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Crystal aggregates in articular cartilage as observed in the SEM

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Scanning electron microscopy, in conjunction with freeze-fracturing and freeze-drying preparation techniques, was used to characterize the morphology and distribution of crystal deposits found *in situ* in the articular cartilage of three *post mortem* human knee joints. Energy dispersive analysis and X-ray diffraction were used to analyse the chemical composition of the individual crystals. Results included a unique observation of an aggregate of bow-shaped monosodium urate monohydrate crystals within the mid-zone of the articular cartilage, and two types of a large aggregate of calcium pyrophosphate dihydrate crystals distributed throughout the thickness of the cartilage. These results provided evidence of an organized crystal distribution and therefore supported the idea that local growth factors are important.

1. Introduction

Crystal deposits containing calcium are known to occur commonly in elderly and osteoarthritic cartilage [1]. Little is known, however, about the organization of these deposits *in situ* in the articular cartilage. It is believed that the distribution, morphology and internal organization of such deposits within the articular cartilage layer have important consequences on the inherent mechanical properties of the diseased tissue, i.e. the articular cartilage with associated crystal aggregates. This raises important questions with regard to the distribution of stresses induced in such tissue, and whether the onslaught of fibrillation, or degeneration, of the articular cartilage could have a mechanical as well as a biochemical component [2–4]. A detailed study of such tissue, in particular the crystal aggregates, was considered necessary to establish the organization of such aggregates within the cartilage matrix.

This study was undertaken to ascertain the gross morphology of large aggregates of crystals within articular cartilage, the distribution of these aggregates within the cartilage layer, the nature of the interface between the crystal agregate and the cartilage matrix, the organization of the individual crystals within the aggregates, and their morphology. The results could be used in conjunction with finite-element analysis to investigate the stresses involved in a system which would model an aggregate, or a distributon of aggregates, within a cartilage matrix. Such an approach may provide some answers to the questions posed above.

Much of the early work on crystal deposits was based on radiographs, which were used to identify linear calcific deposits concentrated in the mid-zone of the articular cartilage. However, the resolution of radiographs used for such purposes is known to be poor [5] and the detailed analysis of such mineral deposits is impossible. Histology, in conjunction with polarized light microscopy, is a standard method used for the identification of crystal deposits within articular cartilage. A recognized problem associated with this method, however, is that individual crystals tend to fall, or be plucked, out of the thin sections during preparation, leaving characteristically shaped holes [1]. This problem is exaggerated if large crystal aggregates are present, as insufficient tissue remains to produce a viable section.

Until recently there have only been limited reports of the use of scanning electron microscopy (SEM) to study crystals *in situ* in the articular cartilage [6–8]. SEM has several advantages compared to optical microscopy. These include higher magnifications (due to enhanced resolution) and a greater depth of field. This has particular relevance to the investigation of individual crystals *in vivo*, which due to their small size, (1–20 μm), are difficult to observe using the optical microscope. In addition a three-dimensional image of the crystals is obtained by using the SEM, compared with a two-dimensional silhouette observed by using the optical microscope.

In this study SEM was used, in conjunction with freeze-fracturing and freeze-drying preparation techniques, to characterize the morphology and distribution of large crystal aggregates *in situ* in the articular cartilage. This method caused minimal disruption of the surrounding tissue and circumvented the problems discussed above of preparing crystal-containing cartilage for optical microscopy. Energy dispersive analysis (EDA) (in conjunction with the SEM) and X-ray diffraction were used to analyse the chemical composition of the crystals [9].

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2. Materials and methods

Samples of articular cartilage were taken *post mortem* from one patella and three femoral condyles of three human knee joints. The anatomical sites of the samples taken, as characterized by Clift *et al.* [10], are given in Table I. The cadavers were of both sexes with an average age of 76 years. The physical appearance of the surface of the cartilage was also graded according to Clift *et al.* [10] by using a modification of the criteria originally proposed by Sokoloff [11]. These are also given for each specimen in Table I.

Following fixation with formal saline, 5 mm sections were radiographed to identify any crystal deposits [12]. Slivers of articular cartilage taken from these crystal-rich areas were then frozen in liquid nitrogen, fractured and freeze-dried, in an Edwards–Pearse Tissue Dryer at $-60\,^{\circ}C$ for 12 h to minimize tissue distortion. The specimens were coated with gold or carbon before examination in one of two scanning electron microscopes, a JEOL JSM-35C or a JEOL-T330, with accelerating voltages of between 10 and 20 kV. EDA, using a Link Systems AN10000 analyser attached to the JSM-35C microscope, was used to analyse the composition of the crystal deposits. X-ray diffraction of isolated crystal aggregates was also used to confirm the composition.

3. Results and discussion

The slivers of crystal-rich articular cartilage taken from the four *post mortem* specimens were examined by using SEM. Large aggregates of crystals were observed in all four specimens. All but one of these aggregates consisted of individual crystals of triclinic and monoclinic calcium pyrophosphate dihydrate (CPPD). However, an aggregate of monosodium urate monohydrate (MSU) crystals was also identified, using EDA, within the mid-zone of the articular cartilage of one of the femoral condyles. The individual MSU crystals within this aggregate were a distinct bow-shape.

Since MSU crystals are not radio-opaque, their presence in articular cartilage cannot be identified by the use of X-rays. The observation of an aggregate of MSU crystals within the cartilage layer was therefore unexpected. It was also surprising to find such an aggregate deep within the mid-zone, as previous reports of MSU crystals present in the articular cartilage quote the superficial zone as the predominant location of these crystals [13, 14]. The morphology of the individual MSU crystals within the crystal aggregate was unique in that they were a distinct bow-shape (Fig. 1). This is in contrast to the needle-shaped crystals usually observed in synovial fluid [15].

TABLE I Specimen details. Anatomical position: P, patella; MC, middle of condyle; TC, top of condyle. Fibrillation: S, severe; M, mild (Clift *et al.* [10])

Specimen number	Sex	Age	Anatomical position	Fibrillation	Crystal identified
23-1	F	80	P	S	CPPD
23-3	F	80	MC	M	CPPD
29-2	F	77	TC	S	CPPD
35-2	M	68	TC	S	MSU

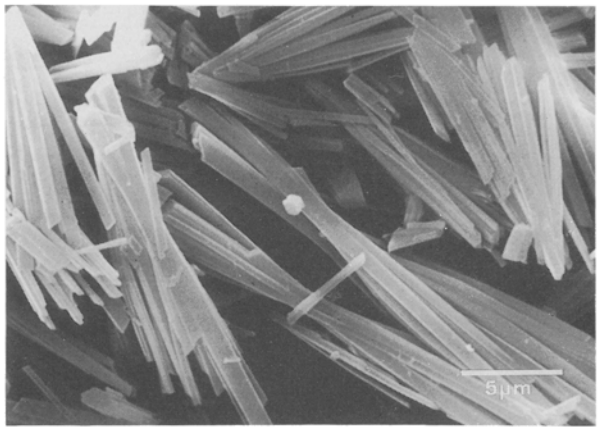

Figure 1 Part of an aggregate of bow-shaped monosodium urate monohydrate crystals which was observed deep within the articular cartilage.

MSU crystals grown by McGill *et al.* [16] *in vitro* in the presence of serum, synovial fluid and the components thereof closely resembled the morphology of the MSU crystals observed *in vivo* in the articular cartilage, whereas those grown without the presence of any biological components were needle-like. McGill *et al.* [16] concluded from their experiments that crystal formation is influenced by the biological milieu present at the time of crystal formation. It is interesting to note that *in vivo* two different morphologies of MSU crystal exist in the articular cartilage and the synovial fluid respectively. This would suggest that the medium in which such crystals are grown is also an important factor in the determination of the formation of the crystals. These observations corroborate the conclusions of McGill *et al.* [16].

Two types of CPPD deposit were observed. The first consisted of dense aggregates of crystals which were approximately spherical or ovoid, up to 700 μm in diameter, and clearly separated from one another by a layer of tissue of variable thickness. A part of one such aggregate is shown in Fig. 2. The sharp demarcation between the edge of the aggregate and the adjacent cartilage matrix should be particularly noted. In

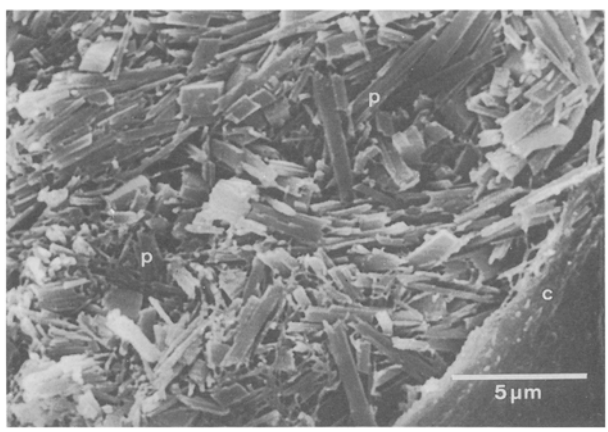

Figure 2 Part of a well-defined, dense aggregate of calcium pyrophosphate crystals (p) situated within the articular cartilage (c).

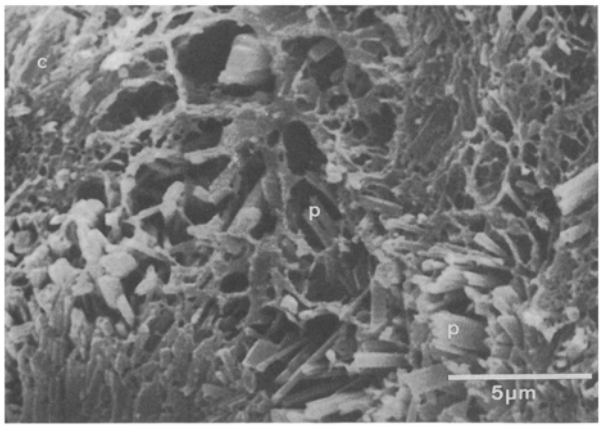

Figure 3 Calcium pyrophosphate crystals (p) with a more random distribution and a less well-defined interface with the surrounding cartilage matrix (c).

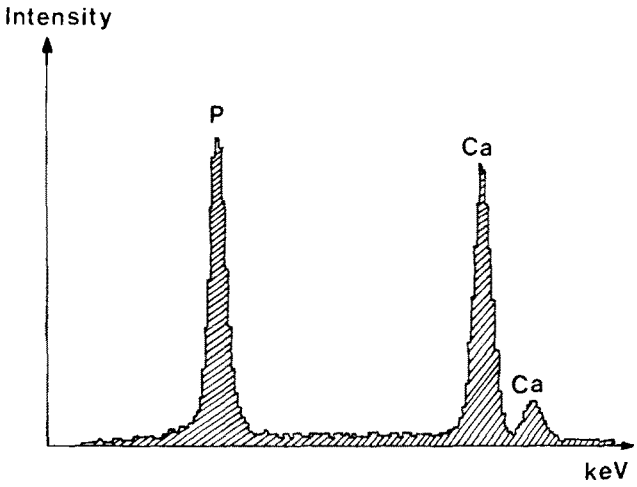

Figure 5 Energy dispersive analysis indicating the presence of significant amounts of calcium pyrophosphate dihydrate.

contrast to this regular arrangement, groups of crystals were observed with a more random distribution and a less well-defined interface with the surrounding organic matrix, as illustrated in Fig. 3. In both cases the aggregates were not confined to the mid-zone of the articular cartilage, as previously reported [5, 8, 17, 18], but were observed throughout its thickness from the calcified to the superficial zone. Aggregates observed near the surface of the articular cartilage may be explained as a consequence of the degeneration of the cartilage and a subsequent reduction in its apparent thickness. In support of this argument, surface aggregates were always observed in association with a fibrillated articular surface (Fig. 4). Individual crystals within both types of aggregate appeared as rods or rhomboids and varied in size from 0.5 to 2.0 μm in width and from 2 to 10 μm in length. X-ray diffraction and EDA (Fig. 5) respectively, confirmed that the crystals in both types of deposit were monoclinic and triclinic CPPD.

The contrasting appearance of the two distinct types of crystal aggregate is difficult to reconcile. One explanation may be that they are at different stages of development. These observations lead one to speculate on the mechanisms leading to the initial formation of these large crystal aggregates, and the role played by the cells and the matrix. The fact that the crystal distribution is not random, but that these large well-defined CPPD aggregates are formed again, leads one to speculate that local growth factors are important.

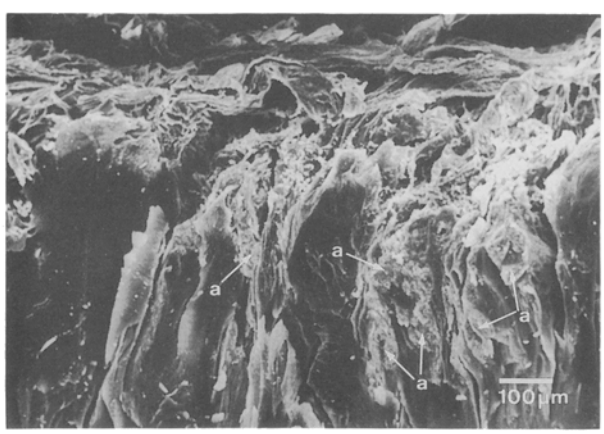

Figure 4 Aggregates of crystals (a) associated with a fibrillated articular surface.

4. Conclusion

Results from this work confirmed previous reports of crystal deposits found in the articular cartilage [7, 12, 19–21], but with several unique observations. These included: (i) an aggregate of *bow-shaped* MSU crystals was observed within the *mid-zone* of the cartilage layer, rather than the superficial zone as previously reported [13, 14], (ii) large aggregates of CPPD crystals were distributed *throughout the thickness* of the articular cartilage and not just confined to the mid-zone as previously reported [5, 8, 17, 18], and (iii) two distinct types of CPPD aggregate were identified with either densely packed, or loosely arranged, crystals.

The fact that the crystal distribution is organised with large well-defined aggregates reinforces the suggestion that local growth factors are important. The observation of bow-shaped MSU crystals, in contrast to needle-like crystals, *in vivo* deep within the cartilage layer also provdes valuable evidence in support of McGill *et al.*'s [16] *in vitro* studies of the growth of MSU crystals, and their conclusion that crystal formation is influenced by the biological milieu present at the time of crystal formation. In conclusion, these detailed observations of large crystal aggregates made using the high-powered magnification capabilities of the SEM, proved invaluable in the analysis of the organization and distribution of large crystal aggregates within the cartilage matrix.

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References

1. P. DIEPPE and P. CALVERT, "Crystals and joint disease" (Chapman & Hall, London, 1983).
2. M. G. EHRLICH, H. J. MANKIN, H. JONES, A. GROSSMAN, C. CRISPEN and D. ANCONA, "Biochemical confirmation of an experiment osteoarthritis model", *J. Bone Jt Surg.* **57A** (1975) 392.
3. R. W. MOSKOWITZ, "The biochemistry of osteoarthritis", *Brit. J. Rheum.* **23** (1984) 170.
4. A. J. BOLLET, "An essay on the biology of osteoarthritis", *Arthritis Rheum.* **12** (1969) 152.
5. H. K. GENANT, "Roentgenographic aspects of calcium pyrophosphate dihydrate crystal deposition disease (pseudogout)", *Arthritis Rheum.* **19** (1976) 307.
6. K. ISHIKAWA, I. MASUDA, T. S. K. OHIRA and M. S. K. YOKOYAMA, "A histological study of calcium pyrophosphate dihydrate crystal deposition disease", *J. Bone Jt Surg.* **71A** (1989) 875.
7. H. U. CAMERON, V. L. FORNASIER and I. MACNAB, "Pyrophosphate arthropathy", *Amer. J. Clin. Pathol.* **63** (1975) 192.
8. J. A. REES, S. Y. ALI and A. Z. MASON, "Scanning electron microscopy and microanalysis of "cuboid" crystals in human articular cartilage", in "Cell mediated calcification and matrix vesicles", edited by S. Y. Ali (Elsevier Science Publishers B.V, 1986) p. 365.
9. H. OZAWA and T. YAMAMOTO, "An application of energy dispersive X-ray microanalysis for the study of biological calcification", *J. Histochem. Cytochem.* **31** (1983) 210.
10. S. E. CLIFT, B. HARRIS, P. A. DIEPPE and A. HAYES, "Frictional response of articular cartilage containing crystals", *Biomaterials* **10** (1989) 329.
11. L. SOKOLOFF, "The biology of degenerative joint disease" (University of Chicago Press, 1969).
12. I. WATT, "Radiology of the crystal-associated arthritides", *Ann. Rheum. Dis.* **42**(supp) (1983) 73.
13. L. SOKOLOFF, "The pathology of gout", *Metabolism* **6** (1975) 230.
14. P. A. DIEPPE, P. A. BACON, A. N. BANJI and I. WATT, "Atlas of clinical rheumatology" (Oxford Medical Publishing, Oxford, 1986).
15. H. PAUL, A. J. REGINATO and H. R. SCHUMACHER, "Morphological characteristics of monosodium urate: a transmission electron microscopic study of intact natural and synthetic crystals", *Ann. Rheum. Dis.* **42** (1983) 75.
16. N. W. McGILL, A. HAYES and P. A. DIEPPE, "Morphological evidence for biological control of MSU crystal formation *in vivo* and *in vitro*", *Brit. J. Rheum.* Abstr. Suppl. (November 1989).
17. D. RESNICK, G. NIWAYAMA, T. G. GEORGEN, P. D. UTSINGER, R. F. SHAPIRO, D. H. HASELWOOD and K. B. WIESNER, "Clinical radiographic and pathologic abnormalities in calcium pyrophosphate dihydrate deposition disease: pseudogout", *Radiology* **122** (1977) 1.
18. A. J. REGINATO, H. R. SCHUMACHER and V. A. MARTINEZ, "The articular cartilage in familial chondrocalcinosis: light and electron microscopic study", *Arth. Rheum.* **17** (1974) 977.
19. D. R. MITROVIC, "Pathology of articular deposition of calcium salts and their relationship to osteoarthritis", *Ann. Rheum Dis.* **42**(supp) (1983) 19.
20. S. Y. ALI, S. GRIFFITHS, M. T. BAYLISS and P. A. DIEPPE, "Ultrastructural studies of pyrophosphate crystal deposition in articular cartilage", *Ann. Rheum. Dis.* **42**(supp) (1983) 97.
21. A. O. BJELLE, "Morphological study of articular cartilage in pyrophosphate arthropathy", *Ann. Rhenum. Dis.* **31** (1972) 449.

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