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Crystal deposition in normal and diseased articular cartilage: an extended report

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Summary
Particles observed on the surfaces of human articular cartilage following trauma and from chondromalacic, osteoarthritic and rheumatoid joints using the scanning electron microscope were analysed by x-ray diffraction technique. The particles contained calcium and phosphorus and they were identified only in structurally abnormal cartilage. These findings suggest that local abnormality of articular cartilage probably underlies crystal-deposition.

Keywords: *Crystals, articular cartilage, scanning electron microscopy, x-ray diffraction analysis.*

Résumé
Les particules observess sur les surfaces des cartilage articulaires chez les humanins apres un traumatisme et a patrtir de malaises chondrotique, osteoarthritique et rhumatismique en utilisant le microscope electronique a scarrer été analyse par une technique rayon-X diffractaire. Les particules contenaient du calcium et du phosphore et aitnte identifices structurellement seulement cartilage chez les anormal. Ces resultats suggerent que lanomalie locale du cartilage articulaire cache le depot de crystal.

Introduction

Crystals of various compositions may be deposited in synovial joints. Their importance lies in the fact that they may cause inflammation and/or joint damage. In the variety known as "crystal-deposition disease"[1], crystals of calcium pyrophosphate, calcium orthophosphate and calcium hydroxy-apatite are found. This clinical entity is still to be fully elucidated, however. In order to provide some relevant data, in this study, normal and diseased joints of various age groups have been examined using scanning electron microscopy (SEM) and x-ray diffraction analysis (XDA) of surface particles.

Materials and methods

Very thin slices were removed from the articular surfaces of 30 joints during orthopaedic operations for various conditions. Specimens were obtained from:

a. 7 *trauma* patients (controls); 2 in their teens and 5 aged 70 or over. The site of trauma in the young patients was the knee in all cases and the hip in the old who were undergoing prosthetic replacement for fracture of the neck of the femur.

b. 9 patients suffering from *chondromalacia patellae* (age range 20-30 years); all were undergoing exploration of the knee for the anterior knee pain syndrome.

c. 8 patients suffering from *osteoarthritis* (age range 65-80) years); 4 were undergoing joint replacement of the knee and 4 of the hip.

d. 6 patients suffering from *rheumatoid arthritis* (age range 50-60 years); 3 of which were undergoing joint replacement of the knee, 2 of the hip and one patient had excision of the head of the radius.

The specimens were kept in sterile water during transit to the laboratory which was usually after a period of three days. They were then fixed in 2% buffered glutaraldehyde for three weeks thereafter, dehydrated in graded alcohol before critical point drying by CO_2. Next, the specimens were mounted onto stubs and carbon-coated in the usual fashion. Thereafter, they were examined, after coating with gold, in the Phillips 501B scanning electron microscope with attachment for energy-dispersive micro-analytical system (Link System 860). Attention was directed to any "free" surface particles and in order to distinguish true observations from artifacts, attempts were made to identify their chemical composition. The results were graphically analysed for all chemical elements using carbon-coated cellotape as the standard.

Results

The radiographs of the joints from which samples were obtained after trauma were otherwise normal.

Electron microscopy

Three general types of surfaces were identified in scanning electron micrographs:

a. *smooth surface* which was cellular and flat or undulating (Figure 1);

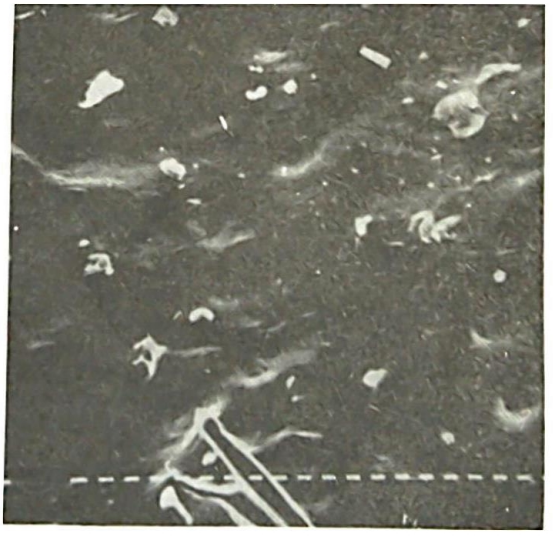

Fig. 1: SEM showing the smooth surface

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b. *scaly surface* which had neither cellular nor Fibrillary structures identifiable (Figure 2); and

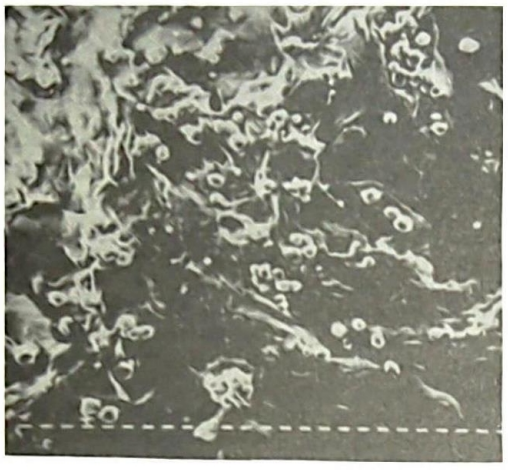

Fig. 2 : SEM showing the scaly surface

c. *fibrillary* surface were no cellular elements could be identified and the fibre pattern had no order (Figure 3).

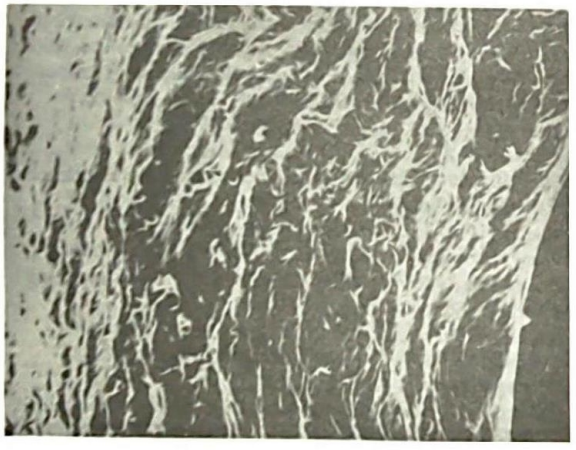

Fig. 3:- SEM showing the fFibrillary surface.

The distribution of the different types of surfaces within the orthopaedic conditions studied is shown in Table 1.

Table 1: The distribution of surface types among the orthopaedic conditions studied.

	Smooth	Scaly	Fibrillary	Total
Trauma (young)	2	-	-	2
Trauma (old)	3	2	-	5
Chondromalacia	4	2	3	9
Osteoarthritis	-	5	3	8
Rheumatoid arthriti	-	6	-	6
Total	9	15	6	30

Crystals were observed on the surface among degenerate cells debris and in relation to cracks and crevices but not obviously within cells. Crystals were also observed among globular particles (Fig. 4).

Fig. 4 : SEM showing globular particles indicating joint effusion.

These particles were often clumped together like bunches of grapes and were present only in joints with an effusion. They were uniformly electron dense and could be distinguished from the red cells which were generally of the same size but more disc shaped with electron opaque centres (Fig. 5)

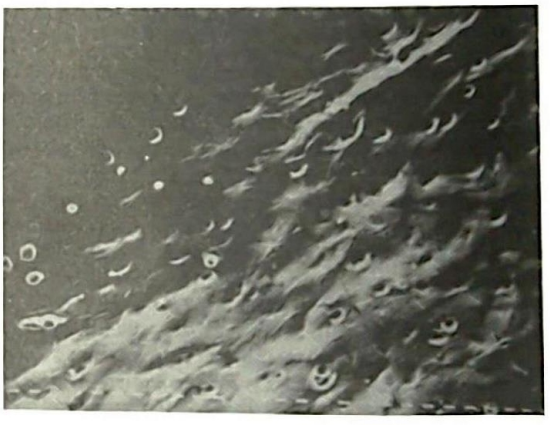

Fig. 5 : SEM showing red blood cells indicating haemarthrosis

X-ray diffraction analysis (XDA)

When compared to carbon-coated cellotape (Fig. 6), three elements most frequently showed peaks on XDA; namely, sulphur, calcium and phosphorus in the order of frequency. A surlphur peak was observed in all specimens.

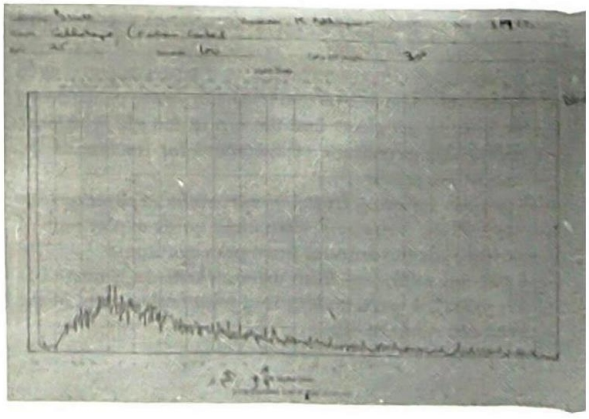

Fig. 6: XDA of carbon-coated cellotape (control)

The distribution of calcium peaks among the specimens is shown in Table 2. Small calcium peaks were identified in 40% of the "old" articular cartilages (Fig. 7)

Table 2: The distribution of calcium peaks among the orthopaedic conditions studied.

	Cartilage without effusion		Cartilage with effusion		
	Ca++ Peak	No Ca++ peak	Ca++ peak	No Ca++ peak	Total
Trauma (young)	-	2	-	-	2
Trauma (old)	2	3	-	-	5
Chondromalacia	2	5	-	2	9
Osteoarthritis	6	-	2	-	8
Rheumatoid arthritis	-	-	6	-	6
Total	10	10	8	2	30

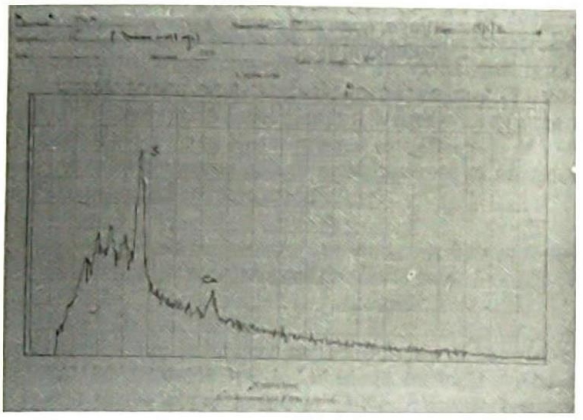

Fig. 7: XDA of 'old' articular cartilage

The two specimens showed some degree of surface disorganisation and scalined characteristic of osteoarthritis although they were not associated with any joint effusions or radiographic evidence of osteoarthritis. 22.2% of the chondromalacia group had small calcium peaks (Fig. 8) and these two specimens also showed features characteristics of osteoarthritis. No calcium peaks were present in the two specimens with joint effusion.

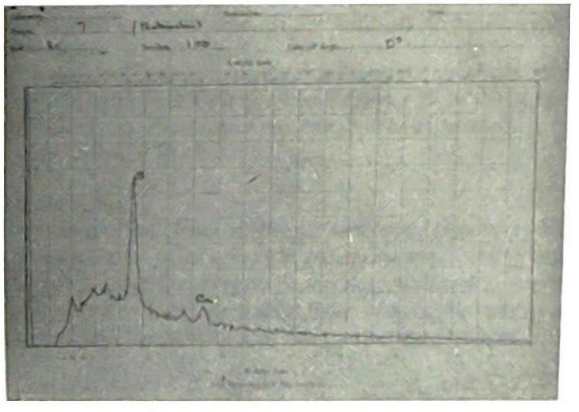

Fig. 8: XDA of chondromalacic cartilage

All the osteoarthritic specimens showed calcium peaks but there were two varieties. "Dry" joints with surface changes mainly of disordered fibre patterns had low calcium peaks. The joints with an effusion, usually associated with very grossly disorganised or scaly surfaces, had very high calcium combined with very high phosphorus peaks. (Figure 9).

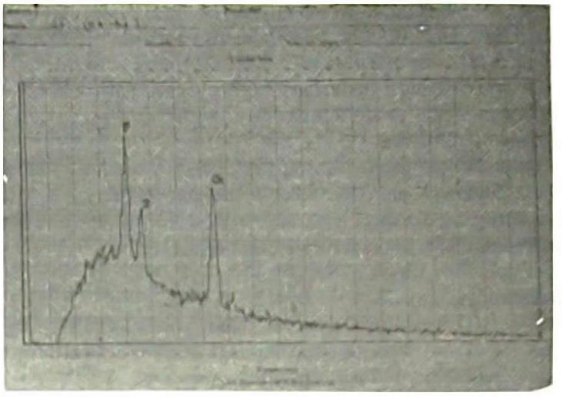

Fig. 9: XDA of osteoarthritic cartilage.

All rheumatoid specimens had very high calcium and phosphorus peaks (Fig. 10).

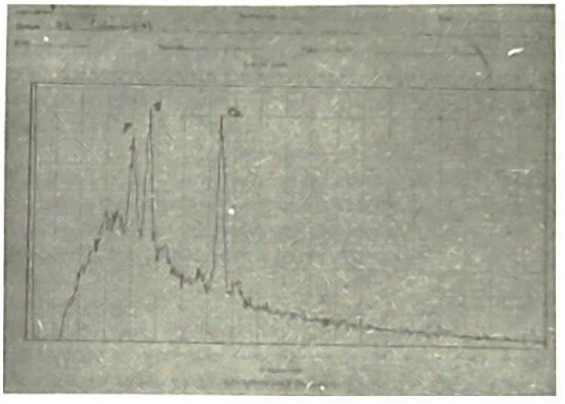

Fig. 10: XDA of rheumatoid cartilage

Discussion

A sulphur peak is presumed to indicate the presence of proteins, hence, sulphur peaks were observed in every specimen regardless of whether crystals were present. The crystals appear to be composed predominantly of calcium and phosphorus salts. Consequently, there was a close correlation between calcium and phosphorus peaks; a phosphorus peak was absent if the calcium peak was low and *vice versa*.

The mechanism by which these calcium crystals are deposited in the articular cartilage is not known. A primary metabolic origin has been suggested [1] and the possible mechanisms have recently been discussed [2]. Apparently, a primary cartilage disease could lead to an exudative reaction or electrolyte imbalance from which calcium salts could then be precipitated and extruded to the surface. On the other hand, the finding from this study of a close correlation between crystal-deposition and surface disorganisation appears to implicate local structural changes [3]. The surface aberration most commonly associated with calcium deposition is very similar to that obtained on scanning osseous elements [4]. This raises the possibility that the subchondral bone is being "exposed" and that it is from this that calcium salts are released into the joint. There was no EM evidence of calcification which confirms the findings of previous studies [5-8]

The articular cartilage is organised into zones [9]. The thin surface layer is cellular and almost devoid of fibres. Immediately deep to this, the fibres are fine and arranged in bundles nearly parallel with the surface. Deeper still, the fibres are coarser and are arranged as tight spirals. The fourth zone which surmounts the subchondral bone is calcified. These descriptions could very easily be applied, respectively, to the three surface aberrations identified in this study. Each surface type appears to represent the depth to which erosion of the articular cartilage has reached. Thus, Figure 1 (*smooth surface*) corresponds to the superficial zone and Figure 2 (*scaly surface*) with the calcified zone while Figure 3 (*fiybrillary surface*) is somewhere in between.

Acknowledgments

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