Human articular cartilage and fibrocartilage: A study with high-angle *x*-ray diffraction

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SUMMARY High-angle *x*-ray diffraction was applied to the study of four meniscal fibrocartilages and 11 articular cartilages from patients suffering from various articular disorders. In eight samples microcrystals were seen, apatite most frequently, CaHPO_4 in two instances, calcium pyrophosphate dihydrate (CPPD) in one. These results confirm the association of various crystals in a single joint, and favour their heterogenous partition on collagen fibres.

Key words: meniscus, apatite, calcium pyrophosphate dihydrate, chondrocalcinosis, crystals.

High-angle *x*-ray diffraction is currently used to determine the molecular arrangement of polymeric compounds in a solid state. This technique is also applied to determine the characteristics of biological substances such as collagen fibres¹ or DNA strands.²

The present work was designed to analyse samples of human articular cartilage and meniscal fibrocartilage to detect possible modifications of collagen structure in pathological situations, or to detect and identify microcrystals known to appear in such pathological articular conditions,³ or both.

Material and methods

Four meniscal fibrocartilages and 11 articular cartilages were studied. They were obtained surgically from three patients with meniscal defects, four with osteoarthritis, and four with rheumatoid arthritis or ankylosing spondylitis. Two patients suffered from a fracture of the femoral neck, one from sporadic chondrocalcinosis, and one from hereditary diffuse chondrocalcinosis.⁴

In all cases the samples of superficial articular cartilage were carefully isolated to avoid possible artefact from the calcified cartilage.

The method used for fibre diagrams was applied to these samples to establish their *x*-ray diffraction pattern. Briefly, the samples, kept humidified with

distilled water, were stretched between the brackets assuring a length increment of 5 to 20%. Each sample was then positioned perpendicular to the *x*-ray beam at a distance of 15 mm. Best orientated areas were determined by polarising microscopy. The distance between sample and *x*-ray film was assessed with calcite. Photographs were obtained with a flat camera, under continuous hydrogen flow and 75% humidity, with Cu K (1.542 Å) (0.1542 nm) radiation.

Results

High angle *x*-ray diffraction of cartilage samples show maximal diffraction at 0.29 nm and 1.1 nm. These characteristics are similar to those of periodic collagen with a triple helical structure.^{5–7} 0.4 and 0.74 spots were observed only with the most elongated samples and were related with the helical structure (Fig. 1b).

The results obtained in this pathological series were similar to those obtained with controls (Fig. 1a) and in other series of pathological extra-articular collagen samples.^{1 5–7}

Additional diffraction rings were observed for seven samples (Table 1). These were characteristic of apatite as shown Fig. 2. Two of these seven samples also displayed diffraction patterns of CaHPO_4 at a few sites (Fig. 3) They had been respectively obtained from a patient with chondrocalcinosis and a patient with rheumatoid arthritis.

The typical pattern of mono- and triclinic

Fig. 1a Control: X-ray diffraction pattern obtained from a stretched rabbit artery, showing the characteristic lines of collagen (►). Calcite diffraction line appears at the bottom (←). 1b Patient 4: X-ray diffraction obtained from stretched pathological cartilage.

Table 1 Clinical features of the patients and major characteristics of the samples studied with high-angle X-ray diffraction (origin, nature of the identified crystals)

No.	Sex	Age	Disease	Sample	Presence of		
					Apatite	CaHPO ₄	CPPD
1	M	62	Meniscal defect	Meniscus	X		
2	M	36	Meniscal defect	Meniscus			
3	M	30	Meniscal defect	Meniscus			
4	F	53	Coxarthrosis	Cartilage	X		
5	F	64	Coxarthrosis	Cartilage			
6	F	22	Coxitis	Cartilage			
7	F	70	Femoral neck fracture	Cartilage			
8	M	55	Knee arthritis	Cartilage			
9	M	60	Chondrocalcinosis	Meniscus	X	X	
10	M	62	Ankylosing spondylitis	Cartilage			
11	F	79	Femoral neck fracture	Cartilage	X		
12	M	68	Rheumatoid arthritis	Cartilage	X	X	
13	M	31	Patella pseudarthrosis	Cartilage	X		
14	M	55	Coxarthrosis	Cartilage	X		
15	F	70	Chondrocalcinosis	Cartilage			X

CPPD=Calcium pyrophosphated dihydrate.

Ca₂P₂O₇ was obtained from the sample of a patient with hereditary diffuse chondrocalcinosis (patient 15).

Ca₂P₂O₇ had previously been identified in a fragment of meniscus from patient 9, suffering from

sporadic articular chondrocalcinosis, by means of scanning electron microscopy and classical powder x-ray diffraction. This patient's sample analysed in high-angle x-ray diffraction failed to display the characteristic pattern of this compound.³

Fig. 2 Patient 13: X-ray diffraction pattern showing the presence of apatite (◄) in a pathological cartilage sample. The largest arrow (►) indicates the major characteristic line of collagen.

Fig. 3 Patient 12: X-ray diffraction pattern showing the presence of apatite (◄) and CaHPO_4 (→) in a pathological cartilage sample. The largest arrow (►) indicates the major characteristic line of collagen.

Discussion

High-angle x-ray diffraction of 15 samples of human cartilage showed the characteristic x-ray diffraction pattern of collagen, as previously described⁵⁻⁷ together with microcrystalline compounds. However, this technique requires an elongation of cartilage samples, which provides a crude orientation of collagen fibres. It therefore provides no information on possible alterations of chondroid collagen fibres in pathological conditions.

The identification of microcrystalline compounds inside the cartilage appears more rewarding. Such crystals were observed in five out of 13 samples from patients with degenerative and/or inflammatory articular disorders without radiological evidence of calcification, and in the two cases of chondrocalcinosis.

These deposits were apatite in six instances (one meniscus, five cartilage samples). Such crystals are usually detectable in elderly patients only. In our series the youngest patient was 31 years old, but suffered from patella pseudarthrosis. In most cases different patterns were obtained from different areas of any given sample, suggesting an irregular partition of microcrystals in the tissue. It should also be noted that the finding of apatite in these six patients is in keeping with previous data on the presence of this compound in synovial fluid⁸ or cartilage⁹ of elderly patients.

The calcium pyrophosphate dihydrate (CPPD) pattern was observed in one of the two patients with chondrocalcinosis. A heterogeneous partition of the crystals is again suggested in this case, since CPPD had been identified by other means in another fragment of the same meniscus of the second patient.

CaHPO_4 was detected in two samples, obtained from a patient with diffuse hereditary chondrocalcinosis and one with rheumatoid arthritis. This type of crystal had been reported previously only in menisci¹⁰ or on the surface of articular cartilage.³

These results confirm that various types of crystals can be found in a single joint.¹¹ However, this technique allows no precise localisation of the crystals among collagen fibres. More sophisticated methods may answer this question in the future.

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