Formation of calcium phosphate crystals in normal and osteoarthritic cartilage

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Introduction

The mechanism of cartilage calcification has been outlined in recent reviews.^{1,2} A study of epiphyseal cartilage calcification has clearly established that the first crystals of apatite that are found in the hypertrophic zone are found in matrix vesicles, in the longitudinal septa.^{1,2} Matrix vesicles are extracellular, membranous particles, about 100 nm in diameter, that are associated with calcification of numerous tissues.³ Over 80% of the alkaline phosphatase activity of epiphyseal cartilage is associated with matrix vesicles, and this enzyme content may facilitate the calcification process initiated by matrix vesicles.⁴ Mitochondria and degenerating chondrocytes may play a part in the second stage of mineralisation and collagen is important in the third stage for retaining the mineral in the matrix.^{1,2}

Research on the pathogenesis of osteoarthritis has led to the conclusion that it may be initiated by various individual anatomical, mechanical, or metabolic factors, but that there is a final common pathway of cartilage degeneration that eventually leads to the total loss of articular cartilage and joint function.⁵ Enzyme analysis and biochemical estimations of human osteoarthritic cartilage led us to postulate a calcification abnormality in the diseased tissue as one such aetiological factor.⁶ This would provide an explanation for a certain form of the disease and would not necessarily explain the various disease processes that lead to different forms of osteoarthritis. Electron microscopical studies of arthritic human articular cartilage have shown apatite-type crystal deposits associated with matrix vesicles and increased alkaline phosphatase activity

in the diseased tissue.^{7,8} These crystal deposits were quite distinct and different from calcium pyrophosphate crystal deposition. In his study of crystal deposits in human joints McCarty had included apatite among the various crystals seen in articular and periarticular tissues in inflammatory arthritis.^{9,10} Apatite crystals have also been observed in the synovial fluid of arthritic patients by Dieppe^{11,12} and by Schumacher and his colleagues.^{13,14} Faure¹⁵ has also observed calcium hydrogen phosphate dihydrate crystals in synovial tissues. We have attempted to elucidate the nature and origin of apatite-type crystals in human osteoarthritic cartilage and have studied the mechanism of their formation and association with matrix vesicles.^{7,8,16,17} Apart from calcium pyrophosphate crystal deposition in chondrocalcinosis, we have found at least three different morphological types of apatite crystals in human arthritic cartilage and have characterised these by electron probe analysis and by cryoultramicrotomy. Some of our results have been published elsewhere¹⁷ and here we shall give further details of the variety of crystals found in fresh, human articular cartilage specimens.

Materials and methods

Fresh human osteoarthritic articular cartilage was obtained from femoral heads resected for total hip replacement. It was degenerate or residual cartilage (type IV in the nomenclature of Ali and Bayliss⁶). Normal specimens were obtained from femoral heads after amputation of hind quarters for osteosarcoma or other malignant growth. Articular cartilage was also obtained from patients who had subcapital fracture, where the cartilage surface and tissue appeared

quite smooth and 'normal'. Small (1 mm³) specimens were fixed in glutaraldehyde only and processed for electron microscopy by conventional techniques as described elsewhere.^{6,18} Unstained sections were used for electron probe analysis, with an energy dispersive system, in a Philips 300 transmission electron microscope. For morphological studies tissue specimens were double fixed in glutaraldehyde and osmium tetroxide and sections were stained with uranyl acetate and lead citrate.^{6,18}

Results and discussion

Examination of more than 12 specimens of human osteoarthritic cartilage has indicated that there are different morphological types of crystals present in different layers of articular cartilage. It is preferable to deal separately with these different types of crystals because the mechanism of their formation, location, and the pathological consequence may all be quite different.

CRYSTAL NODULES IN DEEPER ZONES OF ARTICULAR CARTILAGE

The number of membrane-bound matrix vesicles (50 nm to 250 nm in diameter) is increased in the pericellular area of the chondrocytes in arthritic articular cartilage, especially in the tidemark region, just above the calcified cartilage and subchondral bone. Because of the great number of microscopic matrix vesicles around each chondrocyte and the variation from one area to another, it has not been possible to count the vesicles and assign numbers. It is for this reason that we have previously relied on the quantitative estimation of

the marker enzyme alkaline phosphatase, which is sometimes 30 times as high in osteoarthritic tissue as in the normal cartilage.^{6,7*} Associated with these vesicles, and often originating from them, are dense mineral nodules (0.1 to 0.4 μm in diameter) composed of fine crystals (Fig. 1). Their morphology and electron probe analysis (with a calcium:phosphorus ratio exactly that of hydroxyapatite) indicates that they

are apatite crystals. Their increased presence in arthritic cartilage may imply several pathological consequences, including reduplication of the tidemark, reversion of articular cartilage to growth phase, or a general increased tendency towards tissue calcification. Although such crystals are also present in normal specimens near the tidemark, it is the increase in numbers, distribution, and incidence of occurrence right up to the mid-zone

of articular cartilage that is particularly noticeable.

DENSE, 'CUBOID' CRYSTALS IN THE SURFACE ZONE OF ARTHRITIC CARTILAGE

These microscopic, dense 'cuboid' crystals appear mostly just under the surface of arthritic articular cartilage in the pericellular matrix surrounding chondrocytes (Fig. 2). In some specimens they appear as a band of fine particles in the surface zone. They often appear square shaped in the plane of the section (Figs. 2 and 3) suggestive of a cuboid shape, though this needs further confirmation. These types of 'cuboid' crystals have neither been seen in articular cartilage nor described by anyone else previously, although we have been aware of their presence in arthritic cartilage for some years. They vary in size from 50 nm to 200 nm and are evident in unstained sections and in sections obtained by cryoultramicrotomy,⁸ thus ruling out any preparative artefacts.

Electron probe analysis of these cuboid crystals indicates that they are mainly composed of calcium and phosphorus. Analysis of over 60 cuboid crystals in a section of arthritic cartilage (from a 70-year-old woman) gave a calcium to phosphorus ratio of 1.72:1 while that for an hydroxyapatite standard was 1.79:1 under the same conditions (detailed results will be published elsewhere). This apatite-like Ca:P ratio has been confirmed by analysis of cuboid crystals found in cryosections.¹⁷ It has been difficult to reconcile the apatite-like Ca:P ratio with the cuboid shape of the crystals. There is a small amount of magnesium present with calcium and phosphorus which is indicative of Whitlockite; in their cuboid habit these crystals appear very much like Whitlockite. Electron diffraction and scanning electron microscopical studies are being undertaken to resolve this problem.

These cuboid crystals have now been found in the last six consecutive arthritic specimens that we have examined by electron microscopy. In some arthritic specimens they are present in the surface, intermediate, and deep zones of articular cartilage. They are absent in young articular cartilage but are sometimes present in old 'normal' articular cartilage

Fig. 1 Electron microscopical appearance of human osteoarthritic articular cartilage (from 32 year old woman) showing apatite crystal nodules (type I) and matrix vesicles (arrows) in the deep zone.

Fig. 2 Human arthritic cartilage (from 64 year old man) showing a chondrocyte and dense 'cuboid' crystals (type II) in the pericellular region of the collagenous matrix under the articular surface.

Fig. 3 Higher magnification picture of dense 'cuboid' crystals (type II) and several matrix vesicles closely associated with them (same specimen as fig. 2).

Fig. 4 Surface of arthritic articular cartilage (from 64 year old man) showing needle-shaped crystal clusters and individual crystals of apatite (type III) in an unstained section.

specimens. In one specimen from subcapital fracture of the femoral head some of these cuboid crystals were present adjacent to large areas of pyrophosphate crystal deposits. In some areas of arthritic cartilage they are closely associated with matrix vesicles (Fig. 3). These unique cuboid crystals in arthritic specimens are too small to be viewed by light microscopy and may not show up in radiographs of joints.

NEEDLE-SHAPED CRYSTAL CLUSTERS ON THE SURFACE OF ARTHRITIC CARTILAGE
These fine, needle-shaped crystals occur in clusters on the surface of arthritic cartilage in the acellular amorphous band termed the lamina splendens (Fig. 4). Individual needles in section are approximately 80 nm in length and 8 nm in width. They appear like, and give the same Ca:P ratio as hydroxyapatite. These needle-crystal

clusters on the cartilage surface appear very similar to apatite crystals observed by Dieppe¹¹ and Schumacher¹³ by electron microscopy in synovial fluid, synovial membrane, and synovial phagocytes. Matrix vesicles are not associated with these clusters of fine crystals, which probably originate elsewhere in the joint and are deposited on the surface of articular cartilage as an epiphenomenon. In contradiction to this we have seen these crystals on the smooth surface of a specimen obtained as avascular necrosis of the femoral head where there was no damage or erosion of articular cartilage.

In conclusion, we believe that the presence of these three different types of calcium phosphate crystals and the associated increase in matrix vesicles and alkaline phosphatase activity of arthritic articular cartilage may imply a calcification abnormality and may have aetiological implications in certain forms of osteoarthritis. The discovery of cuboid (Whitlockite) crystals in human articular cartilage is quite new and requires further characterisation.^{16,17} Previously Whitlockite has been reported only in pathological calcification sites in soft tissues such as lung and spleen¹⁸ and in hard tissues such as carious dentine.²⁰

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