A96 The Application of X-Ray Photoelectron Spectroscopy (XPS) to Examine the Surface Chemistry of Cancellous Bone and Medullary Contents to Refine Bone Sample Selection for Nuclear DNA Analysis

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Learning Overview: The goals of this presentation are to: (1) describe the use of XPS for surface chemistry analysis of cancellous bone tissue and medullary contents as a novel approach to decipher biological tissues from diagenetic infiltrations (e.g., soil) among trabeculae; and (2) present the methodology as a potential tool for refining bone sample selection for nuclear DNA analysis.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by improving current understandings of the relationship between nuclear DNA yield and cancellous bone and medullary content chemical composition, thus informing bone-sample selection for nuclear DNA analysis in a forensic context.

Research by Andronowski and colleagues investigated whether differences in 3D bone microstructure may be used to explain differential nuclear DNA yield among bone tissue types (cortical and cancellous bone), with a focus on osteocytes and the 3D quantification of their associated lacunar spaces.[1] Identifying which bone tissue type(s) and/or bone envelope(s) (e.g., periosteal, intracortical, and endosteal) provide the highest nuclear DNA yields will further inform current bone-sampling protocols for human identification and limit the amount of bone tissue necessary for DNA analysis. Results demonstrated that osteocyte lacunar density values were independent of nuclear DNA yield, suggesting an alternative explanation for the higher nuclear DNA yields from bones with high quantities of cancellous bone. A plausible explanation focuses on remnants of soft tissue between trabeculae observed using Synchrotron Radiation-based micro-Computed Tomography (SR micro-CT). Though soft tissue was not present on the surface of the bones, 3D scans consistently revealed probable soft tissues within the medullary cavities of skeletal elements with high cancellous content.

It is hypothesized that residual soft tissues, which likely include endosteum and osteological lining cells, contributed to the higher nuclear DNA yields from cancellous bone. The application of a novel XPS approach has the potential to explain why nuclear DNA yield rates differed among bone tissue types in the specimens used in Andronowski and colleagues original study.[1]

The surface chemical composition of cancellous bone specimens and their medullary contents were assessed (n=24) using XPS. Skeletal elements were retrieved from six adult males at increasing postmortem intervals and included the talus, medial cuneiform, patella, and sixth rib. Three Regions Of Interest (ROIs) per specimen were obtained and comparatively analyzed. All XPS experiments were conducted using a Kratos AXIS Supra system at the Saskatchewan Structural Sciences Centre (SSSC). This system is equipped with a 500mm Rowland circle monochromated Al K-α (1486.6 eV) source and combined Hemi-Spherical Analyzer (HSA) and Spherical Mirror Analyzer (SMA). A spot size of hybrid slot (300x700) microns was used. All survey scan spectra were collected in the 5eV–1,200eV binding energy range in 1eV steps with a pass energy of 160eV. An accelerating voltage of 15keV and an emission current of 15mA was used for the analysis. The data was processed using CasaXPS (version 2.3.18PR1.0).

The chemical composition of the surface of cancellous bone and associated medullary contents revealed high percentages of carbon and oxygen (12.33-30.44% and 56.70-85.29%, respectively), which is consistent with biological material. Trace amounts of phosphorous (0.39%-3.98%), calcium (0.34%-6.45%), and nitrogen (1.25%-9.39%) were further detected in all specimens. Sodium, sulfur, and iron were detected in specimens from one individual, albeit in almost undetectable amounts (<0.5%). Most individuals were recovered from the outdoor environment following three and nine months of natural decomposition. However, one individual was placed in a burial and recovered after nearly three years. This individual's bones were visibly soil-stained and revealed trace amounts of silicon (0.71%-1.69%) and aluminum (0.97%). These inorganic elements are the major components of common soil and their presence is likely the result of diagenetic activity. Results indicate that intertrabecular spaces within cancellous bone provide a safe reservoir for soft tissue, suggesting that areas of high cancellous content are a preferential location for nuclear DNA extraction

Results of this work have broader applications as they offer promise for the development of a refined method for identifying the skeletal elements most likely to yield nuclear DNA. The procurement of small, primarily cancellous bones with associated soft tissues within marrow spaces should be preferentially sampled and no longer dismissed as potential DNA sources in favor of cortical bone tissue.

Reference(s):

1. Andronowski J.M., Mundorff A.Z., Pratt I.V., Davoren J.M., Cooper D.M.L. 2017. Evaluating Differential Nuclear DNA Yield Rates and Osteocyte Numbers Among Human Bone Tissue Types: A Synchrotron Micro-CT Approach. *Forensic Science International: Genetics*. (28): 211-218.

XPS, Nuclear DNA, Cancellous Bone