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Compositional Analysis of Human Skeletal Samples Using Raman Spectroscopy and Correlation to DNA Recovery

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The purpose of this study was to determine if Raman spectroscopy can be used as a rapid and non-destructive screening tool to predict DNA recovery potential for bone, and to explore the correlation between the compositional structure of bone and preservation of DNA. The two primary structural components of bone are calcium hydroxyapatite and collagen.[1,3,4,6,7-11] It was proposed that by analyzing bone using Raman spectroscopy, the presence, state, and distribution of hydroxyapatite and collagen can be measured, which may be useful in predicting if the sample is viable for genetic testing.

Currently, a reliable screening method for DNA extraction from bones does not exist. Previous studies demonstrated that certain skeletal elements (e.g., weight-bearing long bones, molar teeth) are preferred for forensic DNA testing; however, once these skeletal elements are selected, the sampling technique is blind.[1,2,6,9] The heterogeneous nature of bone diagenesis poses a challenge in determining which region(s) of the bone contain the most intact microstructure (and presumably the most DNA).[5] With Raman spectroscopy, it was proposed that the entire diaphysis can be sequentially scanned, and data collected from each scanned region can provide qualitative and quantitative information regarding bone microstructure (i.e., calcium hydroxyapatite, collagen), which may correlate to the quality and quantity of DNA present in each region. Forensic DNA analysis of human remains is time-consuming, labor-intensive, and expensive. This pre-screening tool has the potential to provide analysts with preliminary data to make informed decisions on the location of optimal sampling sites along the diaphysis of a long bone, thereby reducing the amount of bone consumed and destroyed in the testing process. Raman spectroscopy could offer a robust and reliable method to expedite and streamline the processing of unidentified skeletal remains in casework.

As part of my SURF project, I conducted a comprehensive survey containing a variety of questions to gauge the thoughts of participants in the forensic science community. Participants' insights and expertise provided guidance in my quest to develop better methods for identification of human remains. Results from the survey have raised awareness of possible limitations, but respondents' enthusiasm also indicates that this research could potentially change the field of forensic DNA casework as it pertains to unidentified human remains investigations. Because of SURF, I am better prepared to continue this research for my Honors Thesis.

My Honors Thesis will involve the analysis of results to assess the relationship between the decomposition state of bone microstructure and DNA recovery, and to determine the reliability of Raman spectroscopy as a screening method. Preliminary research and survey results suggest that higher quantities of DNA (and better quality DNA) can be recovered from bone samples containing preserved hydroxyapatite and collagen versus samples taken from regions of the same bone that are in a more advanced state of diagenesis. DNA quantification using the Quantifiler™ Trio Human DNA Quantification Kit and the QuantStudio5 Real-Time PCR System will provide data on total DNA recovery from each scanned bone section, as well as a quality index to indicate the degree of DNA degradation present.

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