

Synchrotron FTIR analysis of Articular Calcium Containing Crystals

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Problems in accurately identifying calcium-containing crystals have 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 to study synovial fluid and tissue culture models to identify and characterize calcium-containing crystals.

Discarded synovial fluids from were obtained in accordance with our local IRB. They were examined for CPPD crystals by polarizing 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. Crystal containing material was placed on an IR reflective (visibly transmissive) Kevley slide. Potential crystal-containing areas were identified with light microscopy, and examined with Synchrotron based FTIR Microspectroscopy. The FTIR spectra generated were compared with known spectra of multiple forms of pure calcium-phosphate crystals, as well as spectra generated by FTIR with synthetic CPPD and BCP crystals and cartilage proteoglycans alone and in mixtures.

Both CPPD and BCP crystals were readily identifiable in synovial fluids and in articular cartilage vesicle models using synchrotron FTIR analysis. 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. In spectra from many CPPD crystals, the peak at 1052 cm^{-1} found on the standard spectrum for CPPD was altered. Spectra generated by combining standard spectra for synthetic CPPD and cartilage proteoglycans mimicked the differences seen in this peak.

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. They also support the close proximity of proteoglycans to crystals. This technology should further our understanding of these important pathologic crystals.