SODIUM PHOSPHATE CRYSTALS IN CHONDROMALACIC PATELLAR CARTILAGE

B. F. SHAHGALDI, F. W. HEATLEY

From St Thomas' Hospital, London, England

We report the finding of sodium- and phosphorus-based crystallisation in abnormal human articular cartilage. We prepared five chondromalacic, five osteoarthritic and four macroscopically normal specimens of patellar cartilage by a cryofracturing technique and examined them in a scanning electron microscope.

An energy-dispersive X-ray microanalysis system was used to identify the crystals, which were found in only three of the five chondromalacic specimens. Star-shaped crystals were seen either individually or in clusters in the matrix of the cartilage. They consisted of sodium and phosphorus, and we have found no previous reports of such findings. The calcified zone, the bone, and the articular surface were free from crystals.

RESULTS

Crystals were seen in the articular cartilage of three of the

---

The presence of crystals in synovial joints has been reported since the 18th century (Dieppe and Calvert 1983), the best known being monosodium urate monohydrate in gout (McCarty and Hollander 1961) and calcium pyrophosphate dihydrate in pseudogout (Kohn et al 1962). Less common crystals are those of calcium hydroxyapatite (Dieppe et al 1976) and of cholesterol in effusions from osteoarthritic joints (Fam et al 1981).

The accurate identification of unknown crystals requires both chemical and morphological studies. Scanning electron microscopy, incorporating secondary and backscatter electron imaging and X-ray microanalysis, can be used for morphological and chemical analysis, providing a reproducible technique.

We used scanning electron microscopy to identify crystals containing sodium and phosphorus in specimens of chondromalacic human patellar cartilage prepared by a cryofracturing technique.

MATERIALS AND METHODS

Specimens of patellar cartilage were obtained from five female patients aged from 23 to 44 years who were having surgery for severe chondromalacia. One had a patellectomy; the other four had patellar surface replacement. The five specimens contained degenerate as well as intact surrounding cartilage. Control specimens were obtained from five osteoarthritic patellae from patients aged from 66 to 84 years, and from four macroscopically normal patellae from male and female cadavers aged 53 to 74 years. The presence of macroscopically normal cartilage was established by the Indian ink staining method of Meachim (1972).

Each specimen was bisected. One half was fixed in 2% glutaraldehyde buffered with 0.1 M sodium cacodylate and 0.1 M sucrose followed by partial digestion in bovine testicular hyaluronidase to remove the proteoglycans (0.5 mg/1.5 × 1.5 × 0.5 mm specimen size). The other half was fixed in 2% glutaraldehyde without buffer and without subsequent hyaluronidase digestion to prevent contamination of the tissue with sodium ions from the buffer.

The surfaces for examination were prepared by cryofracture: specimens were frozen in liquid nitrogen and fractured through notches in the subchondral bone to produce fracture planes at 90° to the articular surface (Jeffery et al 1991). Critical point drying was followed by sputter coating with gold or carbon for morphological examination and X-ray microanalysis, respectively. We used a Hitachi S-520 scanning electron microscope fitted with a backscatter electron detector (S-4548, high-efficiency type reflected electron-detective device) and an X-ray analyser (Kevex X-ray energy-dispersive spectrometer, Delta System; Kevex Corporation, Burlingame, California), at an acceleration voltage of 15 kV.

Morphological examination used secondary electrons in a backscatter mode. The Kα characteristic X-ray lines from areas with visible crystallisation were analysed qualitatively using an 'energy-dispersive system' for a counting time of 100 seconds. The specimen tilt, working distance and specimen dimensions were kept constant for all measurements.
five chondromalacic specimens. No crystals were found in the five osteoarthritic or four normal specimens.

Each chondromalacic specimen showed three distinct regions:

1) severely damaged cartilage containing open vertical fissures;
2) cartilage with macroscopically visible surface fibrillation but intact cartilage below the surface, except for closed

Figure 1 – A secondary electron image showing solitary crystals on a bed of collagen in a partly digested specimen.
Figure 2 – A secondary electron image of a partly digested specimen, showing several clusters enclosed in proteinaceous membranes.
Figure 3 – A secondary electron image showing crystal clusters among degenerated cells and cell debris.
vertical fissures in the cartilage deep to the superficial zone; and 3) cartilage with a macroscopically normal surface but containing closed fissures similar to those in 2).

Crystals were not found in severely damaged cartilage or that with a macroscopically normal surface. In all three positive specimens they were in the matrix of cartilage with macroscopically visible surface fibrillation. No crystals were found in the bone or on the articular surface.

The crystals were star-shaped and either solitary or in a cluster enclosed by a proteinaceous membrane. The solitary crystals were identical in shape but varied in size (Fig. 1); the clusters included different sizes and shapes (Fig. 2). Crystals were in localised areas, either within closed fissures or among degenerated cells and cell debris (Fig. 3). X-ray microanalysis of crystals in both enzymatically-digested and undigested specimens showed that they con-

---

**Fig. 4**

Kα X-ray energy-dispersive spectrum of crystals.

---

**Fig. 5**

Kα X-ray energy-dispersive spectrum from the matrix of macroscopically normal cartilage which showed no crystals.
sisted of phosphorus and sodium (Fig. 4).

The lacunae of the cells adjacent to a fissure containing crystals showed mineralisation that had no well-defined crystalline structure, but had peaks of phosphorus and sodium similar to those recorded for crystals. The matrix of the articular cartilage from all three different regions, in the absence of crystals, contained a small amount of sodium and phosphorus (Fig. 5) whether or not sodium cacodylate buffer and digestive enzyme had been used.

**DISCUSSION**

It is uncertain whether crystal formation in human joints is the cause or the effect of disease. Crystals form when generalised or localised metabolic abnormalities cause a build-up of solute (Dieppe and Calvert 1983). Sodium and phosphorus are present in abundance in articular cartilage, but because they are soluble they are largely lost during processing; a small residual amount is detected by X-ray microanalysis. The newly reported crystals did not dissolve during processing, and we deduce therefore that they are complex in nature and probably of sodium pyrophosphate type. Their formation requires overcoming an energy barrier and the thermodynamic equilibrium that exists in normal tissue; this indicates an altered ionic concentration of salts to levels that favour crystal formation.

The chondromalacic cartilage showed extensive structural damage rendering it mechanically inferior to normal tissue, and therefore less able to facilitate fluid transport. This could have led to localised build-up of solute. It seems likely that the crystals were formed in shear-induced closed fissures, which we have previously reported to be early signs of cartilage breakdown in chondromalacia (Shahgaldi, Heatley and Amis 1992). In addition, the positive detection of mineralisation in the lacunae of cells close to sites of crystallisation suggests some involvement of cartilage cells in the early stages of crystallisation.

To test the hypothesis that the crystals were artefacts, we prepared the second half of each specimen in a different manner, eliminating all sodium-ion contamination from the buffer and not using enzymatic digestion. Crystals were found in the same three chondromalacic specimens. It is also of interest that the clusters of crystals were found to be enclosed in a protein membrane. This suggests that they were formed in vivo; membrane encapsulation is a characteristic feature of such crystal formation. Koizin and McCarty (1976) and Keen et al (1991) have also reported proteinous covering of various crystals.

For specimen preparation cryofracturing is much better than knife sectioning because it provides contaminant-free surfaces with minimal structural damage. The knife sectioning may introduce contaminants from the tools and the environment, and the knife edge could distort material within the surface.

Cryofracturing was first used to study articular cartilage by Clark (1985) and more recently by Jeffery et al (1991). We have used the technique extensively (Shahgaldi et al 1992), but these three chondromalacic specimens are the first in which we have found crystals. The use of X-ray microanalysis to identify elements contained in a material is reliable, reproducible and has the advantage that a small area of a large specimen can be analysed (point analysis). Our use of this combined with backscatter and secondary electron imaging enabled us to map accurately the crystals on specimens which included bone and cartilage, and establish their exact location and their relationship to the cells and the matrix.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

**REFERENCES**


