THE MORPHOLOGY OF THE CALCIFICATION FRONT IN ARTICULAR CARTILAGE
ITS SIGNIFICANCE IN JOINT FUNCTION

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Biochemical and histochemical studies have indicated that there is specific cellular activity in the region of the calcification front of articular cartilage implying that a regulation process takes place there. Using scanning and transmission electron microscopy and light microscopy to examine tissue sections of both undecalcified and decalcified articular cartilage in the region of the calcification front, we have looked at its morphology with particular reference to its cellular control. Our observations show that physiological calcification is an active process under cellular control and is related to the presence of extracellular membrane-bound matrix vesicles.

Osteoarthritis is thought of as a non-inflammatory disease resulting from a mechanical failure of the joint. A number of conditions lead to osteoarthritis including congenital dislocation of the hip, slipped upper femoral epiphysis, Perthes' disease, trauma and certain generalised metabolic disturbances such as chondrocalcinosis and ochronosis. When such a cause is evident we refer to the disease as secondary osteoarthritis. However, probably less than a third of the cases can be accounted for in this way. Primary (senile) osteoarthritis remains idiopathic. The most commonly held view is that it is a disease of wear and tear, akin to the wearing out of bearings in a machine. However, this view neglects to consider the cartilage as a living tissue capable of repair.

In any living tissue injury results in responses that re-establish equilibrium conditions. Many of the changes observed morphologically in osteoarthritic joints can be attributed to repair processes. Indeed, sequential radiographs occasionally show morphological improvement in an osteoarthritic hip; cysts and bone sclerosis appear to regress and there is a reappearance of the joint space. These changes are similar to those frequently seen after McMurray's osteotomy for osteoarthritis of the hip (Perry, Smith and Whiteside 1972; Macys, Bullough and Goodfellow 1977; Macys, Bullough and Wilson 1980).

Numerous differences have been documented between the chemistry of cartilage and synovial fluid of arthritic and normal joints (Ali and Bayliss 1974; Mankin 1974; Muir 1980). However, it has proved difficult to establish the cause and effect relationships in these findings.

Understanding pathophysiology is dependent upon knowledge of the normal physiology and of the response to disturbances of the organ system. We would like to present a general survey of normal joint function together with a special consideration of one component of its regulation, namely the calcification of the articular cartilage.

We feel that further elucidation of the feedback mechanisms, biochemical as well as physical, that maintain the homeostasis of the calcification front (tidemark) will be important in understanding the aetiology of osteoarthritis. Normal joint function. Normal joint function depends upon a number of factors including freedom of movement between the articulating surfaces, the stability of the joint during use, and a proper distribution of load across its surfaces. These factors are governed by the interdependent actions of the following: the shape (or geometry) of the articulating surfaces; the biological control of the mechanical properties of the constituent bone, cartilage and periarticular tissues; and the integrity of the supportive tissues of the limb, including the ligaments, muscles and tendons.

Through its effect on the distribution of load, the shape of the joint is a key factor in determining the range of stresses imposed upon its tissues (Goodfellow and O'Connor 1980). The forces normally acting upon the articular cartilage, for instance, must fall into a range suited to the continued vitality and viability of the cartilage cells; that is, sufficient to provide a nutritional supply from the synovial fluid (Maroudas et al. 1968) and to stimulate the chondrocytes to adequate matrix production while not so excessive as to cause pressure necrosis (Salter and Field 1960). Degeneration of the cartilage, as evidenced for example by chondrolysis, may occur when
the stress on the cartilage falls outside the optimal range (Maurer and Larsen 1970).

This paper studies one mechanism of joint-space control, that is, the active calcification of articular cartilage and its ensuing endochondral ossification. The implication of disturbances in these mechanisms in stress-induced degeneration of articular cartilage in osteoarthritis will also be discussed.

**The organisation of joint function.** The normal articulating surface is formed from the expanded cancellous end of the bone which is covered by a thin layer of articular cartilage. The articular cartilage is tethered to the jagged surface of the underlying bone by a thin layer of calcified cartilage that interlocks with the bone. However, the two tissues, bone and cartilage, remain distinct (Fig. 1).

The shape of a joint is mostly determined by the bone, not the cartilage, and while the general shapes of joint surfaces are genetically determined, the specific shape of a particular joint must depend on the environmental forces acting upon it. Wolff's law provides that bone density and the organisation of bone trabeculae correlate with the magnitude and direction of applied load (Murray 1936). In articular bone this means that the subchondral bone trabeculae must also be undergoing a self-regulated modelling that maintains a joint shape capable of distributing the load optimally. In other words the shapes of the bone, including the articular ends, are in a dynamic state that reflects a feedback dependent on mechanical stress.

One way for growth and bone modelling to occur is by endochondral ossification (Ham and Cormack 1979). This process is exemplified in the epiphysial growth plate where calcified cartilage is invaded by blood vessels from the subchondral bone and is replaced by bone tissue derived from osteoblasts in close proximity to the blood vessels.

The replacement of the calcified layer of articular cartilage by bone and its remodelling also occurs through this process. Previous studies have shown that both the number of blood vessels entering the calcified articular cartilage and the rate of endochondral ossification change with age; they generally decline up to the age of 60 years and then increase in old age to levels comparable to those of youth (Lane, Villacin and Bullough 1977). This change in modelling activity of the joint may reflect an increased need for compensatory and stabilising remodelling resulting from a loss of joint stability in older individuals due to neuromuscular degeneration. In fact, radiographs of the ankle joints of young and old adults show that the joint of an older person is deeper and more angular in its outline than that of a young healthy adult. Anatomical studies (Figs 2 to 5) have supported the view that subtle changes in the shape of a joint occur with age (Ogston 1878; Moffett, McCabe and Askew 1962; Goodfellow and Bullough 1967).

The expected result of replacement of the calcified cartilage by new bone is thinning and eventual disappearance of the former. However, histological study of cartilage of various ages shows that this does not happen (Lane and Bullough 1980). Therefore, articular cartilage...
is not a static tissue. The matrix turns over and new cartilage matrix (Maroudas 1980) and cartilage cells are formed throughout life. Animal experiments using tetracycline labelling (the tetracycline is incorporated into areas of active mineral deposition) and the histology of human cartilage have led us and others (Lemperg 1971) to a common belief; namely that calcified cartilage remains much the same thickness throughout life because the calcification front continues to advance into the non-calcified cartilage at a slow rate that is in equilibrium with the rate of absorption of the calcified cartilage by endochondral ossification (Green et al. 1970).

Observations on the calcification front. The calcification front (the tidemark) is marked histologically by a well-defined line seen on sections stained by various dyes including haematoxylin and eosin, and phloxine and tartrazine (Fawns and Landells 1953). Histochemical staining has shown the presence of lipid as well as the enzymes ATPase and alkaline phosphatase in the tidemark region (Dmitrovsky, Lane and Bullough 1978). In addition, there is a marked change in the proteoglycan content of the matrix at the tidemark as demonstrated both by histochemical staining and biochemical analysis. The lipid in the tidemark region has been characterised biochemically as containing calcium phospholipid complexes similar to those found in bone (Boskey, Bullough and Dmitrovsky 1980). These findings indicate specific cellular activity in the region of the tidemark and imply that a regulation process takes place there.

In our study we, therefore, looked at the morphology of the calcification front keeping in mind the observed changes in joint shape, the application of Wolff's law to the articular end of the bone, and the dynamic physiological modelling at the end of the bone through the processes of endochondral ossification and the advancement of the calcification front, in order to understand these processes and their inter-relationships.

MATERIALS AND METHODS

Scanning electron microscopy. Two methods were used to remove the non-calcified organic matrix in order to facilitate visualisation of the calcification front by scanning electron microscopy. Method A. Fixation in formalin for 48 hours was followed by covering all but the face of the articular surface with a perforated snugly-fitting piece of aluminium foil. The foil provided a holder so that the tissue did not have to be handled directly. The specimens were then immersed in 100 millilitres of 85 per cent hydrazine at room temperature on an agitator for 16 hours. The hydrazine was changed at 10 hours and finally removed by placing the specimens into a 3:2:1 mixture of ethanol, xylene and acetone at room temperature (100 millilitres changed three times at intervals of one to two hours). This also served to extract the residual lipid from between the bone spicules. The aluminium foil was carefully removed. The specimens were dried in air at room temperature, mounted onto studs with silver paint (with the cancellous bone downwards), and then coated in gold using a vacuum evaporator.

Method B. Samples fixed in formalin were put in ceramic crucibles. These were placed in an oven for 18 hours at 600 degrees Celsius. The specimens were mounted and coated in a similar way to that described in Method A.

Transmission electron microscopy. The tissues were fixed in four per cent glutaraldehyde in 0.1 molar cacodylate buffer and post-fixed in osmic acid. The samples were then embedded in epon and ultra-thin sections cut with a diamond knife. The sections were examined and photographed using a Phillips 201 Electron Microscope.

Light microscopy. The tissue was decalcified in a five per cent solution of nitric acid, and embedded in paraffin. Sections were cut both tangential and perpendicular to the articulating surface and stained with haematoxylin and eosin.

Tangential-fixed but undecalcified samples for light microscopy were also embedded in methacrylate and sections cut to a thickness of four micrometres using a Jung Model-K Microtome. Sections were cut tangential to the articulating surface and calcification front and then stained for calcium phosphate by the von Kossa method.

RESULTS

Method A and B of removing the non-calcified organic matrix were found to produce similar views of the calcification front and the ultrastructure appeared almost identical on the scanning electron microscope. However, Method B samples, utilising the incineration procedure, were seen to have greater numbers of superficial cracks of 100 to 200 micrometres in the calcification front. Thus, for clarity we have shown photographs of tissue prepared by Method A.

It can be seen from the accompanying scanning electron micrographs that the chondrocytes are embedded in the surface of the calcification front and that around each cell is a small mound of calcified tissue (Figs 6–9). The number of cells visualised in this view (tangential to the articular surface) is more than might be expected from the examination by the light microscope of sections four micrometres thick cut perpendicular to the articular surface (Fig. 10). However, if a line were randomly drawn across the surface of the calcification front, as visualised by the scanning electron microscope in Figure 7, not all the cells would be intersected by the line. In fact, the average distances between cells intersected by such a line would probably correlate closely to the distances between cells along the tidemark as visualised in perpendicular sections by light microscopy.

This suggests that the apparent dissociation of the calcification front and the chondrocytes, as seen in Figure 6—Scanning electron micrograph of the calcified cartilage and bone. The non-calcified cartilage has been removed (see text). The chondrocyte lacunae can be seen in the exposed edge of the calcified cartilage and also embedded in the exposed surface. A blood vessel can be seen penetrating into the calcified cartilage from the bone. (×180.) Figure 7—Scanning electron micrograph of the exposed surface of the tidemark after the non-calcified cartilage has been digested away. The larger holes and dimples indicate the position of the vessels in the calcified cartilage. The smaller holes are the impressions of the chondrocytes embedded in the surface. The cracks, which are artefacts of preparation, tend to run between the vessels. (×60.) Figure 8—Scanning electron micrograph showing a close up of a chondrocyte in the surface of the tidemark. (×3 500.) Figure 9—Scanning electron micrograph showing a closer view of the surface of the tidemark illustrated in Fig. 6. Note that around each of the impressions of the chondrocyte lacunae there is a mound of calcified material. (×150.) Figure 10—Photomicrograph of a section through the articular cartilage of an adult dog. Note the change of staining of the matrix which occurs at the tidemark and the scarcity of cells that are intersected by it (compare with Figs 6 to 9). (Haematoxylin and eosin, ×75.)
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Fig. 6

Fig. 8

Fig. 9

Fig. 10

VOL. 65-B, No. 1, JANUARY 1983
conventional histological sections of cartilage cut perpendicular to the surface, is due to sampling: the cellular distribution at the calcification front as visualised by scanning electron microscopy is sufficient to explain this phenomenon and to suggest the interdependence of the calcification front to the chondrocytes.

Also seen on the exposed surface of the calcification front in the scanning micrographs are evidences of vessels that have perforated into the calcified cartilage from the subchondral bone. Some of these vessels are seen as a dimpling of the surface but many are seen as holes in the calcification front because the overlying layer of calcified cartilage is thin and has therefore collapsed (Fig. 6). (The cracks seen running between these holes are artefacts). In perpendicular sections of undecalciﬁed tissue observed by light microscopy, occasional cells will be observed caught within the tidemark. Using the von Kossa staining method, which stains the hydroxyapatite a very dark brown colour, it can be seen that there is calcium extending around these cells from the calcification front (Fig. 11). In plastic-embedded sections of undecalciﬁed tissue that have been cut tangential to the articular surface at the level of the calcification front, there are individual cells surrounded by hydroxyapatite. In decalcified tissues in sections cut tangential to the articular surface and stained with haematoxylin and eosin, one finds similar merging of basophilic stain from around solitary cells to form a confluent sheet of calcified tissue (Fig. 12). Often this staining shows a granular, almost vesicular, appearance. In transmission electron microscopic examination of chondrocytes at the tidemark (Fig. 13), we have noted that hydroxyapatite crystal formation occurs along the collagen fibres (Fig. 14) and almost always overlies trilaminar membrane-bound extracellular vesicles measuring 0.1 to 0.2 micrometres (Fig. 15).

**DISCUSSION**

Studies of endochondral ossification in epiphysial growth cartilage have suggested that calcification is an active process that is initiated in extracellular membrane-bound vesicles 0.1 to 0.2 micrometres in diameter which are seen in proximity to the chondrocytes (Ascenzi, Bonucci and de Bernard 1981). In a similar fashion, our observations show that the physiological calcification of articular cartilage at the tidemark is an active process and also is associated with the presence of extracellular matrix vesicles. It is not, except in some pathological states, a passive process following death of the cell; that is to say, it is not dystrophic calcification since histological examination shows that the cells here are viable and active.

This process of active calcification and subsequent

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**Fig. 11** Photomicrograph of an undecalciﬁed plastic-embedded section of cartilage stained by the von Kossa method. In this photograph a lacuna is seen in close proximity to the tidemark and calcium can be seen extending up from the front to surround the lacuna (compare with Fig. 81. (×750.) Figure 12. Photomicrograph of an undecalciﬁed plastic-embedded section of cartilage which has been cut almost parallel to the calcification front. Individual cells are seen surrounded by calcium and in the lower half of the picture the calcification has become confluent (compare with Fig. 9). (×450.)
endochondral ossification, we believe, is integral to the shape of the joint and therefore to the distribution of load through it.

As yet, the feedback mechanisms which control the formation and advancement of the calcification front are poorly understood. Certainly, they would seem to us to be in some way related to the loading of the articular surface—to be responsive, that is, to Wolff's law.

From the data available it appears that the cells close to the calcification front produce substances that promote mineralisation: extracellular matrix vesicles that provide sites for hydroxyapatite deposition (Boskey 1979; Anderson 1980); enzymes that increase local calcium and phosphate concentration (Ali, Anderson and Sajdera 1971; Matsuzawa and Anderson 1971; Felix and Fleisch 1976; Fortuna et al. 1979); phosphoproteins (Leaver, Trillitt and Holbrook 1975), glycoproteins (Trillitt and Owen 1973; Termine et al. 1981), proteolipids (Vogel and Boyan-Salyers 1976; Boyan-Salyers 1980), and calcium phospholipid complexes (Boskey and Posner 1976; Boskey 1978) all of which may be involved in the initial mineral deposition. In addition there are other factors that regulate the size and orientation of mineral crystals (Price et al. 1976; Hauschka 1979; Urist and Mikulski 1979).

The calcification front may be assumed to be in a state of dynamic equilibrium, and the above mentioned factors promoting calcification are probably counterbalanced by substances that inhibit or limit the extent of calcification. These inhibiting substances include proteoglycans (Blumenthal et al. 1979); nucleotide triphosphate (Termine and Conn 1976; Blumenthal, Betts and Posner 1977) and pyrophosphate (Felix and Fleisch 1976).

Alterations in the feedback mechanisms controlling the calcification front would affect the shape of the joint and consequently the distribution of load across it. A redistribution of load might be expected to affect the tissues comprising the joint, that is the cartilage and
bone. If the optimal loading levels were exceeded the effects on the tissues would give rise to a pathological state.

The physiological maintenance of shape of the joint, the mechanical properties of the tissue and integrity of the supportive tissues all depend on complicated interrelated feedback systems. We believe that insufficient attention has been paid to understanding the failure of these systems in considering the aetiology and pathophysiology of senile, so-called idiopathic, osteoarthritis.

REFERENCES


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