A9 Analysis of Osteon Pull-Out and Collagen Degradation to Establish Fracture Timing

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After attending this presentation, attendees will understand the phenomenon of osteon pull-out, the effects of collagen degradation on pull-out morphology, and the utility of these morphological changes in distinguishing peri-mortem versus postmortem traumatic events.

This presentation will impact the forensic science community by documenting morphological differences between fracture-induced osteon pull-out during the peri-mortem and postmortem intervals.

It is hypothesized that the histological appearance of peri-mortem fractures exhibits greater degrees of osteon pull-out due to elasticity imparted by Type I collagen. As the organic collagen fibers degrade postmortem, the characteristics of pull-out differ due to the concomitant reduction in elasticity. This study provides a basis for further investigation into the use of osteon pull-out and histological morphology to distinguish between peri-mortem and postmortem fractures.

Fractures may exhibit characteristic features of peri-mortem injuries as long as they occur while the bone is reasonably elastic. The extended time during which bone maintains its elasticity after death creates an exaggerated peri-mortem interval in bone and limits the capacity of macroscopic fracture evaluation to delimit peri-mortem versus postmortem events. Fracture morphology associated with peri-mortem events is related to moisture content, mineral matrix ratios, and osteon fracture patterns.[1,2] This study evaluates fracture microstructure with Scanning Electron Microscopy (SEM) and correlates the histologic features with Accumulated Degree Days (ADD) and collagen degradation.

Thirty-two foot bones (16 metatarsals and 16 phalanges) from a non-reproducing pig (*Sus scrofa)* were obtained on the same day of slaughter. One metatarsal and one phalanx were selected to represent an ADD of 0. Cross-sectional samples were harvested from these two bones at mid-diaphysis with an autopsy saw. These samples were then washed in a phosphate buffer solution and fractured with a quick snapping force. Fractured pieces were fixed using osmium tetroxide, ethanol, and hexamethyldisilazane, then mounted on SEM stubs and sputter coated with gold. The remaining samples were catalogued, tied in porous cloth, and placed in a cage to decompose in an outdoor setting. ADD was recorded using daily temperature averages for Middlesboro, KY, with a minimum threshold temperature of 57°F. Samples spanning a postmortem interval of 0-1,043 ADD were fractured at regular intervals. Histologic morphology was examined with SEM, and postmortem collagen degradation was analyzed with mass spectrometry. Bone material was prepped according to established analytical platform protocols and analyzed using high-resolution (0.2ppm-3ppm mass error) data acquisition with an Orbitrap™ mass spectrometer.[3,4]

The mass spectrometry analysis of collagen revealed expected degradation with advancing ADD. The average OD_{550} of the two initial specimens resulted in an absorbance of 0.36 compared to the final specimen, which yielded an absorbance of 0.23, a difference of approximately 36.1%. Analysis of SEM images revealed significant differences in osteon appearance occurring at approximately 70% of initial collagen levels. Samples containing 70% or less of intact collagen demonstrated marked structural failure along canaliculi channels compared to samples with >70% of intact collagen. This preliminary data suggests that as collagen degrades with increasing ADD, the osteon pull-out mechanism becomes inadequate at dispersing force, and structural failure occurs along canaliculi channels, which may be a source of structural weakness. SEM analysis of fracture morphology coupled with mass spectrometry quantification of collagen degradation may offer a method for more precise interpretation of fracture timing.

Reference(s):

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Bone Histology, Scanning Electron Microscopy, Skeletal Trauma

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