THE QUALITY OF ETCHED ENAMEL IN DIFFERENT REGIONS AND TOOTH TYPES

A Thesis

by

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ABSTRACT

Purpose

In vitro evaluation and quantification of etch quality in various regions and types of human teeth using novel approaches.

Materials and Methods

27 extracted human teeth (3 of each tooth type) were disinfected, sectioned into mesial and distal halves, and randomly allocated to SEM or μCT for evaluations. Buccal surfaces were treated with pumice, etched with 37% phosphoric acid gel etchant for 15 seconds, rinsed, and air dried. SEM samples were dried in 100% ethanol, placed in a vacuum, and sputter coated with gold. Two regions of etched enamel in each third of the teeth (incisal, middle, and cervical) were viewed at 1200x magnification with the SEM. Using BIOQUANT Osteo (Nashville, TN) software, the percentage of enamel remaining to total surface area was calculated for each image. μCT scans were taken before and after etching. Pre- and post-etch mineral densities were calculated from the scans and compared.

Results

Two way analyses of variance showed no statistically significant interaction between jaw and tooth type. While there were no between-jaw differences, there were significant differences between tooth types. SEM analyses showed that premolars and molars had significantly greater remaining enamel than incisors and canines in the middle ($p=.046$) and cervical ($p=.002$) regions. Mineral densities of anterior teeth

demonstrated a superior response to etching than posterior teeth. There were statistically significant regional differences, with the cervical region having greater remaining enamel than the middle ($p=.015$) and incisal ($p=.006$) regions. The cervical region was also significantly less dense than the middle ($p=.004$, $p=.015$) and incisal regions ($p=.002$, $p=.011$) at pre-etch and post-etch time points, respectively.

Conclusions

There are quantifiable differences in etch quality between tooth types and between regions within teeth. There is greater prismless enamel and inferior etch quality in the cervical regions, especially in premolars and molars. Response to etching is greater in anterior than posterior teeth, especially in the incisal and middle regions. Enamel density remains within accepted norms after etching with 37% phosphoric acid etchant for 15 seconds. Quantification of enamel etch quality with BIOQUANT Osteo and μCT software are valid methods of study.

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NOMENCLATURE

CPP-ACP	Casein Phosphopeptide Amorphous Calcium Phosphate
Micro-CT	Micro-Computed Tomography
μCT	Micro-computed tomography
SEM	Scanning Electron Microscope/Microscopy
WSL	White Spot Lesion

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Enamel formation is an intricate and complex process during tooth formation known as amelogenesis. The highly ordered structure and orientation of the enamel prisms is established during this period of development. Aprismatic enamel is located at the dentinoenamel junction and tooth surface due to its formation at the initiation and termination of amelogenesis.[1-3] It is oriented parallel to the tooth surface, rather than perpendicular like the underlying prismatic enamel.[4] Previous studies have shown that etching in the cervical region of teeth failed to result in an ideal prism end pattern, while other studies evaluating etch quality in various tooth types found anterior teeth to have a superior etch quality to posterior teeth.[4-7] These differences are likely due to the aprismatic enamel at the tooth surface, but no study has observed differences between and within all tooth types and regions collectively. Furthermore, all studies have utilized qualitative methods to classify etch patterns, which are subject to observer bias. Thus, it is important to discern the histomorphologic characteristics of enamel between and within all tooth types and to quantify the enamel etch quality. This may lead to an improved understanding of bonding to enamel in dentistry and orthodontics, as well as inherent characteristics that make a tooth type or region more prone to demineralization.

The present study will evaluate how histomorphologic characteristics of enamel affect the tooth surface's response to etching. The study will include novel approaches to quantify enamel etch quality via etch pattern image analysis and mineral density

analysis. Additionally, it will include all tooth types (incisors, canines, premolars, and molars) and all tooth thirds (incisal, middle, and cervical). At present, there are no studies in the literature assessing quality of etch patterns in a quantitative manner across all tooth types and regions.

Histomorphologic Characteristics of Tooth Enamel

Amelogenesis

Enamel is a homogenous structure primarily composed of inorganic matter, organized into hydroxyapatite crystal prim bundles.[1] Ripa et al[4] demonstrated that there are two distinct layers in enamel: the outer "prismless" enamel layer and underlying prismatic enamel layer. The "prismless" enamel layer has a different orientation compared to the underlying enamel, which has an optic axis nearly parallel to the enamel periphery.[4, 5, 8-10] This is in contrast to prismatic enamel which demonstrates distinct rod boundaries oriented perpendicular to the enamel surface. The aprismatic enamel is believed to be formed during tooth development. It is theorized to be due to decreased ameloblast activity and the disappearance of Tomes' processes during the termination of amelogenesis.[11]

Dental enamel formation, known as amelogenesis, occurs during the advanced bell stage of tooth development. Amelogenesis is a complex process which results in the highly organized structure of enamel prisms. Amelogenesis begins with a secretory stage in which the partially mineralized enamel matrix is formed and increases in length. The initial innermost enamel is secreted by secretory ameloblasts prior to formation of Tomes' processes. Tomes' processes are responsible for the enamel matrix orientation

into rods during the secretory phase, and therefore the innermost enamel adjacent to the dentinoenamel junction is prismless. During the secretory phase Tomes' processes are formed and secrete the enamel matrix in a rod orientation and the ameloblasts migrate from the dentin with deposition of the enamel. After the secretory stage is complete, the maturation stage begins in which the ameloblasts serve a new role of transporting proteins to increase the width and thickness of the enamel with removal of organic material and water. This results in the final enamel being primarily inorganic matter. The maturation phase lasts approximately 3-4 years and results in the overall hardening of the enamel. At the end of the secretory phase of amelogenesis, the ameloblasts lose their Tomes' processes, resulting in the outermost layer of enamel also being aprismatic.[1-3]

Enamel Characteristics and Aprismatic Enamel

Enamel Etch Quality and Characteristics in Various Tooth Regions

Scanning electron microscopy images of etched buccal premolar and molar enamel have shown that the cervical region is composed primarily of "prismless" enamel.[6-8] Etched premolar buccal enamel had a pitted feature in the cervical third with no distinct prism pattern, while the incisal and middle thirds had distinct prism-end, honeycomb-type structures in the study by Arakawa et al.[6] Likewise, Galil et al[7] evaluated etch patterns in different regions of extracted molars and premolars and subjectively classified the patterns on a scale of 1 to 5, with 1 being ideal enamel rod exposure, and 5 being an indistinct etch pattern. The study concluded that etch patterns appear to have systematic geographic distribution with types 4 and 5 patterns occurring

predominately in the cervical third. These studies were limited in that they only evaluated premolars and molars and were qualitative in nature.

Akkus et al[12] evaluated mineral content in 11 incisors with Raman Spectroscopy, comparing the various thirds of the incisor. By focusing a laser light on the sample and evaluating how the phosphate group in the mineral scatters the signals Raman analysis can determine mineral content. The phosphate peak in the resulting spectrum is proportional to the amount of mineral content. The study concluded that the incisal and middle thirds did not differ significantly in terms of mineral content from one another, but the cervical region's mineral content was significantly lower. This may cause this region to be more susceptible to demineralization.

Enamel Etch Quality in Different Tooth Types

A study by Hobson et al[13] evaluated etch patterns indirectly in different tooth types of patients. After etching, the teeth (maxillary and mandibular incisors, canines, premolars, and molars) were replicated with a high resolution silicone impression and poured with an epoxy resin. The resin models were evaluated with scanning electron microscopy. The images were subjectively analyzed using a 4-point qualitative etch pattern scale. The area percentage was calculated for each etch pattern type. There was a significant difference in etch pattern quality between different tooth types. Posterior teeth had an inferior quality of etch than anterior teeth, but an ideal etch pattern occurred in less than 5% of all the tooth types' areas. This could have been due to indirectly assessing the etch pattern via impressions, which may not accurately capture the etchant quality.

Likewise, Mattick et al[14] studied differences in etch patterns of maxillary and mandibular, incisors, canines, premolars, and molars with scanning electron microscopy images acquired from the central regions of the teeth. The resulting etch patterns were subjectively classified in terms of well-defined, poorly-defined, and unetched. The area occupied by each classification of etch was calculated. The study concluded that there were no differences in maxillary and mandibular teeth, that anterior teeth had a significantly greater area of better etch, and the tooth with the worst etch was the mandibular first molar. Both of these studies evaluating differences in etch quality between tooth types were limited as they only evaluated the central region of the teeth and were qualitative in nature.

White Spot Lesions

Etiology

WSLs are the initial signs of carious lesions and appear opaque on the tooth surface due to the demineralized subsurface layer scattering light differently than the adjacent intact enamel. They can be detected with visualization and tactile sensation with an explorer and can only be removed with restorative dental treatment. The understanding of WSL formation is based on WD Miller's chemoparasitic theory. It states that the normal oral flora, namely, *Streptococcus mutans* and *Lactobacilli*, ferment carbohydrates and produce an acid-by product that decreases the oral pH below the critical value of 5.5. If the oral environment remains below this critical value for an extended period of time, tooth demineralization occurs via dissolution of calcium and phosphate.[15-20]

Development of WSLs and caries is multifactorial, with biological and social risk factors. The factors include oral bacteria levels, salivary flow, salivary buffering capacity, fluoride exposure, socioeconomic status, education level, dental accessibility, oral hygiene practices, and diet.[17, 20] Orthodontic treatment is also a risk factor.[21]

Microbiota

Intraoral plaque is a complex biofilm composed of bacteria comprising the oral flora. The two species of bacteria within this biofilm that have been frequently associated with development of caries are *Streptococcus mutans* and *Lactobacilli*. These bacteria adhere to one another via polysaccharides and proteins; the biofilm increases over time if not removed daily.[17, 22] Duchin et al[18] evaluated samples of plaque from WSLs on buccal tooth surfaces. The study found that proportions of *Strep mutans* in samples from the WSLs were significantly higher than from the surrounding, intact tooth structure. Additionally, *Strep mutans* preferentially colonized around the gingival area of buccal tooth surfaces – also where WSLs are most commonly observed in patients. The changes in pH as a result of biofilm accumulation and byproducts creates the imbalance intraorally and produces an oral environment favoring demineralization rather than remineralization. This is why a diet low in sugar and complex carbohydrates and diligent oral hygiene is paramount for preventing WSLs.

Orthodontics and White Spot Lesions

Studies report that 2% to 96% of orthodontic patients develop WSLs during treatment.[23-31] This wide range is due to different protocols and study settings used to classify WSL presence (Table 1). The most frequent reported location of WSL

development is on the labial surfaces of the maxillary anterior teeth, but this has varied across multiple studies.[23, 30, 32, 33] Furthermore, the gingival areas were the most affected.[24, 27, 31] These unsightly lesions can occur in as little as 4 weeks – often less than one appointment interval in orthodontic patients.[28]

The teeth most commonly affected by WSLs vary from study to study. Julien et al[23] assessed upper and lower canine-to-canine digital photographs of 885 patients treated in a university setting. They found that the maxillary laterals and canines and the mandibular canines were the most susceptible. Ogaard et al[34] likewise concluded that maxillary laterals, mandibular canines, and mandibular premolars were most commonly affected in orthodontic treated patients and control subjects with no orthodontic treatment. Khalaf et al,[27] evaluated photos of 45 patients, found the highest incidence of WSLs on the maxillary lateral incisors and canines, as well as on the maxillary and mandibular premolars and molars. The maxillary lateral incisors had the highest incidence of WSLs occurring after orthodontic treatment in the study by Gorelick et al,[24] followed by the mandibular canines and mandibular posterior teeth. Geiger et al[35] showed the highest occurrence of WSLs on maxillary laterals, followed by the maxillary canines (Figure 1). While the specific teeth may vary between studies, there is a general consensus of WSLs occurring in the esthetic zone.

In studies evaluating various risk factors for WSL formation during orthodontic treatment, pre-existing WSLs and poor oral hygiene posed the highest risks.[23, 25, 27] Other risk factors include: increased treatment time, lack of fluoride supplements, consumption of soft drinks and sugary food items.[27, 35, 36] Fixed orthodontic appliances themselves

cause plaque traps and lead to an increased accumulation of plaque, decreased pH, and elevated levels of Streptococci and Lactobacilli, which increases the patient's risk for decalcification.[21, 37]

Tufekci et al[38] have suggested that there is a sharp increase in the number of WSLs during the first six months of treatment, followed by a slower rate of occurrence over the next 6 months. This makes implementing a daily oral hygiene routine at the beginning of orthodontic treatment of utmost importance. It is also important to identify high risk patients early to prevent these unsightly scars. While WSLs can be prevented with good oral hygiene and/or topical fluoride application, these regiments require compliance, which is often lacking in orthodontic patients. This leads to preventable WSLs manifesting themselves during and after orthodontic treatment, and makes the use of a non-compliant product imperative.

Treatment of White Spot Lesions

Several methods have been suggested to decrease and/or treat WSLs; however, the effectiveness of the treatments is dependent on the severity of the lesion and if remineralization can occur. Treatment options include regular fluoride application via fluoridated rinses and toothpaste, fluoride varnish application, casein phosphopeptide amorphous calcium phosphate (CPP-ACP), microabrasion, resin infiltration, and external bleaching.

Fluoridated Toothpaste and Mouth Rinse

Fluoride dentifrices including toothpaste and mouth rinses have been shown to remineralize WSLs in some studies and to have no effect in others. O'Reilly and

Featherstone[28] showed that brushing with a 1.1% sodium fluoride toothpaste and using a 0.05% sodium fluoride mouth rinse could provide a means of remineralization. In contrast, Akin et al[39] showed that there was not a significant difference in WSLs surface area reduction between patients using a low-fluoride mouth rinse and control patients.

Fluoride Varnish

A randomized controlled trial by Du et al[40] evaluated the effectiveness of a 5% sodium fluoride varnish every 4 weeks during the first six months after debonding and found a significant reduction in DIAGNOdent readings after 3 and 6 months of varnish treatment. They concluded that fluoride varnish should be considered effective at reversing WSLs; however, in these studies the visual improvement was not always reported. This indicates that remineralization may have occurred and lesions appear smaller, but may not be removed completely from visual detection. He et al[41] concluded that fluoride varnish treatment of WSLs resulted in a significant decrease in the volume, area, and fluorescence of the lesions. However, of 361 lesions treated with either fluoride varnish or fluoride film, only 2 showed complete healing. Fluoride varnish application eliminates patient compliance but does require frequent office visits for application.

Casein Phosphopeptide Amorphous Calcium Phosphate (MI Paste)

CPP-ACP application has also shown controversial results when it comes to treating WSLs. One study has shown that it provides and facilitates the absorption of calcium and phosphate into enamel to aid remineralization.[42] Andersson et al[43] compared the fluorescence and visual appearance of WSLs treated with either CPP-ACP

and fluoridated toothpaste for 3 months each, or use of a 0.5% fluoridated mouthwash and fluoridated toothpaste for 6 months. The lesions were evaluated visually and via laser fluorescence at 1, 3, 6, and 12 months. There were no significant between group differences, with both groups showing improvements in the lesions over time. However, the CPP-ACP group resulted in significantly more lesions with total disappearance based on visual evaluation. When compared to a placebo cream, the CPP-ACP resulted in significant improvement of WSLs present in patients after debond, based on clinical detection and examination after 12 weeks of treatment.[44] Other studies, have shown improvements in lesion area, appearance, or fluorescence level after 4-12 weeks of treatment, but no significant differences when compared to normal oral hygiene with a fluoridated toothpaste.[45, 46] Huang et al[47] compared the effectiveness of CPP-ACP (MI Paste) or fluoride varnish to a control group. They performed a randomized controlled clinical trial of patients with a WSL present on their incisors after debonding. The study found there was a wide range of improvements, but that neither treatment was more effective at improving WSL appearance after orthodontic treatment than regular home care. Despite its promising results in reducing and treating WSLs, CPP-ACP (MI Paste) application requires patient compliance. This is problematic because patients with WSLs have already demonstrated lack of compliance with regular oral hygiene as evidenced by the presence of the lesions in the first place.

Microabrasion

Enamel microabrasion was first introduced as a treatment for fluorosis. Due to its ability to remove the most superficial layer of enamel and superficial

staining/discoloration, it has since been used as a treatment modality for post-orthodontic WSLs.[48] Studies have shown that the use of an 18% hydrochloric acid and fine pumice slurry improves the visual appearance of the WSLs in 83-99% of cases; it was more effective in mild to moderate lesions than severe lesions.[39, 49, 50] A risk associated with microabrasion however is the chance of uncovering the cavitation with removal of the thin surface enamel overlying the demineralized area.[39] These studies did not evaluate the depth of the lesions, only their visual size and surface area. Future studies are needed to evaluate the use of microabrasion in combination with a fluoride treatment such as MI paste.

Resin Infiltration

Resin infiltration is another method of treating for existing WSLs. The procedure involves etching the enamel surface with hydrochloric acid, followed by infiltration of a low viscosity resin which is light cured. The resin fills the porous surface, which can improve the visual appearance of the lesion while also preventing further demineralization of the lesion.[51, 52] Kim et al[53] evaluated WSLs treated with resin infiltration and assessed the change in appearance of the lesions. 61% of the samples had lesions that were considered completely masked, 33% which were partially masked, and 6% were unchanged. While resin infiltration has been shown to improve the appearance of WSLs and even arrest the demineralization, it has not shown 100% effectiveness in improving the lesion visually, and the acid used to prepare the lesion for the infiltration can be detrimental to surrounding tooth enamel.

External Bleaching

External bleaching after orthodontic debonding is another method to camouflage the opaque white appearance of WSLs. Knosel et al[54] evaluated WSLs and adjacent enamel with a colorimeter to determine the difference after sessions of in-office bleaching and at-home bleaching. The surrounding, healthy enamel had a significantly greater increase in lightness post-bleaching than did the WSL, allowing for less contrast between the two enamel surfaces and an improved visual appearance. While external bleaching may improve the appearance of the WSL relative to the color of the surrounding enamel, it does not facilitate remineralization of the lesion.

Due to the inconsistent results and inability to formulate a standardized methodology to treat WSLs, prevention should be the priority of every clinician to help ensure healthy and successful orthodontic outcomes.

Prevention of White Spot Lesions

Oral Hygiene

Younger age groups (pre-adolescent), poor oral hygiene, and active decay were associated with more WSLs and increased severity of the WSLs.[32, 55] Likewise, Julien et al[23] concluded that a lack of fluorosis, poor oral hygiene, declining oral hygiene status during treatment, and pre-existing WSLs were associated with patients developing demineralization during orthodontic treatment. High risk orthodontic patients who receive more frequent professional cleanings have significantly fewer WSLs than control high risk patients. However, the number of WSLs in high risk patients who received more frequent professional cleanings was still significantly greater than the number of

WSLs among low risk patients with good at-home oral hygiene practices.[56] Good patient compliance relates to a significant decrease in the total number of WSLs, regardless of tooth type.[35] This reiterates that good home care and patient education can decrease the occurrence of demineralization, the need for extra dental visits, and extra costs associated with preventing WSLs.

Saliva

Saliva is the body's natural form of protection against caries. It serves as a buffer to resist pH changes, which ensures that patients remain in the demineralization state for less time after ingesting carbohydrates.[57, 58] The mechanism driving this buffer system is the carbonic acid-bicarbonate in saliva, which is responsible for maintaining the oral cavity pH between 6-8.[20] Leeper[37] showed that orthodontic patients' saliva buffer level was low during treatment, which may predispose them to higher risk for developing WSLs. In addition to acting as a buffer, saliva contains various proteins and minerals including, calcium and phosphate, which can aid in remineralization of the dentition after an acid attack. The flow of saliva also serves as a cleansing mechanism. Buffer capacity, remineralization, and cleansing are each dependent on saliva flow rate, which is why an increased flow rate is associated with a lower caries risk.[59-61]

Fluoride

Fluoride has often been used and studied for its ability to prevent caries. Its role is based on the formation of fluorohydroxyapatite and calcium fluoride intraorally, which integrates into the enamel structure. The critical pH value of fluorohydroxyapatite is lower than that of hydroxyapatite (4.5 vs. 5.5), which provides a protective mechanism

against demineralization if the pH does not drop below 4.5.[62] Fluoride ions can also combine with calcium to form calcium fluoride, which can integrate into the dental plaque and attract phosphate molecules, which serves as a reservoir of fluoride ions and aids in remineralization of the tooth surface.[63, 64] However, F- ions must be present for fluorohydroxyapatite and calcium fluoride formation to occur during an acid challenge, making exposure to fluoride, within acceptable daily limits, necessary to have an anti-cariogenic effect. The study by Ogaard[65] concluded that availability of fluoride ions in the oral cavity was more important than the fluoride incorporated into the enamel, emphasizing the importance of daily exposure to fluoride via water, toothpaste, mouth rinses, and/or varnish.

Concomitant with diligent oral hygiene is the use of fluoridated dentifrices. Stratemann et al[66] compared orthodontic patients who used a 0.4% stannous fluoride gel with a control group who did not. They found that 2% of the experimental group experienced decalcification during treatment, compared to 58% in the control group. Additional studies by Geiger et al[67] and Robertson et al[68] had significant reductions in WSL formation after daily use of a fluoridated mouth rinse or daily application of MI paste, respectively.

Brushing, rinsing, and applying fluoridated gels or MI Paste all require compliance from the patient, which is lacking in individuals most prone to WSLs. Thus, fluoride varnish, fluoride releasing orthodontic bonding agents, and bonded sealants have been studied for use in non-compliant patients. Todd et al[69] evaluated the area and depth of demineralization in vitro, using extracted canines and premolars with bonded

orthodontic brackets. They compared the application of a non-fluoridated varnish and fluoridated varnish to a control. The study showed that fluoridated varnish use had a 50% reduction in the amount of demineralization. Fluoride varnish has, in vivo, decreased the occurrence of WSLs on incisors - the esthetic area where WSLs are most visible – making it useful for preventing WSLs in non-compliant patients.[70, 71] Application of the varnish does require frequent office visits for re-application.

During bonding, glass ionomer cement is sometimes used due to its fluoride releasing properties. Systematic reviews have concluded that glass ionomer cements prevent WSL occurrence compared to conventional bonding composite, but the study designs and overall evidence was weak.[72, 73] Additionally, glass ionomer cements have a high fluoride release capability at initial bonding, which tapers to lower levels for the duration of treatment unless recharged with regular fluoride application.[74]

Sealants

A preventive method that does not require patient compliance is the use of sealants bonded on the buccal tooth surfaces.[33, 75] Despite their placement on buccal enamel, sealants do not significantly alter bracket bond strength.[76-79]

Several in vitro studies have evaluated the ability of sealants to protect against demineralization. Hess et al[80] sealed the right side of extracted human teeth and subjected them to a simulated carious challenge of *Strep mutans* in a nutritive media and assessed the decalcification changes using DIAGNOdent. The study demonstrated that tooth halves with filled resin sealants had less demineralization than the unsealed controls. Another in vitro study compared a filled sealant to fluoride varnish, an unfilled

sealant, and untreated control teeth. The group treated with the filled sealant decreased the depth of demineralization by 97% and performed significantly better than the other products at preventing WSL formation.[75] In vivo studies have shown similar results.[76] The in vivo study by Benham et al[33] demonstrated that sealed teeth were approximately four times less likely to develop WSLs than unsealed teeth and that WSLs that developed on sealed teeth were smaller and less severe.

The Reliance Orthodontic Products sealant, Pro Seal, is utilized as an orthodontic armamentarium to assist in preventing WSLs. Pro Seal is a filled, light-cured fluoride sealant that also contains a fluorescing agent to monitor the absence of sealant clinically. The filler prevents wear from daily activities such as brushing. Van Bebber et al[81] studied the effect of filler concentration on the durability of the bonded sealant. The 18% filled sealant had the least amount of sealant lost. As the filler content increased the wear resistance and retention of the sealant also decreased. Additionally, in vitro studies have concluded that teeth treated with Pro Seal had harder enamel than those treated with fluoride varnish, etchant only, or unfilled resin and that the depth and visual appearance of WSLs was significantly reduced in teeth with Pro Seal applied.[75, 82]

The benefits of sealants appear to justify their use in orthodontics, but their long-term retention is a major issue. Chau et al[83] demonstrated that the majority of sealant loss occurs around the edges of the teeth. In addition, Anderson,[84] evaluated sealant retention in vivo and concluded that the gingival regions of teeth had 6-10% greater sealant loss than the overall tooth surface. The gingival location is where sealant retention is crucial due to the increased prevalence of WSLs in this area. Therefore, there

is a need to improve sealant retention and improve the protective benefits of sealants against WSL occurrence.

Placement of sealants alone can prove challenging due to salivary flow, hyperplastic gingiva, gingival crevicular fluid, and poor quality of etched enamel. Even after successful placement, sealants are subjected to the acidic oral environment and mechanical abrasion from chewing food and brushing. Van Bebber et al[81] showed that a simulated acid challenge had no effect on the sealant retention. Likewise, Anderson[84] concluded that placement of gingival retraction cord provided a statistically significant, but not a clinically significant, improvement in sealant retention. These studies indicated that factors other than the acidic oral environment and contamination during bonding affect sealant loss and hinder an orthodontist's attempts to prevent WSLs. Based on the studies regarding enamel etch quality, it elicits the question if the presence of aprismatic enamel affects sealant retention, particularly in the cervical third.

Orthodontic Bracket Bonding

Bracket bonding failure provides an indirect measure of etch-quality. Linklater et al[85] found that there are significant differences in the bracket bond strength of the different tooth types. The results showed that mandibular bonds fail more often than maxillary bonds. They suggested that factors responsible for the failures include: masticatory forces, poor moisture control, and/or a difference between maxillary and mandibular tooth morphology.

Hobson et al[86] evaluated the relationship between acid-etch patterns in the optimal bracket area (a 4x4-mm area centered at the clinical crown's buccal midpoint),

and orthodontic bond survival in vivo. They concluded there was a statistically significant relationship between the quality of etch and the bond survival length.

Multiple studies have demonstrated that posterior teeth have a significantly greater bond failure and the quality of etch decreases progressively in the more posterior teeth (Table 2).[14, 85-90] Thus, the etching and bonding characteristics of one tooth type cannot be extrapolated to all tooth types comprising the human dentition.[6, 14] Due to molars and premolars having shorter crown heights than canines and incisors, a portion of the bracket base may be bonded to cervical enamel, which may be more susceptible to bond failures due to the aprismatic enamel in the cervical region.

Etching and Bonding to Enamel

Etching and Bonding

Bonding to enamel has been a widely investigated historically. In the 1950's Buonocore was the first to describe the use of a phosphoric acid etchant to alter the enamel surface and improve the retention of bonded resin.[91] The etchant creates a porous enamel surface by selectively dissolving the hydroxyapatite crystals. The ideal depth of etch is proposed to be between 5 and 50μm.[92] The act of etching dental enamel provides an increased surface area and greater exposure of the enamel's organic components, producing a surface favorable for bonding.[91] This allows for the penetration of the resin into the porosities, which is retentive due to a micromechanical bonding of the resin tags.

Adhesive bonding in dentistry has evolved over time, beginning with non-etch to total-etch to self-etch systems. These systems are utilized with either an etch-and-rinse,

self-etch, or a resin-modified glass ionomer technique. Studies have shown that etch-and-rinse bonding systems produce a higher resin bond strength when compared with self-etching adhesive systems.[93, 94] Etching with phosphoric acid etchant also results in a greater surface roughness when compared to self-etching primers, indicating a surface more amenable to bonding.[94] Dental adhesives themselves are composed of hydrophilic and hydrophobic groups, curing initiators, inhibitors or stabilizers, solvents, and inorganic fillers.[95] In orthodontics, the 5th generation, two-step etch-and-rinse system is often used. The tooth surface is etched, rinsed, dried and then the sealant is applied. The sealant then serves as a primer/adhesive prior to bonding the bracket, and also as a protective barrier.

Etch Time

Historically, teeth were etched for 60 seconds, but etching time has decreased over the years. Studies have shown that increasing etch time from 15 seconds (as per the manufacturers recommendations) to 60 seconds, with the etch-and-rinse systems, does not produce significant differences in resin bond strength to enamel.[4, 93, 94, 96] Additionally, a 15 second etch time with 37% phosphoric acid does not make the enamel more prone to demineralization.[97] To determine the quality of etch achieved based on the etch time, Johnston et al[98] evaluated molars with SEM. They classified the etch pattern on a 3-grade scale after etching for 15, 30, 45, or 60 seconds. They found that a 15-second etch time failed to produce an optimal etch pattern on the buccal surface of the molars, but this could differ between tooth types due to potential differences in presence of aprismatic enamel and a greater proportion of molar enamel being composed of

cervical enamel. Therefore, this data cannot be extrapolated to the remainder of the dentition.

Etch Concentration

A phosphoric acid etchant, with a concentration of 30-50%, has been used clinically in bonding procedures both in dentistry and in orthodontics. The background for choosing higher etchant concentrations derives from the study by Chow et al,[99] who found that etchant concentrations greater than 27% result in monocalcium phosphate formation, which is more soluble than dicalcium phosphate dehydrate, formed at concentrations less than 27%. The solubility allows the monocalcium phosphate to be washed away from the enamel surface more effectively, better preparing the surface for bonding. However, Sadowsky et al[96] found that there was no significant difference in orthodontic bracket bond failure when a 15% phosphoric acid etchant was used compared to the standard 37% phosphoric acid etchant. The study, however, did not include molars, which are frequently associated with bond failures, and the 15% etchant had more than double the number of debonds as the 37% group, which could be considered clinically significant despite the lack of statistical significance.

Dental Restorations in the Cervical Third of Teeth

Aprismatic enamel may also play a significant role in microleakage often associated with Class V dental restorations. Studies have shown that microleakage is a common problem along the margins of Class V dental restorations, which are placed in the cervical region of teeth.[100-102] Crim et al[100] demonstrated that the microleakage along the gingival margin was greater than that of the occlusal margin in Class V restorations.

Bonding failures in the cervical region, often attributed to flexion of the tooth during functioning, could also be attributed to prismless enamel observed in this region, which may be more prone to failure due to inability to achieve an ideal etch pattern.

Aprismatic Enamel

Etching is supposed to remove the outer layer of aprismatic enamel and then expose the underlying prismatic rods.[103] Based on the lack of a distinct etch pattern in the cervical region, the aprismatic layer may not completely be removed in these regions, resulting in a poor quality of etch and bonding failure.

At present, there are a limited number of studies evaluating the quality of etched enamel. Additionally, there are no studies evaluating all tooth types and regions collectively. All studies presently have been qualitative in nature, which is at risk of subject and experimenter bias. Quantifying the quality of etch in all tooth types and regions could provide a better understanding of etch effectiveness, enamel bonding preparation, the difficulty with orthodontic sealant retention near the gingival margin, and the occurrence of WSLs. Thus, the present study aims to quantify the quality of etch between and within tooth types and to validate novel approaches to quantify enamel etch quality.

CHAPTER II

MATERIALS AND METHODS

Extracted human teeth from various oral surgery offices were collected, disinfected in a 10% sodium hypochlorite solution for 24-48 hours, sorted by tooth type, and stored in a 0.1% thymol solution.[104-106] Maxillary and mandibular central incisors, lateral incisors, canines, premolars, and molars were included. To be included, the teeth had to have intact buccal enamel surfaces free of restorations, caries, decalcification, fluorosis, and enamel defects. The study was approved by the Institutional Review Board and faculty advisors of Texas A&M University College of Dentistry.

Twenty-seven tooth samples (3 of each tooth type) that met the inclusion criteria were sectioned bucco-lingually and mesio-distally. The roots were removed using a hand piece and diamond disc and discarded following protocol. The buccal right and left halves of the crowns were utilized for the present study. Each half was randomly assigned, using random numbers generated with Microsoft Excel, to either the scanning electron microscope (SEM) or micro-computed tomography (micro CT) aspects of the study.

The surfaces were cleaned with a slurry of non-fluoridated flour of pumice and water with hand-held rubber cup denticators, rinsed, and air dried. A 37% phosphoric acid gel etchant (Reliance Orthodontic Products, Itasca, IL) was then applied to cover the entire buccal surface of each sample, left in place for 15 seconds, copiously rinsed with water, and then dried with an oil- and moisture-free syringe until a frosted appearance of the enamel surface was visually apparent (Figure 2).[81, 84, 93, 94, 97]

SEM Protocol

After etching, samples viewed with SEM were placed in a 100% ethanol solution for 1 hour and then placed in a vacuum overnight to allow for adequate drying.[107] They were mounted on aluminum stubs with conductive tape, with the buccal surface facing upward and parallel to the surface of the aluminum stub. The samples were then sputter coated with gold for 2 minutes and the etchant patterns were viewed with the SEM (Figure 3).[7, 10, 11, 14, 98]

Three regions of etched enamel were viewed with a JEOL (Tokyo, Japan) JSM-6010LA InTouchScope™ Analytical Scanning Electron Microscope, including the incisal, middle, and cervical third of each sample. The images were acquired at 1200x magnification (Figure 4A).[108] Image contrasts were standardized using Preview by Apple photo editing software (Cupertino, California), and then analyzed with BIOQUANT Osteo (Nashville, TN) software. The area of enamel remaining after etching was selected to be included in the image analysis to calculate the percentage of enamel present in the image via the ratio of bone volume to total volume (BV/TV) (Figure 4B). A lower percentage of enamel indicated a better quality of etch. Each image was processed three times and the mean recorded for each tooth. Intraclass correlation for average measures was 0.962 and method error ranged from 2.5 to 2.8.

μCT Protocol

Samples designated for viewing with μCT were prepared by placing a notch 1 mm from the sectioned edge with a diamond disc, which served as a reference for measurements during region of interest selection in the final scans (Figure 5).[109] The

samples were first scanned in a 12.3 mm diameter viewing tube filled with distilled water. They were placed in a vertical (incisal-apical) orientation prior to the etching protocol previously described to serve as a baseline. After etching, samples were again placed in viewing tubes with 70% ethanol, so as to not disrupt the etch patterns, in a vertical (incisal-apical) orientation. The scans were completed using a μCT 35 Desktop Micro CT Scanner (Scanco Medical, Switzerland) with the following parameters as per manufacturer recommendation for dental tissue samples: energy/intensity of 70 kVP, 114 μA, 8 W; medium resolution; field of view/diameter of 12.3mm; and voxel size of 6.0 μm. The incisal, middle, and cervical regions of the samples were defined on the scout scan at a size of 230 slices each (Figure 6). The scans of each region were then completed and produced in a cross-sectional orientation (Figure 7A).

The scans were then post-processed by defining a region of interest that was approximately 50 μm deep in each region of the tooth (incisal, middle, cervical). This was done for both the pre- and post-etch scans (Figure 7A, Figure 7B). This allowed a 600 micron 3D reconstruction of the samples (Figure 7C).[92, 110] The data analysis for bone mineral density (BV/Density bone) was then completed using the μCT 35 version 6.1 software (Scanco Medical, Switzerland) with threshold levels for enamel set between 580 and 1000.[111, 112] Material and apparent densities were recorded for each sample. Apparent density is the ratio of the mass of the mineralized tissue and the total volume of the tissue, including voids present in the sample. Material density is the ratio of the mass of the mineralized tissue and volume of the tissue, excluding any voids that may exist within the sample.[113] As such, apparent density would be expected to decrease as

etching removes the less dense aspects of the hydroxyapatite crystals and creates more voids within the enamel structure. In contrast, material density would be expected to increase due to etching removing the less dense aspects of the enamel. By excluding the voids created with the removal of less dense enamel, material density should be greater than apparent density after etching.

Fifteen μCT samples were randomly selected to re-trace the region of interest for calculation of the apparent and material densities. No systematic differences were detected for either, material or apparent, density. The single measures intraclass correlation was 0.767 and 0.944 for the apparent and material densities, respectively. The average measures intraclass correlation was 0.868 and 0.971 for the apparent and material densities, respectively. The method error for apparent density was 58.95 while the method error for material density was 20.18.

Statistics

To ensure standardization of the procedures, the same investigator performed all of the post-processing of the sample images acquired from the SEM and likewise for the scans acquired from the micro-CT. The examiner was blinded during post-processing as to the tooth type and region the image or scan was acquired from.

Data collected, was coded and entered into SPSS (IBM SPSS Statistics, Inc., Chicago, IL) for statistical analysis using a significance level of 0.05 ($p< 0.05$). Skewness and kurtosis statistics showed normal distributions. For the SEM data, paired T-tests were utilized to observe differences between the various tooth regions. Two way analyses of variance and a Fisher's least significant difference post-hoc test were used to

analyze for differences between jaw (maxilla vs. mandible) and tooth type (incisor, canine, premolar, and molar).

For the μCT data, paired samples T-test were used to evaluate the difference in material and apparent densities before and after etching in the various tooth regions. Independent samples T-tests were used to analyze the difference in material and apparent densities before and after etching between anterior (incisors and canines) and posterior (premolars and molars) teeth.

CHAPTER III

RESULTS

Scanning Electron Microscope/BIOQUANT Osteo Analysis: Percentage of enamel present after etching

SEM qualitative image analysis:

The SEM images showed obvious differences in the quality of etch in various teeth and tooth regions. Not all images/regions demonstrated the ideal key-hole pattern that indicates exposed enamel rods. Molars consistently showed a poorer etch quality in all regions, while incisors typically showed an ideal etch pattern in the incisal and middle regions, and occasionally in the cervical region. There appeared to be an inferior etch quality in the cervical regions across tooth types (Figure 8, Figure 9).

SEM quantitative image analysis with BIOQUANT Osteo (Nashville, TN) software:

Maxillary vs. mandibular teeth:

The mean percentage of enamel remaining in the various tooth types and regions in the sample ranged from 63.6% to 78.8% (Table 3), with greater percentages of enamel present representing a lesser quality of etch (i.e. fewer open enamel rods). Overall, the cervical regions consistently had a greater percentage of enamel present in both the maxillary and mandibular teeth (Figure 10). Maxillary teeth showed a tendency for lower percentages of enamel in all regions than mandibular teeth. However, analysis of variance evaluating tooth and arch differences showed no statistically significant interactions between the two factors and no statistically significant differences between the arches (Table 4).

Anterior vs. posterior teeth:

When differences between tooth types were evaluated independent of the arch involved, premolars and molars had greater percentages of enamel remaining after etching than the incisors and canines in all regions (Figure 11). Analysis of variance showed that there were significant differences in etch quality between tooth types in each region (Table 5). Fishers least significant difference tests showed that molars and premolars had a significantly different quality of etch than incisors and canines. More specifically, incisors and canines had significantly greater etch qualities than molars and premolars ($p<0.05$) in the cervical region. In the middle region, incisors and canines had significantly greater etch qualities than premolars ($p<0.05$). In the incisal region, incisors had significantly greater etch qualities than premolars and molars ($p<0.05$) (Table 5). Overall, there was consistently a significant difference between anterior and posterior teeth.

Differences in tooth regions:

The cervical region showed higher mean percentages of enamel remaining after etching than the middle and incisal regions in all tooth types (Table 6, Figure 12). When the teeth were considered collectively, the cervical region showed the greatest percentage of enamel remaining after etching, followed by the middle region, and then the incisal region (Table 6, Figure 12).

There was a significant overall difference between the cervical and incisal regions and the cervical and middle regions ($p<0.05$), but no significant differences between the middle and incisal regions (Table 7). The incisal region was significantly

different ($p<0.05$) from the cervical region in incisors specifically as well. No other regions were significantly different from one another; however, there was a trend that the cervical region had the highest percentage of enamel remaining after etching, indicating an inferior etch quality (Figure 12).

Microcomputed Tomography Analysis:

μCT apparent and material densities of enamel pre- and post-etch

Anterior vs. posterior teeth:

The apparent densities at T1 (pre-etch) did not differ significantly between anterior and posterior teeth (Table 8). Likewise, there were no significant differences between anterior and posterior teeth material densities at T1 (Table 8).

There were no significant differences at T2 (post-etch) between anterior and posterior teeth apparent densities (Table 9), but anterior and posterior teeth material densities were significantly different ($p<0.05$) in the cervical region at T2 (Table 9).

The anterior and posterior cervical regions were the least dense (material density) of all the regions at T1. The posterior cervical region remained the least dense at T2, while the anterior cervical region showed the greatest increase in material density after etching (Table 8, Table 9).

When changes in apparent and material densities from T1 (pre-etch) to T2 (post-etch) for anterior and posterior teeth were compared, there was a greater change in material density for anterior teeth than posterior teeth (Table 10, Figure 13). There were no significant differences in the apparent density changes from T1 to T2 between anterior and posterior teeth, but there was a significant differences ($p<0.05$) between

anterior and posterior teeth material density changes in the middle and cervical regions (Table 10, Figure 13). The anterior teeth had a greater change in the density from T1 to T2.

Differences in tooth regions:

When densities were compared between the regions of teeth, independent of tooth type, material densities were greater than apparent densities for all regions and time points (Table 11, Figure 14). Paired samples t-tests showed that the cervical region differed significantly ($p<0.05$) from the middle and incisal regions at T1 and at T2 for apparent density (Table 12). Apparent density was greatest in the incisal region, less in the middle region, and least in the cervical region at both T1 and T2. The decrease from T1 to T2 in each region, however, was not significantly different. For the material density of the various regions, there was a significant difference between the incisal and cervical regions and the middle and cervical regions at T1 (Table 12). The material densities for the incisal and middle regions at T1 and T2 were similar but both the T1 and T2 densities were less for the cervical region (Table 11). The material density increased from T1 to T2 in all regions and this increase was significant ($p<0.05$) for all three regions (Table 13, Figure 14).

The apparent densities decreased from pre-etch to post-etch in all regions, while the material densities increased (Figure 14). This is expected due to the method of calculating apparent and material densities with inclusion and exclusion of voids in the sample, respectively.

CHAPTER IV

DISCUSSION

Maxillary vs. Mandibular Teeth

There are no differences in etch quality between maxillary and mandibular teeth. Mattick et al[14] and Hobson et al,[114] are the only studies to evaluate etch patterns in various tooth types qualitatively and likewise shared this conclusion.

Anterior vs. Posterior Teeth

Posterior teeth exhibit an inferior etch quality compared to anterior teeth. In the present study, the SEM etch patterns showed qualitative and quantitative differences between the anterior and posterior teeth. The etch quality in posterior teeth (premolars and molars) was significantly inferior to that of anterior teeth (incisors and canines). Previous studies investigating etch pattern quality have focused on specific teeth and tried to extrapolate their results to the entire dentition.[6, 7, 115] One study evaluated differences between tooth types, but focused on small (4x4 mm) areas at the center of the teeth;[14] another study assessed etch patterns indirectly via impressions of etched tooth surfaces produced with an epoxy resin also within a small (4x4 mm) area at the center of the teeth.[114] Both studies also showed significantly inferior etch patterns in the posterior than anterior teeth, but the differences were not quantified.[14, 114] Aprismatic enamel remaining after etching may explain why the posterior teeth have an inferior etch quality. Whittaker et al[116] concluded that incisors have thinner layers of aprismatic surface enamel than posterior teeth prior to etching. If the etch does not remove the entire thickness of aprismatic enamel, it would inherently result in an inferior etch

quality. This may explain why posterior teeth consistently have been shown to have poorer etch patterns.

Posterior teeth have an inferior response to etching than anterior teeth. Material density, as measured by μCT, indicated that anterior teeth respond better to etching than posterior teeth. Anterior teeth exhibited greater changes in material density from pre-etch (T1) to post-etch (T2) than posterior teeth. This indicates that anterior teeth had a greater response to etching than posterior teeth, which confirms the previous SEM results in regards to anterior teeth having a significantly superior etch quality than posterior teeth. Lower quality etch and inferior response of posterior teeth may be related to bracket bond failure in orthodontics. It has been well established that posterior teeth have a significantly greater number of bracket debonds than anterior teeth.[85, 87-89, 117]

Tooth Regions

Cervical enamel has an inferior etch quality than the middle and incisal regions. In the present study, the SEM images demonstrated qualitatively and quantitatively that the etch patterns in the cervical region were poorer than those of the middle and incisal regions. This is in agreement with previous studies which qualitatively demonstrated that the cervical region does not exhibit a prism-end pattern after etching.[6-8] These studies were limited; however, in that they only examined one specific tooth type and/or region. The present study confirms this relationship across all tooth types and regions. The aprismatic enamel explains the inferior etch quality in the cervical region. Unlike the underlying prismatic enamel, aprismatic enamel is orientated parallel to the enamel

surface rather than perpendicular.[4, 8-10] It is located on the surface of teeth and may be removed when the enamel is etched. If the thickness of prismless enamel is greater than the penetration ability of the etchant, it will result in a poorer etch quality due to remaining prismless enamel at the tooth surface. This explains why pre-treatment mechanical abrasion of the tooth enamel resulted in an improved etch pattern quality.[7] The pre-treatment mechanical abrasion removed the aprismatic enamel, exposing the underlying prismatic enamel, and therefore produced a superior etch quality. The presence of aprismatic enamel after etching possibly explains why sealant retention is poor in the gingival region of teeth.[83, 84] Aprismatic enamel may also be related to orthodontic bracket debond rates. Because of their shorter crown heights, a greater percentage of the brackets are bonded in the cervical regions of molars and premolars, where etch patterns were inferior. Having a less-than-ideal etch quality predisposes brackets to debonding from the tooth.

The cervical region is consistently less dense than the middle and incisal regions. Prior to etching, the material and apparent densities of the cervical enamel were significantly lower than the incisal and/or middle regions. A previous study that examined mineral content of incisors without etching reported that the incisal and middle thirds did not differ significantly from one another, while the cervical region showed significantly lower mineral content than the other two regions.[12] This is consistent with the present study's findings that cervical enamel is less dense. The lower density in the cervical region could be due to the presence and orientation of aprismatic enamel.[4] Oral histology and development studies show that enamel development and

mineralization extends outward from the DEJ, but it also begins at the cusp tips or incisal areas and extends apically towards the cervical region.[3, 118] Due to the cervical region being the last region formed during amelogenesis, it may make the enamel more prone to being aprismatic and less dense at the termination of enamel formation and mineralization. In addition to the cervical region being the last area of enamel formed, it is also the thinnest. Enamel is thickest in the working areas of the teeth, such as the occlusal and incisal aspects, and tapers to a knife-edge at the cemento-enamel junction.[119] Thus, the cervical regions is composed of a greater percentage of aprismatic enamel, which could inherently result in the cervical region also being less dense. The finding that the cervical region is less dense than the incisal and middle regions has clinical significance because a lower enamel density in the cervical region would make this region more susceptible to demineralization, and may contribute to WSLs being more common in the gingival third of teeth.[24, 27, 31]

Methodology

The quantitative analyses with BIOQUANT Osteo and μCT software provide novel approaches for assessing the quality of enamel etch. No other studies have attempted to quantify the quality of enamel etch patterns directly. The BIOQUANT Osteo (Nashville, TN) software is traditionally used for bone biology research. Due to enamel being the most mineralized material in the body and its similarity to human bone, the program was utilized for the analysis of enamel remaining after etching. Additionally, the μCT analysis was used for calculation of mineral density in the various tooth types and regions using appropriate thresholds for hydroxyapatite. The quantitative

results in the present study are consistent with the visual assessments showing inferior etch quality in cervical enamel, both in the present and past studies.[6, 7, 114] They are also consistent with previous studies showing significant differences between anterior and posterior teeth etch quality.[14, 114]

The apparent density decreases from pre-etch to post-etch, while the material density increases. Apparent density is the ratio of the mass of the mineralized tissue divided by the total volume occupied by the tissue, including the voids present within the sample. Material density is the ratio of the mass of the mineralized tissue divided by the total volume occupied by the tissue, excluding any voids present within the sample.[113] As such, apparent density would be expected to decrease if etching removes the less dense aspects of the hydroxyapatite crystals and creates more voids within the enamel structure. In contrast, material density would be expected to increase if etching removes the less dense aspects of the enamel. By excluding the voids created with the removal of less dense enamel, material density should be greater after etching. The increase from pre-etch to post-etch in material density was significant in all of the tooth regions, indicating that etching enamel removes a significant amount of less dense enamel, but there was not a significant change in apparent densities. This is due to the greater amount of method error and variability in region of interest selection via greyscale pixilation due to apparent density's inclusion of voids (greyscale pixels) in the calculation.

Etching enamel for fifteen seconds with 37% phosphoric acid gel etchant does not remove critical amounts of calcium and phosphate from the tooth surface. He et al,[111]

evaluated the hydroxyapatite density of enamel, reported the mean buccal enamel density to be 2228.1 +/- 85.5 mg/cm3. The enamel material densities acquired in the present study fall within this range, both prior-to and after etching. Since the post-etch material density remains within the accepted norms, this indicates that etching with 37% phosphoric acid etchant for 15 seconds does not remove a critical amount of mineral from the enamel surface. It has been previously shown that etching alone does not make a tooth more susceptible to demineralization.[97]

Limitations and Future Studies

Ideally, the SEM portion of this study should have compared post-etch to pre-etch patterns. This would have provided baseline comparisons. However, this was not possible due to the need to sputter coat the samples (i.e. the same sample cannot be viewed via SEM at two time points). However, a portion of the tooth could have been etched and then compared to an adjacent unetched region, but the tooth samples used in the present study had already been sectioned, making only a limited area of buccal surface enamel available. The curvature of teeth was also a limiting factor during the acquisition of the SEM images because orientation affects the visibility of etched enamel prisms. This may explain why incisors, which have a flatter surface, had ideal etch patterns compared to premolars and molars, which have greater buccal surface curvatures. The samples in the present study were oriented on an aluminum stub with their surfaces parallel with the mounting stub. Previous studies have polished enamel to provide a flat surface, but this requires removal of surface enamel.[8, 10, 116, 120] Removal of

enamel could bias the results, suggesting an improved etch quality, when in fact it was due to the removal of the aprismatic surface enamel.

The regions of interest selected and sample sizes used in the present study were also limitations. There was opportunity for random error when selecting the regions of interest for the μCT portion of the study. The region of interest was selected on the pre- and post-etch scans by outlining pixels and the apparent and material densities were calculated based on greyscale threshold values for hydroxyapatite norms for the area defined in the region of interest.[111] Varying the pixels included in a region of interest could result in apparent density differences. Apparent density was less reliable than material density due to the inclusion of voids/empty space in its calculation. Thus, selection of slightly more grey pixels within the border of the region of interest would affect the apparent density calculation if these pixels fell within the threshold range set for hydroxyapatite. A more accurate way to assess the material and apparent densities would be to outline the region of interest multiple times and calculate the mean densities from multiple measurements. The small number of teeth per tooth type used in the present study was also a limitation. This was due to the difficulty in obtaining high quality extracted anterior teeth, which may have effects on categories that were approaching significance in the present study's results.

Clinically, this study implies that the cervical region of teeth may require a different etching protocol than the middle and incisal regions. The cervical regions, particularly in posterior teeth, may require a longer etching time or a more concentrated etch to remove more of the aprismatic enamel. This could produce a better surface for

bonding the brackets. A study could also be performed to determine if fluoride exposure and application has an impact on the density of the tooth enamel, which may make the teeth less susceptible to WSLs.

CHAPTER V

CONCLUSIONS

1. There are no differences in etch quality between maxillary and mandibular teeth.
2. Posterior teeth have an inferior etch quality and poorer response to etching than anterior teeth.
3. Cervical enamel has an inferior etch quality and is less dense than the middle and incisal regions.
4. Surface enamel density, after etching for 15 seconds with 37% phosphoric acid etchant, remains within accepted norms for hydroxyapatite density.
5. BIOQUANT Osteo (Nashville, TN) software and μCT mineral density analysis are valid methods to quantitatively assess etch quality.

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APPENDIX A

FIGURES

Figure 1. Teeth commonly affected by white spot lesions.

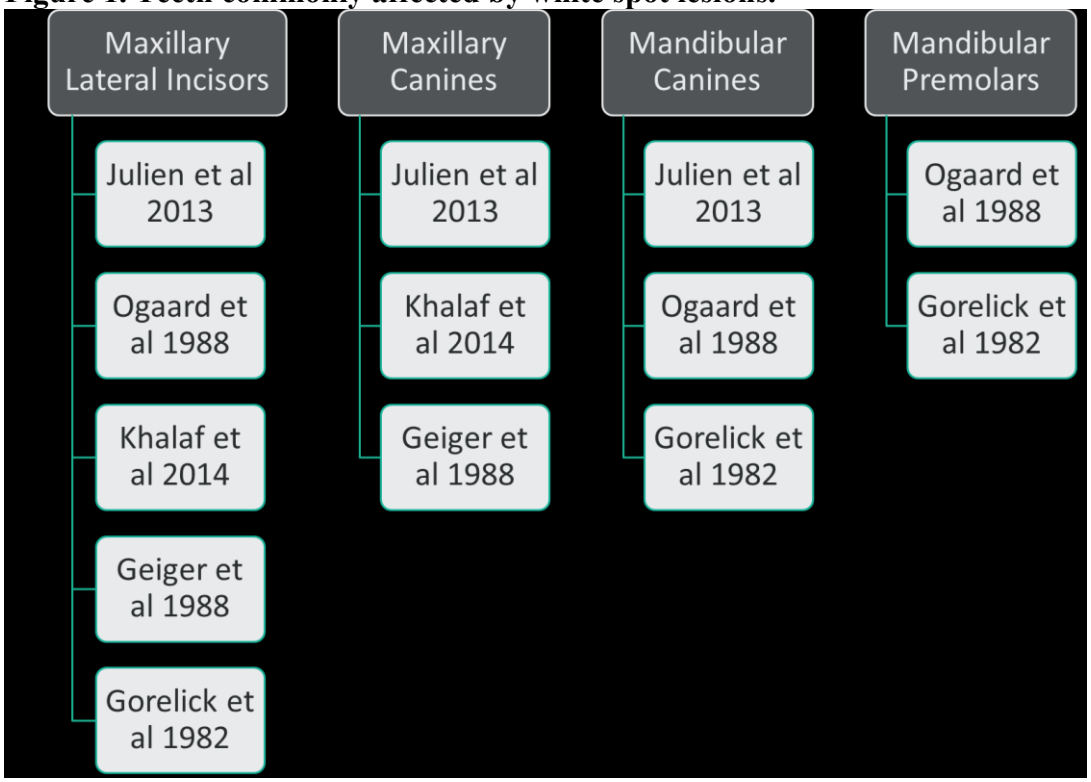


Figure 2. (A) Pre-etch, (B) mid-etch, and (C) post-etch appearance of treated samples.

Figure 3. (A) Gold sputter coating machine and (B) mounted sample after sputter coating.

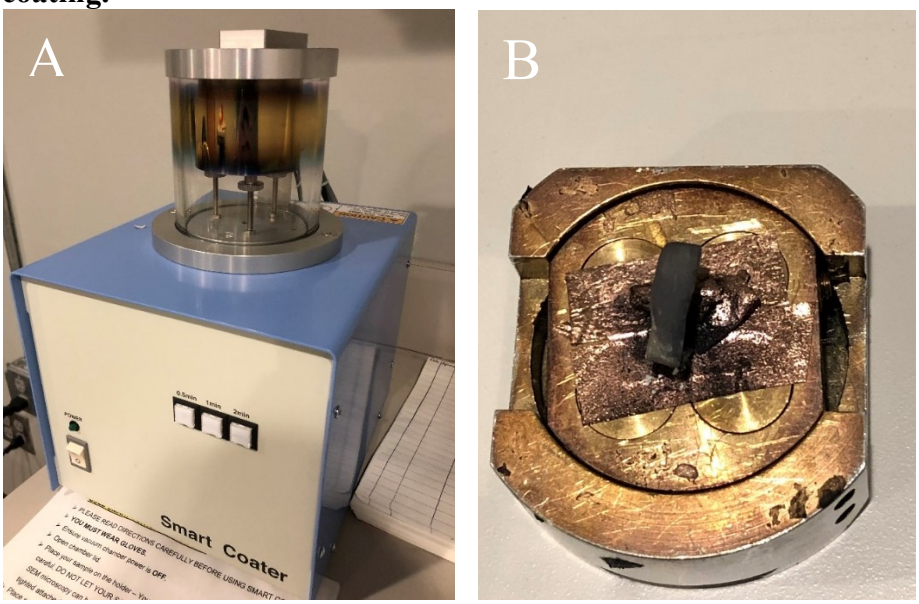


Figure 4. (A) Etch pattern from sample. (B) BIOQUANT Osteo software selection of enamel present in the image for calculation of percentage of enamel remaining after etching (BV/TV)

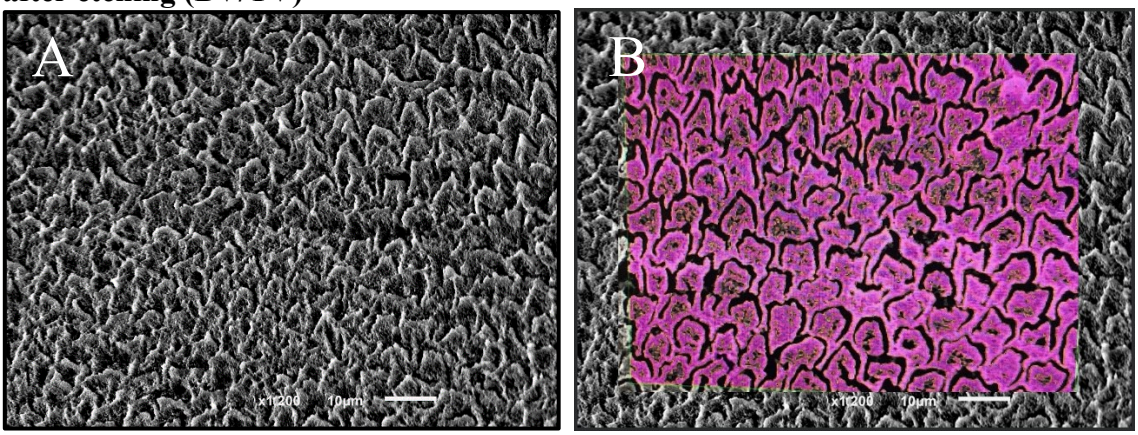


Figure 5. Reference groove placed in micro-CT samples to allow for repetitive measurements during region of interest selection of scans.

Figure 6. Microcomputed tomography scout scan selection of final scan area in the (A) incisal, (B) middle, and (C) cervical thirds of the sample.

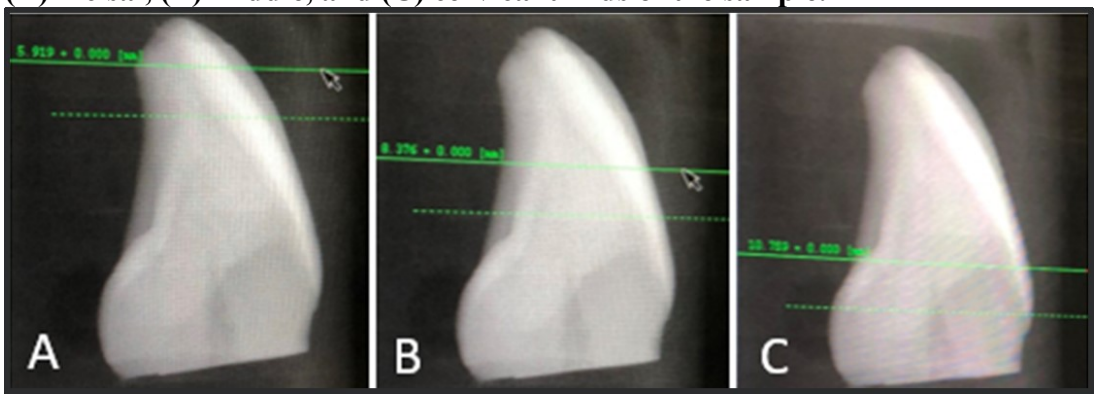


Figure 7. Micro-CT (A) final cross section scan, (B) region of interest selection, and (C) three-dimensional reconstruction of region of interest for density calculations.

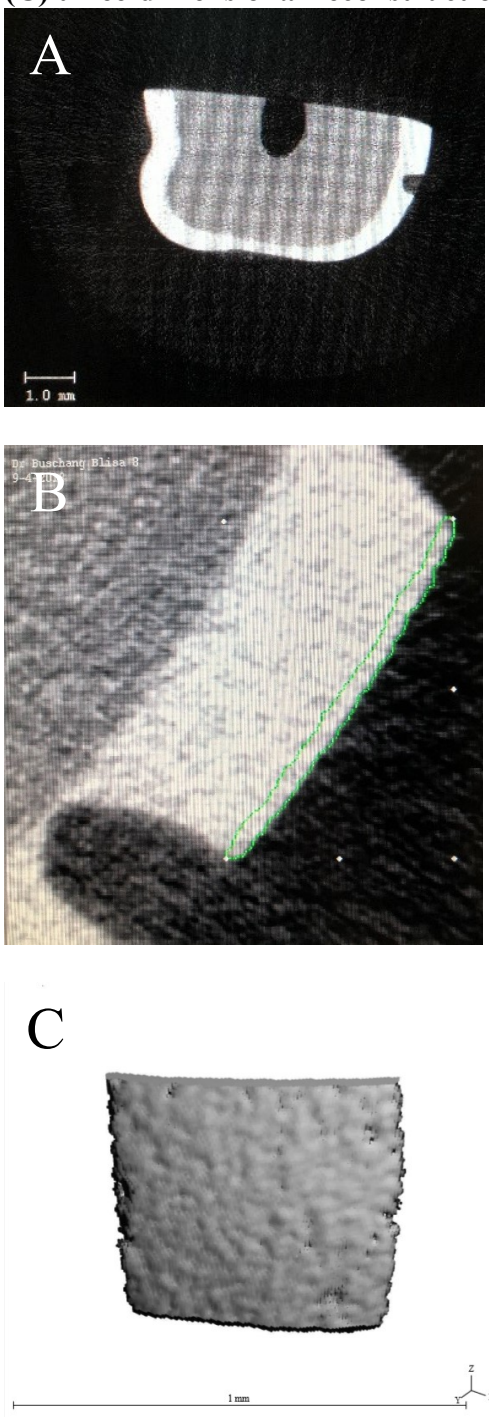


Figure 8. SEM images (1200x magnification) for maxillary teeth and regions.

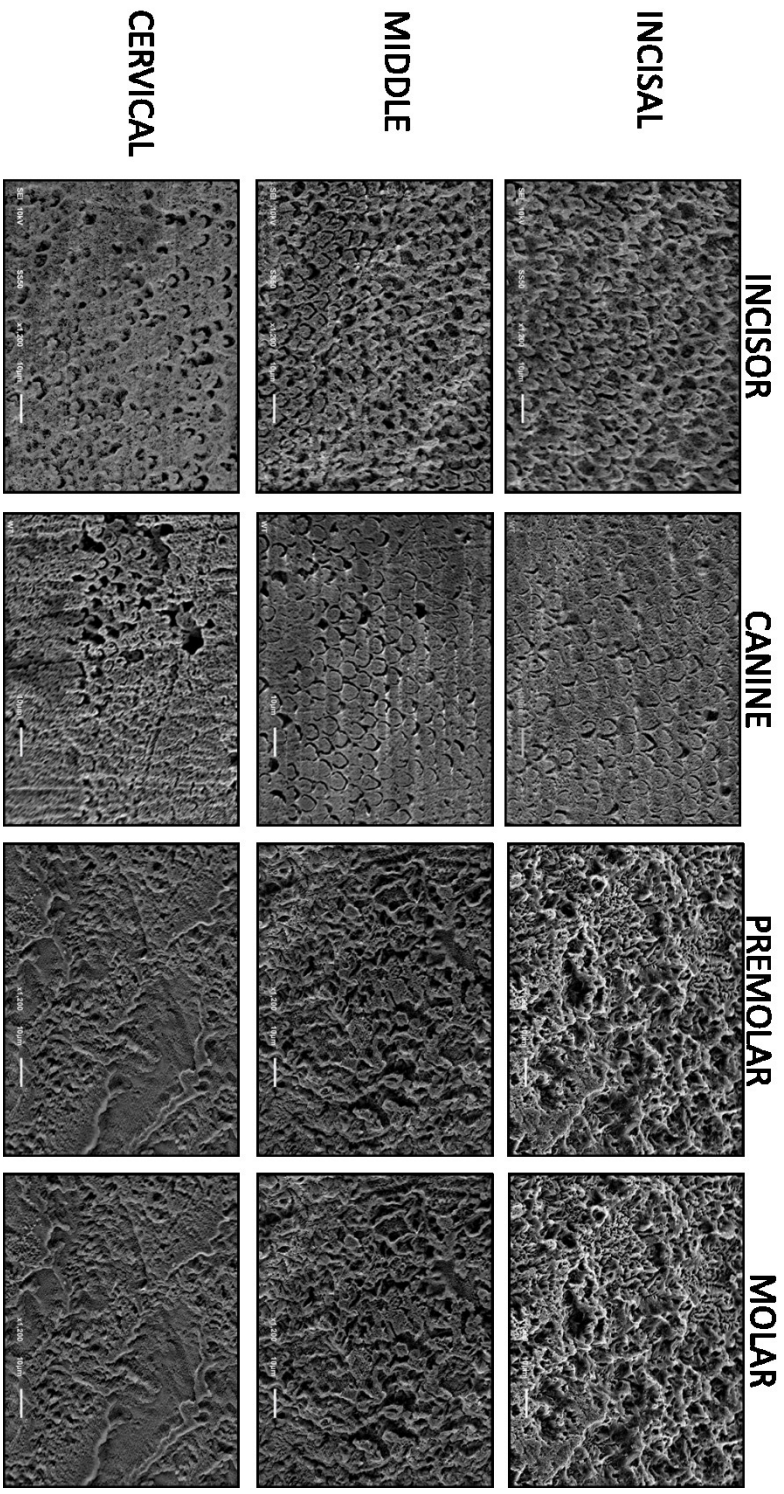


Figure 9. SEM images (1200x magnification) for mandibular teeth and regions.

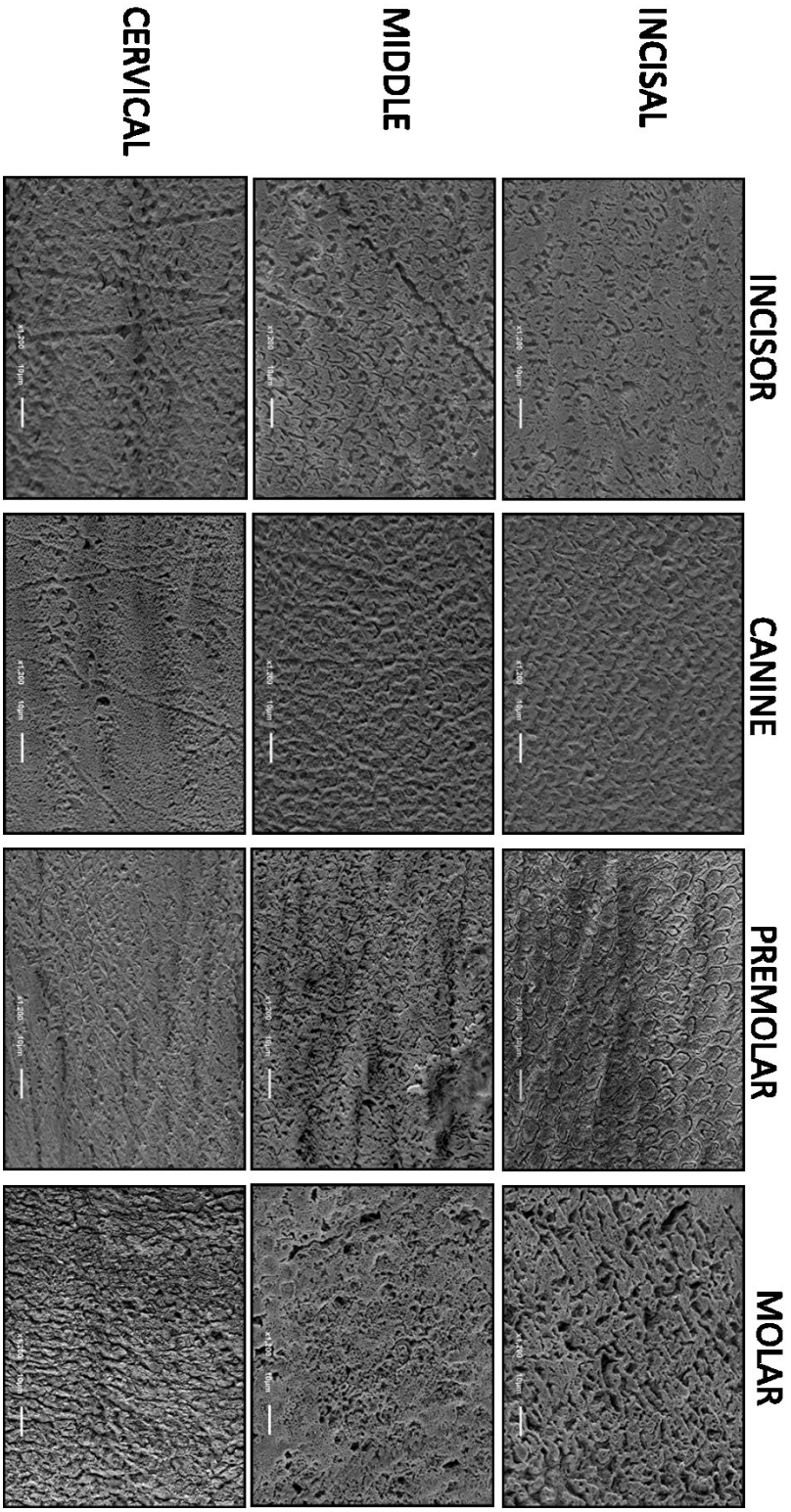
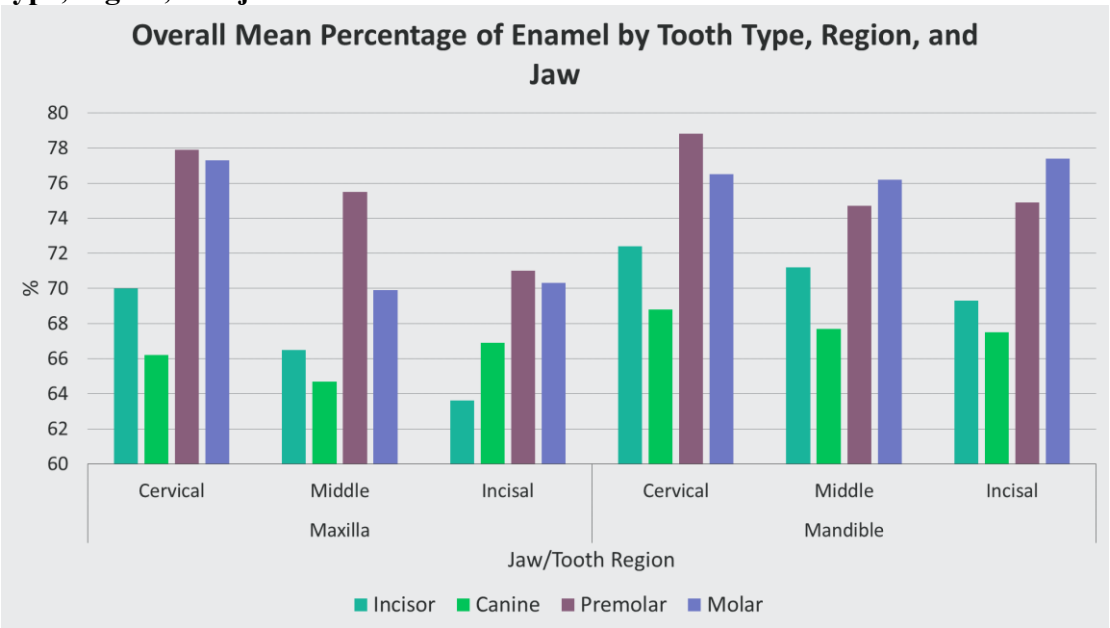
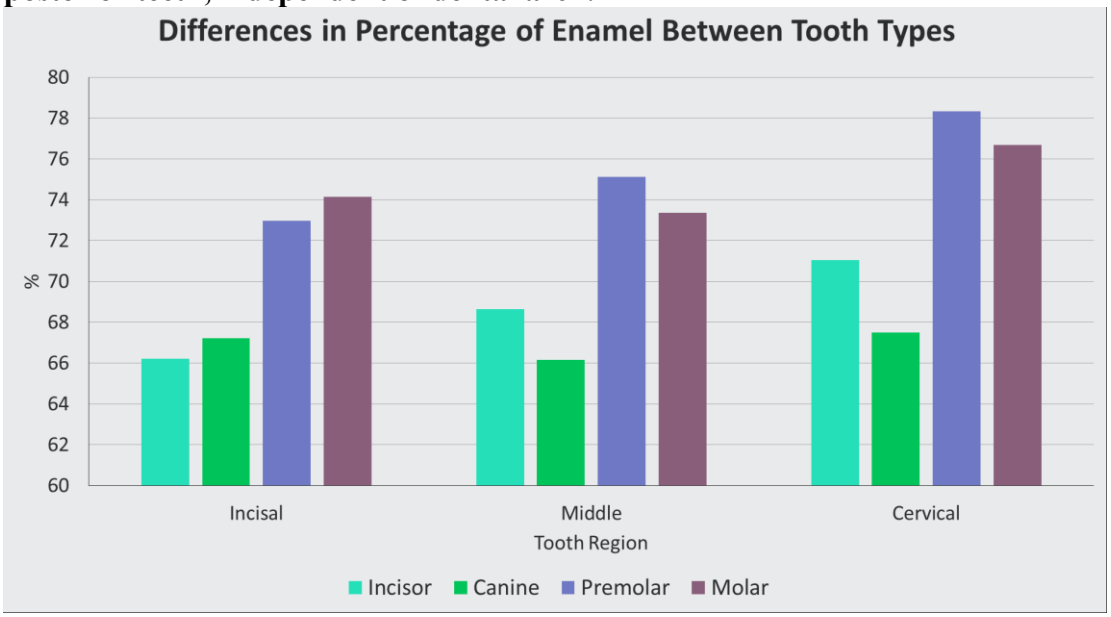


Figure 10. SEM and BIOQUANT mean percentage of enamel (BV/TV) by tooth type, region, and jaw.



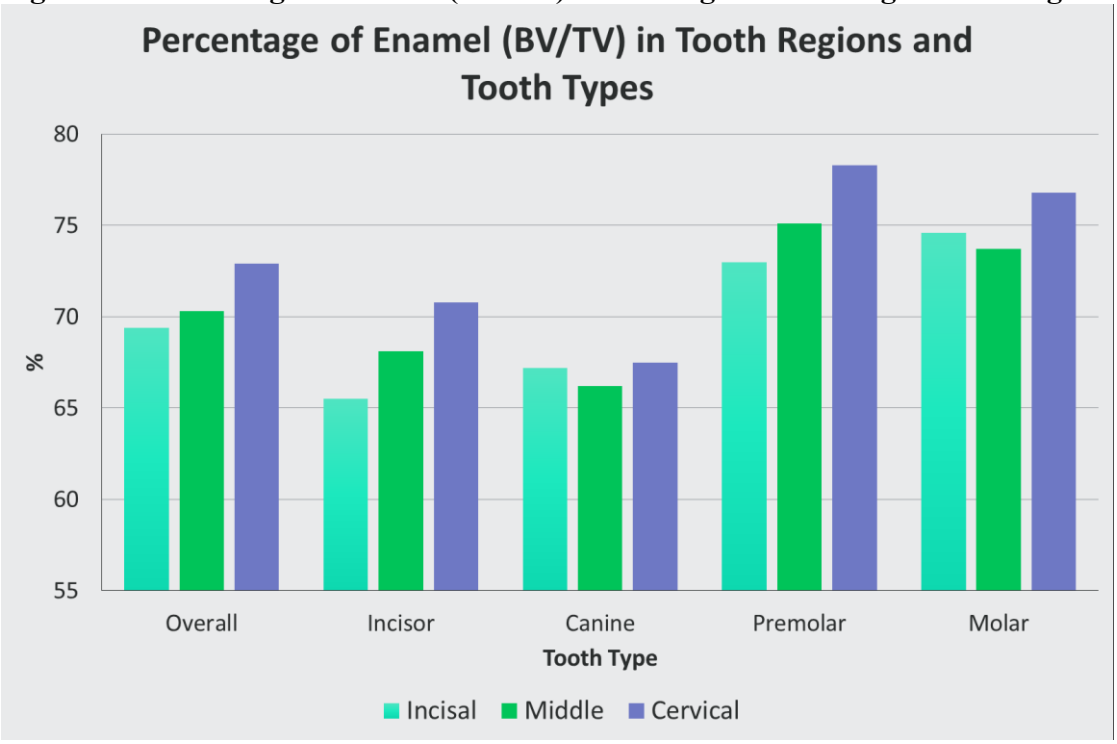
BV = bone volume, TV = total volume

Figure 11. Percentage of enamel (BV/TV) remaining after etching in anterior and posterior teeth, independent of dental arch.



BV= bone volume, TV = total volume

Figure 12. Percentage of enamel (BV/TV) remaining after etching in tooth regions.



BV = bone volume, TV = total volume

Figure 13. Changes in apparent density from pre-etch (T1) to post-etch (T2) for anterior and posterior teeth.

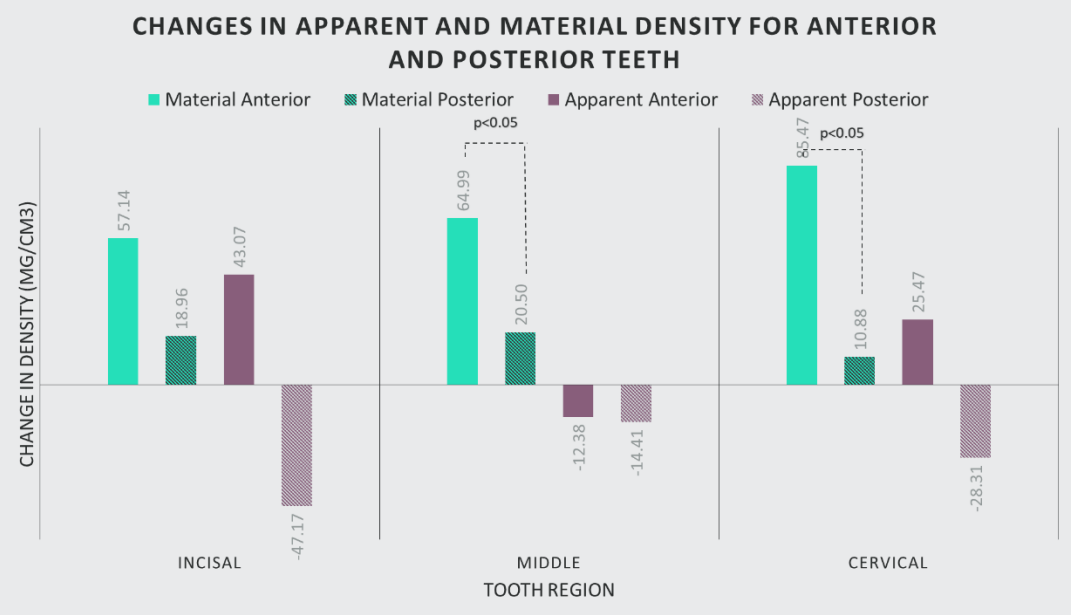
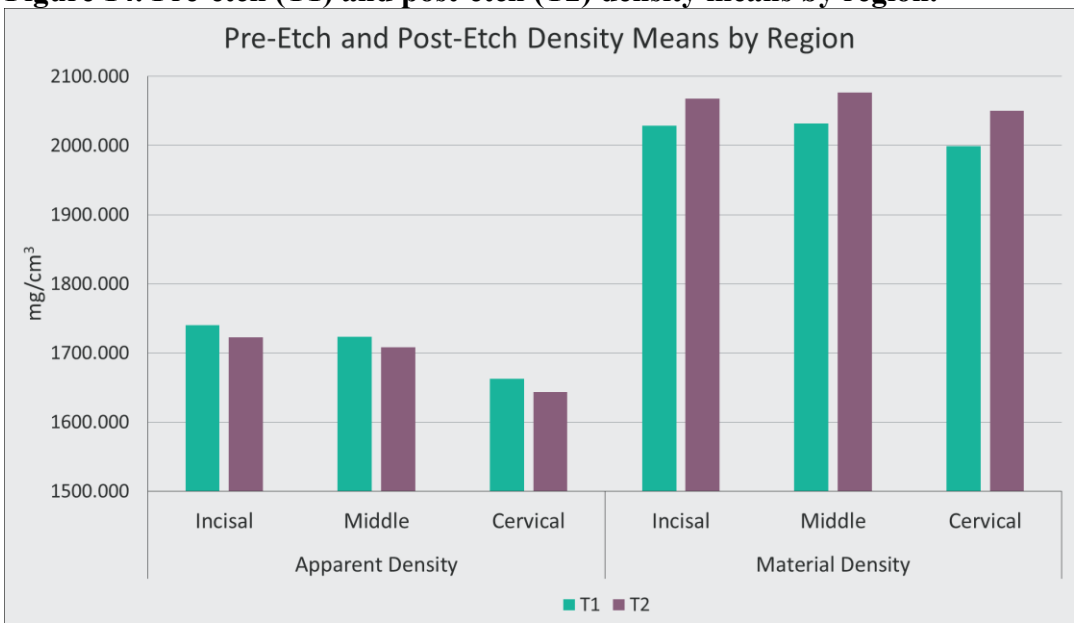


Figure 14. Pre-etch (T1) and post-etch (T2) density means by region.



APPENDIX B

TABLES

Table 1. Prevalence of white spot lesions in orthodontic patients and methods of evaluation.

Study	Prevalence	Teeth	Method of Study
Gorelick et al 1982	50%	U/L 6-6	Private Practice; Pre- and Post-Treatment Clinical Examination
Mizrahi et al 1982	84%	U/L 6-6	Private Practice; Clinical Examination; Compared with Untreated Controls
Zachrisson et al 2010	89%	U/L 6-6	Private practice; Pre- and Post-Treatment Clinical Examination
Ogaard et al 1989	96%	U/L 6-6	Private Practice; Clinical Examination 5 years Post-Treatment
Julien et al 2013	23%	U/L 3-3	Education Setting; Pre- and Post-Treatment Photographs
Lovrov et al 2007	24.9%	U/L 6-6	Private Practice; Pre- and Post-Treatment Photographs
Brown et al 2016	28%	U/L 5-5	Private Practice; Pre- and Post-Treatment Photographs
Chapman et al 2010	36%	U2-2	Education Setting; Pre- and Post-Treatment Photographs

Table 2. Tooth types with greatest number of orthodontic bracket debonds.

Study	Teeth Evaluated	Tooth Type with Greatest Number of Debonds
Adolfsson et al 2002	Incisors, canines, premolars	Mandibular premolars
Kinch et al 1988	Incisors, canines, premolars	Premolars
Linklater et al 2003	Incisors, canines, premolars, molars	Mandibular premolars and molars
Sunna et al 1998	Incisors, canines, premolars	Premolars
Zachrisson et al 1977	Incisors, canines, premolars, molars	2nd premolars and molars

Table 3. SEM/BIOQUANT mean percentage of enamel (BV/TV) remaining by tooth type, region, and jaw.

		Incisor	Canine	Premolar	Molar
Maxilla	Cervical	70.0	66.2	77.9	77.3
	Middle	66.5	64.7	75.5	69.9
	Incisal	63.6	66.9	71	70.3
Mandible	Cervical	72.4	68.8	78.8	76.5
	Middle	71.2	67.7	74.7	76.2
	Incisal	69.3	67.5	74.9	77.4

BV = bone volume, TV = total volume

Table 4. ANCOVAR for percentage of enamel (BV/TV) in tooth regions by jaw, controlling for tooth type.

	Maxilla		Mandible		Group Difference
Region	Mean	SE	Mean	SE	Probability
Cervical	72.66	1.32	74.13	1.36	0.447
Middle	69.19	1.60	72.46	1.65	0.169
Incisal	68.00	1.68	72.28	1.74	0.091

ANCOVAR = analysis of covariance, BV = bone volume, TV = total volume, SE = standard error

Table 5. ANCOVAR for percentage of enamel in regions (BV/TV) by tooth type, controlling for jaw.

	Incisor		Canine		Premolar		Molar		Group Difference
Region	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Probability
Cervical	71.04	1.61	67.50	1.93	78.33	1.93	76.69	2.12	0.002
Middle	68.64	1.94	66.17	2.33	75.11	2.33	73.37	2.57	0.046
Incisal	66.21	2.05	67.22	2.46	72.97	2.46	74.14	2.71	0.068

ANCOVAR = analysis of covariance, BV = bone volume, TV = total volume, SE = standard error

Table 6. Percentage of enamel (BV/TV) for tooth regions.

	Cervical		Middle		Incisal	
Tooth Type	Mean	SD	Mean	SD	Mean	SD
Overall	72.9	6.2	70.3	6.6	69.4	7.1
Incisor	70.8	4.5	68.1	7.4	65.5	6.7
Canine	67.5	4.1	66.2	6.1	67.2	5.8
Premolar	78.3	5.4	75.1	2.5	73	5.5
Molar	76.8	4.8	73.7	5	74.6	6.8

SD = standard deviation, BV = bone volume, TV = total volume

Table 7. Paired differences in percentage of enamel (BV/TV) in tooth regions.

	Tooth Region	Mean	SD	Probability
Overall	Cervical, Middle	2.59	5.06	0.015
	Cervical, Incisal	3.57	1.19	0.006
	Middle, Incisal	0.98	4.99	0.325
Incisor	Cervical, Middle	2.70	4.41	0.103
	Cervical, Incisal	5.30	4.48	0.008
	Middle, Incisal	2.59	3.48	0.056
Canine	Cervical, Middle	1.33	5.85	0.600
	Cervical, Incisal	0.28	1.57	0.866
	Middle, Incisal	-1.06	6.19	0.694
Premolar	Cervical, Middle	3.22	5.29	0.196
	Cervical, Incisal	5.36	9.80	0.238
	Middle, Incisal	2.14	5.37	0.374
Molar	Cervical, Middle	3.13	6.33	0.331
	Cervical, Incisal	2.27	4.76	0.347
	Middle, Incisal	-0.87	5.38	0.737

SD = standard deviation, BV = bone volume, TV = total volume

Table 8. Pre-etch (T1) densities (mg/cm^3) of the anterior (incisors, canines) and posterior (premolars, molars) teeth, along with statistical comparisons of difference.

Density	Region	Tooth	T1 Mean	SD	Mean Diff	SE	Probability
Apparent	Incisal	Anterior	1690.46	159.01	-66.90	52.36	0.214
		Posterior	1757.36	93.64			
	Middle	Anterior	1708.31	84.12	-30.70	40.31	0.454
		Posterior	1739.02	111.47			
	Cervical	Anterior	1649.60	103.27	-27.28	43.35	0.535
		Posterior	1676.88	113.50			
Material	Incisal	Anterior	2013.33	104.80	-33.02	31.82	0.312
		Posterior	2046.35	52.32			
	Middle	Anterior	2013.61	92.70	-40.28	26.08	0.142
		Posterior	2053.89	28.17			
	Cervical	Anterior	2000.42	75.68	1.98	27.30	0.943
		Posterior	1998.43	59.68			

SD = standard deviation

Table 9. Post-etch (T2) densities (mg/cm^3) of the anterior (incisors, canines) and posterior (premolars, molars) teeth, along with statistical comparisons of difference.

Density	Region	Tooth	T2 Mean	SD	Mean Diff	SE	Probability
Apparent	Incisal	Anterior	1733.52	123.79	23.33	47.34	0.627
		Posterior	1710.19	116.09			
	Middle	Anterior	1693.88	113.72	-30.73	42.72	0.479
		Posterior	1724.61	102.18			
	Cervical	Anterior	1639.02	189.98	-9.54	66.67	0.887
		Posterior	1648.57	141.46			
Material	Incisal	Anterior	2070.47	74.44	5.16	25.08	0.839
		Posterior	2065.31	48.19			
	Middle	Anterior	2078.60	73.34	4.21	20.63	0.841
		Posterior	2074.39	22.29			
	Cervical	Anterior	2085.88	86.14	76.57	32.25	0.026*
		Posterior	2009.32	76.80			

SD = standard deviation

Table 10. Density (mg/cm^3) changes (T1-T2) of the anterior (incisors, canines) and posterior teeth (premolars, molars) by tooth region, along with statistical comparisons of difference.

Density	Region	Tooth	T2-T1 Mean	SD	Mean Diff	SE	Probability
Apparent	Incisal	Anterior	43.07	136.74	90.23	51.38	0.092
		Posterior	-47.17	122.98			
	Middle	Anterior	-12.38	99.67	2.03	47.24	0.966
		Posterior	-14.41	129.80			
	Cervical	Anterior	25.47	156.50	53.77	57.75	0.361
		Posterior	-28.31	129.61			
Material	Incisal	Anterior	57.14	68.46	38.18	21.79	0.092
		Posterior	18.96	33.93			
	Middle	Anterior	64.99	71.20	44.48	20.23	0.043
		Posterior	20.50	23.76			
	Cervical	Anterior	85.47	53.38	74.57	26.54	0.010
		Posterior	10.88	81.00			

SD = standard deviation, SE = standard error

Table 11. Pre-etch (T1) and post-etch (T2) material and apparent densities (mg/cm^3) of the incisal, middle, and cervical regions.

Density	Time Point	Region	Mean	SD
Apparent	T1	Incisal	1739.797	111.538
		Middle	1723.665	97.842
		Cervical	1662.690	106.910
	T2	Incisal	1722.756	118.490
		Middle	1708.060	107.528
		Cervical	1643.429	166.121
Material	T1	Incisal	2028.572	84.83773
		Middle	2032.202	72.36637
		Cervical	1999.502	68.01184
	T2	Incisal	2068.087	62.530
		Middle	2076.657	54.955
		Cervical	2050.545	89.268

SD = standard deviation

Table 12. Apparent and material densities (mg/cm^3) paired differences between tooth regions at pre-etch (T1) and post-etch (T2).

Density	Time Point	Pair	Mean Difference	SD	Probability
Apparent Density	T1	Incisal, Middle	16.13	92.24	0.400
		Incisal, Cervical	74.53	108.06	0.002
		Middle, Cervical	50.99	78.77	0.004
	T2	Incisal, Middle	14.70	113.66	0.516
		Incisal, Cervical	79.33	147.61	0.011
		Middle, Cervical	64.63	126.39	0.015
Material Density	T1	Incisal, Middle	-3.63	53.07	0.730
		Incisal, Cervical	29.07	64.68	0.031
		Middle, Cervical	32.70	49.64	0.003
	T2	Incisal, Middle	-8.57	49.90	0.390
		Incisal, Cervical	17.54	85.92	0.308
		Middle, Cervical	26.11	80.77	0.112

SD = standard deviation

Table 13. Change from pre-etch (T1) to post-etch (T2) in material and apparent densities (mg/cm^3) of the incisal, middle, and cervical regions.

Density	Region	Mean Difference	SD	Probability
Apparent	Incisal	-1.42	135.95	0.958
	Middle	13.39	113.18	0.568
	Cervical	0.34	143.87	0.991
Material	Incisal	-39.52	57.63	0.002
	Middle	-44.45	58.28	0.001
	Cervical	-51.04	76.20	0.002

SD = standard deviation