A25 Evaluating Differential Nuclear DNA Yield Rates Among Human Bone Tissue Types: A Synchrotron Radiation Micro-Computed Tomography (SR micro-CT) Approach

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After attending this presentation, attendees will understand whether differences in bone microstructure may be used to explain differential nuclear DNA yield among bone tissue types.

This presentation will impact the forensic science community by improving current understanding of the relationship between nuclear DNA yield and osteocyte lacunar abundance, thus informing bone-sample selection for nuclear DNA analysis in a forensic context.

Molecular human identification has conventionally focused on DNA sampling from dense, weight-bearing cortical bone tissue from femora or tibiae. A comparison of skeletal elements from three contemporary individuals demonstrated that elements with high quantities of cancellous bone yielded nuclear DNA at the highest rates, suggesting that preferentially sampling cortical bone is suboptimal.[1] Despite these findings, the reason for the differential DNA yields between cortical and cancellous bone tissues remains unknown. Evidence from bone microarchitecture may help explain this variation and enrich the understanding of bone microstructural features.

The primary goal of this research is to determine whether the 3D examination of osteocytes and the quantification of their associated cellular spaces (lacunae) can be used to explain differential nuclear DNA yield among cortical and cancellous bone tissue types. Osteocytes and other bone cells are recognized as housing DNA in bone tissue, thus examining the density of their lacunae may explain why nuclear DNA yield rates differ among bone tissue types.

Methods included visualizing and quantifying osteocyte lacunae using SR micro-CT at the Canadian Light Source synchrotron facility in Saskatchewan, Canada. Forty-three bones were selected for SR micro-CT imaging from the 55 elements per skeleton sampled for DNA by Mundorff and Davoren. Representatives from each skeletal element type were chosen and bones from the left side only were sampled. Regions Of Interest (ROIs) from cortical and cancellous bone tissues (n=129) were comparatively analyzed.

Osteocyte lacunae were separated from the high-density bone using global thresholding and segmentation. Despeckling was conducted to remove noise (structures <10μm^3). Features >2,000μm^3 were assumed to be canals and remaining structures were designated as lacunae. Standard nomenclatures for lacunar indices were applied for the analysis of 3D lacunar parameters within the ROIs. The variables measured included: Total ROI Volume (TV), total Canal Volume within ROI (Ca.V), average Canal Diameter (Ca.Dm), total Number of Lacunae (N.Lc), and average Lacunar Volume (Lc.V). To determine lacunar density per mm^3 (N.Lc/BV), Bone Volume (BV) was calculated as TV minus Ca.V (TV-Ca.V). BV for cancellous bone ROIs was calculated as TV minus the volume of the marrow spaces.

Statistical analyses tested the primary hypothesis that the abundance and density of bone's cellular spaces vary between cortical and cancellous bone tissue types. Bones identified by Mundorff and Davoren to yield more DNA

per mass of sample were directly correlated against the number of lacunae and osteocyte lacunar density data.

Results demonstrated that osteocyte lacunar abundance and density varied between cortical and cancellous bone tissue types, with cortical bone ROIs containing a higher lacunar abundance and density than cancellous bone. The osteocyte lacunar density values are independent of nuclear DNA yield, suggesting an alternative explanation for the higher nuclear DNA yields from predominantly cancellous bones.

At the time of this writing, this work represents the first examination of inter-element variation in osteocyte lacunar properties from cortical and cancellous bone tissues in various human skeletal elements. The use of SR micro-CT allowed for a scale of analysis that revealed a high range of variation in lacunar abundance in both tissue types and soft tissue remnants within marrow spaces. As such, it is hypothesized that soft tissue remnants, only observed via SR micro-CT, within the medullary cavities of primarily cancellous skeletal elements are driving the high nuclear DNA yields.

The results have implications that improve current understandings of the relationship between nuclear DNA yield and osteocyte lacunar abundance, and normal variation of osteocyte lacunar parameters. Results of this work also have broader applications as they offer promise for the development of a refined method for identifying the bone tissue type 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. Mundorff A.Z., Davoren J.M. 2014. Examination of DNA yield rates for different skeletal elements at increasing post mortem intervals. *Forensic Sci Int: Genetics.* (8):55-63.

3D Imaging, Nuclear DNA, Osteocyte Lacunae