Structural consequences of traumatising articular cartilage

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SUMMARY Articular cartilage-on-bone has been subjected to repeated impact loading in vitro and the associated structural changes occurring in the general matrix examined by optical and transmission electron microscopy (TEM). The study shows that repeated trauma transforms the pseudorandom arrangement of fibrils comprising the general matrix of normal articular cartilage into a structural configuration strongly aligned in the radial direction and displaying a prominent waveform or crimp. This *stress-induced* structural transformation can be predicted from the application of a recently developed structural model of articular cartilage. Further, this altered structure bears a close resemblance to that commonly observed in articular cartilage exhibiting both non-progressive degeneration and osteoarthritic changes.

Key phrases: repeated impact loading in vitro, stress-induced transformation of collagen architecture.

Although a number of investigators have examined the response of articular cartilage to impact loading or trauma, most of these studies have provided only a superficial description of the structural changes induced in the cartilage matrix. For example, earlier work by Radin and Paul¹ showed a dramatic loss of cartilage thickness when joints were subjected in vitro to a combination of sustained high static loads and periodic impaction. Repo and Finlay² reported the development of radial fissures extending from the articular surface down into the deep zone in cartilage/bone samples subjected to a critical level of impact loading. Their scanning electron microscopy studies showed a tearing apart of the collagen fibrillar structure. Dekel and Weissman³ observed damage to the superficial layer of cartilage and detachment to expose the underlying matrix after repeated overloading in vivo.

In the author's view more thorough studies of the relationship between mechanical trauma and structural changes have in part been thwarted by an inadequate understanding of the structural principles governing the function of articular cartilage. In a series of recent papers the author has developed a number of new structural concepts for this tissue, in particular the relationship of the collagen fibrils to each other and their overall orientation throughout

the various functional zones comprising the full cartilage depth.^{4–6} These studies viewed articular cartilage as a composite biological system in which the collagenous architecture provides a three-dimensional meshwork within which the highly deformable water swollen proteoglycans are trapped, and showed that the manner in which the collagenous structure is developed will have a profound effect on the tissue's ability to resist compressive loads, i.e., as a braced structure possessing adequate strength in shear. Further, degradative changes in cartilage involve significant alterations in the collagenous arrangement, and it has been shown that for these changes to occur in a structurally consistent manner a rather specific mode of development of the original fibrillar architecture is required.⁷

There is clearly an important need for metabolically passive trauma studies to be carried out in view of the fact that many skeletal fractures must involve the transmission of abnormally high impact loads through the articulating joints, thereby subjecting articular cartilage to potentially damaging levels of stress.^{2 8 9}

The aim of the present study was simply to traumatise cartilage-on-bone and examine the pattern of matrix response in the light of these more recent structural concepts developed by the present author. The study concerns only the immediate structural response of cartilage to impact without

Fig. 1 Full depth slice of cartilage (articular surface uppermost) showing gross structural changes observed in traversing from undamaged (A) to damaged regions (B and C). ($\times 30$).

reference to more long-term changes in the matrix that might occur through an altered cellular expression or metabolic response. Such studies have in fact been carried out by other workers.^{10 11}

Materials and methods

Cartilage-bone solid segments were sawn from the medial femoral condyles of more than 20 mature bovine animals whose stifle joints were ostensibly free of any degenerative changes. The sawn subchondral base of each composite segment was carefully ground to ensure that it was flat. They were then mounted on a simple impact loading device. This consisted of a 2.4 kg pendulum of radius 0.65 m which could be released manually from a range of heights to deliver a variable energy of impact of 2–12 J to the cartilage/bone sample via a smooth slightly convex 20 mm diameter PVC impactor.

Samples were impacted with a succession of blows 10 to 300 in number delivered at approximately one second intervals. The impaction process was carried out nearly continuously but terminated at the first visible signs of disruption to the articular surface under the impactor.

After traumatisation the layer of articular cartilage directly under the impactor together with some adjacent undamaged control cartilage was removed from the subchondral bone by undercutting. During the entire procedure the articular surface was kept moist with Ringer's solution.

Perpendicular slices of cartilage incorporating the full cartilage depth and ≈ 0.1 – 0.2 mm in thickness were cut from the cartilage layers by methods previously described by the author.⁵ These slices were examined in their wet functional condition by differential interference contrast light microscopy (DIC).⁴

Slices from four cartilage layers were processed for transmission electron microscope examination by standard TEM procedures.¹² Thin sections, which incorporated in their plane the radial direc-

tion of the original cartilage layer, were cut and examined.

Results

Although more than 20 cartilage/bone samples, each taken from different animals, were in fact traumatised, only 10 showed visible disruption within the range of impacting conditions used in the present

Fig. 2 Typical ground-glass like texture of mid-zone of untraumatised articular cartilage incorporating chondrocyte columns aligned in radial direction (arrow). (DIC, $\times 590$).

study. This disrupted tissue showed significant mechanical softening relative to adjacent control cartilage when gently probed. Examination of the undisrupted impacted samples by light microscopy showed no obvious alteration in any of their functional zones when compared with the control cartilage. Of the 10 samples that did manifest a visibly disrupted surface, no consistent pattern could be discerned relating the onset of damage to energy of impact. It is however possible that some energy threshold does exist above which damage is always inflicted in articular cartilage. However, much closer attention would need to be given to preparing a highly uniform configuration of cartilage/bone test piece and eliminating anatomical variations between animals. The wide range of impacting conditions able to induce disruption in the 10 samples would largely reflect the relatively crude sampling procedures used.

OPTICAL MICROSCOPY

Fig. 1 shows at low magnification a typical pattern of gross structural change observed by traversing from the full thickness of undamaged articular cartilage (region A) into an extensively disrupted region

where the upper zone of tissue has been completely removed by the impacting process (region C). In the transition region (B) between these two extremes the cartilage has retained its full zonal thickness, but

Fig. 3 Variety of matrix textures in middle and deep zones of traumatised tissue: (a) fine radial fibrous arrays with mild crimp in vicinity of chondrocyte column; (b) relatively coarse, crimped radial structure, adjacent to fissure extending from articular surface into deep matrix; (c) radial structure with acute crimp. Arrows indicate radial direction. (All DIC, $\times 590$).

its lateral continuity has been broken by the formation of radial clefts or fissures penetrating from the articular surface down into the middle and deep zones.

When the tissue is viewed optically at much higher resolution the single most dramatic consequence of repeated trauma on each of the 10 cartilage/bone samples was that a fundamental change occurred in the character of the middle and deep matrix. Whereas in the unimpacted control cartilage the matrix consistently showed a 'ground-glass' like appearance with no obvious texture or preferred orientation (Fig. 2), the impacted matrix was characterised by the presence of an easily resolved radial arrangement of fibres showing a variable waveform or crimp (Fig. 3). Although variable in its extent in any one impacted sample, this radial texture occupied a significant portion of the general intercellular matrix. It was not merely confined to the structurally more specialised pericellular regions; regions where in fact previous studies have drawn attention to the occasional presence of a fine but optically resolvable radial arrangement of fib-

Fig. 4 Exposed mid-zone of traumatised tissue showing radial tufts consisting of fibrous aggregates. Arrow indicates radial direction. (DIC, $\times 212$).

Fig. 5 Crimped radial fibrous structure (C) at root of cleft (see dashed outline) penetrating down into mid-zone. Arrow indicates radial direction. (DIC, $\times 212$).

rous structure in the deep zones of normal mature articular cartilage.^{13 14}

Where the middle zone was exposed directly to the impacting surface it showed a series of radial tufts comprising loose aggregates of aligned and crimped fibres (Fig. 4). In regions where the original cartilage depth had been preserved but penetrated by deep radial clefts (as in region B in Fig. 1) the radial texture was assumed to be associated with a focus of stress acting at or near the roots of these clefts (Fig. 5). Further beyond the cleft root (shown in Fig. 5) the crimped fibrous texture is replaced by matrix which appears normal.

In the most severely traumatised cartilage (see for example region C in Fig. 1) the middle and deep zones showed a significant reduction in optical density as compared with the less damaged or adjacent control regions. This increased matrix translucency was associated with a more acutely crimped radial structure (see Fig. 3c) and at times a distinct loss of coherency in the fibrous arrangement (Fig. 6).

Fig. 6 Traumatized deep zone showing increased confusion of fibrous arrangement. Arrow indicates approximate radial direction. (DIC, $\times 590$).

TRANSMISSION ELECTRON MICROSCOPY

The four cartilage layers from which samples were examined by TEM were taken from the group of 10 cartilage/bone segments showing visible disruption at their impacted sites. These four were considered representative of this larger group.

The ultrastructure of the middle and deep matrix in the respective control regions was characterised by a dense arrangement of fibril segments generally orientated with varying obliquity about a well defined radial mean (see Fig. 7).

As anticipated from the optical microscopy the middle and deep zones of the traumatised cartilage were characterised by a distinct though variably crimped in-phase arrangement of the collagenous fibrils. Fig. 8 is an example of acute deep zone crimp. Note the tendency for the individual fibrils to aggregate into aligned bundles separated by regions considerably depleted in collagen. This fibril aggregation and concomitant matrix depletion is even more prominent in Fig. 9, a mid-zone matrix structure situated closer to the exposed surface. Here the fibrils have a less acute waveform and are considerably fewer in number than in the deep zone structure in Fig. 8. A more incipient form of fibril

crimp is shown in the mid-zone matrix in Fig. 10 where aggregates of fibrils have become locally kinked without the development of the more extended in-phase relationship as seen in Figs 8 and 9.

An example of mid-zone ultrastructure which when viewed under the light microscope exhibited a distinct loss of optical density and an increasingly confused texture (as in Fig. 6) is shown in Fig. 11. The most distinguishing ultrastructural feature is a greatly increased spatial separation and more random orientation of the fibrillar elements.

The tufted structure (see Fig. 4) developed in the mid-zone exposed directly to the impactor is shown ultrastructurally in Fig. 12. The fibrils comprising this optically distinct structure have aggregated into closely aligned arrays and show marked continuity along their individual lengths.

Discussion

Many other structural details could be incorporated in this presentation as reflecting the spectrum of

Fig. 7 Typical pseudorandom arrangement of collagen fibrils in mid-zone of control cartilage. Note the varying orientations of individual segments about a radial mean (see arrow). (TEM, $\times 14\,000$).

Fig. 8 *Traumatised deep zone showing ultrastructure of acute crimp similar to that shown optically in Fig. 3c. Arrow indicates radial direction. (TEM, $\times 14\,000$).*

ultrastructural changes resulting from the repeated traumatisation of articular cartilage. However, there was one major aspect of structural modification observed in all of the visibly disrupted samples subjected to varying levels and numbers of impacts that could be interpreted consistently at both the optical microscope and ultrastructural level. Whereas the matrix comprising the middle and deep zones of the control cartilage was characterised by an arrangement of discrete fibril segments variously orientated about a mean radial direction, on repeated impact it was transformed into a structure comprising fibrils strongly aligned in the radial direction and displaying a variable waveform or crimp geometry.

Earlier studies by the author have provided convincing experimental evidence that the three-dimensional collagen network in the middle and deep zones of articular cartilage is developed from a principally radial arrangement of relatively continuous fibrils which, over short distances, are repeatedly deflected sideways to create a crude type of zig zag configuration.⁴⁻⁷ As an integral part of this

fibrillar configuration some sort of interfibril linkage would tie neighbouring fibrils into a pseudorandom coherent whole within which the hydrated proteoglycan complexes could be constrained (termed the 'deflected radial fibril model' in this paper).¹⁵

The above concept contrasts with the generally accepted view that the three dimensional network in the cartilage general matrix is developed from fibrils which may be continuous in a particular direction over reasonably large distances within the matrix and which lie in a range of orientations about a radial mean¹⁶⁻¹⁸ (termed the 'oblique fibril model' in this paper).

Although both these structural concepts describe a braced system able to sustain compressive loading they predict quite different structural responses to mechanical 'overload'. In the oblique fibril model high energy impact would probably result in a progressive flattening of those fibrils lying initially at orientations oblique to the radial direction, i.e. the direction of compression, thus encouraging the development of a more transverse fibrous texture. However, with the deflected radial fibril model high energy compressive impact would tend to disrupt first the interfibril linkages, presumably by overextension of the matrix in the transverse direction, thereby destroying either partially or totally the pseudorandom arrangement of each oblique fibril segment. Such fibrils no longer constrained in this

Fig. 9 *Ultrastructure of traumatised mid-zone exposed directly to the impacting surface as a result of the complete removal of the upper layer of cartilage. Arrow indicates radial direction. (TEM, $\times 5600$).*

Fig. 10 *Traumatised mid-zone showing several sites where more localised aggregation and in-phase collapse of collagen fibrils has occurred (see open arrows). Radial direction see solid arrow. (TEM, $\times 12\,200$).*

Fig. 11 *Ultrastructure of traumatised mid-zone showing a largely random and dispersed collagenous arrangement. (TEM, $\times 14\,000$).*

short-range braced configuration most suited to resisting the swelling pressure of the hydrated proteoglycans could then revert to a low energy configuration namely a parallel radial arrangement: a prediction entirely confirmed in the traumatised articular cartilage (Fig. 3). The total length of each fibril previously accommodated within the repeatedly deflected segments of the zig zag configuration would now incorporate a degree of redundancy. This excess length could then be absorbed within the now parallel arrays by means of an in-phase collapse. Again this is consistent with the incorporation of a well defined waveform or crimp in the radial arrays observed in the traumatised cartilage. Further, with a breakdown in the interfibril connections fibrils will be increasingly separated by any additional swelling of the now less constrained proteoglycans (see Figs 9 and 11).

The present experimental results therefore provide further substantial support for the structural model proposed by the author.

It is not yet clear to the present author what the precise mechanism is whereby the upper layer of articular cartilage is disrupted and removed by the traumatisation process. That it does occur as a direct consequence of trauma is consistent with both in-vivo and in-vitro trauma studies of other workers.^{1 3} This upper layer which incorporates the articular surface will, by virtue of its in-plane arrangement of

Fig. 12 Ultrastructure of portion of tufted matrix similar to that shown in Fig. 4. (TEM, $\times 13\,900$).

collagen fibrils, have a pronounced strain-limiting influence on the matrix extensions induced in the transverse direction. Thus provided that structural continuity is maintained across this upper layer, it should be difficult to create any substantial damage in the underlying general matrix. The pattern of structural change observed in all samples examined was always associated with major disruption or removal of the articular surface, and this is consistent with such a prediction.

The regions of middle and deep matrix showing both a confused optical texture and a more random and dispersed fibrillar arrangement at the ultrastructural level (Fig. 11) were observed in samples of cartilage where the upper zone had been completely removed by impacting. Such changes in the matrix probably represent the end effect of repeated trauma—the aligned radial arrays becoming further impacted and collapsing into a less coherent configuration.

Whether or not the proteoglycans remain intact after trauma remains unresolved. Even if they do their role in the compressive load-bearing process

will always be determined by the extent to which they are constrained within the collagenous meshwork. Once proteoglycans are released from such constraints, a situation resulting from the trauma-induced structural transformation described above, they would be redistributed relatively freely by any applied load: hence the consistent pattern of mechanical softening observed in the traumatised cartilage (see 'Results').

RELEVANCE OF THE PRESENT STUDY TO IN-VIVO CARTILAGE BREAKDOWN

There are at least two areas where the results of this investigation may have relevance to the general problem of in-vivo cartilage degeneration. Firstly, there is a striking similarity between the crimped radial texture consistently induced in the traumatised cartilage and that of the softened cartilage obtained from the central weight-bearing region of the tibial plateaus of large mammals⁴—a region not covered by the meniscus and therefore in direct contact with the femoral condyle (Fig. 13). These matrix changes occurring in the human knee joint

Fig. 13 Crimped radial texture commonly observed in mid-zone of bovine tibial plateau articular cartilage. Arrow indicates radial direction. (DIC, $\times 590$).

from early adulthood onwards have been described as non-progressive degeneration.^{19 20} Examination by the present author of more than 40 bovine and canine knee joints has shown that in each case the occurrence of a prominent crimped radial texture was almost invariably associated with a disrupted articular surface. It could therefore be conjectured that this degenerate articular cartilage in the knee joint is the result of repeated mechanical overloading sustained during normal joint function. The present experimental methodology cannot establish whether or not the disruptive potential of a limited number of high energy impacts can be equated with a much greater number of lesser impacts. This would be difficult to demonstrate in an in-vitro experiment in view of the loss of tissue viability with extended intervals of time. However, the very close structural similarity between the traumatised matrix and that obtained from the tibial plateau region leaves this as a distinct possibility.

Secondly, articular cartilage derived from human femoral heads classified clinically as osteoarthritic

frequently shows a similar crimped radial texture easily resolved at the light microscope levels⁶—Fig. 14 shows such an example. While it must be acknowledged that there is little agreement in the literature as to the primary factors which lead to the osteoarthritic breakdown of cartilage, there is a general acceptance that mechanical stress has a significant role in its development.^{21 22} Again it is conceivable that these strong radial arrays are a mechanically induced derivation of the original pseudorandom fibril architecture—this original arrangement being rendered more susceptible to repeated mechanical loading by other complex biological changes.

In summary, this study shows experimentally that in-vitro trauma can induce a systematic change in the structure of articular cartilage, which is consistent with the concept of a 'deflected radial fibril model' developed earlier by the present author. This altered structure closely resembles that commonly associated with degenerate forms of cartilage, thus reinforcing the view that mechanical overloading in vivo is likely to have a significant influence on the rate of functional deterioration of this tissue.

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Fig. 14 Crimped radial texture commonly observed in mid-zone of osteoarthritic human femoral head articular cartilage. Arrow indicates radial direction. (DIC, $\times 590$).

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