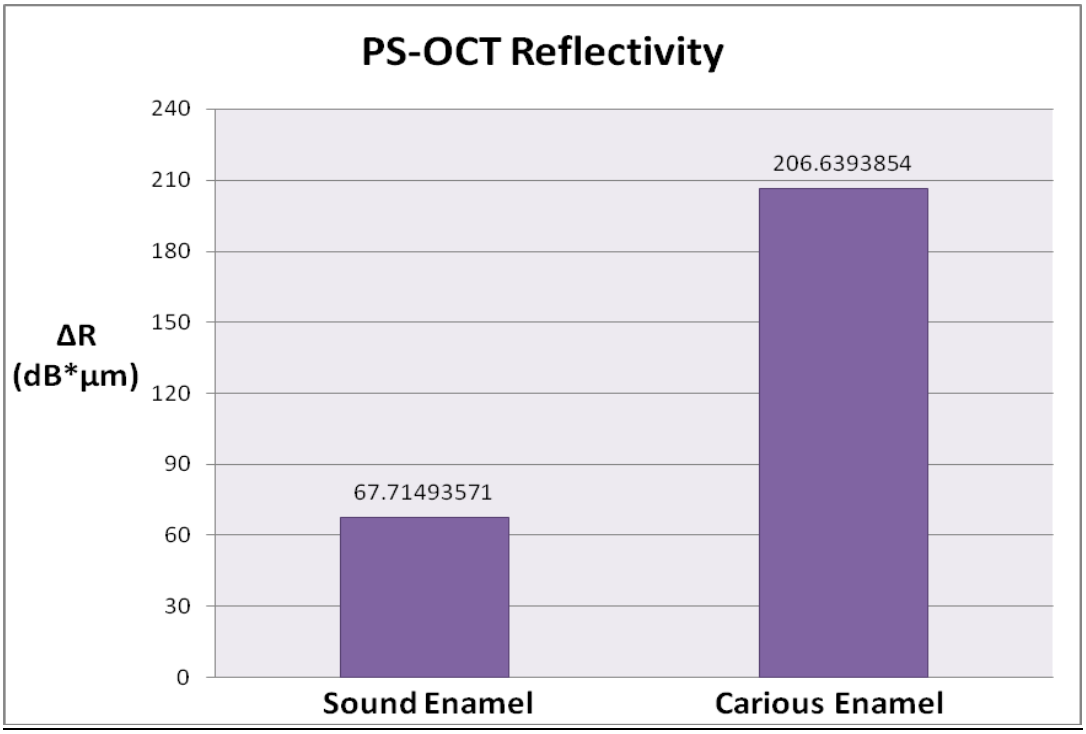

Figure 10. The ΔR value for both of the two groups were calculated. An unpaired t-test statistic showed a statistically significant difference between the two groups, $p < 0.05$.

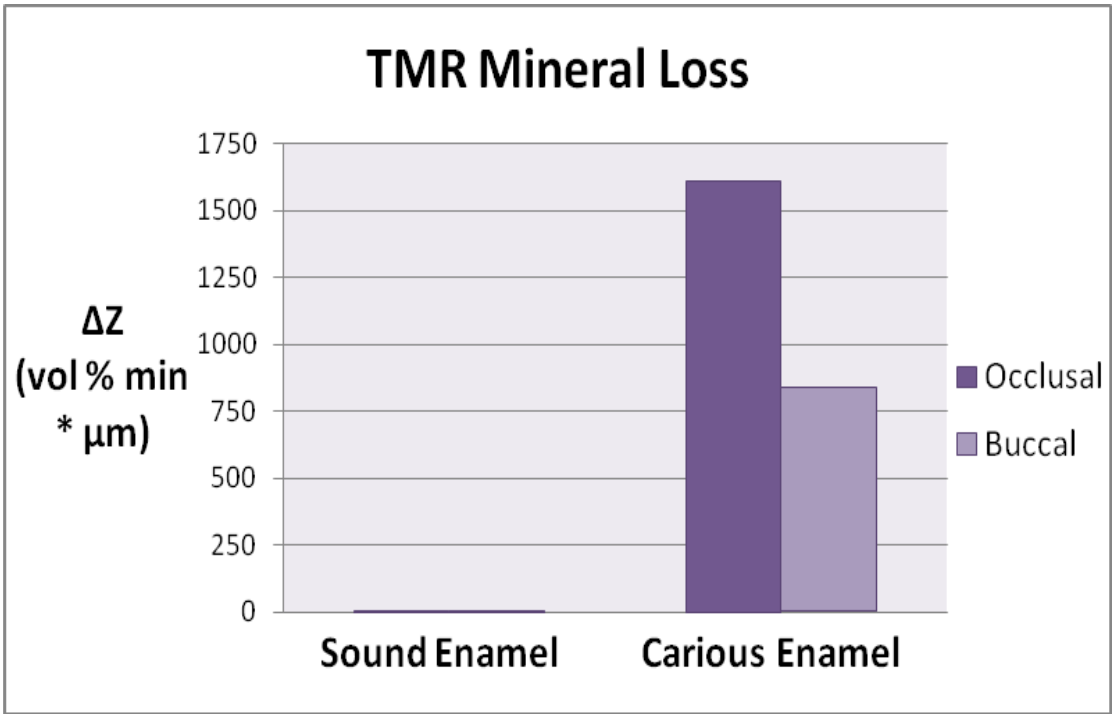

Figure 11. There was a statistically significant difference between sound and carious enamel groups in the TMR ΔZ analysis of both the occlusal and buccal surfaces, $p < 0.05$.

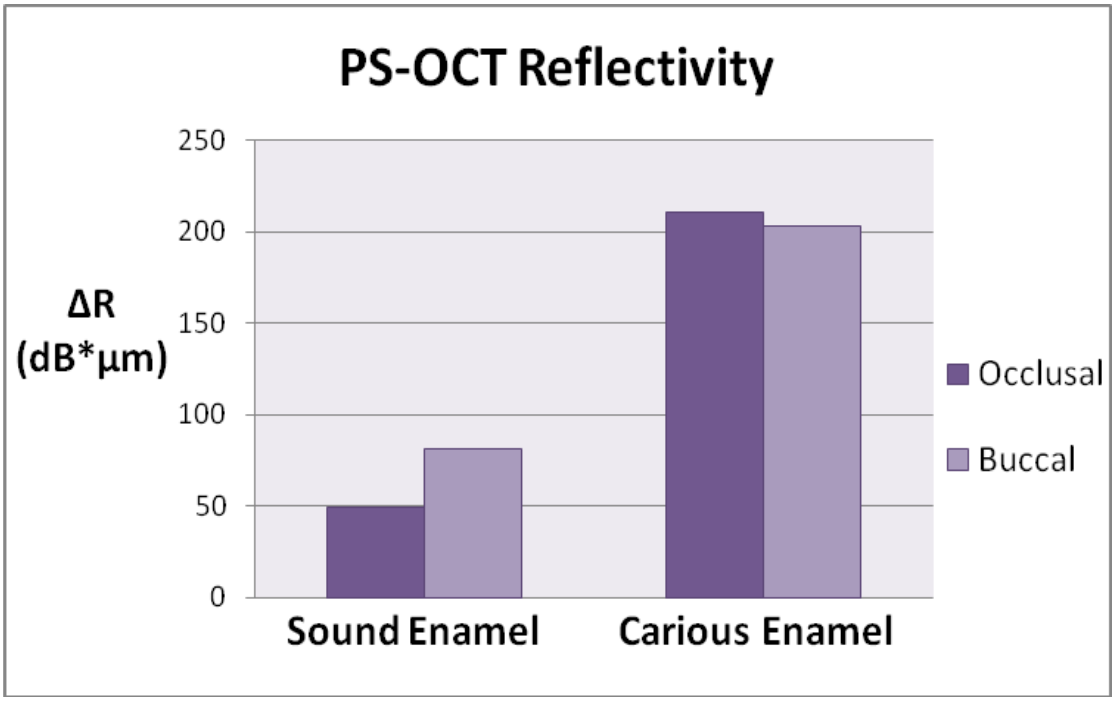

Figure 12. There was a statistically significant difference between sound and carious enamel groups in the PS-OCT ΔR analysis of both the occlusal and buccal surfaces., $p < 0.05$.

		TMR		
		no lesion	lesion	Total
PS-OCT	no lesion	30	26	56 (70%)
	lesion	3	21	24 (30%)
	Total	33 (41%)	47 (59%)	80 (100%)

Table 2. Kappa statistic to show if the TMR and PS-OCT findings agree. A Kappa value of 0.324 reveals a fair agreement between the TMR and PS-OCT findings.

II. Lesion Depth: PLM and PS-OCT

The lesion depth of the demineralized areas was measured in the same area of each sample for both the PLM and PS-OCT analysis. Both PLM and PS-OCT lesion depth measurements showed a statistically significant difference ($p < 0.05$) between the sound and carious enamel groups. (figure 13 & 14)

We then divided the occlusal and buccal surfaces to evaluate if we could detect a significant difference between both the sound and carious enamel groups in each surface. We calculated each surfaces' respective PLM and PS-OCT lesion depth values to determine the amount of demineralization in both of these enamel groups. We found that for the occlusal surface, there was a statistically significant difference between the sound and carious enamel groups in both the PLM and PS-OCT lesion depth measurements, $p < 0.05$. (figure 15) However, for the buccal surface, there was only a significant difference between the two enamel groups, when the lesion depth was calculated with PLM. When the lesion depths on the buccal surfaces were measured in the PS-OCT images, there was not a statistically significant difference between the sound and carious enamel groups, $p > 0.05$. (figure 16)

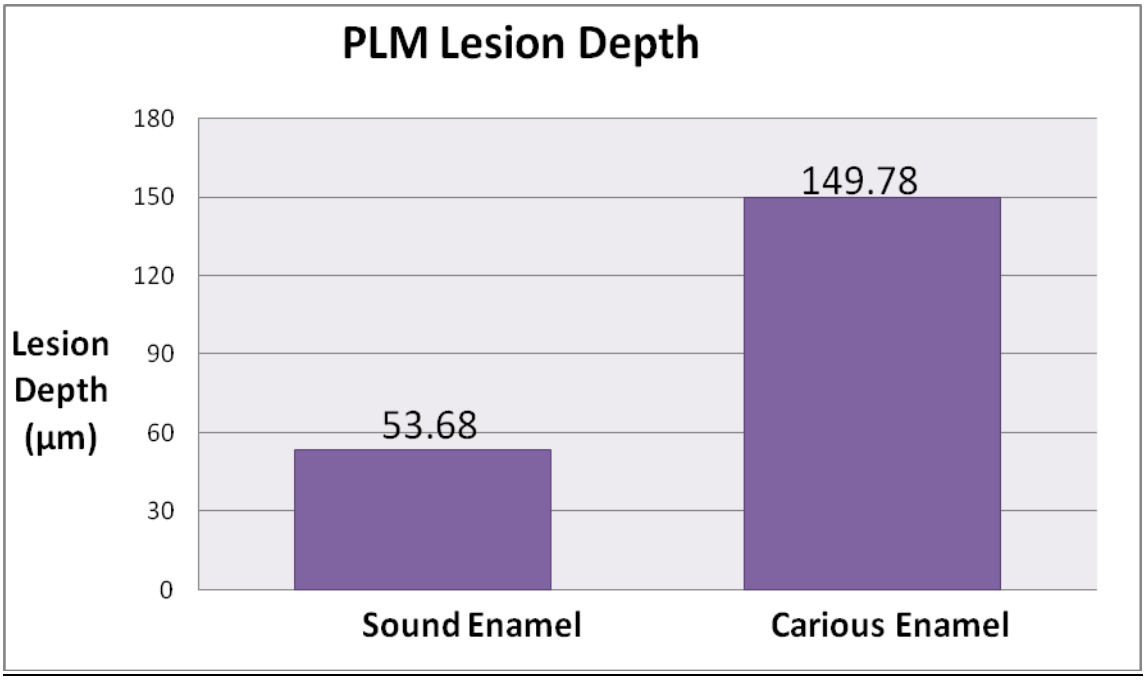

Figure 13. PLM lesion depth measurements showed a statistically significant difference between the two groups, $p < 0.05$.

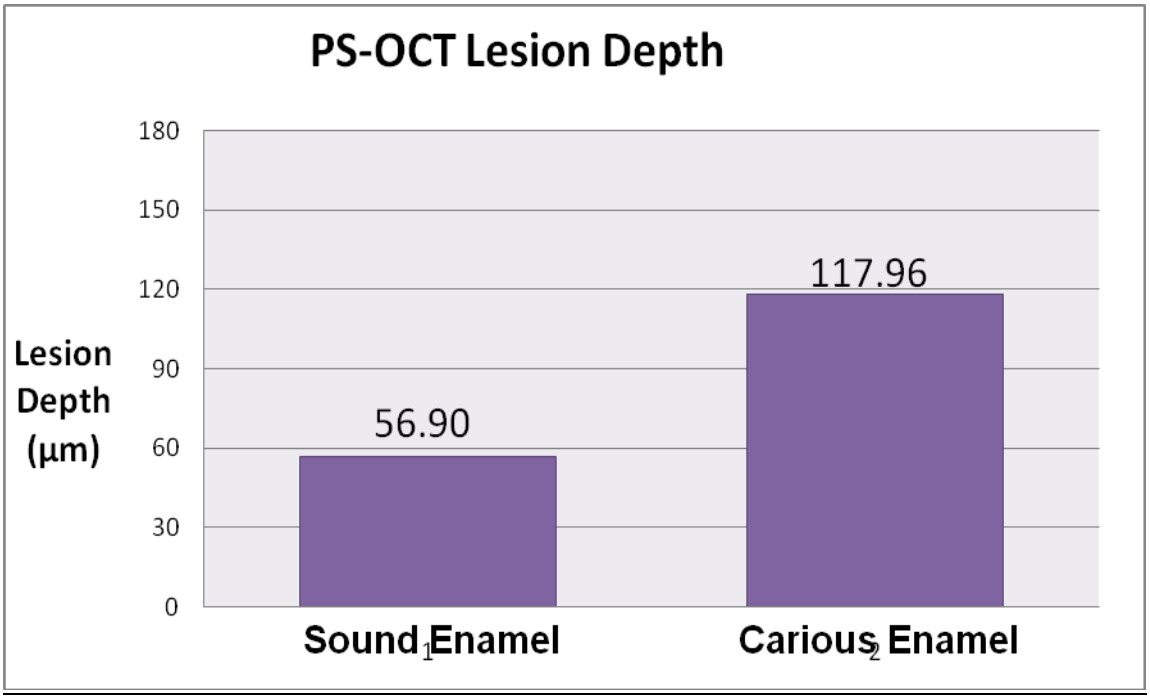

Figure 14. PS-OCT lesion depth measurements showed a statistically significant difference between the two groups, $p < 0.05$.

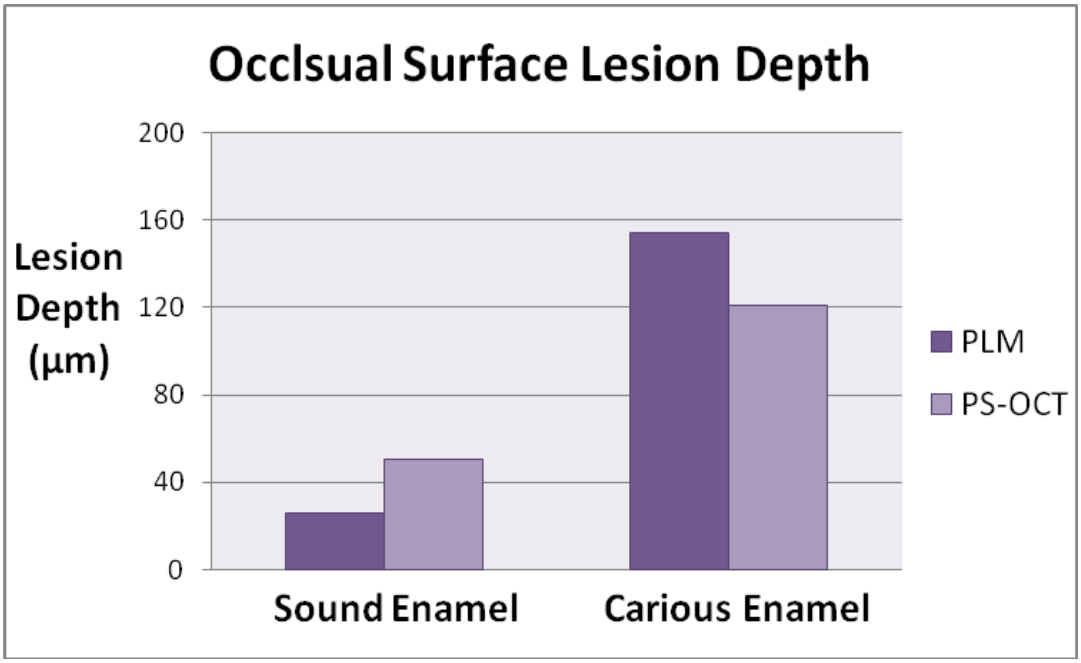

Figure 15. There was a statistically significant difference between sound and carious enamel groups in both PLM and PS-OCT analysis of the occlusal surfaces., $p < 0.05$.

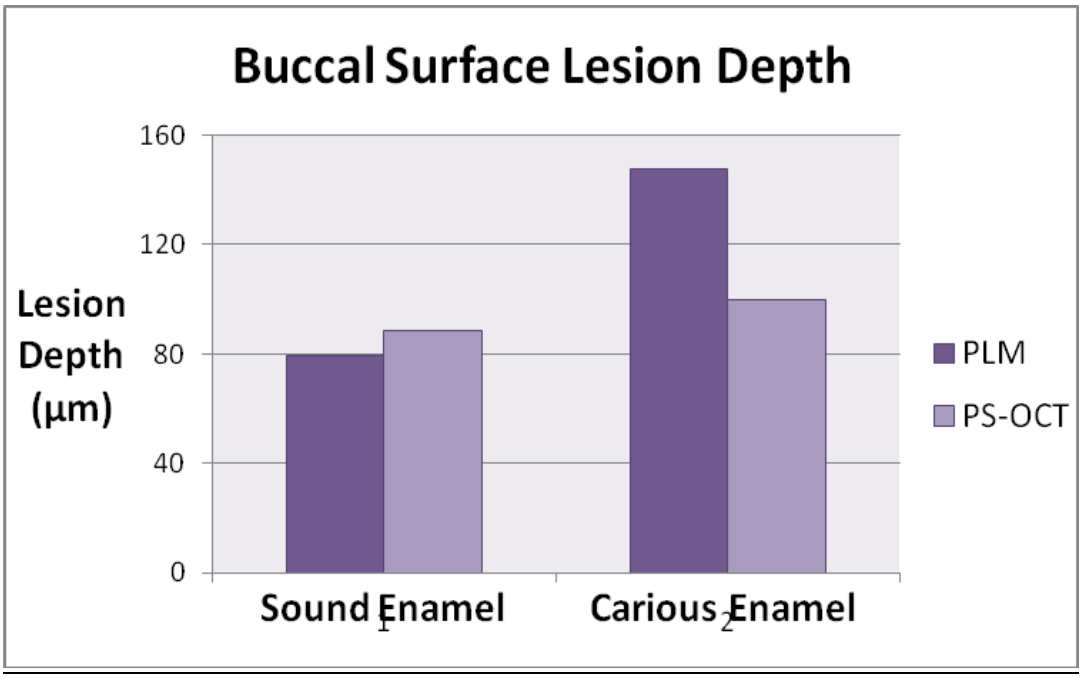

Figure 16. There was a statistically significant difference in the lesion depth on the buccal surfaces, between sound and carious enamel groups when measured with PLM, $p < 0.05$. However, there was not a significant difference in the buccal lesion depth between the two groups when measured with PS-OCT, $p > 05$.

Fluoride Varnish Treatment versus Control

Our results show that there is not a statistically significant difference between the fluoride varnish treatment group and the control group in both the gold standard, TMR, and PS-OCT tests. An unpaired t-test was performed, and a p-value > 0.05 was found. (figure 17 & 18)

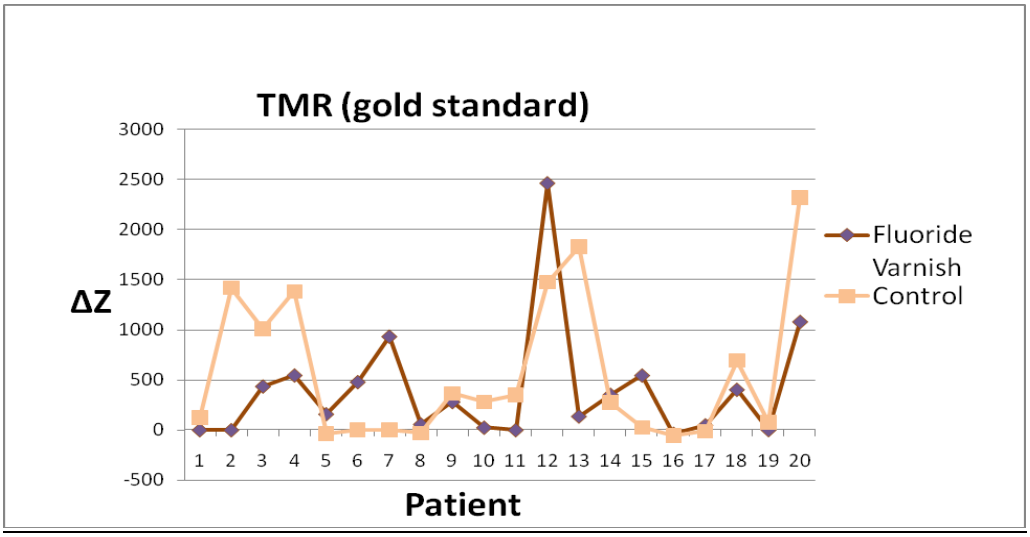

Figure 17. TMR ΔZ comparison between the buccal surfaces treated with fluoride vanish and the controls were performed. The test and control groups were found to be not significantly different, p > 0.05.

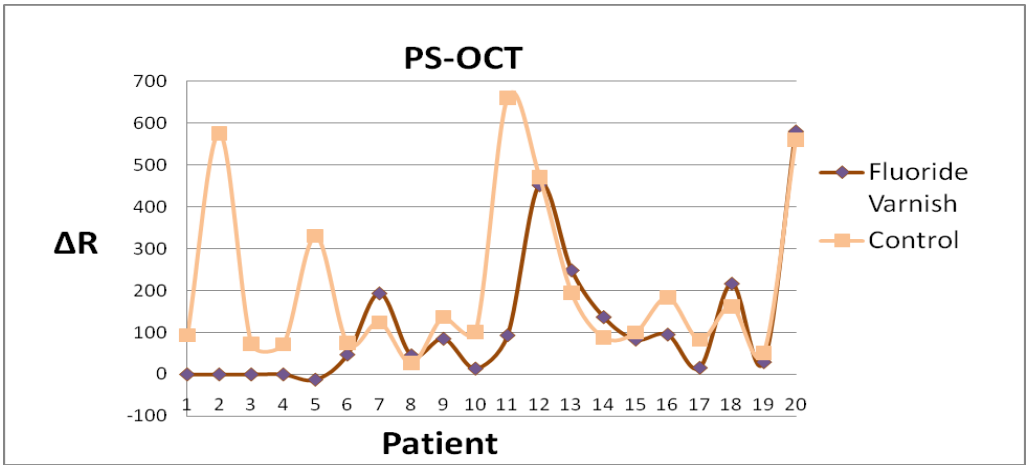

Figure 18. PS-OCT ΔR comparison between the buccal surfaces treated with fluoride vanish and the controls were performed. The test and control groups were found to not be significantly different, p > 0.05.

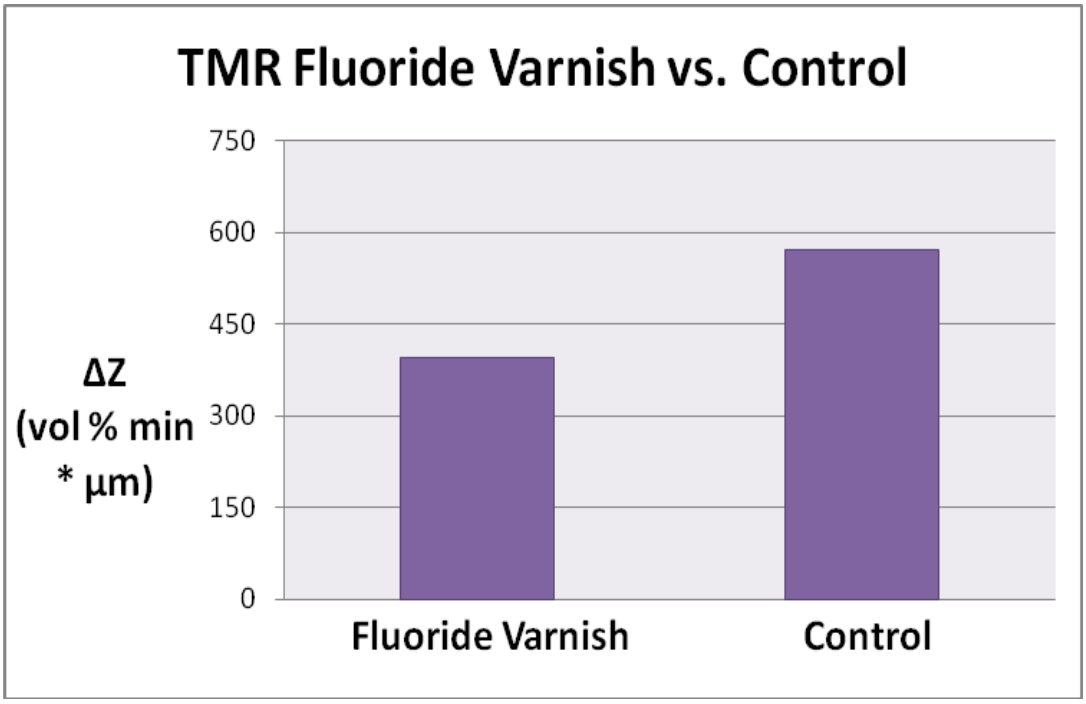

Figure 19. Comparison between the buccal surfaces treated with fluoride vanish and the controls revealed that these two groups were not statistically significantly different when analyzed with TMR, p > 0.05.

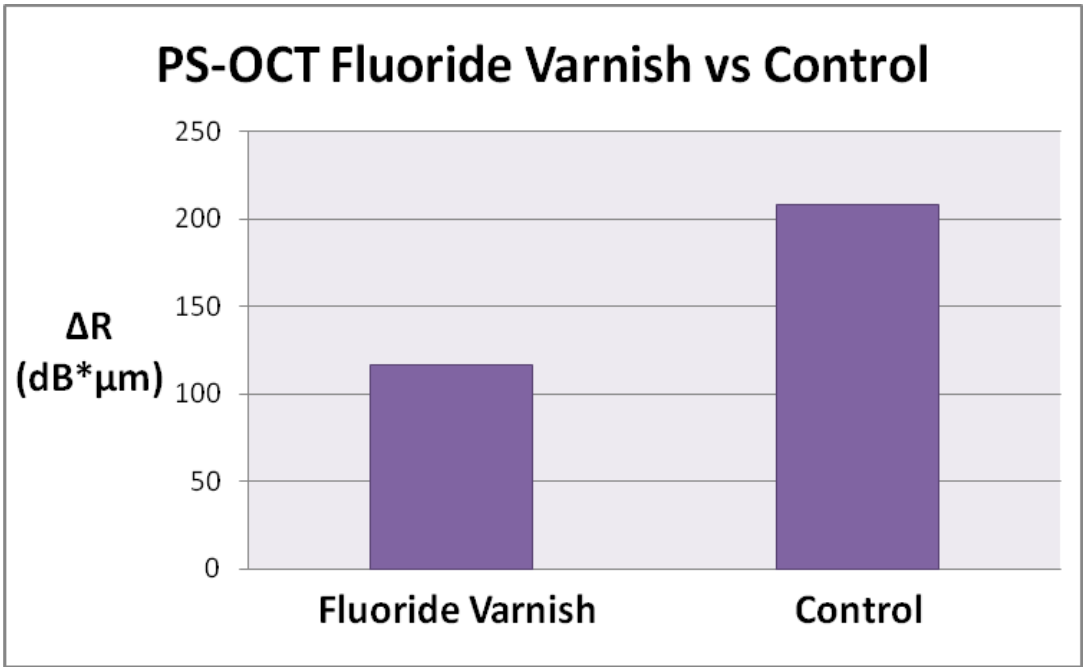

Figure 20. Comparison between the buccal surfaces treated with fluoride vanish and the controls revealed that these two groups were not statistically significantly different when analyzed with PS-OCT, p > 0.05.

DISCUSSION

I. PS-OCT compared to PLM and TMR

High quality PS-OCT images were acquired of both occlusal and buccal surfaces *in vivo* with excellent optical penetration through the sound enamel to the dentinal enamel junction. In addition, it was shown that the severity of early enamel demineralization under orthodontic bands and on occlusal surfaces can be quantified *in vivo* with PS-OCT. The similar results comparing sound and carious enamel areas, found between the gold standard, TMR, and PS-OCT analysis, validate the ability of PS-OCT to directly quantify demineralized lesions. These in vivo results are in agreement with the findings from an *in vitro* study in 2006 by Jones et al, who found that the correlation between ΔZ and ΔR was very strong, $r = 0.755$.[38]

When dividing our data between occlusal and buccal surfaces, it was shown that there is a significant difference between the sound and carious enamel groups when using ΔZ and ΔR to compare. This suggests that PS-OCT is a reliable method to be used *in vivo* on both buccal and occlusal surfaces to detect the amount of mineral loss in demineralized lesions. However, unpaired t-test revealed when using lesion depth calculated from the PS-OCT images, to compare sound and carious enamel groups, there was not a statistically significant difference. This can be due to the inherent limitations in the PS-OCT system.

II. Limitiations

Possible factors that might attribute to not being able to detect a significant difference in the lesion depth between the sound and carious enamel groups with the PS-OCT scans are that: the lesioned areas might have remineralized with saliva, the surface was initially fluorosed, the occlusal surface had a previous sealant or composite restoration, or that the 30 day test period was not long enough to sufficiently allow enamel demineralization to develop.

It was beneficial to have the patients receive their extractions the same day as their second PS-OCT scans, to ensure that the remineralizing effects of saliva did not occur. Eighteen out of the 20 patients received their extractions the same day, however one received their extractions 3 days later and another received their extractions 24 days later. Ogaard et al. in 1994 showed that lesion regression by saliva was a speedy process complete within a few weeks in nearly all cases.[41] This is of great importance in the orthodontic practice since white spot lesions developed between one appointment and the next (eg. 4 weeks), may regress rapidly by removing a loose band for some weeks. In our study however, it was advantageous to have our patients receive the extractions the same day as their debonding appointment, as lesion regression by saliva was not our objective. Remineralization of the lesions would make the detection of demineralization with our PS-OCT system negligible. Despite the fact that two patients did not receive their extractions on the same day, when our results are recalculated to eliminate the data from these two patients, our results remain unchanged.

Another limitation of PS-OCT is that, previously fluorosed enamel surfaces will show up as demineralized areas in PS-OCT images. This false demineralization reading,

which is actually fluorosis, will be more resistant to demineralization. Therefore, the detection of the formation of demineralization with PS-OCT, in samples that are fluorosed will be negatively affected.

The hand-held PS-OCT system used in this study has some limitations when being used clinically. Such limitations include difficulties in repositioning the probe at the same measuring point on different occasions, and difficulties in taking images of the same exact area with both the PS-OCT and TMR systems. A direct correlation between PS-OCT ΔR and TMR ΔZ values was not possible due these drawbacks. The images acquired from the PS-OCT scans were *in vivo*, however the images from the TMR scans were taken *in vitro* from thin slices of our sample. It is therefore impossible to perfectly match the single slice imaged *in vivo* with the exact slice imaged with the TMR.

In addition, there are some methods available that can be applied by a busy clinician to evaluate demineralization, whereas others are more suitable for the researcher undertaking a clinical trial. The PS-OCT system used in this study can present a problem clinically because its image interpretation is complicated and quantification would not be practical for chair-side analysis. However, this method is a valuable technique for the researcher undertaking a clinical trial because it presents a way to accurately detect demineralization and to assess new products or interventions, which in turn will help prevent the appearance of white spot lesions during orthodontic treatment.[24]

III. Fluoride Varnish Treatment versus Control

Both TMR and PS-OCT tests similarly revealed there was not a statistically significant difference in the amount of demineralization between the premolars treated

with fluoride varnish and the control groups, $p > 0.05$. This agreement between the gold standard, TMR, and PS-OCT verifies the reliability of PS-OCT to detect demineralization clinically.

There has been controversy in literature as to the efficacy of using fluoride varnish before band cementation during orthodontic treatment. In 1998, van der Linden et al, studied the effects of a fluoride containing varnish (Fluor Protector), under orthodontic bands and found that this application reduced white spot formation significantly when a glass inomer was not applied, however in combination with glass ionomer cement, it did not contribute to the reduction in WSL.[42] However, in 2002, Schmit et al.[20] showed fluoride varnish to be beneficial in reducing the depth of a demineralized lesion by 35% and demonstrated a greater efficacy when using resin-modified glass ionomers, with a 50% depth reduction of the demineralized area compared to conventional composite resins.

Our study used Cavity Shield® as the fluoride varnish on the buccal surfaces, before cementing the bands with Band-Lok, a glass ionomer cement on their mesial, distal and lingual surfaces. The fluoride treatment group had lower demineralization values in both the PS-OCT and TMR analysis, however it did not break statistically. (figure 19) It is possible that there was not a statistically significant difference between treating the buccal surfaces with fluoride varnish and not, due to the fact that a fluoride-releasing glass ionomer was also used on the mesial, distal, and lingual surfaces to cement the bands. In a study by Gorton and Featherstone in 2003, they showed that the cariostatic effect of fluoride-releasing glass ionomer cements is localized to the area around the orthodontic appliances, and is statistically significant after four weeks.[14]

Perhaps the beneficial effect of the fluoride-releasing glass ionomer was extended toward the buccal surface to moderately inhibit demineralization in the 30 day time period to both the test and control samples. In addition, it is also possible that improved patient compliance with oral hygiene can negate the beneficial effects of fluoride varnish. However, the cariostatic effects of an in-office application of fluoride varnish should be advocated for the less compliant orthodontic patient.

IV. Future Direction

Newly developed Fourier domain systems (FD-OCT), such as Swept-OCT and Spectral-OCT, have been reported to acquire real-time high-resolution three dimensional (3-D) images in the range of several seconds. Being able to take a whole volumetric 3-D image of the tooth will provide more comprehensive data, and will aid in its comparisons with the gold standard, TMR. These systems are also available with a handheld probe option and have been investigated for imaging dental caries.[43,44] In 2006, the systems were applied for early caries detection in tooth, for diagnostics of tooth condition after operational tooth treatment, and for diagnostics of the alveolar bone structure on two patients *in vivo*.

CONCLUSION

It has been successfully demonstrated that PST-OCT is a reliable and noninvasive system for early detection of demineralization clinically. In addition, PS-OCT shows that there is a significant difference between sound and demineralized enamel in its amount of light reflectivity, which is confirmed with the gold standard, TMR. PS-OCT is able to nondestructively measure early demineralization *in vivo.* There is no significant difference in the fluoride varnish treatment group and the control, in both the PS-OCT and TMR values.

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