A134 The Use of Laser Scanning Confocal Microscopy in Distinguishing Between Peri-Mortem Trauma and Postmortem Damage Using Histotaphonomic and Histochemical Techniques

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After attending this presentation, attendees will better understand the techniques used to implement laser scanning confocal microscopy in assessing whether bone damage occurred peri- or postmortem. While this pilot project used bone damaged approximately 24 hours after death and five years after death, this presentation will demonstrate that the methods introduced can be used to distinguish peri-mortem from early postmortem damage occurring just days after death.

This presentation will impact the forensic science community by adding to the body of knowledge on modern histological techniques used in forensic science and anthropology. This study will also add to the body of knowledge used in timing bone damage as either peri-mortem or postmortem. Present techniques in timing bone damage relies on macroscopic variations, particularly with regard to break margin morphology and color variation. By understanding the microstructural and histochemical variation between bone fractured at the time of death and bone damaged after death, one can better reconstruct the death and depositional events pertaining to human remains.

The purpose of this research was to determine if it is possible to distinguish bone damaged peri-mortem from damage occurring five years postmortem using microstructural and histochemical techniques. This research used laser-scanning confocal microscopy to quantify the degradation of two non-collagenous proteins, osteocalcin and osteopontin, as well as the presence of osteoclasts at margins of peri-mortem fractures and postmortem breaks. Additionally, this project analyzed the microstructural aspect of the fracture and break margins using qualitative and semi-quantitative methods and further quantified the natural autofluorescence.

The peri-mortem sample for this research derived from bone sections excised from commercially reared domestic pig (*Sus scrofa*), while the postmortem sample originated from elements from purpose-bred *S. scrofa* left over from a decompositional research study conducted five years ago with a known date and time of death, and documented environmental factors. The break margins were excised and sectioned to 10µm using a Buehler® IsoMet® low-speed saw. After sectioning, the samples were divided into three approximately equal sections and differentially stained for visualization of the specific proteins and the osteoclasts. The "protein sections" were stained using basic fuchsin as a base stain and specific Enzyme-Linked Immuno-Sorbent Assay (ELISA) kits to quantify their differential presence. The osteoclast sections were stained using Alexa Fluor® 488 and an osteoclast antibody kit. Imaging was conducted using a Carl Zeiss™ LSM-510 META® Laser Scanning Confocal and Multi-Photon Microscope with a 4',6-Diamidino-2-Phenylindole/Green Fluorescent Protein (DAPI/GFP) base setting and an Argon 2 and Helium/Neon (HeNe) 1 and 2 lasers. Image acquisition was achieved using the Carl Zeiss™ Zen 2009® Image software that was tied to the microscope, with image analyses conducted using ImageJ v1.50i, and Volocity® v6.3. A one-way Analysis of Variance (ANOVA) was conducted, with the levels of osteopontin, osteocalcin, and the ratio between the two set as the dependent variable, and the subject class as the independent variable. Further, a linear regression analysis was conducted to determine the relationship of osteopontin:osteocalcin ratio to time. A one-way ANOVA was conducted to evaluate the variance between the levels of osteoclasts present in the "peri-mortem" and

postmortem samples. Lastly, a 3D analysis was conducted from the z-stack results in order to ascertain the surface structural variation between the two sample groups.

The results of this study found that laser scanning confocal microscopy can be used to distinguish between the peri-mortem trauma group and the postmortem group. In particular, higher protein levels were seen in the peri-mortem trauma group as opposed to the postmortem, and a higher osteoclast level was also observed. Additionally, the postmortem group generated a higher amount of natural autofluorescence. Lastly, the margin of the peri-mortem group appeared more jagged than the postmortem group.

Laser Scanning Confocal Micros, Taphonomy, Skeletal Trauma