G3 The Correlation Between Root Translucency, Calcium Hydroxyapatite Content, and DNA Preservation in Teeth for Unidentified Human Remains (UHR) Investigations

Paul M. Yount, University of New Haven, West Haven, CT 06516-2048; Virginia M. Maxwell, DPhil, University of New Haven, West Haven, CT 06516; James M. Lewis, DMD, Madison, AL 35758; Thomas J. David, DDS, Marietta, GA 30062; Murray K. Marks, PhD, University of Tennessee, Knoxville, TN 37920; Paula Brumit, DDS, Nocona, TX 76255; Angie Ambers, PhD, Henry C. Lee Institute of Forensic Science, West Haven, CT 06516*

Learning Overview: The goal of this presentation is to provide attendees with an understanding of tooth microstructure, an overview of the pattern of increased root translucency with chronological age, a summary of diagenetic processes that impact the integrity of a tooth's mineral matrix, and an assessment of the potential correlation between these variables and DNA preservation.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating that knowledge of tooth microstructure—and understanding changes that occur to the mineralized matrix over time—can guide scientists in making informed decisions regarding optimal sampling from highly decomposed or skeletonized human remains for forensic DNA testing.

Human teeth are composed of three hard tissue layers (enamel, dentin, and cementum) that surround an inner chamber filled with vascularized soft tissue (pulp). Although pulp (like most soft tissues) is subject to rapid decomposition postmortem, hard tissues are more resistant to environmental insults. The endogenous genetic material protected by a tooth's rigid, mineralized hard tissues is often targeted for forensic DNA testing in missing persons and UHR investigations. One particular component of teeth—the inorganic mineral matrix (calcium hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$)—is purported to play a major role in postmortem DNA preservation. Numerous research studies demonstrate that electrostatic interactions occur between positively charged calcium residues in hydroxyapatite and negatively charged phosphate groups in the DNA backbone. These interactions with the hydroxyapatite matrix are believed to protect DNA from damage. However, although hydroxyapatite is thermodynamically stable under antemortem physiological conditions, changes to this mineral matrix occur over time as a decedent's remains are exposed to destructive environmental factors such as heat, humidity, ultraviolet light, microbial attack, and acidic soil. Alterations to the hydroxyapatite mineral matrix occur during a process called diagenesis and can involve substitution of calcium ions (Ca^{2+}) with carbonate (CO_3^{2-}) residues. When this occurs, DNA molecules dissociate from the mineral matrix and become more susceptible to damage.

The goal of this research is to determine if an established, validated forensic odontological method of age estimation for decomposed or skeletonized human remains (i.e., evaluation of tooth root translucency) can also be a reliable predictive parameter for downstream DNA typing success. Approximately 70% of dentin in the roots of human teeth is composed of hydroxyapatite crystals, and it has been asserted that there is a direct relationship between increased tooth root translucency and decalcification of hydroxyapatite. There may also be a direct relationship between tooth root translucency and DNA preservation within a tooth's microstructure. This research involved: (1) microscopic examination, measurement, and photo-documentation of root dentin translucency in intact and sectioned teeth (n=54) from human subjects of known chronological age; (2) X-ray Diffraction (XRD) of pulverized tooth powder to assess the degree of carbonate substitution within the calcium hydroxyapatite matrix of each tooth; and (3) DNA extraction from tooth matrices to assess the correlation between degree of root dentin translucency and the quantity/quality of the DNA recovered.

The Crystallinity Index (CI) of each tooth was calculated using XRD data. Analyses for this sample set (n=54) indicate that the CI decreases as the existence of carbonated (calcium-deficient) hydroxyapatite increases; similarly, a general trend of lower CI values were observed for teeth exhibiting greater degrees of root translucency and with increasing chronological age of the donor. Research is ongoing to generate additional data to determine if a direct correlation indeed exists between higher levels of carbonated (calcium-deficient) hydroxyapatite and decreased DNA recovery. Although weight-bearing long bones (femora, tibiae) and molar teeth are the current preferred skeletal sample types for forensic DNA testing (due to the protection afforded by the compactness and rigidity of their macrostructure), changes to a bone's or tooth's microstructure should also be considered in the development of Standard Operating Procedures (SOPs) or "best practices" models for human remains identification. Molecular changes to tooth microstructure due to the chronological aging process and postmortem environmental exposure—and the correlation between these changes to the quantity/quality of recoverable endogenous DNA—has not yet been the subject of investigation. Empirical data from this study could provide the framework to develop general guidelines for forensic odontologists in the field to use in evaluating a tooth's potential for DNA profiling success based on root translucency.

Tooth Root Translucency, Hydroxyapatite, Unidentified Human Remains

*Presenting Author