COLOR OF DENTINE AS AN AGE INDICATOR

FOR HISPANIC POPULATIONS IN SAN ANTONIO, TEXAS

THESIS

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For Mom, Dad, and Georgie- my constant sources of inspiration.

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CHAPTER I

INTRODUCTION

In the field of forensic anthropology it is important to estimate the sex, age, forensic ancestry, stature, and the postmortem interval of an individual in order to positively identify human skeletal remains. While several methods exist to estimate these factors (e.g. analysis of the morphological features of the innominate in order to assess sex, examination of the pubic symphysis to estimate age, and software such as FORDISC to estimate forensic ancestry) one of the goals of a forensic anthropologist is to use the most accurate and precise methods to improve the chances of identifying a deceased individual. The purpose of this thesis is to present an objective method for measuring tooth tissue color in an effort to identify trends in shading that may provide an estimation of age at death in individuals of Hispanic ancestry. I hypothesize that there will be a quantifiable change in the shade of dentine as the individual gets older.

In skeletonized remains, cranial or post-cranial elements can be used to pinpoint certain markers to provide evidence of the sex, age, forensic ancestry, and stature of the individual. Specifically, dental analysis can yield significant results when estimating age. In the case of sub-adults, rates of dental development and eruption are often an accurate way to estimate the age of the child. In adults, examining the tooth degeneration, such as degree of abrasion (i.e. tooth wear) can aid in age estimation. Dental aging methods, a

well as non-dental methods, are not as accurate in adults as they are for sub-adult because sub-adults have predicable rates of development, while adults vary in the degree of degeneration that their body experiences throughout their mature life (Komar and Buikstra 2008).

Dentition can be helpful when estimating sex and ancestry, but to a much lesser extent. The overall shape of the teeth can be used to distinguish between males and females as a result of sexual dimorphism (Foster and Harris 2009). This method is particularly challenging if a tooth is isolated and lacks a basis for comparison. Furthermore, it is important to consider the population from which the individual derived when employing this method. Chinese males, due to their typical small physique, may share size features in common with a European female. In terms of ancestry, differences in the shape of the palate, features of the incisors (e.g. shovel-shaped), features of the molars (e.g. Carabelli's cusp), as well as metric variation of major human populatons can aid in assessing the forensic ancestry of an individual (Hanihara and Ishida 2005).

Research on dentition and its application in age estimation appears to be focused around similar themes: tooth eruption in infants and children (Moorrees et al. 1963, 2005; Demirjian et al. 1973, 1976, 1980; Kent et al. 1978), dental degeneration in adults (Gustafson 1950; Johanson 1971; Lamendin 1992), and occlusal attrition (Brothwell 1981; Smith 1984; Richards and Miller 1991; Grauer 1991). Although age estimation in children is quite accurate because of known development and eruption standards, much remains when it comes to adults. When the human body reaches maturity (e.g. dental development and epiphysis fusions are complete), it becomes increasingly challenging to differentiate the age of an individual. This is one of the reasons why more precise age

estimation methods in adults are necessary. These methods must also meet the *Daubert* criteria in order to be accepted in a court of law if the instance ever arises. The *Daubert* criteria are federal standards specified in *Daubert v. Merrell Dow Pharmaceuticals* (92-102, US 579, 1993). The ruling outlines four criteria dealing with the introduction of scientific evidence at trial: (1) whether the theory or technique has been or can be tested, (2) whether the method has been subject to peer review and publication, (3) the known or potential error rate of the technique, and (4) the degree of acceptance of the theory or technique in the relevant scientific community (Komar and Buikstra 2008:286).

The focus on Hispanic populations, the largest minority population in the United States (US Census Bureau 2001), is of particular importance due to the fact that there is limited information in the application of biological profiling methods with regard to individuals who are considered Hispanic. This paucity of data and lack of methodology may be the result of the challenge in defining the term "Hispanic," which changes depending on the geographic location. For instance, the Hispanic population in Florida is composed of people likely of Cuban or Puerto Rican descent, while the Hispanic population in Texas is overwhelmingly of Mexican descent. Additionally, age estimation techniques for Hispanic populations in San Antonio, Texas is of particular importance to forensic anthropology practitioners in the United States, especially those tasked with providing positive identifications of deceased individuals from border crossing locales. Of all the southern border states, Texas has the largest number of incoming crossings in the United States (RAND Texas 2008). The following table (Table 1), created by using the statistics provided by RAND Texas, compares the number of crossings in terms of

incoming passengers in personal vehicles, buses, and pedestrian crossings in Texas to New Mexico, Arizona, and California in 2007.

Table 1. Number of Crossings from Mexico to Four U.S. Border States.

Category	**Texas**	**New Mexico**	**Arizona**	**California**
Incoming Passengers in Personal Vehicles	84,407,511	2,541,925	19,593,375	57,991,451
Incoming Passengers in Buses	1,808,452	40,430	309,531	1,230,642
Incoming Pedestrians Crossings	20,914,686	264,851	11,806,206	16,553,220

Source: RAND Texas© 1998-2009 All rights reserved.

Although there have been several reports stating that violence in Mexico does not spill into Texas border cities (Tedford 2009), the south Texas border is still considered a principal drug smuggling corridor between the United States and Mexico (NDIC 2008). In addition to drug-related violence, the harsh weather conditions of the Texas-Mexico border may also be a source of human fatalities in that area, especially to those who are trying to cross the border illegally. According to the U.S. Citizenship and Immigration Service (2000), Texas has an estimated 1,041,000 illegal immigrants living within its boundaries, second only to California with an estimate of 2,209,000 illegal immigrants.

It is likely that a significant number of deceased individuals found at the Texas-Mexico border were victims of crime or exposure deaths due to heat. It is also likely that these individuals are Mexican nationals. For this reason, it is crucial for forensic anthropologists to have population-specific age estimation (as well as sex and stature

estimations) methods in order to yield accurate results while, in turn, helping law enforcement officials identify remains.

The purpose of this research is four-fold: (1) to determine whether dentine shade measurably changes with age in individuals of Hispanic ancestry and therefore reject the null hypothesis that there is no change in the shade of dentine; (2) to propose a new and objective technique utilizing a portable color measurement scanner to quantify color of dentine; (3) to address one component of the data deficiency regarding Hispanic populations via examination of the dental hard structures and their relationship to age; and (4) to discuss future applications of this technique utilizing other dental and skeletal tissues (i.e., enamel and cementum) for age estimation in forensic anthropological and odontological settings.

Dental Anatomy

Teeth have played a significant role in forensic science, particularly in the anthropological and odontological aspects. A large number of positive identifications have been achieved when the teeth of a deceased individual are compared to antemortem dental records (Takashi et al. 2005). Such comparative identification requires the person employing the technique, typically a forensic odontologist, to identify similarities and discrepancies between the ante- and postmortem dental radiographs. An example of a discrepancy is a tooth present in the postmortem radiograph but absent in the antemortem one. Also, due to teeth's resistance to the most rigorous postmortem situations, Hillson states that "teeth provide one of the most protected environments for preservation of biochemical information on diet and biological affinities" (Hillson 1996:294). In other

words, information on diet can be inferred from analyzing the morphological features of a tooth (e.g. severe attrition on high carbohydrate diets) and DNA can be extracted from existing organic material that may be found within the tooth. This is particularly important in archeological settings and their study of past populations. Because teeth persist long after other parts of the skeleton have disappeared, dentition is an ideal element to utilize. In addition, dental development is affected far less than other tissues by endocrinopathies (e.g. type 1 diabetes and autoimmune thyroid disorders) and other developmental insults (Smith 1991; Sweet and Dizinno 1996). Another positive aspect of using dentition is that identified teeth are easily available. As a result, tooth samples can be a great asset when studying age estimation methods, since one can only test the efficacy of age indicators through the analyses of known age samples (Jackes 2000).

The human tooth is comprised of several structures (Figure 1). The dentine forms the main mass of the tooth, enamel and cementum the outer covering layers, and the pulp the delicate core (Schroeder 1991:314) (Figure 2). The portion of the dentine that is covered with enamel is called the anatomical crown (as opposed to clinical, or the clinically visible coronal portion of the tooth), while the portion covered with cementum is called the anatomical root (as opposed to clinical, or the tooth portion embedded in tissue). The region where the enamel meets cementum is called the cementoenamel junction, most commonly referred to as CEJ. The following section will briefly describe these as well as some additional dental structures.

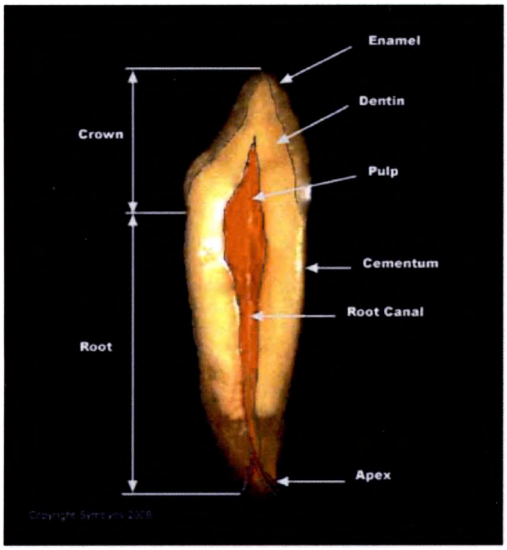

Figure 1. Basic tooth anatomy. Copyright © 2008, Symbyos all rights reserved.

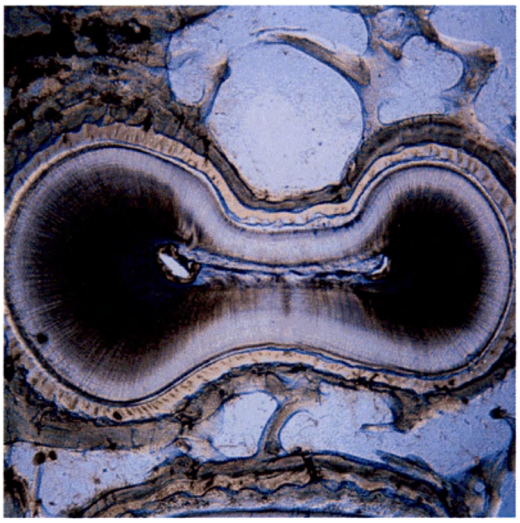

Figure 2. Light micrograph of a premolar tooth (horizontal section). Magnification: x5 at 6 x 7cm size. Copyright®2009 Nature Publishing Group all rights reserved.

The long and thin center is the root canal; the black and white area surrounding it is the mineralized dentine; the dentine is covered by a thin layer of cementum; the area in light yellow is the periodontal ligament.

Dental enamel is a crystalline structure and its formation is based on three processes: the formation of an enamel matrix and its initial mineralization, the resorption of this matrix, and the maturation of the crystalline structure (Schroeder 1991:38).

Dentine, which has a yellowish intrinsic color, is formed by odontoblasts during the process of odontogenesis. Once this process is complete, the odontoblasts continue to form irregular, or secondary, dentine at a lower rate (Solheim, 1998). Dentine is significantly softer than enamel, but harder than bone or cementum (Schroeder 1991:118). Radicular cementum is a mineralized, non-homogenous connective tissue that covers the entire dentine of the root from the cementoenamel junction to the apex, and aids in the attachment of the tooth to the alveolus (Schroeder 1991:144). There are two types of cementum, namely, acellular cementum which is the portion that does not incorporate cells, and cellular cementum which contained cememtocytes and it is found primarily in the apical third of the root (AAP 2001). Pulp tissue is a specialized connective tissue that is comprised of cells, an intercellular matrix, and collagen fibers; it also contains blood vessels and nerves (Schroeder 1991:130). Pulp tissue has many functions. Those related to this study involve the pulp's responsibility for the nourishment of the odontoblasts and, thus, indirectly responsible for the formation of primary and secondary dentine (Shroeder 1991). In addition, the pulp contains the nerves that register pain when the dentine is affected by factors such as trauma, heat or cold, and pressure (Schroeder 1991). The pulp tissue is housed in the pulp cavity, which has two parts, namely the coronal pulp chamber and root canal (Schroeder 1991). The apex, located at the inferior surface of the root canal, allows the blood vessels and nerves to enter and exit the pulp (Schroeder 1991).

Dentine is preserved in most cremations, but is strongly affected by the heat. At high temperatures, the peritubular and intertubular dentine boundaries are obliterated, the dentine has a granular texture, and the tubules are distorted (Hillson 1996:197). This is

important to keep in mind in cases where cremains are in question, and where a close analysis of the available dentine, depending on the degree which is affected, can be of use in estimating the age of the individual. In addition, fully fossilized teeth have better dentine preservation than much younger archaeological material (Hillson 1996), likely as a result of the birefringence and cross-links of dentine collagen fibers (Wojtowicz 1998). Another positive aspect of using dentine is that in forensic applications, dentine is more accurate than enamel when employing the method of racemisation- a process which uses the optical properties of the biochemical compounds of the tooth to estimate age (Rösing and Kvaal 1998). This is due to the stable temperature of dentine. Although dentine is a favorable substance to study, there are several drawbacks to keep in mind in terms of the technique being utilized, availability of tools, and necessary consults with individuals well-versed in oral structural biology.

Often the examination of dentine requires that the tooth go through a sectioning procedure that is destructive and time consuming. This is especially true when a large number of teeth are to be analyzed. Also, the tools used in this procedure (See Materials and Methods) are not easily available unless one has access to a dental clinic or a forensic odontology lab. Because dentine is not often studied by those outside of the field of odontology, except perhaps for dental anthropologists, consultations with knowledgeable dental professionals are highly recommended.

There are certain intrinsic factors which can affect the color of dentine that must always be taken into account throughout the period of analysis. Dr. Jorge C. Muñoz, D.M.D., states that one of these factors is when the individual suffers from a medical condition which affects the coloration and calcification of the dentine, with one of the

most common being *dentinogenesis imperfecta*, which can be caused by genetic factors. Trauma is the intrinsic second factor which can affect dentine coloration, in cases where the color of the dentine color changes when blood fills the dentinal tubules. This is not to be confused with postmortem pink teeth phenomenon in which dentine is colored due to an increase of intracranial blood pressure leading to a hemorrhage in the pulp chamber, which has been anecdotally reported in cases of asphyxia, drowning, or strangulation (Pessoa Soriano et al. 2009), but is not absolute medicolegal evidence of these causes and/or manners of death. Pink teeth may occur antemortem as a result of an accident (e.g car accident or fall) (J. Muñoz, personal communication, October 12, 2009), or occur postmortem as a result of perimortem or postmortem events (e.g. drowning or gravity). A third factor occurs in cases when an individual is on specific medication, which can turn the dentine a gray shade. The most common of these medications is tetracycline, which is a broad spectrum antibiotic used to treat upper respiratory infections and acne. The effects of such antibiotic are more pronounced during the period of tooth development. High-concentration fluoride is also known to affect the shade of dentine in severe cases where the individual drinks a large amount of un-processed mineral and/or well water over a period of time. Extrinsic factors such as smoking, staining of the teeth due to dark soda or tea, and bleaching have little or no effect on this substance because it is embedded within the tooth (J. Muñoz, personal communication, August 28, 2007).

CHAPTER II

LITERATURE REVIEW

Gustafson (1947) developed one of the first systematic methods for age estimation using 41 permanent teeth from individuals of known age. In a blind test to match isolated teeth, Gustafson estimated age from a general impression of secondary dentine deposition, cement thickness, and periodontosis (currently referred to as periodontitis). He then assigned point values (0-3) to each of these according to degree. Gustafson noticed that an increase in point value corresponded with an increase in age. Although Gustafson's original method was devised over sixty years ago, current techniques have remained fairly static. Several methods and techniques exist to determine age in both the outer part and the inner part of the tooth in adults, namely the number of teeth in the dentition, tooth color and fluorescence, attrition, periodontal recession, cementum apposition, root resorption, amount of secondary dentine, root translucency (root dentine sclerosis), thickness of peritubular dentine, and racemisation among others (Rösing and Kvaal 1998). Gustafson (1950) revised his system to employ six of these age-related factors rather than the three he originally utilized in his 1947 study.

There have been several studies that have tested and revised Gustafson's technique of age estimation. Johanson's method (1971) analyzed six dental variables,

namely secondary dentine deposition, abrasion, cementum deposition, root resorption, periodontal recession, and root translucency in order to estimate age. Johanson evaluated the ground sections of 162 teeth from 31 males and 15 females and found that multiple regression analysis produced better results than Gustafson's (1950) with a distinct decrease in the standard deviation (±10 years in 95.7% of all estimations).

Burns and Maples (1976) employed Gustafson's dental variables, with the exception of root resorption due to its low correlation with age, alongside multiple regression analysis to estimate the age of 355 teeth from 167 individuals ranging from 10 to 90 years of age. They concluded that Gustafson's technique is a sound method and that the addition of four additional variables (tooth position, race, sex, and periodontal disease) is significant for age estimation.

In their study of age estimation using two dental features, namely periodontis and root translucency, Lamendin et al. (1992) compared their technique to Gustafson's. Their sample was comprised of 306 teeth that were extracted during the autopsy of 208 French individuals with ages ranging from 22 to 90 years. With the use of the formula $A = 0.18 \times P + 0.42 \times T + 25.53$, where A = age in years, P = periodontosis height × 100/root height, and T = transparency height × 100/root height, Lamendin et al. concluded that their method worked best for individuals between 40 and 80 years of age, with a mean error of 8.9±2.2 years, which is lower than Gustafson's 14.2±3.4.

Prince and Ubelaker (2002) applied Lamendin's technique to the Terry skeletal collection in order to test the accuracy, precision, and applicability of the method. Their application of the Lamendin method, with the addition of sex- and ancestry-specific equations, produced mean errors between 6.24±4.97 and 9.19±7.17 years, and was most

accurate with the 30 to 69 year-old group. They concluded that sex alone, as apposed to ancestry and sex combined, has a significant effect on estimating age using Lamendin's technique.

Gonzáles-Colmenares et al. (2007) compared the accuracy of the Prince and Ubelaker (2002) as well as Lamendin (1992) methods on 79 teeth from recent skeletal remains of individuals from Granada, Spain. In addition, the authors aimed to develop a new formula based on their results to be applied to a racially mixed population from Colombia who were undergoing autopsy. They concluded that the Prince and Ubelaker method demonstrated a higher accuracy for both the Spanish and Colombian population when compared to the Lamendin method.

Brkic et al. (2008) tested the accuracy of Johanson's (1971) method on 140 permanent teeth of ages ranging from 18 to 80 years. They concluded that the Johanson method showed a strong correlation between chronological and dental age of individuals. In an earlier study, Brkic et al. (2006) estimated the age of 160 extracted teeth of known age and sex by analyzing the translucency of the root dentine, the root and root canal from x-rays, and six parameters on each tooth. They concluded that all of the methods were in significant correlation with the real age of the individual.

Another age estimation method involving dentition is that of counting cemental annuli. Charles et al. (1989) used a sample of 73 premolars from cadaver and clinical extractions in order to analyze growth layer groups in cementum and its relationship to age. A growth layer group consists of one hypercalcified layer and one hypocalcified layer. With the use of reduced major axis regression statistics, the authors found an apparent decrease in growth layer groups with age. Charles et al. related this decrease in

layers to the decline in cementogenesis activity or to the increase difficulty in counting the layers as they become thinner. Although this technique shows potential, the authors stated that there was an increase in error estimation with increasing age that resulted in high error estimates for all age ranges.

Although the present study is not the first to use dentine as an age estimation indicator, to the author's knowledge, it is the first time that this specific technological device has been applied towards assessing and quantifying its shade. Previous research examining dental tissue color includes studies by Martin-da la Heras and colleagues (2003), who analyzed the color of dentine using spectroradiometry. They concluded that their technique is a potentially useful and objective method to estimate age in adults. Similarly, tooth root color has been digitally recorded by Laskarin and colleagues (2006), with their resulting data showing that there is a correlation between the obtained RGB (red, green, blue) color values and age.

Although many researchers make use of the advanced technology available to them, there are others who stand by simpler technology such as radiographs. In his longitudinal assessment of age-related change in the dental pulp chamber, McBride (2007) used dental radiographs in order to estimate age. Six qualitative criteria evident in oral radiographs taken during a routine dental examination from 1991 to 1999 were developed from 37 subjects represented on three occasions each. Age was estimated from averaged criterion scores and incorporated longitudinal information for prediction with cross-sectional data. This method showed a correlation 82% of the time. In addition, McBride points out that reliable age indicators in oral radiographs are persistent in an individual in their eighties and nineties. Although this method appears to be

accurate, it has not been used with much frequency, likely as a result of the fact that it is fairly recent and has not had time to become widely accepted in the scientific community- one of the criteria required by *Daubert*.

One notable difference between the current and older techniques is the technology available to the researcher. Dobberstein et al. (2008) systematically analyzed collagen and DNA in order to test reliability of age estimation based on aspartic acid racemization and genetic analyses in teeth. The data showed a remarkable stability of collagen dental proteins as well as a high potential for dental DNA to be successfully extracted. The authors point out that after a large postmortem interval, or the period of time from the death of an individual to discovery, and extreme postmortem conditions, age determination based on aspartic acid racemization and genetic analyses lose their reliability.

Dental analyses have also been applied to paleodemography and archaeological settings, where estimating the age of skeletons might pose a challenge. Brothwell (1989) identified a high correlation between the total molar wear scores ($M_1 + M_2 + M_3$) and dentally assessed age in a sample of 328 early British individuals of Bronze Age to Saxon date. Brothwell pointed out that although the wear score in relation to age may vary from group to group, the pattern in the frequencies of adult total wear scores would at least establish fluctuations in the ages at death of a particular population sample.

Walker et al. (1991) estimated the age of archaeological populations by looking at their tooth wear, or attrition. The authors concluded that wear rates vary widely among prehistoric populations depending on the nature of their diet and culinary practice. In

addition, the authors pointed out that sex differences in wear rates are a potentially significant source of error when attempting to estimate age in archeological populations.

Although Charles et al. (1989) stated the method of counting cemental annuli for age estimation yielded high error estimates for all age ranges, Cipriano-Bechtle et al. (1996) compared cemental annulations to macroscopic methods of adult age estimation using early medieval skeletons from a Bavarian cemetery. They concluded that the cemental annulations technique provides a distribution that has apparent biological validity, in contrast to the macroscopic methods, and also yields a smoother age distribution extending beyond 60 years. It is likely that the seven-year gap between these studies allowed for the advances in accuracy of the technique.

Constandse-Westermann (1997), in her study on a known sex and age sample of Dutch individuals from Zwolle (Netherlands) who died between 1819 and 1828, found that it is possible to obtain higher number of age estimates, as well as more accurate ones, than methods involving the auricular surface, pubic symphysis, and cranial suture closer when using attrition and alveolar resorption. The difference from real age was 3.8 years in males and 6.3 years in females. This method not only emphasizes the efficacy of dentition for age estimation, it also adds a useful method to employ in studies of paleodemography.

Sengupta et al. (1998) measured the root dentine translucency in human teeth of varying antiquity. The focus of their study was to establish a standard protocol of dental age estimation for human teeth found at any depositional phase. They concluded that the lower canine was the most reliable tooth for this particular method.

Jeong et al. (2008) also used attrition in the age estimation of three mummies excavated in Korea between 2002 and 2004. With the use of a nondestructive method (e.g. three dimensional reconstructed images of the total tooth), standard methods using serial sectioning (Johanson 1971), as well as examination of degree of attrition (Gustafson 1950), the authors estimated the mummies to be 23, 51, and 64 years of age. In addition, Jeong et al. concluded that the three-dimensional reconstruction of the tooth using a CT scan can be a valuable method because it minimizes the damages made to the specimens and it yields similar ages to that of Gustafson's method.

Due to the fact that there is little to no literature that is directly related to the shade of dentine and association with the age of an individual, this pilot study drew upon previous research such as the ones outlined in this section, and utilized those research methods and statistical analyses that best fit the current study.

CHAPTER III

MATERIALS AND METHODS

Tooth Collection

Teeth removed from the oral cavity from a known and documented sample of molars from 72 modern Hispanic adult individuals were used in order to conduct this study. The sample was comprised of 34 males and 38 females with overall ages ranging from 12 to 83 years with a mean age of 38.93 and a standard deviation of 19.66. The molars were extracted by Drs. Jorge C. Muñoz, Francisco Arroyo, and Andrea Rodríguez at the "Smiles of San Antonio" dental clinic located in San Antonio, Texas. The extractions took place from Spring 2007 to Fall 2008. Careful collection and data recording were employed in this study. Each extracted tooth was coded with its own identification card containing only the individual's age, sex, forensic ancestry, dental/medical history, the location of the molar, and date of extraction.

This study was limited to molars due to the limitations imposed by the requirements of the color scanner. The lens of the scanner was large enough for molars (Figure 3), but not for smaller teeth such as a canines or incisors, since these would not have covered the entire lens and light would have penetrated resulting in an accurate reading.

Figure 3. Illustrating the size of the lens versus the size of the molar. Photo by author.

Storage

Following Dr. Muñoz's suggestion, the extracted molars were individually kept in a small glass container with a screw top to avoid contamination by foreign materials. The molars were kept in a dry environment in order to maintain a stable condition of storage. The glass container was then placed in a re-sealable plastic bag along with its identification card.

Procedure

On December 31, 2008 at "Smiles of San Antonio" dental clinic in San Antonio, Texas, Dr. Muñoz cross-sectioned each molar. The author was responsible for drying and color scanning each specimen because, unlike the cross-sectioning, these two procedures did not require any prior experience. A scaler was used in order to scrape away any calculus or any other material found on the outside of the molar. Each molar was sectioned horizontally along the cementoenamel junction with a Master II air-driven

high speed hand piece by Henry Schein Dental® that utilized a No. 699 high-speed carbide burr (Figure 4). This particular bur used distilled water (as opposed to mineral) to avoid the heat that is created as a result of friction. Heat, along with other factors such as trauma and certain medications, may affect the color of dentine. Following the sectioning, the molars were cleaned and dried with a three-way air-water-spray syringe, and also allowed to air dry thoroughly.

Figure 4. Burr placed just below the horizontal sectioning region along the cementoenamel junction. Photo by author

The scanning was done using HunterLab's MiniScan XE Plus® portable color measurement scanner, manual version 2.4 (Figures 5 and 6). The shade was taken from the root portion of the tooth. In addition to the color scanner, a Vita® manual shade guide (Figure 6), commonly used in dental offices to assess enamel shade, was utilized in order to compare the reliability and effectiveness between the computerized and visual methods of measuring the shade of dentine. The manual shade was obtained by visually examining the root portion of the molar, as was done with the scanner, and matching the color to the one that resembled it the most from the shade guide. Both the color scanning and the manual shade assessment were done by the author in order to minimize inter-

observer error. Most of these tools, with the exception of the color scanner, can be found in a dental clinic, and at dental or forensic odontology laboratories. Each step was carefully documented and close-up pictures were taken to facilitate future replication of the experiment.

Figure 5. HunterLab's MiniScan XE Plus®. Photo by author.

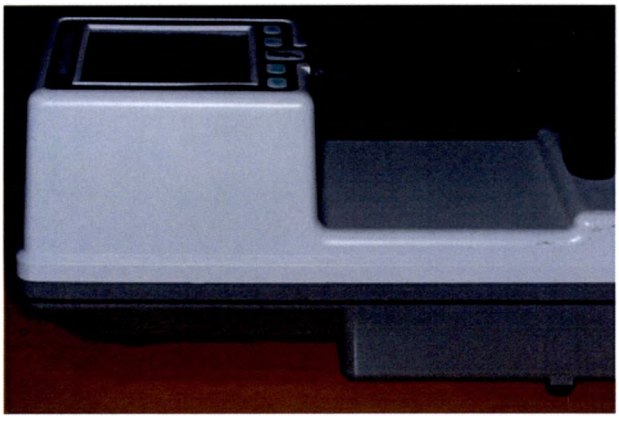

Figure 6. HunterLab's MiniScan XE Plus®, profile view. Photo by author.

Figure 7. Vita® manual shade guide. Photo by author.

In order to properly measure the color of the dentine in this study, the molar was placed directly on the color scanner lens as flat as possible to prevent light from penetrating and thus affecting the shade (Figure 8). Each scan yielded three different shades using the L, a, b Hunter scale. In this scale, L measures lightness and varies from 100 for perfect white to zero for black; 'a' measures redness when positive, gray when zero, and greenness when negative; 'b' measures yellowness when positive, gray when zero, and blueness when negative (HunterLab 2006:10-2). The L, a, b Hunter scale relates to the XYZ tristimulus values established by the Commission Internationale de l'Eclairage (CIE) in 1964 (HunterLab 2006). The relationship between the Hunter scale and the XYZ tristimulus values is as follows:

$$L = 100\sqrt{Y/Y_n}$$

$$a = K_a (X/X_n - Y/Y_n) / \sqrt{Y/Y_n}$$

$$b = K_b (X/X_n - Z/Z_n) / \sqrt{Y/Y_n}$$

Where X, Y, and Z are CIE tristimulus values; X_n, Y_n, and Z_n are tristimulus values of the illuminant (i.e. a table of spectral distribution as close as possible to a natural light source, usually daylight, to be duplicated (HunterLab 2008:Glossary)); Y_n is equal to 100.00; K_a and Kb, the chromaticity coefficients for the illuminant, and X_n and Z_n are listed below (Tables 2 and 3) (HunterLab 2008:3):

Table 2. CIE 2° Standard Observer.*

Illuminant	X_n	Z_n	K_a	K_b
C	98.04	118.11	175.0	70.0
D_{65}	95.02	108.82	172.30	67.20

*Standard Observer refers to a numerical representation of what the average human eye sees. The degrees refer to the field of view (HunterLab 2008:1).

Table 3. CIE 10° Standard Observer.*

Illuminant	X_n	Z_n	K_a	K_b
C	97.30	116.14	174.30	69.40
D_{65}	94.83	107.38	172.10	66.70

These color values were entered into a Microsoft Office XP Excel spreadsheet along with the specimen number, age and sex of the individual, tooth number, and shade obtained from the Vita® manual shade guide (Appendix A).

Figure 8. Placing of molar on the lens of the color scanner. Photo by author.

Once all of the shades were obtained, a yellowness index (YI) per ASTM method E313-96 was formulated from the L, a, b scale (HunterLab 2006). Yellowness indices are used to measure degradation caused by light, chemical exposure, and processing. These indices are often used in textile, paint, and plastic industries, but may be used for measurement of any nearly white or nearly colorless object (HunterLab 2006). The index is calculated as follows:

$$YI\ E313\text{-}96=\frac{100\ (C_xX-C_zZ)}{Y}$$

Where X, Y, and Z are the CIE tristimulus values and the coefficients depend on the illuminant and observer indicated on the following table (Table 4) (HunterLab 2008):

Table 4. Illuminant and Observer Values.

Coefficient	C/2°	D65/2°	C/10°	D65/10°
C_x	1.2769	1.2985	1.2871	1.3013
C_z	1.0592	1.1335	1.0781	1.1498

HunterLab's Universal Software V4.10 automatically calculated the yellowness index (Appendix B). This particular software also allows the user to display the results in a graph in order to observe how each molar's YI compares to one another.

After calculating the yellowness index of the individuals, the indices were entered into SPSS Statistics 17.0 in order to run two regression analyses on the data. One analysis tested whether both sex and age had a significant effect on the shade of dentine, while the other analysis tested the significant effect of age on the shade of dentine. In order to evaluate whether the use of a color scanner is a more reliable method than a visual assessment, a second color trial was done on eight specimens (four males and four females) chosen at random four months following the original scanning. The data were then entered in the SPSS 17.0 Statistics® program and analyzed using a Kappa statistic, which measures the agreement (i.e. reliability) between tests by the same observer or between two observers (Komar and Buikstra 2008). Kappa uses the following formula in order to remove the probability of random error:

$$K = \frac{\text{probability of agreement} - \text{probability of random error}}{1 - \text{probability of random error}}$$

In addition to the Kappa statistic, a paired t-test also tested the intra-observer error in the use of the color scanner in this particular study.

Limitations

The majority of molars in the sample were third molars. Due to the fact that these molars display the greatest variability in size, shape, and eruption rates even within single

individuals, their reliability may not be as accurate as other tooth types (Guerisoli et al. 1998). It is not clear whether color is affected by this variability.

In addition, because the sample utilized in this study is of living individuals, the results might not be as accurate when applied to archeological specimens where severe desiccation might influence the shade of dentine. Future studies comparing the shades of dentine obtained from severely desiccated teeth will help identify whether there is a significant difference in the correlation with shade of dentine and age in desiccated and non-desiccated teeth.

CHAPTER IV

RESULTS

Statistical analyses of the yellowness index (YI) of the overall sample yielded a positive correlation between the shade of dentine and the age of the individual. In other words, the shade of the dentine gets darker as the individual gets older.

The first regression analysis for the sample showed that age ($df = 71$, $r = .327$; $t = 2.117$, $p = .038$) and not sex ($df = 71$, $r = .327$; $t = -.517$, $p = .607$) is the significant factor.

In order to test the degree of correlation between age and the yellowness index, a second regression analysis was performed. The analysis showed that age is significantly correlated ($df = 71$, $r = .317$; $f = 7.837$, $p = .007$) with the yellowness index. The lightest yellowness index ($YI = 13.05$) was observed in a twenty year-old individual, and the darkest ($YI = 41.83$) in an eighty three year-old individual.

A formula was established in order to predict the age of the individual with the yellowness index:

$$Age = 17.1 + .851\ (YI)$$

A general prediction interval formula was also established (Kutner et al. 2004):

$$\pm 2 * \sqrt{352.695\ (1/72 + (YI - 25.67)^2 / 53.723 * 71}$$

The age prediction formula tends to underestimate older individuals and overestimate younger individuals due to the nature of the sample. Although most decades are represented in the sample (i.e. 12 to 83 years old) most of the individuals were in their twenties and thirties. This can be better observed in figure 9 (Appendix C).

Testing for Intra-Observer Error

Kappa Statistic

Due to the fact that this particular statistical analysis benefits from having categories, four categorical variables were created in order to represent the agreement between the first and second trial: High-High, two High-Low, Low-Low. These categories were based on the yellowness index. A median of 34.50 was established in order to distinguish between high and low yellowness indices. The results of the analysis utilizing the Kappa statistic yielded an agreement 7 out of 8 times with no significant change from the first to second trial (Appendix D, table 5), demonstrating that the use of the color scanner is a reliable and objective method with a low standard of error (.042).

Paired T-Test

Utilizing a paired t-test yielded the following statistics ($df = 7$, $t = -.756$, $p = 0.474$). Due to the fact that the p-value is larger than .05, the null hypothesis ($H_0 = 0$) is therefore accepted. In other words, there is no significant difference between the two measurements taken in the first and second trials.

Both the Kappa statistic and the paired t-test agree that the intra-observer error in the use of the color scanner is non-significant. This method has the potential of being highly reliable even in cases where the person using the scanner is a novice.

The manual shade guide, as opposed to the scanner, requires a degree of subjective guessing on behalf of the observer. In order to emphasize the difference in reliability between the scanner and the manual method, a simple non-statistical analysis was employed. The results of the first trial were compared to those of the second trial on a one-to-one basis (Appendix D, table 6).

Only one of the specimens (#9) matched in shade from the first to the second trial. The shades for the remaining seven specimens differed from those of the first trial. These results indicate that this method of visual assessment using the manual shade guide should not be employed in matters of forensic interest due to its poor precision and lack of reliability. It would be interesting to analyze in a future instance whether the human eye has the ability to distinguish between the different shades of yellow included in the manual guide. Such a study might also determine the degree of reliability of an experienced observer when assessing the shade of dentine.

CHAPTER V

DISCUSSION

It is unknown why dentine darkens with age, but there are two possible potential factors to consider. Toto et al. (1971) found that there was a significantly greater loss of water from younger to older teeth following dehydration of dentine at 105° C. This loss of moisture may be one factor that causes the dentine to get darker with age. Another factor may be the progressive mineralization of dentine with age (Schroeder 1991). The wide array of mineral, organic, and trace elements that comprise the dentine, and their subsequent increase (e.g. mineral) or decrease (e.g. organic) with age, depending on the component, may explain the darkening of dentine. A third factor may be the combination of the first and second factors.

To my knowledge, this is the first time where a correlation between the shade of dentine and age has been identified. Previous studies (Brudevold 1957; Brudevold et al. 1960) have found that enamel has a tendency to become darker with age. The latter study proposed that the darkening occurred as a consequence of certain trace minerals accumulating in the outer portion of the enamel with age. Age estimation from enamel may not be accurate because it is affected by extrinsic factors such as soda and coffee intake, smoking, and oral hygiene in general among others (J. Muñoz, personal communication, August 28, 2009). A study by Ten Cate et al. (1977) found that the tooth

root color becomes progressively yellow with age, and that such yellow discoloration may be the result of increasing thickness of the root cementum and the increasing mineralization of the root dentine.

It is not surprising that the results of this study show that the sex of the individual is not a significant factor when it comes to the shade of dentine. Richards and Miller (1991), in their study of tooth wear and age, found no significant differences and therefore no need to separate the sexes when employing their regression equations. Johanson (1971) found practically no difference between the sexes in the values obtained from his study. Hojo (1954), on his studies on attrition of modern Japanese skulls, found that there is no sex difference in the rate and degree of attrition. Lunt (1978) also emphasized the unimportance of sex differences in his study of molar attrition in medieval Danes. However, several studies have suggested that sex may be a factor in dental attrition.

Brothwell (1989) proposed that male and female jaw size, chewing stresses, and diet may lead to differences in dental wear. Jackes (2000) performed a study where Spitalfields individuals were coded for attrition using a grading system derived from Brothwell (1981) and analyzed using exact maximum likelihood statistics. The results show that male attrition is greater than female attrition. She attributed this difference to the forces developed under male masticatory muscle strength, as opposed to dietary or activity factors. The ambiguities of the role of sex in dental age estimation methods should be explored further.

Following the positive correlation between age and the shade of dentine found in this study, future studies should attempt to establish more clearly defined and narrower

age ranges with the availability of a larger sample size. Although the current sample of 72 molars allowed for the calculation of an age prediction formula, a larger sample with a wider age distribution will likely establish a subsequent formula with a higher degree of accuracy than the one provided in this study.

It is important to use caution when applying age ranges to groups other than the sample from which the ranges where derived from. A misapplication may result in invalid results. Teeth from modern Hispanic individuals from San Antonio, Texas were used in this study. Therefore, it is acceptable to apply the findings on such data to forensic cases in the south-central region of Texas, as well as neighboring areas. Several studies have indicated that the environment, both physical and cultural, plays a significant role in human adaptability (Garruto 1995; Stinson 2000). Genetics also have an effect on human adaptability but to a lesser extent (Lasker 1969). It is likely that Hispanic individuals living in Texas differ both culturally and biologically from those living in Puerto Rico, Colombia, or other Latin American countries as a result of the adaptation to their particular environment. Upcoming studies should employ the proposed technique using the color scanner when analyzing the dentine of other populations. In order to ensure accuracy, these impending age ranges should remain population-specific.

Forensic anthropologists and odontologists working in the south-central region of Texas may benefit from this method since it focuses on the specific Hispanic population that they are most likely to come across in their forensic cases. This technique may be applied to skulls found along this area, assuming that they have molars present, in order to estimate the age-at-death of the individual in question.

The technique of dentine analysis employed in this study may be one more method of age estimation to add to the existing repertoire utilized by bioarcheologists. As previously discussed, teeth have a high degree of preservation and, as a result, may be found frequently in archeological settings. Although Lucy et al. (1995) noted that it is impossible to use color and translucency when assessing archeological teeth, their study focused on root translucency in particular, and therefore, dentine should not be excluded in terms of its use in archeological remains. I believe that if a tooth is found, even with a slight degree of occlusal wear or minor fracture, it can be sectioned just below that fracture (if below the CEJ) and its dentine can then be analyzed in the same manner as the molars in this study. Theoretically, due to the strong nature of enamel, the dentine within the teeth of archaeological specimens should be well preserved. Although this method may be efficacious in archeological settings, the archeologist must be willing to destroy a tooth in order to analyze the shade of its dentine due to the destructive nature of the technique.

Researchers wishing to employ this objective technique must use a color scanner which yields the three shades (l, a, b) used to formulate a yellowness index. In addition to the color scanner, a software that yields the numerical indices is also needed. Hunter Associates Laboratory, Inc. provides both the scanner and the universal software. Once a yellowness index is formulated, the researcher can either enter the yellowness index in the formula provided in the "RESULTS" section or refer to Appendix E for a table with predicted ages and lower and upper ranges for a yellowness index ranging from one to fifty in order to estimate the age which their particular individual falls under. The use of a manual shade guide is not recommended in the absence of a color scanner due to the

low reliability of the method. Furthermore, formulating the yellowness index without software may result in faulty calculations thus lessening the objectivity of the method.

CHAPTER VI

CONCLUSION

The findings of this pilot study reject the null hypothesis that there is no quantifiable change in the shade of dentine, and support the alternate hypothesis that there is a quantifiable change in the shade of dentine as the individual gets older. The correlation between the shade and the age of Hispanic individuals in San Antonio, Texas is significant. The sex of the individual is not a significant factor in this case. This study also found that the color scanner is not only an objective method of estimating age but a reliable one as well. On the contrary, manual shade guides are not reliable and should not be used to estimate the shade of dentine.

The significant correlation between the shade of dentine and the age of the Hispanic individuals in this study delivers a promising topic for future research. Further study on this topic should be undertaken on the basis of the following:

1. Improvements in technology occur at a very fast pace. An updated version of the color measurement scanner might allow for the scanning of not only molars, but incisors and canines as well. On that note, the software for the scanner might be updated into an even more user-friendly tool. The method employed in this

research, namely the use of a portable color measurement scanner, leaves subjectivity aside and yields a quantified shade value. Due to the fact that the shade was obtained through a color scanner and not the human eye, this method is much stronger in comparison to others that rely on visual observations alone.

2. Future research should aim for larger sample sizes in order to better observe trends. One way to build up a larger sample size is to contact several dental clinics and let them know about the purpose of the research. As long as they do not have to provide a patient's personal information other than age and sex, there is a chance they will be willing to help. Once a clinic is recruited, provide them with all of the storage and data collecting materials (e.g. small glass beakers and identification cards).

3. Research on the chemical composition of dentine should be done alongside those who specialize in dental structures in order to pinpoint the cause(s) of variation in dentinal shade.

4. Future studies should also be conducted on other dental structures that are not easily affected by extrinsic factors such as cementum to determine if a correlation exists between shade and age. This will enable those in the field of forensics, particularly those in the odontological and anthropological aspects of the field, to explore new methods of age estimation using dentition in order to add practical and reliable techniques to the existing repertoire. The human dentition may provide a haven for extensive research not only in regards to age estimation, but possibly in regards to sex and forensic ancestry estimation as well.

5. Due to the limited amount of information available on individuals considered

Hispanic, studies in age, sex, ancestry, and stature estimation of Hispanic populations should be explored further.

This method can be applied in modern forensic contexts to modern Hispanic individuals, particularly those from the south-central region of Texas, due to the high degree of objectivity and reliability. This technique should also be measured against modern American Blacks, American Whites, Native Americans, and others in order to determine the usefulness and reliability of the technique across other groups.

This method may also show promise in bioarcheological settings where teeth are often recovered due to their high degree of preservation. With further testing, it is anticipated that this technique may aid in estimating the age-at-death of the individual.

With the results verifying that the shade of dentine gets darker as the individual gets older, as well as the establishment of an age prediction formula according to the yellowness indices of the current sample, this technique can be employed by forensic anthropologists, forensic odontologists, and bioarcheologists who wish to add an additional method of age estimation to their existing tool kit.

APPENDIX A

TOOTH SAMPLE WITH AGE, SEX, SCANNER AND MANUAL SHADES

Age	Sex	Shade L	Shade A	Shade B	Manual Shade
15	M	64.09	-3.12	15.24	B3
16	M	89.32	-0.86	9.94	B2
19	M	79.75	-0.19	9.99	B1
20	M	85.32	0.59	11.59	B2
20	M	87	-0.17	6.6	B1
20	M	90.2	0.48	7.37	B1
20	M	84.77	1.75	12.68	B2
24	M	91.36	-0.91	11.66	B1
30	M	92.42	-0.62	9.5	B1
34	M	81.36	1.92	14.17	B2
35	M	85.22	1.14	19.18	A3.5
35	M	81.44	0.82	17.94	A3.5
41	M	87.09	1.72	16.78	B4
42	M	81.01	0.13	15.15	B2
45	M	85.78	0.99	11.8	B3
48	M	76.21	1.47	13.52	A4
48	M	88.92	-7.00E-02	9.6	B1
48	M	84.45	-0.16	12.27	B3
48	M	86.04	-0.26	11.62	B2
50	M	74.73	2.21	19.34	A3.5
53	M	76.39	0.75	11.67	B3
56	M	74.33	2.34	14.44	B3
58	M	82.72	0.59	10.38	B3
61	M	89.28	0.14	17.81	B3
62	M	91.49	-0.22	11.58	B3
63	M	86.78	0.05	10.61	B2
65	M	77.5	1.64	12.47	B3
65	M	80.89	1.26	16.29	C4
65	M	84.99	0.57	17.45	B4
69	M	87.84	0.34	10.09	B4
73	M	76.57	1.29	15.48	A4
73	M	87.51	0.71	14.08	B4
73	M	88.27	-7.00E-02	12.07	A3.5
83	M	78.23	1.98	19.93	A4

Age	Sex	Shade L	Shade A	Shade B	Manual Shade
12	F	73.41	1.44	13.65	C2
17	F	76.98	0.74	8.31	C1
17	F	76.93	0.82	10.21	B3
18	F	82.33	0.27	9.97	B1
18	F	82.59	-0.48	8.47	B1
18	F	80.67	-0.03	8.48	B1
18	F	79.68	0.59	9.97	B2
19	F	71.7	-0.35	12.57	B2
19	F	72.77	-0.15	15.56	B3
19	F	82.09	-0.7	10.47	C1
22	F	85.76	-0.9	10.74	B2
22	F	82.2	0.28	11.28	B3
22	F	81.88	-0.02	10.43	B3
22	F	83.41	0.27	12.01	B3
22	F	84.66	-0.16	9.64	B3
22	F	78.87	0.13	11.01	B1
24	F	82.45	-4.70E-01	16.2	B3
25	F	83.98	-0.67	12.83	B4
25	F	83.19	-0.08	12.91	A3.5
25	F	78.98	-0.04	13.83	B3
27	F	78.7	0.25	12.47	B2
27	F	85.18	-0.7	13.39	B2
27	F	76.17	0.14	15.03	B3
28	F	78.41	-1.05	7.51	B2
28	F	84.58	0.75	13.28	B4
34	F	85.16	-0.29	10.64	B4
38	F	83.58	-0.15	8.24	B2
38	F	87.51	0.11	8.98	B1
40	F	80.17	0.62	12.03	B3
42	F	85.86	0.34	9.73	B3
42	F	78.39	1	10.64	C4
53	F	79.25	0.43	10.29	B1
56	F	65.89	4.80E+00	20.88	A4
61	F	82.97	1.46	14.06	B4
61	F	82.42	-0.34	9.56	C2

APPENDIX B

TOOTH SAMPLE WITH AGE, YELLOWNESS INDICES, AND SEX

Age	Yellowness Index	Sex
15	33.15	M
16	18.42	M
19	20.84	M
20	23.41	M
20	13.05	M
20	14.63	M
20	26.53	M
24	21.1	M
30	17.32	M
34	30.33	M
35	37.3	M
35	36.13	M
41	33.14	M
42	30.65	M
45	24.04	M
48	30.2	M
48	18.5	M
48	24.22	M
48	22.59	M
50	42.4	M
53	25.82	M
56	33.42	M
58	21.68	M
61	32.94	M
62	21.48	M
63	20.85	M
65	27.93	M
65	33.76	M
65	33.94	M
69	19.93	M
73	33.68	M
73	27.5	M
73	23.11	M
83	41.83	M

Age	Yellowness Index	Sex
12	31.3	F
17	18.84	F
17	22.82	F
18	20.69	F
18	17.06	F
18	17.81	F
18	21.52	F
19	27.89	F
19	33.74	F
19	20.86	F
22	20.49	F
22	23.24	F
22	21.42	F
22	24.34	F
22	19.25	F
22	23.34	F
24	31.6	F
25	24.92	F
25	25.76	F
25	28.61	F
27	26.34	F
27	25.64	F
27	31.88	F
28	15.29	F
28	26.82	F
34	20.93	F
38	16.74	F
38	17.74	F
40	25.45	F
42	19.62	F
42	23.51	F
53	22.1	F
56	52.11	F
61	29.27	F
61	19.35	F
66	41.78	F
72	21.75	F
80	21.22	F

APPENDIX C

FIGURE OF PREDICTED AGE WITH LOWER AND UPPER RANGES

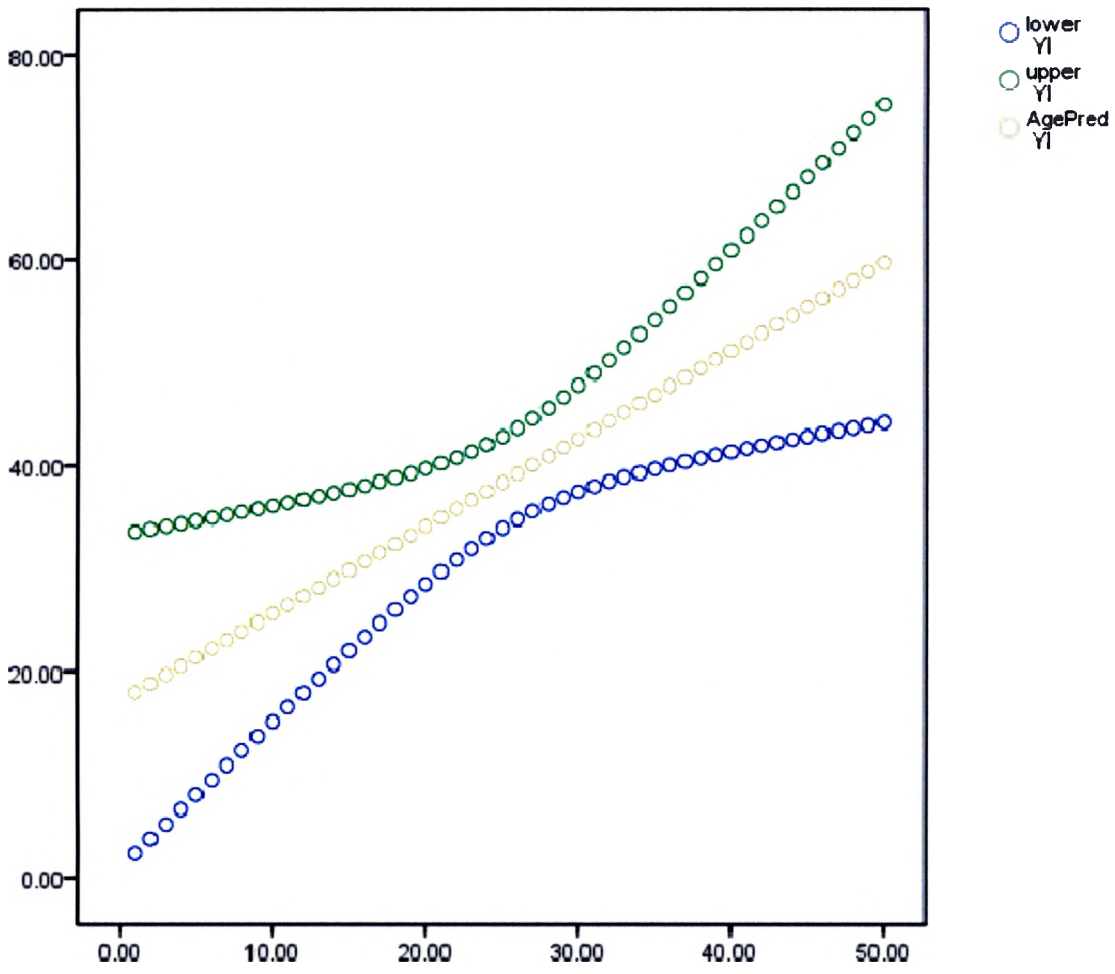

Figure 9. Predicted age with lower and upper ranges.

Note: Yellowness index on the x-axis and predicted age on the y-axis

APPENDIX D

TABLES FOR FIRST AND SECOND COLOR TRIALS: COLOR SCANNER AND MANUAL SHADE GUIDE

Table 5. First and Second Trial with Respective Yellowness Index and the Difference between Trials.

Specimen	First Trial YI	Second Trial YI	Difference*
9	17.32	22.11	4.79
11	37.3	36.98	-0.32
16	30.2	33.73	3.53
18	24.22	21.28	-2.94
41	21.52	19.23	-2.29
57	31.88	22.97	-8.91
59	26.82	35.07	8.25
61	16.74	29.23	12.49

*The difference is not significant

Table 6. First and Second Trial Utilizing the Manual Shade Guide.

Specimen	1st Trial	2nd Trial
9	B1	B1
11	A3.5	A1
16	B3	A1
18	B1	A1
41	B1	A1
57	B2	A1
59	B3	B1
61	B4	B1

Note: The letter-number combination is the shade given by the Vita® manual guide

APPENDIX E

TABLE WITH PREDICTED AGES AND LOWER AND UPPER RANGES FOR A YELLOWNESS INDEX RANGING FROM 1 TO 50

YI	PREDICTED AGE	LOWER	UPPER
1	17.95	2.31	33.59
2	18.8	3.74	33.86
3	19.65	5.17	34.13
4	20.5	6.6	34.41
5	21.36	8.03	34.68
6	22.21	9.45	34.96
7	23.06	10.87	35.24
8	23.91	12.29	35.53
9	24.76	13.7	35.82
10	25.61	15.1	36.12
11	26.46	16.5	36.42
12	27.31	17.89	36.73
13	28.16	19.28	37.05
14	29.01	20.65	37.38
15	29.87	22.01	37.72
16	30.72	23.36	38.08
17	31.57	24.68	38.45
18	32.42	25.99	38.85
19	33.27	27.26	39.27
20	34.12	28.51	39.73
21	34.97	29.71	40.23
22	35.82	30.86	40.78
23	36.67	31.96	41.39
24	37.52	32.98	42.07
25	38.38	33.93	42.82
26	39.23	34.79	43.66
27	40.08	35.58	44.58
28	40.93	36.28	45.58
29	41.78	36.91	46.65
30	42.63	37.48	47.78
31	43.48	37.99	48.97
32	44.33	38.47	50.2
33	45.18	38.9	51.47
34	46.03	39.31	52.76
35	46.89	39.69	54.08
36	47.74	40.05	55.42
37	48.59	40.4	56.78
38	49.44	40.73	58.15
39	50.29	41.05	59.53

40	51.14	41.37	60.91
41	51.99	41.67	62.31
42	52.84	41.97	63.72
43	53.69	42.26	65.12
44	54.54	42.55	66.54
45	55.4	42.83	67.96
46	56.25	43.11	69.38
47	57.1	43.39	70.8
48	57.95	43.66	72.23
49	58.8	43.94	73.66
50	59.65	44.21	75.09

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