When Bone Lights Up: A Novel Way of Labeling Proteins and Cells and Its Potential Uses

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In forensic anthropological research, histology has been a staple mode for analyses. Much of this research, however, has been limited to transmission white-light microscopy. When converting to laser-scanning confocal microscopy, additional analyses can be conducted. This poster will focus on a method which can be used in laser scanning confocal microscopy.

When examining bone, bone is not a singular material but is rather made up of constituent parts, specifically hydroxyapatite and collagen. However, when we go deeper into bone, we see that it is made up of additional elements, including various proteins and cells. Two specific bone proteins, osteocalcin and osteopontin, are necessary for the binding of the hydroxyapatite to the collagen. Specifically, osteocalcin binds hydroxyapatite to osteopontin, and osteopontin binds osteocalcin to type I collagen. In addition to these bone proteins, various cells play a role in the development and maintenance of bone. This poster will also touch on the labeling and imaging of bone cells in undecalcified samples.

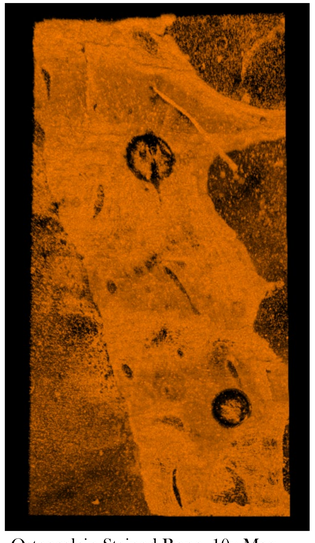
While labeling of proteins is nothing new in bone and mineral research, its use in forensic science is still somewhat novel. Further, almost universally, such labeling is conducted using decalcified bone; a process by which the hydroxyapatite has been chemically removed leaving the collagen matrix behind. The method presented here uses undecalcified bone and can be applied to forensic samples with limited pre-treatment before labeling and imaging. The same can be said for the osteoclast imaging, though admittedly the common research protocol (Coxon, 1) does not mention the decalcification process, thus it is believed that undecalcified bone was used.

Methodology

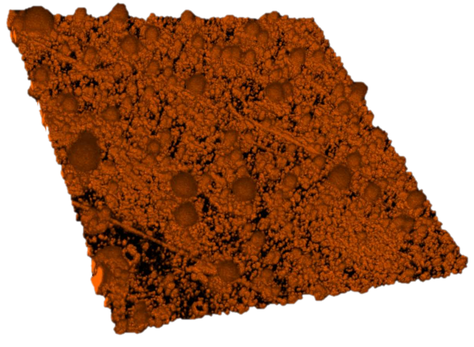
Osteoclast Labeling

The osteoclast labeling is a relatively simple methodology with only a few steps and a lot of waiting. Once labeled, this research has found that, though designed for osteoclasts, this method also works well with osteoblasts and even osteocytes within lacunae. It should be noted that with the AlexaFluor 488 it must be handled in a relatively dark room as the label is light sensitive and can "wear out" and become unusable after a while.

1) Incubate for 15 mins in PBS solution of 0.5% (v/v) Triton X-100
2) Stain with 10% Bovine Serum with MitoTracker Red FM Dye in PBS and allow to rest for 1 hour
3) Highlight with Goat anti-rabbit IgG AlexaFluor 488
4) Allow to air dry in dark room 40 1 hour
5) Rinse in PBS solution
6) Allow to dry 24-48 hours in dark area
7) Place cover slip with non-fluorescent mounting medium

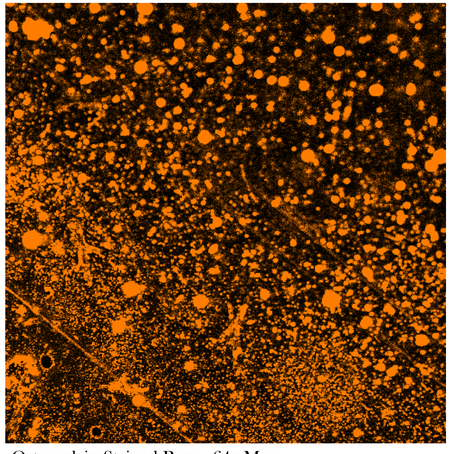


Osteocalcin Stained Bone, 10x Mag



Osteopontin Stained Bone, 64x Mag, FIJI/ImageJ© 3D Model

Please scan for additional images and videos, as well as a copy of this poster, and full author contact information



Osteocalcin Stained Bone, 64x Mag

Image of Osteoblast stained following protocol, 40x Mag, 3-Channel Image

Methodology

Osteopontin/Osteocalcin Labeling

When labeling for the bone proteins, again the researcher will want to be in a darker room due to the fluorophore label used. This method allows one to visualize the proteins without fluorescing the underlying hydroxyapatite matrix. While this project uses AlexaFluor 555, it is possible to combine the proteins labels and then antibody label with both AlexaFluor 488 and 555, with a rinse between each process thus allowing your to visualize both the osteocalcin and the osteopontin within the same sample.

1) Hydrate and sterilize in PBS for 30 mins
2) Stain with Primary Antibody
   a. Stain using Osteocalcin Polyclonal Antibody per manufactures instruction (≈ 2µL) *OR*
   b. Stain using Osteopontin Monoclonal Antibody per manufacturers instruction (≈ 2µL)
3) Incubate 30 mins
4) Rinse in PBS
5) Label with goat anti-mouse IgG, AF555 (10µ/mL)
6) Allow to air dry in a dark room for 1 hour
7) Rinse in PBS
8) Allow to air dry in a dark space for 24-48 hours

Use and Conclusion

There are a number of areas in which this methodology can be employed in better understanding forensic anthropological science. Among the research being conducted is using protein separation and osteoclast migration as a means of calculating injury/damage timing (perimortem *vs* postmortem). This is particularly useful in those select cases where postmortem damage occurs in bone, though the bone is still "wet" or otherwise maintains properties of living bone. By calculating the cellular migration along with the protein degradation, we can add an additional scientifically rigorous method in distinguishing perimortem injuries from early postmortem damage.

In addition to the injury timing, calculating the perimortem interval, specifically calculating protein and cellular degradation. Protein degradation is being examined as a means of calculating the postmortem interval in remains found in an advanced state of decomposition or early skeletalization. In a living individual the ratio of *OC*:*OPN*, which in a living individual is 1:1. A change in this ratio can be used to calculate the PMI

Additional research being conducted includes the comparison of samples from embalmed and unembalmed remains to see if embalming ultimately effects the bone as well as calculation of age via proteins along with the assessment of sex and other markers of health and biological profile information in highly fragmented remains.

1. Coxon FP. Fluorescence imaging of osteoclasts using confocal microscopy. In: Helfrich MH, Ralston SH, editors. Bone Research Protocols. 2nd ed. New York: Springer; 2012;401-24.