Degradation of Osteocalcin and Osteopontin for Use in Postmortem Interval Estimation During the Early Postmortem Period

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25 March 2024 Anatomy Connected, American Association for Anatomy, Toronto, ON

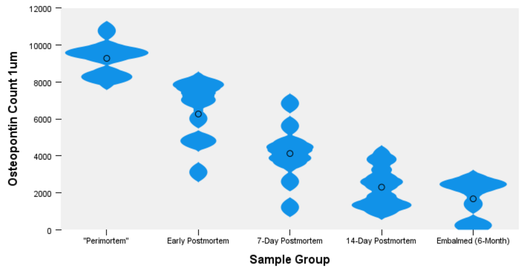
One of the more difficult tasks biological anthropologists and pathologists are tasked with is assessing the postmortem interval in early skeletonized remains. During this period, the assessed postmortem interval can be rather wide, lasting weeks to months in range. Often this assessment is made on the decomposition of soft tissue and the degradation of the skeletal elements.

In the extra cellular matrix (ECM) two non-collagenous proteins: an acidic glycosylated phosphoprotein known as osteopontin (OPN), and a γ-carboxylated protein, osteocalcin (OC), connects the organic and inorganic matrices. OPN is primarily derived from osteoclasts, acting as an anchor for these clasts to the bone matrix, though also has genesis in osteoblasts and osteocytes. OC, on the other hand, is predominately produced by osteoblasts, though like OPN, also has genesis with osteocytes as well.

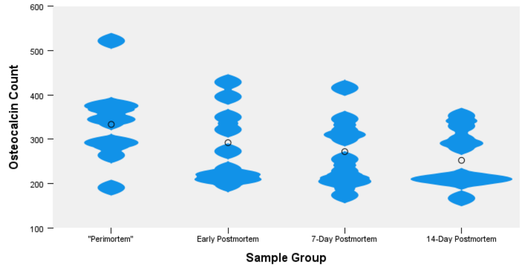
Once the metabolic functions have ceased following somatic death, the complexes binding the OPN and OC along with the osteonectin (which binds to the Type III, Type VI, and Type V collagen) begin to degrade. One of the quantitative portions of this research assesses the differential degradation of OPN to OC. The role of OC in diagenesis has been reported in the osteoarcheological literature, but the lack of information regarding the role of OP in diagenesis suggests that the two degrade at different rates with OP decomposing faster than OC (1-4). In 2014, Boaks and colleagues (5) found that collagenous proteins degrade at consistent rate. If the same holds true for the non-collagenous proteins, then mapping and quantifying the degradation of OC and OPN can help determine the postmortem interval (PMI).

Methodology

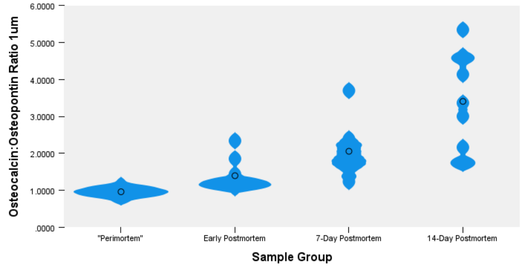
- Iliac bone
- 25 Individuals
  - 9 Biopsy (Perimortem)
  - 9 Cadaveric (Postmortem)
    - <48hrs (Early Postmortem)
    - 7-Days Postmortem
    - 14-Days Postmortem
  - 7 Embalmed anatomical
- Thin sectioned to 100µm
- Labelled
  - Osteocalcin primary antibody
  - Osteopontin primary antibody
  - IgG Alexafluor 555 secondary antibody



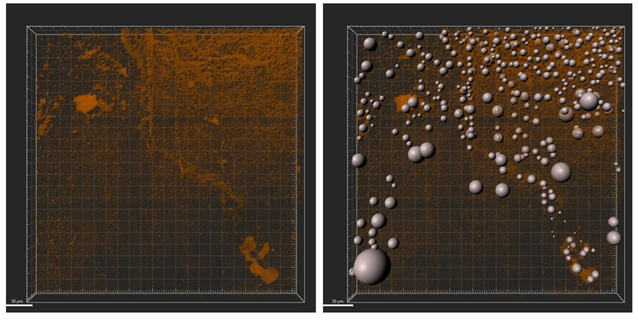
Mean Osteopontin Complex Count



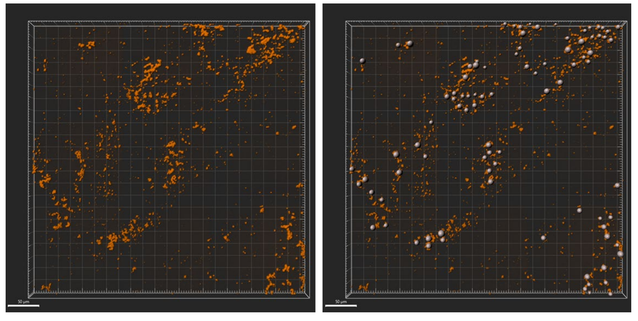
Mean Osteocalcin Complex Count



Ratio of Osteopontin : Osteocalcin Complex Count



Perimortem Osteopontin locations



14-Day Postmortem Osteopontin locations

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Methodology

Imaging and Analysis

- Carl Zeiss LSM800 Laser Scanning Confocal Microscope
- 400x Total magnification per frame
  - 9 frames per image
  - Total tile array 447.23µm^2
- 50 Optical slices
- AF555 Channel setting
  - 0.2% Power
  - 30µm Pinhole

Analysis

- Bitplane™ Imaris® v.9
  - Spot feature
  - 0.5µm threshold
- Data Analysis
  - One-Way ANOVA
  - Pearson Correlation Test
  - Logistic Regression
- Osteopontin
- Osteocalcin
- Osteopontin : Osteocalcin

Results

The results of this study found that the osteocalcin remained relatively stable throughout ($p=0.198$, $f=1.649$). The osteopontin count saw significant decrease, and that there was a strong correlation with these changes ($p<0.001$, $f=24.1$; $r=-0.832$). The results of the logistic regression likewise demonstrated a significant result with an r2 value of 0.723. When the 6-month sample is included the results of the ANOVA was $p<0.001$, $f=26.836$, and the Pearson correlation being $r=-0.848$. The pixel intensity and standard deviations analyses found a significant variation in the standard deviations, while the mean intensity was not found to be significant ($p=0.097$, $f=2.120$). The results of the ratio analysis were also found to be significant at $p=0.035$. When taken all together, the results of this study have demonstrated that the degradation of osteopontin, and the ratio of osteocalcin to osteopontin can potentially be used as a means of assessing the postmortem interval in bone from remains within a 6-month postmortem window.

1. Buckley M, Anderung C, Penkman K, Raney BJ, Götherström A, Thomas-Oates J, Collins MJ. Comparing the survival of osteocalcin and mtDNA in archaeological bone from four European sites. Journal of Archaeological Science. 2008;35:1756-64.
2. Collins MJ, Gernaey AM, Nielsen-Marsh CM, Vermeer C, Westbroek P. Slow rates of degradation of osteocalcin: green light for fossil bone protein? Geology. 2000;28(12):1139-42.
3. Ostrom PH, Gandhi H, Strahler JR, Walker AK, Andrews PC, Leykam J, Stafford TW, Kelly RL, Walker DN, Buckley M, Humpula J. Unraveling the sequence and structure of the protein osteocalcin from a 42 ka fossil horse. Geochimica et Cosmochimica Acta. 2006;70(8).
4. Smith CI, Craig OE, Prigodich RV, Nielsen-Marsh C, Jans MME, Vermeer C, Collins MJ. Diagenesis and survival of osteocalcin in archaeological bone. Journal of Archaeological Science. 2005;32:105-13.
5. Boaks A, Siwek D, Mortazavi F. Temporal degradation of bone collagen: a histochemical approach. Forensic Sci Int. 2014;240:104-10.