Chemical Imaging of In Vitro Models of Calcium Crystal Formation

Eric Mattson¹, Carol Hirschmugl¹, PhD, Claudia M. Gohr, BS², Ann K. Rosenthal, MD²

¹ Physics Department, University of Wisconsin-Milwaukee, Milwaukee, WI
² Medical College of Wisconsin and Zablocki VA Medical Center, Milwaukee, WI

Problems in accurately identifying calcium-containing crystals has slowed progress towards understanding the role of these important particulates in osteoarthritis. Synchrotron FTIR analysis has been useful in studying mineral formation in bone. We set out to determine if it could be used in synovial fluid and tissue culture models to identify and characterize calcium-containing crystals.

Discarded synovial fluids were obtained and were examined for CPPD crystals by polarized light microscopy. Calcium-containing crystals were also generated in vitro using well-characterized models of crystal formation, including a tissue culture based model with porcine articular chondrocytes and a model utilizing porcine articular cartilage vesicles. Potential crystal-containing areas were identified with light microscopy, and examined with synchrotron based IR microspectroscopy. The results were compared spectra from multiple forms of pure calcium-phosphate crystals (e.g. CPPD and BCP), and cartilage proteoglycans, and various mixtures of the above. Both CPPD and BCP crystals were readily identifiable in synovial fluids and in articular cartilage vesicle models. The IR spectrum of crystals embedded in ACV’s matched known standard spectra of both CPPD and BCP crystals. Similarly, crystals present in synovial fluid matched known standards of CPPD and BCP. Most CPPD crystals were monoclinic. CPPD crystals identical to those seen in synovial fluids were also readily identifiable in articular chondrocyte monolayers incubated with ATP. Most of the BCP crystals generated in vivo and in vitro were consistent with hydroxyapatite, rather than other calcium phosphate crystals known to be present in BCP. Interestingly, careful examination of different regions of a single human or porcine crystal often revealed both CPPD and BCP containing areas in a single crystal. IR maps of articular cartilage vesicles incubated with ATP and B-glycerophosphate show CPPD-like crystals in close proximity to BCP crystals.

This exciting technology allows for careful and accurate identification of calcium-containing crystals. These data support the presence of mixtures of calcium–containing crystals in a single region, and prove the validity of the monolayer model. This technology should further our understanding of these important pathologic crystals.

Figure 1: Crystals measured in Articular Cartilage Vesicle models incubated with ATP.