H28 XRD and FTIR: A Diagnostic Tool to Determine Whether or Not a DNA Profile Can Be Successfully Generated From Heat Treated Bone Prior to DNA Extraction

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After attending this presentation, attendees will understand the effects that temperature has on the material and physical properties of bone and their subsequent correlation with DNA amplification and typing.

This presentation will impact the forensic science community by demonstrating that XRD and FTIR can be used to predict whether or not a DNA profile can be obtained from heat treated bone, prior to DNA extraction.

Deoxyribonucleic Acid (DNA) from skeletal tissue can be invaluable, as it is often the only available source of information for individual identification. Bone is considered a tri-phase composite, made up from collagen (protein), hydroxyapatite (mineral) and water. Unlike soft tissue, osteocyte cells, which are the source of DNA in bone, can be protected from external environmental factors by the protein-mineral matrix. However, when skeletal tissue is exposed to extreme conditions, including high temperature or long periods of submersion in water, the potential for generating a useful 'DNA profile' can be adversely affected. Although heating has been shown to cause damage to DNA through oxidation and hydrolysis, there has been very little detailed research into the amplification of DNA from bone compromised by heat. Studies have often lacked appropriate controls or were based on case studies where accurate environmental parameters, such as temperature and/or the exposure period were unknown.

To date, DNA-based identification from badly decomposed remains has often been reliant on the use of mitochondrial DNA (mtDNA), which exists within cells in much higher abundance than nuclear DNA (nDNA). However, mtDNA is only inherited through the maternal line. This reduces the usefulness of identifications made using mtDNA, as matrilineal relatives cannot be distinguished from one another. nDNA profiling of such samples would greatly improve the specificity of identification.

By comparison to soft tissue, the protocols for extracting DNA from bone are often time-consuming and laborious, usually requiring a demineralization step prior to extraction, which can take a number of days. In the case of skeletal tissue that has been compromised by environmental insults, even more time can be 'wasted' as not all the samples extracted will successfully produce a useable DNA profile. In individual cases, time may not be a critical issue. However, in cases where hundreds or thousands of samples are processed, such as in the World Trade Centre attacks (2001) where 13,000 samples were processed, a large amount of time and money was wasted on unsuccessful profiles.

The goal of this project is to develop a diagnostic tool that can reliably predict the likelihood of successfully obtaining a useable DNA profile from a compromised skeletal tissue sample. Mechanical properties, such as hardness and elastic modulus and material properties, such as collagen content and mineral content, of bone that has been compromised through heat treatment, will be correlated with the results of nDNA profiling.

Using *Bos taurus* as an animal model, sections of femora were heat treated, using a muffle furnace, at 50 °C intervals to 600 °C for periods of one and two hours. Post treatment, Vicker's hardness was recorded, and the Crystallinity Index (CI) of samples' mineral content measured using X-ray diffraction (XRD) and Fourier Transform Infrared imaging Spectroscopy (FTIR). These properties were then correlated with the presence of detectable nDNA as determined by the PCR amplification of 100, 300 and 500 base pair fragments and STR based DNA profiling.

The results of this study show that the characteristics of bone change when heat-treated. XRD and FTIR findings showed that the crystallinity of hydroxyapatite increased with temperature, while Vicker's hardness was seen to increase until 200 °C then suddenly decrease before a rapid increase at 300 °C. The ability to amplify DNA and hence obtain a DNA profile genotyping was lost above 200 °C. Using logistic regression both FTIR and XRD were shown to produce a CI value that could be used to successfully predict whether or not a DNA profile could be obtained.

The ultimate goal of this study is to produce a diagnostic tool that can be performed quickly and cheaply, in order to determine whether subsequent DNA profiling is a viable/cost effective option for identification purposes. Such a tool would not only save time and money, but would increase the overall success rate of DNA profiling.

Predicting DNA, HeatTreated Bone, Temperature

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