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Sexual dimorphism refers to differences between males and females of the same species. A general pattern of sexual dimorphism is displayed in humans. Across mammalian populations, males generally have a larger body than females. Many studies have shown that, as body mass increases, tooth volume increases isometrically (Ungar, 2014). For example, on average, males have larger teeth than females (Schwartz and Dean, 2005). However, despite clear gross dental size differences, some studies have suggested that males and females also exhibit divergent quantities of enamel (Saunders et al., 2007). Females have been shown to have relatively thicker enamel, and a larger enamel cap area than males (Smith et al., 2006). This reveals an ambiguity. Although males possess overall larger teeth, studies suggest that male dental composition contains less relative enamel than would be expected given their tooth size. Similarly, females have been shown to exhibit smaller teeth than males, but a greater relative amount of enamel.

This study has developed a methodological protocol for taking volumetric measures on dental microstructure using computed tomography (CT) scans. This protocol will help future studies evaluate the relative quantity of enamel and dentin in teeth. A better understanding of the volumetric differences in the dental microstructure is imperative to the understanding of dental sexual dimorphism. We examined the dental microstructure using 3D modeling from individuals in the Point Hope population. This population has been known to use their teeth as tools in several ways. We understand the microstructure of the dentition within the sample population may be impacted by some wear and use of dentition within this community.

The dental microstructure was examined using 3D modelling in 14 individuals from a sample of the Point Hope population including eight adult females and six adult males of

unknown age. Three hundred and twelve total teeth were examined. One hundred and eighty-six teeth came from female subjects, and one hundred twenty-six teeth came from males. The 3D models of the dentition and microstructure of enamel and dentin was generated using the software. Due to a small sample size, analysis of gross dental size and enamel volumes were performed using a one-tailed, non-parametric Mann-Whitney U-Test (a=0.10). Volumetric measurements of the total tooth volume, enamel volume, and dentin volume were recorded for each subject.

Our results suggest that there are divergent quantities of enamel between males and females within the mandibular teeth. Specifically, the central incisors, lateral incisors, canines, first premolars, second premolars, and second molars within the mandible showed that there are statistical differences between sexes in dental composition. Males possessed a greater amount of enamel in their mandibular central incisors, lateral incisors, canines, first premolars, second premolars, and second molars. Additionally, the maxillary dentition also demonstrated notable statistical difference in enamel quantity within the second premolars between males and females. Males had a larger enamel quantity within their second molars than females. Males also demonstrated larger average total dental volume (345.59 mm^3) than females (342.02 mm^3).

We have successfully generated a protocol in which future studies could quantify dental microstructure and add to the understanding of dental sexual dimorphism. Further research implementing this protocol for segmenting CT scans and utilizing the segmentation to gather volumetric measurements of dental microstructure should use higher resolution micro-CT scans and a larger sample population.

SEXUAL DIMORPHISM IN PERMANENT HUMAN DENTITION

Sara Aldeeb, B.S.

APPROVED:

_____

Emma K. Handler, Ph.D., Major Professor

_____

Rachel A. Menegaz, Ph.D., Committee Member

_____

Scott D. Maddux, Ph.D., Committee Member

_____

Caroline Rickards, Ph.D., Graduate Advisor

_____

Jonathan Tune, Ph.D., Chair, Physiology and Anatomy

_____

J. Michael Mathis, Ph.D., Dean, Graduate School of Biomedical Sciences

SEXUAL DIMORPHISM IN PERMANENT HUMAN DENTITION

INTERNSHIP PRACTICUM REPORT

Presented to the Graduate Council of the

Graduate School of Biomedical Sciences at

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In Partial Fulfilment Requirements

For the Degree of

MASTER OF SCIENCE

By

Sara Aldeeb, B.S.

Fort Worth, Texas

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Table of Contents

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION TO THE STUDY

The following practicum report was performed as a requirement for the Master of Science in Medical Sciences Anatomy Track program, from May 2020 – May 2021, at the University of North Texas Health Science Center (UNTHSC). The study was conducted under the direct supervision of Emma Handler, Ph.D., in the Department of Physiology and Anatomy at UNTHSC.

Sexual dimorphism refers to differences between males and females of the same species. A general pattern of sexual dimorphism is displayed in humans. Across mammalian populations, males generally have a larger body than females. Many studies have shown that, as body mass increases, tooth volume increases isometrically (Ungar, 2014). For example, on average, males have larger teeth than females (Schwartz and Dean, 2005). However, despite clear gross dental size differences, some studies have suggested that males and females also exhibit divergent quantities of enamel (Saunders et al., 2007). Females have been shown to have relatively thicker enamel, and a larger enamel cap area than males (Smith et al., 2006). This reveals an ambiguity. Although males possess overall larger teeth, studies suggest that male dental composition contains less relative enamel than would be expected given their tooth size. Similarly, females have been shown to exhibit smaller teeth than males, but a greater relative amount of enamel.

Several studies have attempted to identify the differences between sexes through the measurements of dental size, dental morphology, and dental composition. However, there is a lack of consistent data regarding sexual differences in the internal structure of teeth. For example, there has been a vast diversity of results in dental literature regarding dental microstructure dimensions. A possible reason for these diverse results may be due to the

variation in data collection. Many studies examining dental composition have relied on radiographic images, which provides only a two-dimensional approach to measuring internal dental structures, thus rendering this approach inadequate to accurately quantify microstructure volumes (Monalisa et al., 2018). 3D computed tomography (CT) has been demonstrated to more accurately depict the microstructure of human dentition (Grine, Stevens, and Jungers, 2001)

The focus of this practicum was the development of a methodology for segmenting and creating 3D models of human dentition from CT scans. This methodology was then applied to a human sample in order to quantify enamel volumes. The first aim is to generate a protocol to quantify dental microstructure utilizing 3D imaging software and CT scans. A protocol for quantifying dental microstructure will be useful in contributing to the growing body of consistent evidence that there is sexual dimorphism in the quantity of enamel in humans. In addition, it will allow for more consistent results of evidence of sexual dimorphism within dental microstructure. Further, this protocol can be used to measure quantities of dental microstructure in general to compare size and composition of teeth. The second aim is to determine the rate of sexual dimorphism of gross dental size in the sample population. We hypothesize males will have larger tooth volumes than females. The third and final aim is to compare the ratio of enamel quantity to gross dental volume in males compared to females. In this aim, the different scaling relationships of enamel volume of males compared to females will be demonstrated. We hypothesize that males will exhibit less enamel relative to gross tooth size and females will exhibit more enamel relative to gross tooth size.

BACKGROUND AND LITERATURE

Section 1: Dental Development

Section 1.1: Anatomy of Teeth

Teeth are the hardest substances in the human body. They are essential for chewing and play an important role in speech as well. Teeth are composed of four types of dental tissues. Enamel, dentin and cementum are considered hard tissue and have a similar structure to that of bone. The residual fourth tissue is pulp, which is soft non-calcified tissue. The pulp chamber primarily contains nerves, blood vessels and connective tissue (Reichenmiller and Klein, 2007). Enamel is the hardest substance in the body and contains low amounts of protein and a high amount of minerals (Chun, Choi and Lee, 2014). Dentin is harder than bone or cementum, but it is softer than enamel (Chun, Choi and Lee, 2014). This is due to the fact that dentin contains fewer minerals than enamel. Cementum is a calcified layer of bone-like tissue that covers the dentin of the tooth root, and its formation occurs during root development and throughout the life of a tooth (Reichenmiller and Klein, 2007). One key feature of enamel is that once formed, it is not subject to further remodeling following its formation (Reichenmiller and Klein, 2007). Crown measurements are often taken to ascertain dental size due to hard tissue making up the majority of the crown of the tooth (Chun, Choi and Lee, 2014).

Section 1.2: Tooth Development

In order to better understand enamel and dentin formation, it is necessary to cover a synopsis of tooth development. One of the major tooth development stages is the bell stage. The bell stage usually begins at eleven weeks during prenatal development. During this stage, the

cusps on each crown begin to form and the size of the crown begins to increase (Lesot and Brook, 2009). In addition, cells such as ameloblasts and odontoblasts begin to differentiate. Ameloblast cells form enamel, the hard, dense outermost surface of the crown. Odontoblast cells form dentin, the bone-like tissue beneath the enamel (Lesot and Brook, 2009). The formation of the tooth shape begins here through the formation of the hard dental tissues. Following the bell stage is the appositional stage. In this stage the enamel and dentin are formed. Odontoblasts elongate and migrate downwards forming the dentin matrix while the ameloblasts migrate forward and upward, forming the enamel matrix (Lesot and Brook, 2009). The pulp chamber does not expand and is instead the residual cavity. The two main tissues which make up the crown and tooth size are enamel and dentin (Chun, Choi and Lee, 2014).

Section 1.3: Enamel Formation

The ectoderm gives rise to ameloblasts. Ameloblasts are cells present during tooth development which deposit the enamel and outermost surface of the tooth, forming the crown (Reichenmiller, Klein, 2007). As mentioned earlier, enamel is not subject to further remodeling following its formation. The main non-collagenous structural protein in amelogenesis is amelogenin. The amelogenin protein exists on both the X and Y chromosome and is produced by alternative splicing within the amelogenin gene (Pfeiffer and Brenig 2005). A previous study conducted by Pfeiffer and Brenig in sheep found the gene and protein to be useful in sex identification. The remaining non-collagenous structural proteins in amelogenesis are made up of proteins such as proline, leucine, histidine, and glutamate (Antoine and Hillson, 2016).

Section 1.4: Dentin Formation

Neural crest cells give rise to odontoblasts which form dentin as well as all connective tissue within dentition (Schoenwolf et al., 2009). There are three types of dentin that emerge depending on the stage of development: primary, secondary, and tertiary. Primary dentin makes up the majority of dentin and forms until the growth of the root is completed. Secondary dentin is formed in lesser quantities and only forms after the tooth has erupted and the initial growth of the tooth is complete. Tertiary dentin is formed as a result of a stimulus, usually an injury, to the tooth (Reichenmiller and Klein, 2007). Therefore, unlike enamel, dentin is prone to remodeling following its formation. Cementum is a calcified layer of bone like tissue that covers the dentin (Schoenwolf et al., 2009).

Section 2: Sexual Dimorphism in Teeth

Section 2.1: Dental Sexual Dimorphism

Sexual dimorphism refers to differences in size, appearance, and stature between males and females (Banarjee et al., 2016). Many forensic experts have used teeth as an additional tool for sex determination due to their resistance of post-mortem destruction (Zorba, Moraitis and Manolis, 2011). Consistency of these variations is extremely valuable in the identification of sex. Multiple studies have shown that there is sexual dimorphism in various aspects of human dentition such as size, morphology, and composition. On average, males possess larger tooth crowns than females in human populations (Schwartz and Dean, 2005). It is also well established that dental size is well correlated with body size (Gingerich, Smith and Rosenberg, 1982). In the case of humans, this means that males tend to have larger teeth than females.

Section 2.2: Sexual Dimorphism in Enamel

Some authors have argued that sexual dimorphism occurs in dental composition as a result of differences in length of gestation and initiation of dental development *in utero* between males and females (Antoine and Hilson, 2016). For example, studies have shown that males possess a greater crown size and dentin quantity due to a longer bell stage of tooth development (Monalisa et al., 2018). Studies have also found varying amounts of enamel based on tooth type and development initiation times (Antoine and Hilson, 2016). Tooth type has been shown to increase dimorphic variability and it has even been suggested that overall size of the tooth may have the greatest impact on enamel thickness variation (Hall et al., 2007). Others have attributed this distinction due to the influence of the X and Y chromosomes. The X chromosome is active in amelogenesis, the process of enamel development, and promotes enamel and dentin formation (Alvesalo, 1997). It has been shown that approximately 90% of the genetic material responsible for enamel production is located on the X chromosome and the remaining 10% is found on the Y chromosome (Monalisa et al., 2018). Thus, it is suggested that these differences in the effects of X and Y chromosome may help explain these sexual dimorphisms in size, morphology and composition.

Section 3: Previous Studies on Dental Microstructure

Data on dental microstructure has been collected using an array of collection methods, which has led to inconsistency in reported results. For example, dental data has been collected via radiographs, CT scans, and dental casts in attempts to quantify tooth composition. These variations in data collection methodologies exacerbate the already lacking understanding of sexual dimorphism in dental composition. Radiographs, although used less frequently in recent years, provide a static, two-dimensional image. Several studies have used radiographs in attempt

to quantify the amount of enamel in a given tooth. In addition, studies have shown measurements made from radiographs do not indicate accurate dental microstructure and instead may under or overestimate the true value (Monalisa et al., 2018). Most recently, CT scans have been more widely used to examine the internal structure of teeth. This is due to the fact that sectioning teeth utilizing CT scans has been shown to give more accurate measurements of dental microstructure (Belgin, Serindere, Orhan, 2019; Monalisa et al., 2018). Despite this, there is still a dearth of CT scans-based research on the dynamic of sexual dimorphism in enamel and dentin. The variety of tools used to collect internal dental data is expansive, but CT scans are a cost-effective way to ascertain the volume of teeth. Thus, generating a structured protocol for sectioning teeth using CT scans and taking volumetric measurements may help guide future studies and lead to more consistent results.

Section 4: Dentition of Sample Population

As will be discussed in materials and methods within this practicum, the sample in this study includes individuals from the Point Hope population. The Point Hope population is a sub-population within the artic Inuit population.

Section 4.1: Inuit Diet

The staple cuisine of the Inuit populations consisted primarily of meat, fish, walrus, and seals. These were often eaten raw, dried, or frozen (Howells, 1942). In the short artic summers, the meat would be roasted if resources to do so were available. Cooked meat was then preserved by drying it on stones. In the winter, meat was preserved by freezing it (Wood, 1992). Due to consuming frozen, often unthawed, meat, the tough exterior of the meat, and the practice of

drying the meat, substantial demands were placed on the teeth and jaws of this population (Wood, 1992). Additionally, the artic temperatures as well as frozen food hardness may contribute to the chipping of teeth or breakage of their teeth. Due to climatic constraints, very few vegetables could be cultivated or were naturally occurring. Thus, little vegetation was likely consumed (Giffen, 1930). Dental evidence suggests the Inuit population, including those living in Point Hope often engaged in extramasticatory behaviors, occupational behaviors that use teeth as tools to accomplish non-mastication-based tasks. Specifically, individuals are thought to have gripped meat in their teeth in order to manipulate it with both hands. Consequently, significant wear is often present on central and lateral incisors (De Poncins, 1941; Wood 1992).

Section 4.2: Inuit Tooth Use and Sexual Division of Labor

Inuit groups, and the Point Hope population specifically, have been known use their teeth as tools in several ways. Moreover, there is documented evidence of a developed sexual division of labor within the Inuit populations (Wood, 1992). Wood (1992) has suggested that, with the assumption that diet is similar for females and males within this population, any differences in wear of teeth between sexes may be attributed to the use of masticatory function between females and males (Wood, 1992). The author goes on demonstrate dental wear and abrasion differences between sexes due to the sexual divisions of labor and use of teeth as tools (Wood, 1992).

Males within the Inuit communities focused time dedicated to food acquisition primarily on hunting. It has been shown that males used their anterior teeth mostly to utilize a bow drill. The bow drill is a simple hand-operated type of tool used to catch fish and seals while hunting (Wood, 1992). This drill was often guided by means of a mouthpiece secured into place by their

anterior teeth; specifically, their incisors (Howells, 1942). Many males also used their teeth as a method for catching prey. This demanding grip often resulted in tooth loss (Wood, 1992).

Females' duties revolved around managing the household and processing animal skins to make clothing. The main activities involving tooth use in female duties included preparing and maintaining animal skins to create clothing (Giffen, 1930). They often used their teeth to stretch the skins and to sculpt the clothing piece. These clothing pieces were used to maintain warmth during artic temperatures. Some anthropologists also noted that females in Inuit populations have greater masticatory musculature and developed more wear than males in the same population (Leigh, 1925). Although the female tasks were generally less physically demanding, they were more repetitive in nature and therefore caused marked wear in teeth (Wood, 1992).

SPECIFIC AIMS

Section 1: (Aim 1) Generate a protocol to quantify dental microstructure utilizing 3D imaging software and CT scans.

A protocol of quantifying dental microstructure will be useful in contributing to the growing body of evidence that there is sexual dimorphism in the quantity of enamel in humans. Developing this protocol will lead to additional studies utilizing CT scans to quantify dental microstructure which will lead to more consistent data. This data could eventually enhance the understanding of dental development, dental genetics, sexual dimorphism, and what roles each of these play in the variation in tooth composition between males and females.

Section 2: (Aim 2) Determine the rate of sexual dimorphism of gross dental size in the sample population.

Hypothesis 2: We expect males will have larger teeth than females.

Section 3: (Aim 3) To compare the ratio of enamel in males and females. In this aim, we will measure whether different scaling relationships are present in enamel volume of males and females relative to their total dental volume.

Hypothesis 3: We expect that males will exhibit less enamel relative to gross tooth size and females to exhibit more enamel relative to gross tooth size.

MATERIALS AND METHODS

Section 1: Sample Population & Methods

The CT scans used in this study were obtained from adult subjects from a Point Hope Inuit sample. These 14 CT scans were a part of a larger collection used in a previous study at the University of North Texas Health Science Center. The CT scans had the following parameters: (1) acquisition matrix of 512 x 512 pixels, (2) pixel size of 0.46 x 0.46 mm, (3) voxel depth of 0.5 mm, (4) tube current value of 0.07 mA, and (5) x-ray tube voltage value of 110 kV.

Fourteen individuals from the Point Hope Inuit population were examined: eight adult females and six adult males of unknown age. Three hundred and twelve total teeth were looked at. One hundred eighty-six teeth were examined from females, and one hundred twenty-six teeth were examined from males (Table 1).

Table 1. Sample size of number of individuals included and total teeth included.

	n	**Teeth**
Female	8	186
Male	6	126
Total	14	312

Section 2: Measurements and Statistical Analysis

Segmentations for gross dentition, enamel and dentin were created in the module "segment editor" within the 3D slicer program. Volumetric measurements were automatically calculated using the "segmentation statistics" module. Scalar volumes (volume^2) of the gross dentition layer, enamel layer and dentin layer for each tooth were recorded. Because dentin is less mineralized than enamel, the quality of the CT scans made it difficult to segment out the dentin layer. As a result, the dentin models generated were excluded from statistical analysis. The protocol generated is appropriate and can be used with any CT scan. However, with regards to the dentition, it will yield better results when using a micro-CT scan. For that reason, this study will focus on enamel and gross dental size for the statistical analysis.

Due to previous research demonstrating that males have larger teeth than females (Schwartz and Dean, 2005), a one-tailed nonparametric two sample t-test (Mann-Whitney U-Test, $a = 0.10$) was performed. While $a = 0.05$ is most commonly used in biological sciences, we chose to use an alpha set to 0.10 in the non-parametric analyses due to the small sample size.

To compare total tooth volume between sexes (Aim 2), we performed a Mann-Whitney U-Test on all teeth including central incisors (I1), lateral incisors (I2), cuspids (C), first premolars (PM2), second premolars (PM2), first molars (M1), second molars (M2), and third molars (M3) (See a list of abbreviations in Table A.1 of Appendix A). This was done on both

mandibular and maxillary dentition for each of the 14 subjects. If a subject was missing a tooth from both left and right sides (for example, right and left maxillary cuspids), that subject was excluded from analysis for that specific tooth. If only one tooth was present (for example the right maxillary cuspid was present, but the left maxillary canine was absent), that was the only tooth that was included in the analysis. If both right and left sided teeth were present (for example, both the right and left maxillary cuspids were present), an average of the two teeth was taken. This process was conducted to ensure the assumption of independence in the Mann-Whitney U-tests. All scalar total volume measurements for each tooth were recorded from "segment statistics" module on 3D slicer and analyzed using a 90% confidence interval.

A similar process was used to compare enamel quantity between sexes (Aim 3). We performed a Mann-Whitney U-Test on all teeth (See a list of abbreviations in Table A.1 of Appendix A). This was also done on both mandibular and maxillary dentition for each of the 14 subjects. If a subject was missing a tooth from both left and right sides, that subject was excluded from analysis for that specific tooth. If only one tooth was present, that was the only tooth that was included in the analysis. If both right and left sided teeth were present, an average of the two teeth was taken. This process was conducted to ensure the assumption of independence in the Mann-Whitney U-tests. All scalar enamel volume measurements for each tooth were recorded from "segment statistics" module on 3D slicer and analyzed using a 90% confidence interval.

To further examine the rate of sexual dimorphism using the full dentition volume within the sample, the average size of male and female individual teeth was compared. The coefficient of sexual dimorphism was examined by taking the average volume of each tooth for males and females and dividing the male mean by the female mean. This coefficient of sexual dimorphism was calculated for the maxillary and mandibular individual teeth between sexes.

To further examine the rate of sexual dimorphism using enamel quantity volume within the sample, the average enamel of male and female individual teeth as also compared. The coefficient of sexual dimorphism was examined by taking the average enamel volume of each tooth for males and females and dividing the male mean by the female mean. The coefficient of sexual dimorphism was calculated for the maxillary and mandibular individual teeth between sexes. In addition to calculating the coefficient of sexual dimorphism for both enamel quantity and total volume, the total dental average and total enamel volume average for each sex was also calculated and compared.

Finally, enamel volume was regressed against total volume for each sex. Analysis of covariance (ANCOVA) was then conducted to assess how potential slope and y-intercept differences influence the significant results using PAST statistical software (Hammer, Harper, and Ryan 2001).

Section 3: Limitations

The semi-automated segmentation of teeth introduces certain variability in the resulting enamel volumes, especially where differentiation between the enamel and dentin volume is difficult. Using a micro-CT scan in place of a CT scan, with much a greater resolution, may resolve this limitation in a future study. Additionally, due to lack of resources and time, we acknowledge the sample size is relatively small and does not adequately represent the Point Hope population. As has been demonstrated in previous studies, diet and age play a role in the wear and tear of dentition. As a consequence of the lack knowledge on diet and age for each subject, wear and tear of enamel cannot be assessed accurately utilizing the current methods.

PROTOCOL

Section 1: Data Collection Protocol (Aim 1).

A protocol was successfully generated to quantify dental microstructure. Using this protocol, future studies can be done on dental microstructure which will enhance our understanding of dental development, dental genetics and sexual dimorphism and what roles each of these have in the variation of tooth composition between males and females.

Section 1.1: Model Creation Overview

This protocol details how 3D Slicer software (Kikinis and Vosburgh, 2014) should be used to generate 3D models of each tooth in order to measure total volume and enamel volume. Using 3D Slicer, a model of each subject's total dentition was generated by first segmenting the CT scan in a semiautomated fashion. The skeletal cranial CT scans were loaded into 3D Slicer using a DICOM file. "Volume rendering", an automated process on 3D Slicer, was used to display image volumes of the dentition in each subject. Each CT scan is cropped to only display the maxilla and mandible utilizing the region of interest feature on "volume rendering". In addition, utilizing the "cropped volume" module, each file is cropped to display the newly generated regions of interest. Utilizing "segment editor" module, a total of three layers was generated for each tooth. The first layer was gross tooth segmentation. The second layer was an enamel segmentation of that tooth. The third layer was dentin segmentation.

Section 1.2: Gross Tooth Segmentation

Through the "segment editor" module on 3D Slicer, using the paint and threshold tools, the full dentition of each subject was drawn or selected (Figure 1A). The threshold value of approximately 1800 for each gross dentition layer. If any regions of the mandible or maxilla are accidently highlighted, a Wacom drawing tablet with a pen was used to erase all undesired highlights. The gross dental segmentation included the full dentition including the enamel and dentin of each tooth. However, a separate layer of segmentation was created for the enamel region utilizing the paint or threshold tools on "segmentation editor". A similar process is repeated for the enamel and dentin segmentation of each tooth for each subject.

Section 1.3: Enamel Segmentation

A similar process to the gross dental segmentation is used to enhance the enamel segmentation (Figure 1B). When segmenting out the enamel, the threshold tool was applied to narrow the threshold value of approximately 1000. If necessary, a tablet was used to draw and enhance the enamel region and erase any undesired highlights.

Section 1.4: Dentin Segmentation

Dentin regions were erased if accidently selected by the semi-automated threshold tool within the enamel layer. Enamel regions were excluded from the dentin layer by utilizing the masking feature under the threshold tool. While segmenting the dentin layer, it was observed that the segment quality of dentin layer was not ideal. This is likely due to the low-resolution CT scans used in this sample. As a result, dentin segmentations were excluded from statistical analyses in order to avoid skewing results due to inaccurate data.

The newly made three-dimensional models of gross dentition, enamel and dentin were then exported as an .STL file for analysis. Then, a projected volume measurement of enamel for each tooth selected was recorded using the “segment statistics” module in 3D Slicer.

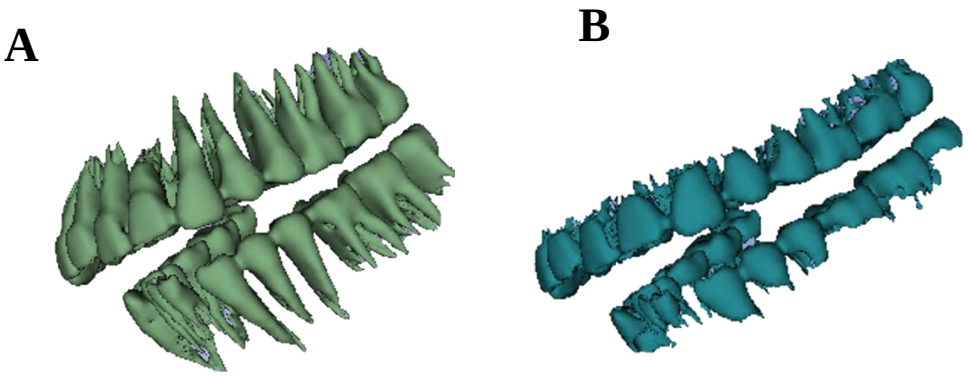

Figure 1. Example of Segmentation models of the full dentition (A) and enamel (B).

RESULTS

Section 1: Protocol (Aim 1)

As described above, a protocol was successfully generated to quantify dental microstructure. It was found that this protocol would best be suited with micro-CT scans. Due to dental microstructure such as dentin containing much less mineralization than enamel, regular CT scans are not optimal. A micro-CT scans with greater resolution are required. Therefore, in the future, studies should utilize this protocol using micro-CT scans. This protocol will contribute to growing body of evidence that there is sexual dimorphism in the quantity of enamel in humans.

Section 2: Dental Sexual Dimorphism (Aim 2).

The results of average full tooth volume in males and females support the hypothesis that males do have larger teeth than females within this sample. As shown in Table 2, males had a larger tooth average than females, albeit only slightly. The results of average enamel volume in males and females demonstrate that males contain more enamel than females (Table 2). Males exhibiting larger teeth infers a greater quantity of enamel in males. However, due to males only slightly exhibiting a larger average tooth volume and the vast difference of enamel volume between males and females, infers there are factors of sexual dimorphism at play within the dental composition of this sample.

Table 2. Enamel and full dental volume averages.

Sex	Enamel Volume	Full Tooth Volume
Male	208.72	345.59
Female	150.94	342.02

Sexual dimorphism was further explored by comparing the average size of male and female individual teeth. The coefficient of sexual dimorphism was ascertained by taking the average volume of each tooth for males and females and dividing the male mean by the female mean. As can be seen in Table 3, male maxillary central incisors are 21% larger than female central incisors. In addition, males also possessed larger maxillary dentition in their second molars (14% larger), and third molars (22% larger). Female demonstrated larger maxillary dentition in lateral incisors (4% larger), canines (6% larger), first premolars (7% larger), second premolars (16% larger), and first molars (17% larger).

Within mandibular dentition, males generally had larger dentition than females. Male central incisors were 18% larger than females. Lateral incisors were larger by 31% than female lateral incisors. Canine teeth and first premolars were 9% larger in males than in females. Second molars were also 19% larger in males than in females. Females demonstrated larger mandibular dentition in second premolars (10% larger), first molars (21% larger), and third molars (8% larger). Overall, males demonstrated a general pattern of larger full dental volume than females in both mandibular and maxillary teeth, albeit not in every tooth.

Table 3. Coefficient of sexual dimorphism using full dentition volume. *M* = average.

Maxillary	$\boldsymbol{M_m/M_f}$
I^1	1.21
I^2	0.96
C	0.94
PM^1	0.93
PM^2	0.84
M^1	0.83
M^2	1.14
M^3	1.22
Mandibular	$\boldsymbol{M_m/M_f}$
I_1	1.18
I_2	1.31
C	1.09
PM_1	1.09
PM_2	0.90
M_1	0.79
M_2	1.19
M_3	0.92

A Mann-Whitney U-test was preformed to examine significant values of full volume comparisons of males and females. As shown in table 4, the results from a Mann-Whitney-U test revealed that males exhibited significantly larger mandibular central incisors ($p = 0.05$), lateral

incisors (p = 0.05) and second molars (p = 0.07). As shown in table 5, males also exhibited significantly larger maxillary third molars (p = 0.08).

Table 4. Full volume comparison between male and female mandibular dentition. Values listed are from a Mann-Whitney-U test.

Tooth (#)	Sex	*n*	μ	Std. Dev.	z-value	*p*-value
I1 (24-25)	Females	6	102.7	73.4	1.70	**0.05**
	Males	4	131.1	30.5		
I2 (23-26)	Females	6	136.7	59.24	1.70	**0.05**
	Males	4	188.2	86.36		
C (22-27)	Females	7	243.96	73.51	0.89	0.22
	Males	5	270.43	60.60		
PM1 (21-28)	Females	7	215.8	73.678	0.56	0.32
	Males	5	240.0	73.018		
PM2 (20-29)	Females	7	247.0	122.1	0.08	0.46
	Males	5	242.9	81.52		
M1 (19-30)	Females	6	605.2	125.1	-1.50	0.91
	Males	5	484.3	86.05		
M2 (18-31)	Females	6	244.7	93.9	1.49	**0.07**
	Males	4	338.9	68.2		
M3 (17-32)	Females	5	253.2	35.3	-0.44	0.67
	Males	3	256.3	76.9		

Table 5. Full volume comparisons between male and female maxillary dentition. Values listed are from a Mann-Whitney-U test.

Tooth (#)	Sex	*n*	μ	Std. Dev.	z-value	*p*-value
I1 (8-9)	Females	6	205.6	48.2	0.43	0.38
	Males	4	221.7	122.3		
I2 (7-10)	Females	6	153.9	53.7	0.21	0.46
	Males	4	166.2	78.9		
C (6-11)	Females	8	308.9	81.7	-0.65	0.71
	Males	6	280.9	137.5		
PM1 (5-12)	Females	8	258.8	51.8	-0.39	0.65
	Males	6	224.5	108.3		
PM2 (4-13)	Females	8	256.5	57.2	-1.42	0.92
	Males	6	212.1	96.2		
M1 (3-14)	Females	8	604.9	124.2	-1.29	0.88
	Males	6	481.3	140.8		
M2 (2-15)	Females	8	567.2	91.9	0.26	0.43
	Males	6	557.7	155.2		
M3 (1-16)	Females	6	459.9	135.4	1.49	**0.08**
	Males	4	585.7	96.6		

Section 3: Sexual Dimorphism of Enamel (Aim 3).

In order to broadly examine quantities of enamel between males and female, a coefficient of sexual dimorphism using enamel volume was calculated. Within both maxillary and mandibular dentition males exhibited a greater quantity of enamel than females (Table 6).

In maxillary dentition, males exhibited greater enamel quantity within central incisors (38% larger), lateral incisors (29% larger), cuspids (34% larger), first premolars (42% larger), second premolars (20% larger), first molars (19% larger), second molars (35% larger), and third molars (19% larger).

As shown in table 6, in mandibular dentition, males exhibited greater enamel quantity within central incisors (84% larger), lateral incisors (82% larger), cuspids (55% larger), first premolars (43%, second premolars (54% larger), first molars (5% larger), second molars (38% larger), and third molars (1%). The third molars exhibited the least increase of enamel quantity; however, males still exhibited a greater quantity in comparison to females.

Table 6. Coefficient of sexual dimorphism using enamel volume. *M* = average.

Maxillary	$\boldsymbol{M_m/M_f}$
I^1	1.38
I^2	1.29
C	1.34
PM^1	1.42
PM^2	1.20
M^1	1.19
M^2	1.35
M^3	1.19
Mandibular	$\boldsymbol{M_m/M_f}$
I_1	1.84
I_2	1.82
C	1.55
PM_1	1.43
PM_2	1.54
M_1	1.05
M_2	1.38
M_3	1.01

A Mann-Whitney U-test was preformed to examine significant values of divergent quantities of enamel between males and females. The results of the Mann-Whitney U-test revealed that there are divergent quantities of enamel between sexes within the mandibular dentition. As seen in Table 7, many mandibular teeth including central incisors ($p = 0.06$), lateral incisors ($p = 0.04$), canines ($p = 0.03$), first premolars ($p = 0.08$), second premolars ($p = 0.07$) and

second molars (p = 0.07), all showed that there are different quantities of enamel between males and females.

Table 7. Enamel volume comparison between male and female mandibular dentition. Values listed are from a Mann-Whitney-U test.

Tooth (#)	Sex	*n*	μ	Std. Dev.	z-value	*p*-value
I1 (24-25)	Females	6	50.6	44.5	1.49	**0.06**
	Males	4	93.3	38.6		
I2 (23-26)	Females	6	56.3	30.2	1.80	**0.03**
	Males	4	102.4	73.5		
C (22-27)	Females	7	102.6	35.3	1.86	**0.03**
	Males	5	158.9	53.8		
PM1 (21-28)	Females	7	95.5	36.4	1.38	**0.08**
	Males	5	136.9	53.9		
PM2 (20-29)	Females	7	111.6	66.8	1.46	**0.07**
	Males	5	171.4	83.8		
M1 (19-30)	Females	6	240.9	76.8	0.18	0.43
	Males	5	253.6	59.1		
M2 (18-31)	Females	6	244.7	93.9	1.49	**0.07**
	Males	4	338.9	68.2		
M3 (17-32)	Females	5	253.2	35.3	-0.44	0.67
	Males	3	256.3	76.9		

There were also different quantities of enamel within maxillary second premolars (p = 0.09) between males and females (Table 8). Males had significantly more enamel quantity in their maxillary second premolars. No other maxillary dentition showed statistically different quantities of enamel between males and females. As a general pattern, females portrayed relatively less enamel in their maxillary central incisors than males. A similar pattern in the second premolars is shown in both mandibular and maxillary dentition.

Table 8. Enamel volume comparisons between male and female maxillary dentition. Values listed are from a Mann-Whitney-U test.

Tooth (#)	Sex	*n*	μ	Std. Dev.	z-value	*p*-value
I1 (8-9)	Females	6	94.7	31.5	0.64	0.30
	Males	4	130.7	93.1		
I2 (7-10)	Females	6	69.2	21.5	0.85	0.19
	Males	4	89.1	42.8		
C (6-11)	Females	8	140.2	43.4	0.87	0.18
	Males	6	187.5	187.5		
PM1 (5-12)	Females	8	104.3	31.9	1.17	0.12
	Males	6	148.1	72.8		
PM2 (4-13)	Females	8	105.6	24.9	1.31	**0.09**
	Males	6	127.2	45		
M1 (3-14)	Females	8	225.9	66.2	0.73	0.23
	Males	6	269.9	104.7		
M2 (2-15)	Females	8	255.2	33.8	0.58	0.28
	Males	6	305.8	176.8		
M3 (1-16)	Females	6	238.8	74.5	-0.65	0.25
	Males	4	283.6	85.8		

Linear regression analysis revealed that in both males and females, enamel volumes were highly correlated (r_f= 0.9410, r_m =0.9649, p = < 0.0001) with volume of the tooth (Table 9). This indicates that the amount of enamel present on a given tooth is dependent on the size of the tooth.

The ANCOVA analysis revealed that the y-intercepts of the two regression lines in this comparison were significantly different ($F_{1,16}$ = 19.22, p = 0.0001), but that the slope of the regression lines were not significantly different ($F_{1,16}$ = 6.31, p = 0.018). This confirms that while males tend to have greater quantity of enamel on their teeth relative to females (Fig. 2), the discrepancy is not robust. Part of this may be due to the small sample size.

Table 9. Linear regression results for female and male enamel volume versus total volume.

Sex	*n*	Correlation (r)	Coefficient of Determination (r^2)	Slope	*p*-value
Females	16	0.9410	0.8854	0.5810	< **0.0001**
Males	16	0.9649	0.9311	0.4204	< **0.0001**

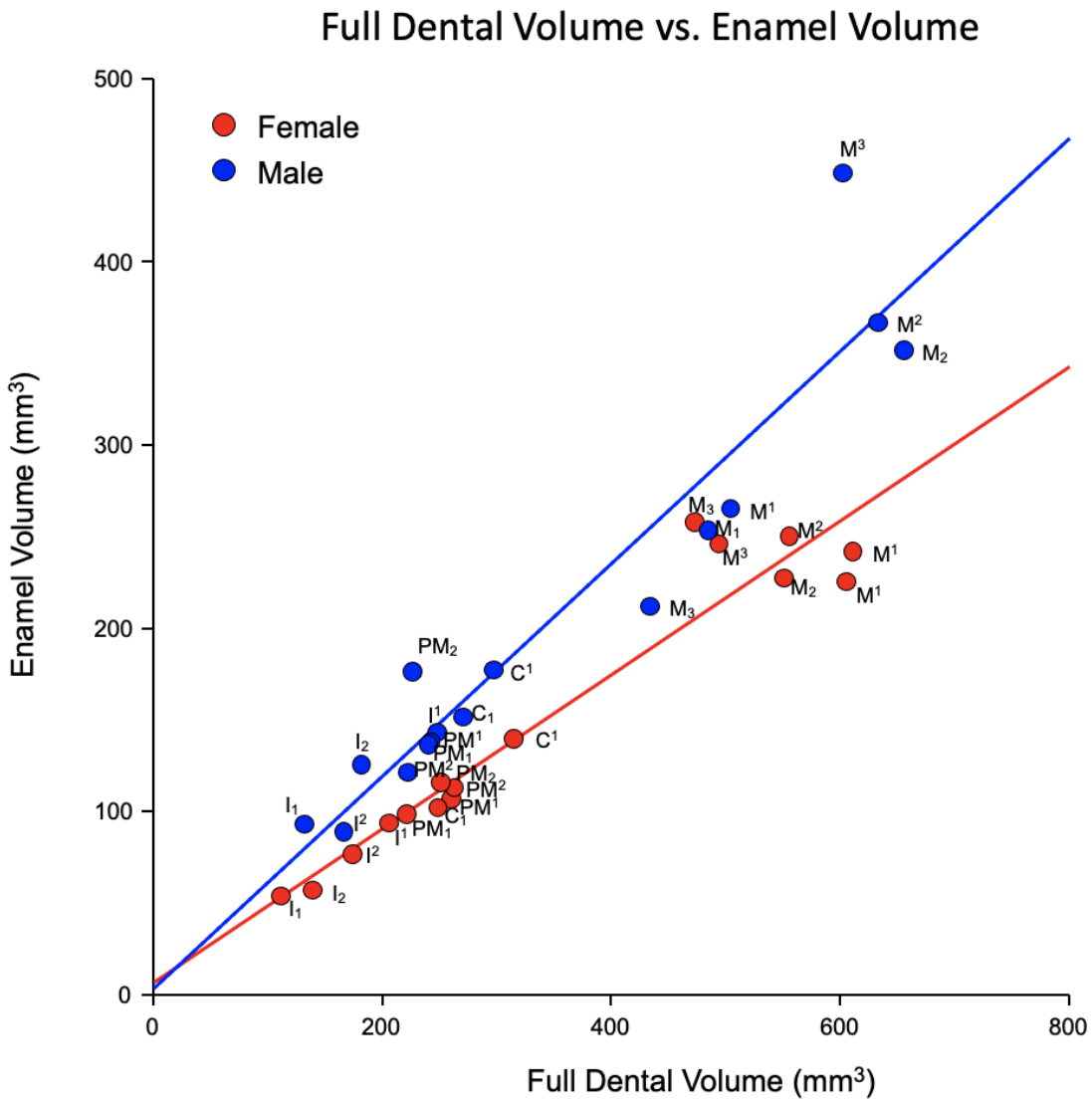

Figure 2. Linear regression of enamel volume plotted against total tooth volume in females and males. Males are shown in blue, and females are shown in red.

DISCUSSION

The Mann-Whitney-U test for total volume revealed that males exhibited significantly larger mandibular central incisors, lateral incisors and second molars. Males also exhibited significantly larger maxillary third molars. As a general trend however, based on the coefficient of sexual dimorphism of total volume, it was found that males exhibited larger teeth. This was expected. Although not many significant values were found, the small sample size may have contributed to this discrepancy.

As previously mentioned, dental evidence suggests that this sample population, often engaged in extramasticatory behaviors, and occupational behaviors that use teeth as tools to accomplish non-mastication-based tasks such as gripping food with their teeth. Therefore, there is significantly more wear often present within central and lateral incisors in this sample (De Poncins, 1941; Wood 1992).

This pattern is demonstrated in Table 7, females have significantly less enamel in the central and lateral incisors in comparison to their canines, premolars, and molars. The same pattern is also seen in males demonstrating less enamel quantity in their central and lateral incisors relative to their other teeth.

Males also exhibited significantly more enamel than females in their anterior teeth. This may be attributed to the frequency of use of anterior teeth within females in this population. As mentioned previously, although female tasks were less physically demanding within the oral cavity, they were more frequent in nature. Often females used their anterior teeth to stretch animal skin as pieces of clothing to keep warm (Wood, 1992). This may explain how the central incisors, lateral incisors and canines all demonstrated significantly less enamel quantity in females than in males in this sample (Table 7).

On average, the first and third mandibular molars demonstrated similar volumes of enamel across both males and females. However, females had significantly less enamel than males in the second molars. Overall, however, males tend to have significantly more enamel quantity than females in this sample. As previously noted by some anthropologists have stated, females in this population were shown to have developed more wear and abrasions than males in the same population (Leigh, 1925). The sexual division of labor within this population, the additional repeated stressors of female tasks, along with the diet in this population, could have contributed to the significantly fewer enamel volume within mandibular second molars in females than males. Therefore, these discrepancies are not robust. Increasing the sample size in the future may aid in demonstrating a better relationship of the enamel quantity between males and females.

The linear regression analysis indicates that the amount of enamel on the tooth is highly correlated with overall tooth size, but that males and females do not exhibit significant differences in the relative amount of enamel on teeth. This latter finding is in contrast to previously published data suggesting that, in humans, females tend to exhibit greater amounts of enamel relative to their male counterparts. Further, the present findings suggest that males, as opposed to females, exhibit more enamel on a given tooth (Figure 2). As previously noted, both sexes showed low amounts of enamel on the anterior teeth, likely as a result of common extramasticatory behaviors. It is possible that due to the small sample size and the high rate of wear on the anterior teeth, these results may not reflect the biological structure of the original tooth.

SUMMARY AND CONCLUSIONS

The focus of this practicum was the development of methodology for creating 3D models of dentition using CT scans and 3D Slicer software to quantify dental microstructure and explore the rate of sexual dimorphism within a sample (Aim 1). Other research to date has focused primarily on radiographic images, which provide only a two-dimensional approach to measuring internal dental structures. Measurements taken from radiographs have led to inconsistent data on sexual dimorphism within dental microstructure (Monalisa et al., 2018). Therefore, the methodology developed in this practicum will help shed light and contribute to the growing body of evidence that there is sexual dimorphism in the dental microstructure within humans. Moreover, this study demonstrates that even CT scans provide low quality results when taking volumetric data. Therefore, we suggest that micro-CT scans be used in future studies of volumetric dental comparisons.

For Aim 2, we hypothesized males would possess a larger tooth volume than females. As expected, males possessed a larger tooth volume average than females. Males exhibited significantly larger mandibular central incisors, lateral incisors and second molars. As shown in table 5, males also exhibited significantly larger maxillary third molars. To broadly compare the sexual dimorphism of the total tooth volume between genders, a coefficient of sexual dimorphism was also calculated. A general pattern of male exhibiting larger total tooth volumes than females was observed. Given that we do not know the body size of the individuals in the study, we cannot determine the rate of overall sexual dimorphism within the sample to see if the rate is comparable between body size and dentition. Similarly, the small sample size may also

obscure the full picture. For these reasons, we acknowledge that the rate of sexual dimorphism present in the dentition may not be a representative sample of the population at-large.

For Aim 3, we hypothesized that males would exhibit less enamel relative to gross tooth size and the females to exhibit more enamel relative to gross tooth size. To broadly compare the sexual dimorphism of enamel quantity between males and females, a coefficient of sexual dimorphism of enamel was calculated. The results revealed aa greater quantity of enamel in males for all teeth examined, both mandibular and maxillary. Mann-Whitney U- test revealed many mandibular teeth including central incisors, lateral incisors, canines, first premolars, second premolars and second molars, all showed significantly different quantities of enamel between males and females. In each of these cases, males had greater enamel quantity than females. There were also different quantities of enamel volume within the maxillary second premolars between males and females. Males also possessed significantly greater enamel quantity in maxillary second premolars. Overall, across both mandibular and maxillary dentition, males possessed greater amounts of enamel. This is in direct contrast to previous research (e.g., Antoine and Hilson, 2016). Again, the small sample may reflect a pattern that is not representative of the whole population. However, males have larger teeth and necessarily greater volumes of enamel as a result.

When comparing teeth within the female sub-sample, they showed less enamel in mandibular central and lateral incisors in comparison to their mandibular canines, premolars, and molars. A similar trend is also shown in males. This may be attributed to the fact that as dental volume increases, enamel volume also increases. However, it may also be attributed to the functionality of the anterior dentition in this population.

The pattern of increased wear and decreased enamel quantity within the incisors was expected in this sample. The Point Hope population frequently placed substantial demands on their anterior teeth and often engaged in extramasticatory behaviors (De Poncins, 1941; Wood 1992). These behaviors and tendencies, along with their diet often contributed to chipping and breaking of their anterior teeth (Giffen, 1930; Wood 1992). Although teeth with visible breakage were not included in this sample, it was difficult to ascertain the level of wear of the anterior teeth using these relatively low-resolution CT scans.

In addition to these patterns of increased demand and force on anterior dentition, the roles of sexual division of labor may have also led to some unexpected results. The female demands and tasks within this sample although not as physically demanding were more frequent in nature (Wood, 1992). The females in this population often tended to household duties and helped make clothing pieces to keep warm. One of the best ways they did this was by utilizing animal skin. Females would have to stretch the animal skin utilizing their dentition (Wood, 1992). Therefore, these frequent stressors on female dentition may have led to these overall decreased quantities of enamel.

The linear regression analyses on both males and females demonstrated that the amount of enamel is highly correlated with the size of the tooth, as expected. Previously, we hypothesized that males would exhibit a negative allometric relationship, while females would exhibit a positive allometric relationship. This means that as dental volume increased, males would portray less relative enamel than would be expected for their given tooth volume. However, males had a slope closer to 1.0 than did females. As shown in the ANCOVA analysis, the best-fit-regression lines in males and females were significantly different, but that the slope of the regression lines were not significantly different. Males portrayed a greater quantity of

enamel on their teeth relative to females. Although, this discrepancy is not robust. Part of this may be due to the small sample size. Despite these findings being contradictory to our initial hypothesis, these results provide evidence that dental wear may play a substantial role in the amount of enamel relative to the total tooth size. Although intuitive, this study demonstrates that high amounts of dental wear can skew expected results. In the future, care should be taken to ascertain the amount and location of dental wear when considering which teeth to compare for microstructural compositional differences in adult teeth.

In the future, studies should employ larger sample sizes in order to detect significant differences in the rate of sexual dimorphism of gross dental size and dental microstructure. In addition, utilizing micro-CT scans with a greater resolution, may provide significant differences within enamel volume between sexes which were not detected in this study. Also, if examining dental microstructure, micro-CT scans would be best suited due to increased resolution. The protocol developed in this study should be refined using micro-CT scans and will likely yield more reliable results. Finally, it is imperative to conduct additional studies such as this one so we can get a better understanding of dental development and what roles genetics, tooth development, and sexual dimorphism play within these variations of tooth composition between males and females.

In conclusion, this study has created a protocol to quantify dental microstructure utilizing 3D imaging software and CT scans. This study has also demonstrated that CT scans do not provide high enough resolution to conclusively answer questions about dental microstructure. Compared to the rest of the skeleton, teeth are small. Large features in the skeleton can be captured by the regular CT scan, but the microstructure of the teeth is too small to be captured by the same scan. Micro-CT scanning would resolve this issue. For this reason, it is suggested that

future studies employing this protocol use micro-CT scans. Moreover, studies interested in exploring comparisons of dental microstructure are encouraged to use micro-CT scans.

INTERNSHIP EXPERIENCE

This internship practicum was performed at the University of North Texas Health Science Center in Fort Worth, TX, under the direct supervision of Emma Handler, Ph.D., over the course of one year as a partial requirement for the degree of Master of Science in Medical Sciences. In May of 2020, I transferred to the Medical Sciences Research Track program. In June of 2020, Dr. Handler introduced me to this project and her previous work. From June 2020 through September 2020, Dr. Handler and I held weekly meetings to discuss previous research and literature in order to build a foundation upon which I could build this project. In early December 2020, I presented my practicum research proposal as a "Work-in-progress" seminar in the Center for Anatomical Sciences.

The sample of CT scans utilized in this study were from a previous research study conducted here at the University of North Texas Health Science Center. I gained basic 3D Slicer proficiency using the tutorials and manuals offered by 3D Slicer and Dr. Menegaz.

As the focus of this practicum is methodology, from November through February of 2021, I focused on developing methodology to develop 3D models of total dentition, enamel, and dentin. Also, in February 2021, I completed Head and Neck Anatomy course. The course included gross laboratory requirement and lecture-based learning.

Upon completion of data acquisition, all volumes for each tooth segmented were recorded, calculated and analyzed with Dr. Handler and Dr. Maddux. The results of which have

been reported in this practicum report. An abstract of the work done in this practicum was accepted for the 2021 UNTHSC Research Appreciation Day (March 22-26th) in January of 2021.

This internship has introduced me to the rigorous field of graduate level research which has ultimately helped me grow as a scientist and as a student. I am extremely thankful for the mentorship provided by Dr. Handler, Dr. Menegaz, and Dr. Maddux.

APPENDIX A

Abbreviations

Table A.1. List of abbreviations.

Tooth	Description
I^1	Maxillary central incisor
I^2	Maxillary lateral incisor
C	Maxillary cuspid (canine)
PM^1	Maxillary first premolar
PM^2	Maxillary second premolar
M^1	Maxillary first molar
M^2	Maxillary second molar
M^3	Maxillary third molar
I_1	Mandibular central incisor
I_2	Mandibular lateral incisor
C	Mandibular cuspid (canine)
PM_1	Mandibular first premolar
PM_2	Mandibular second premolar
M_1	Mandibular first molar
M_2	Mandibular second molar
M_3	Mandibular third molar
I1	Average of central incisors
I2	Average of lateral incisors
C	Average of cuspids (canines)
PM1	Average of first premolars
PM2	Average of second premolars
M1	Average of first molars
M2	Average of second molars
M3	Average of third molars

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