Calcifications in Human Osteoarthritic Articular Cartilage: Ex Vivo Assessment of Calcium Compounds Using XANES Spectroscopy

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1. Introduction
Osteoarthritis (OA) affects the entire joint. In addition to articular cartilage destruction, OA induces changes in other joint components including the bone, menisci, synovium, ligaments, capsule, and muscles. Multiple factors including the genetic background, mechanical overload, ageing and intra-articular calcium (Ca²⁺)-containing microcrystal (CC) deposition contribute to the pathogenesis and progression of OA.

CCs encountered in human joint crystal deposition-associated joint diseases include Ca²⁺ pyrophosphate dihydrate (CPPD) and basic Ca²⁺ phosphate (BCP) crystals. CPPD crystals (Ca₉P₂O₇·2H₂O) are found to be triclinic and/or monoclinic forms. BCP crystals encompass OCP, carbonated apatite (CA), tricalcium phosphate, and whitlockite crystals. A pathogenic role for CC-associated microcrystalline stress in OA is supported by recent clinical and experimental findings. CCs have been identified in 60% of OA synovial fluids and in 100% of OA cartilages obtained during total knee or hip joint replacement. CCs are associated with destructive forms of OA, as well as with chondrocyte differentiation toward a pro-mineralising phenotype.

The mechanisms underlying articular cartilage mineralisation during OA are incompletely understood. Briefly, calcification can occur when the balance between inhibitors and pro-mineralising factors is disrupted by any of multiple influences such as genetics, ageing, extracellular matrix modifications, dysregulation of inorganic pyrophosphate (PPi) and phosphate (Pi) metabolisms, changes in extracellular Ca²⁺ levels, and chondrocyte phenotype alterations. However, little is known regarding the state of Ca²⁺ compounds in human OA articular cartilage.

The objective of this study was to use XAS performed at the Ca K-edge (XANES) to compare the state of Ca²⁺ compounds between human OA articular cartilage areas with CCs versus areas without CCs.

2. Experimental design

2.1. Patients
Specimens of knee joint cartilage were obtained from 6 patients undergoing total knee replacement surgery for OA at a single orthopaedic department. Patients were selected at random and included prospectively. One rheumatologist (CN) reviewed the medical file of each patient. Clinical data (age, sex, body mass index [BMI]; and presence of genu varum or genu valgum) and preoperative plain knee radiographs were obtained and analysed. Kellgren and Lawrence staging was used to characterize OA severity. Patients with secondary OA (inflammatory disease or trauma) were not included.

2.2. Cartilage sample preparation

Figure 1
Knee joint specimen obtained during arthroplasty and schematic representation of the sample collection protocol. The specimen included femoral condyle and tibial plateau cartilage from both the medial and the lateral compartments (A). Cartilage areas were labelled as follows: 1, medial condyle; 2, lateral condyle; 3, medial tibial plateau; 4, lateral tibial plateau; S, superficial layer; D, deep layer (B).

3. Discussion

Although the presence of CCs in osteoarthritic cartilage was recognised several decades ago, the relationship between CCs and OA remains controversial. Several recent studies support a pathogenic role for CCs in OA, thus confirming many earlier clinical and experimental studies, and suggest that articular cartilage mineralisation may be a consistent feature of end-stage OA. However, the role for Ca²⁺ compounds in the mineralisation process remains poorly understood.

We first used FT-IR spectroscopy to distinguish cartilage areas with CCs from those without in samples that were initially selected based on absence of calcifications by macroscopic examination. CCs were present in 7 of 12 specimens, from 4 of 6 patients, suggesting that FT-IR spectroscopy was sensitive for detecting calcifications in our biological samples. FT-IR reflects only the overall chemical composition of the species contained in the sample at the molecular scale, including lipids, proteins, and PO₄ groups associated with Ca²⁺.

Figure 2
Absorption spectra were obtained for biological cartilage samples Red solid line, carabapate; red dotted line, amorphous carbonated Ca²⁺ apatite; black solid line, Ca²⁺ pyrophosphate dehydrate; and blue solid line, non-diffusible tissue Ca²⁺. (a) Patient 1; (b) Patient 3; (c) Patient 4; (d) Patient 5; (e) Patient 6; (f) Patient 2.

Therefore, we also used XANES spectroscopy at the Ca K-edge to specifically characterize Ca²⁺ compound-containing species. We found at least two distinct types of Ca²⁺ compounds in OA articular cartilage. One type was found in OA cartilage areas without CCs and seemed composed of tissue Ca²⁺ compounds, i.e., not linked to mineral compounds, as the XANES spectrum was similar to that found in hydrated tissues. In these areas, tissue Ca²⁺ was the main Ca²⁺ compound, whereas Ca²⁺ involved in CCs was absent or less abundant. The other type of Ca²⁺ compound was identified in CCs-containing OA articular cartilage areas and had a XANES spectrum specific of CPPD crystals.

4. Conclusion
In summary, in the present study, we show that XANES spectroscopy can be used to accurately characterize sparse CCs in human OA cartilage. We find that Ca²⁺ compounds differ between calcified and non-calcified cartilage areas. In calcified areas, they appear to be mainly involved in calcifications, namely Ca²⁺ crystals. Further application of SR-related technologies should bring additional information about other important chemical elements involved in these calcifications.