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The organisation of collagen fibrils in the superficial zones of articular cartilage

JOHN M. CLARK

Department of Orthopaedics, RK-10, University of Washington School of Medicine, Seattle, WA 98195, USA

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INTRODUCTION

In the classic Benninghoff model of cartilage structure, the tangential surface fibres are presented as an extension of radial fibres which have arched in the transitional zone (Benninghoff, 1925). This relationship has never been demonstrated by direct observation of fibrils but is inferred from polarised light microscopy (Zambrano *et al.* 1982; Dunham *et al.* 1988). Some investigators regard the surface as an independent layer resting upon, but not functionally continuous with, a random network in the middle zones (Redler, Mow, Zimny & Mansell, 1975; Lane & Weiss, 1975; DeBont *et al.* 1986; Inoue, 1981).

Using the scanning electron microscope (SEM), we have observed previously that articular cartilage contains well-defined vertical fibres in the middle layers but were then unable to trace them through the transitional zone (Clark, 1985). In the present study, multiple-plane fractures were examined to solve this problem.

MATERIALS AND METHODS

The articular cartilage from the tibial plateaus, patellae, femoral heads and medial femoral condyles of four mature dogs (physes closed), ten mature rabbits (ages one to two years) and twelve adult human cadavers (ages 38 to 72 years) provided the basic material for this study. The human cartilage was removed fresh at autopsy. The animals were killed by sodium pentobarbitone injection. All of the animal joints were free of degenerative changes. The medical history of the human subjects was unknown but the human material studied showed no local signs of systemic disease or any condition which would affect the joint, such as inflammation or disuse. Some of the human cartilage exhibited mild softening, dullness and focal areas of superficial fibrillation, but no erosions were present. Each joint surface, including a thin layer of subchondral bone, was fixed whole by immersion in cacodylate-buffered 2·0% glutaraldehyde and then dehydrated into ethanol.

Using the 'cryofracture' technique (Humphreys, Spurlock & Johnson, 1974, 1975), each specimen was broken apart with a broad, sharp chisel to create clean surfaces along predetermined planes for SEM viewing (Fig. 1). The joint surfaces were first subdivided by fractures made perpendicular to the articular surface and in the sagittal plane of the body. The resulting pieces were then fractured vertically again, at a right angle to the first fracture, and then tangentially, parallel to the surface. The tangential fractures were made as close to the surface as possible, but usually crossed several layers obliquely. Ideally, orthogonal surfaces in three planes were available for

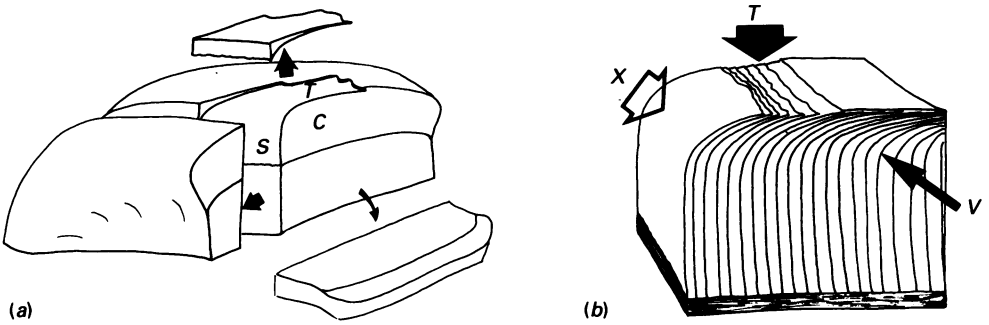


Fig. 1 (*a–b*). The fracture method used to expose the fibrous matrix of articular cartilage. (*a*) Two vertical fractures were made perpendicular to one another. The initial fracture was made in the sagittal (*S*) and the second (*C*) in the coronal plane. A third fracture, parallel to the surface created a tangential section (*T*). (*b*) The fracture sequence exposed two surfaces in which the passage of radial fibres through the transitional zone could be traced: *V*, a vertical section through the fibres where they bent; and *X*, a curved section which followed the plane of the bend. Where surface '*X*' cleanly separated overlapping sheets, an *en face* view of the bending fibres was presented.

viewing. Control of the direction of the vertical fractures was improved by first scoring the subchondral bone with a thin saw. The specimens were held in a foil tray filled with ethanol when immersed in the liquid nitrogen. The mantle of solid alcohol helped prevent crushing and bending artifacts. The chisel was precooled to reduced local warming.

The specimens were critical point dried, mounted on Cambridge mounts and sputter-coated with gold. All were viewed in a JEOL JSM 35C scanning electron microscope. High resolution studies were performed using an Hitachi HFS II with field emission.

In this paper, a collagen fibril is the smallest fibrillar unit (roughly 40–60 nm in diameter) visible to the SEM. A fibre is a group of parallel fibrils. The nomenclature for the zones of articular cartilage follows that used by Benninghoff (1925) and Lane & Weiss (1975).

RESULTS

With practice, creating clean vertical fractures in the desired direction was straightforward. Tangential fractures were more difficult to control and few of these stayed in one plane. For a specimen to yield useful information about collagen fibre configuration and continuity, it was necessary for at least one fracture to run parallel to the fibrils. The orientation and relationship of fibrils transected in one plane could then be reconstructed by observation of the surface in which they were intact (Figs. 2, 3).

Fig. 2 (*a–b*). Fracture combinations. (*a*) Rabbit patella. Here, the vertical fractures meet at right angles and the tangential fracture (*T*) is quite superficial. The specimen presents each of the surfaces shown in Figure 1. On the right (*V*), the fracture remains flat and vertical where it meets the surface. On the left (*X*), the fracture turns where the fibres bend in the transitional zone. Bar, 100 μm. (*b*) Canine tibial plateau. The tangential fracture plane (*T*) in this specimen is oblique and transects collagen fibres at different levels. Where the tangential fracture intersects the vertical fracture (*V*), the relationship between the radial fibres and the details on the tangential surface can be reconstructed. Bar, 100 μm.

Fig. 3 (*a–b*). Vertical fractures showing the continuation of radial fibres through the transitional zone (surface '*V*' in Fig. 1 *b*.) (*a*) Rabbit patella. As seen here, most of the vertical fibres were broken before crossing into the surface. In isolated areas, (arrows), the fibres were intact and their structure in the transitional zone could be studied. Bar, 100 μm. (*b*) Human tibial plateau. In this specimen the vertical fracture (*V*) intersects the tangential fracture surface (*T*), so that the relationship of the fibrils can be seen where they turn (to the left) and overlap in the surface. The surface layers (arrows) are one to three fibrils thick. Bar, 10 μm.

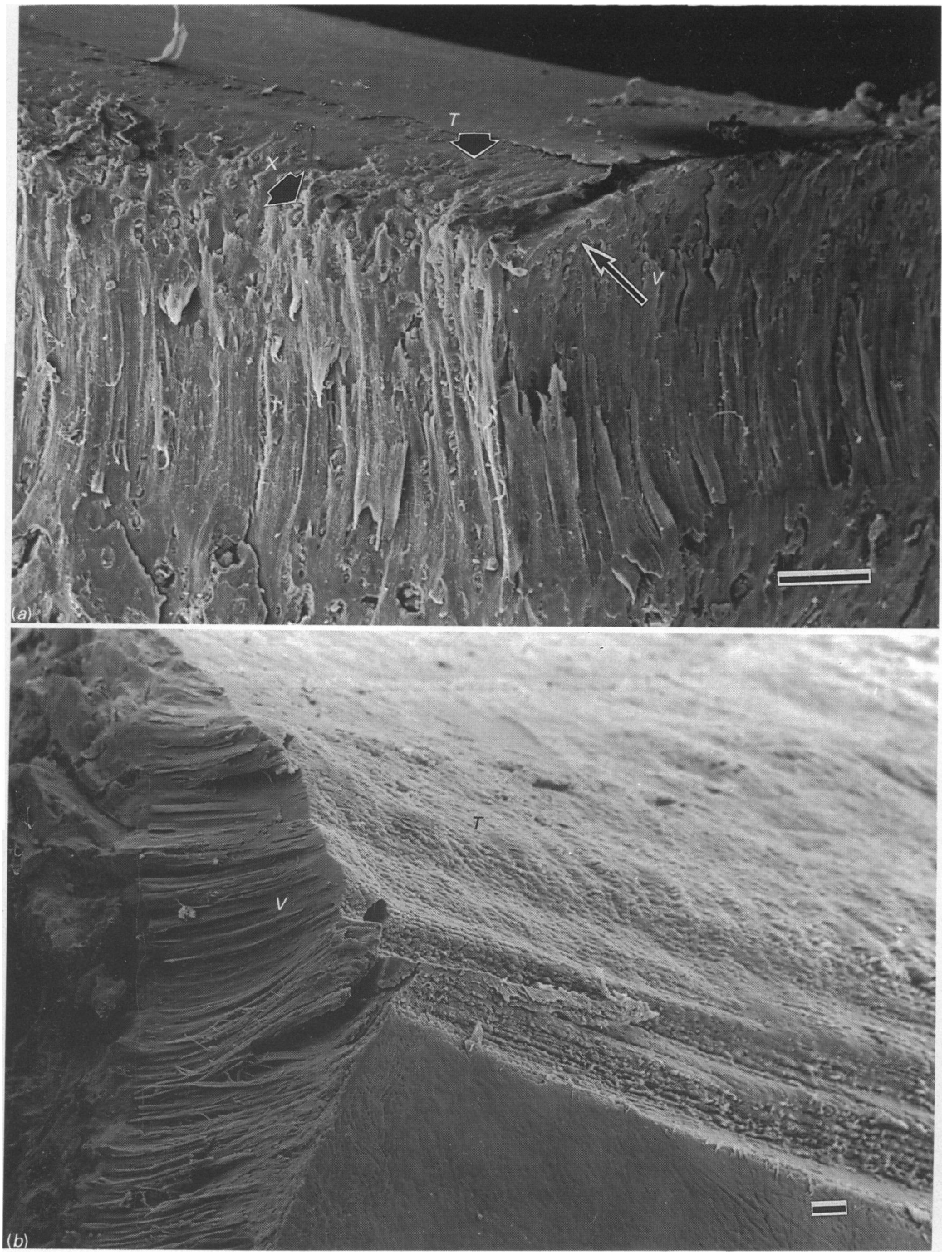


Fig. 2. For legend see opposite.

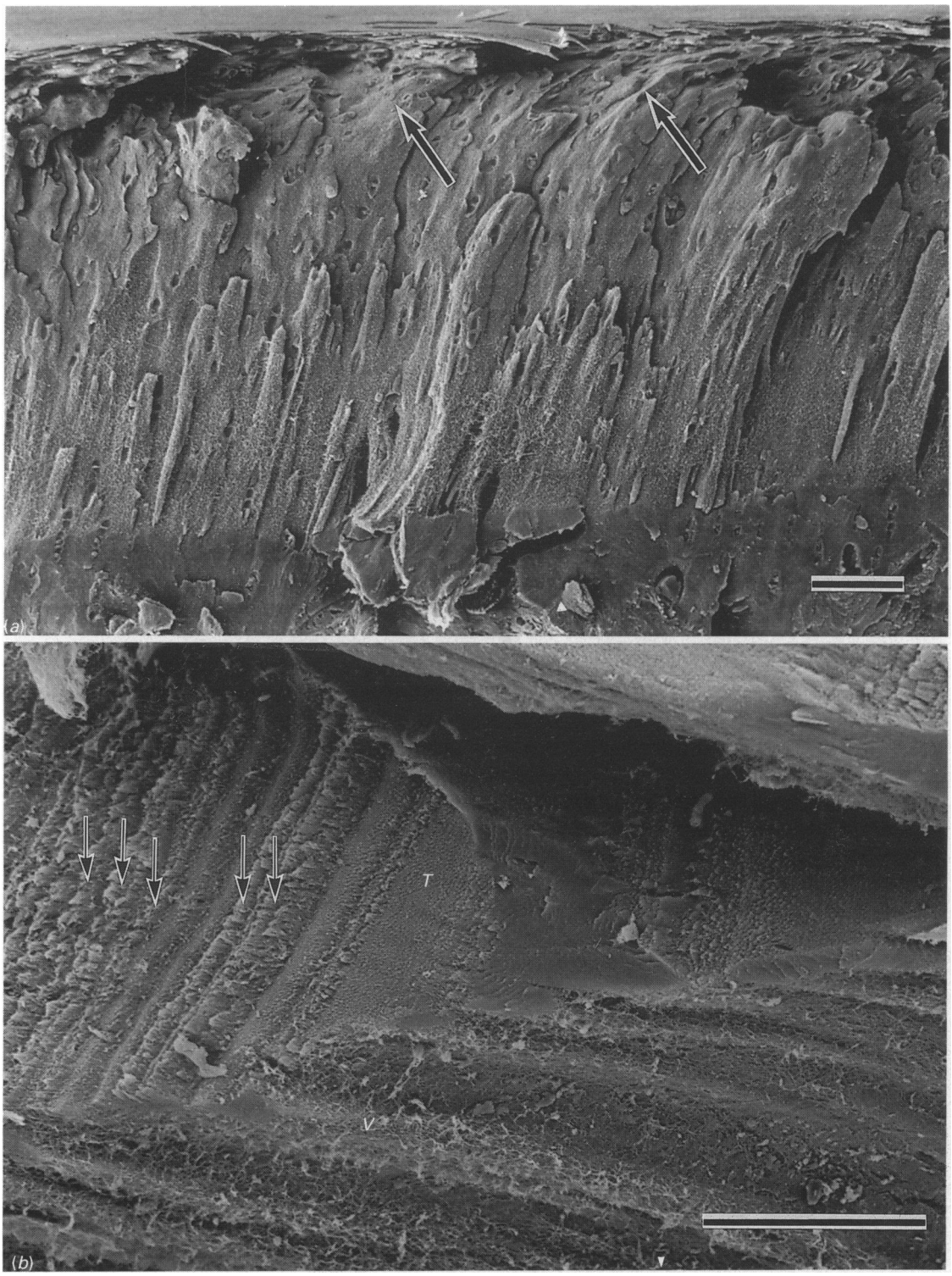


Fig. 3. For legend see p. 118

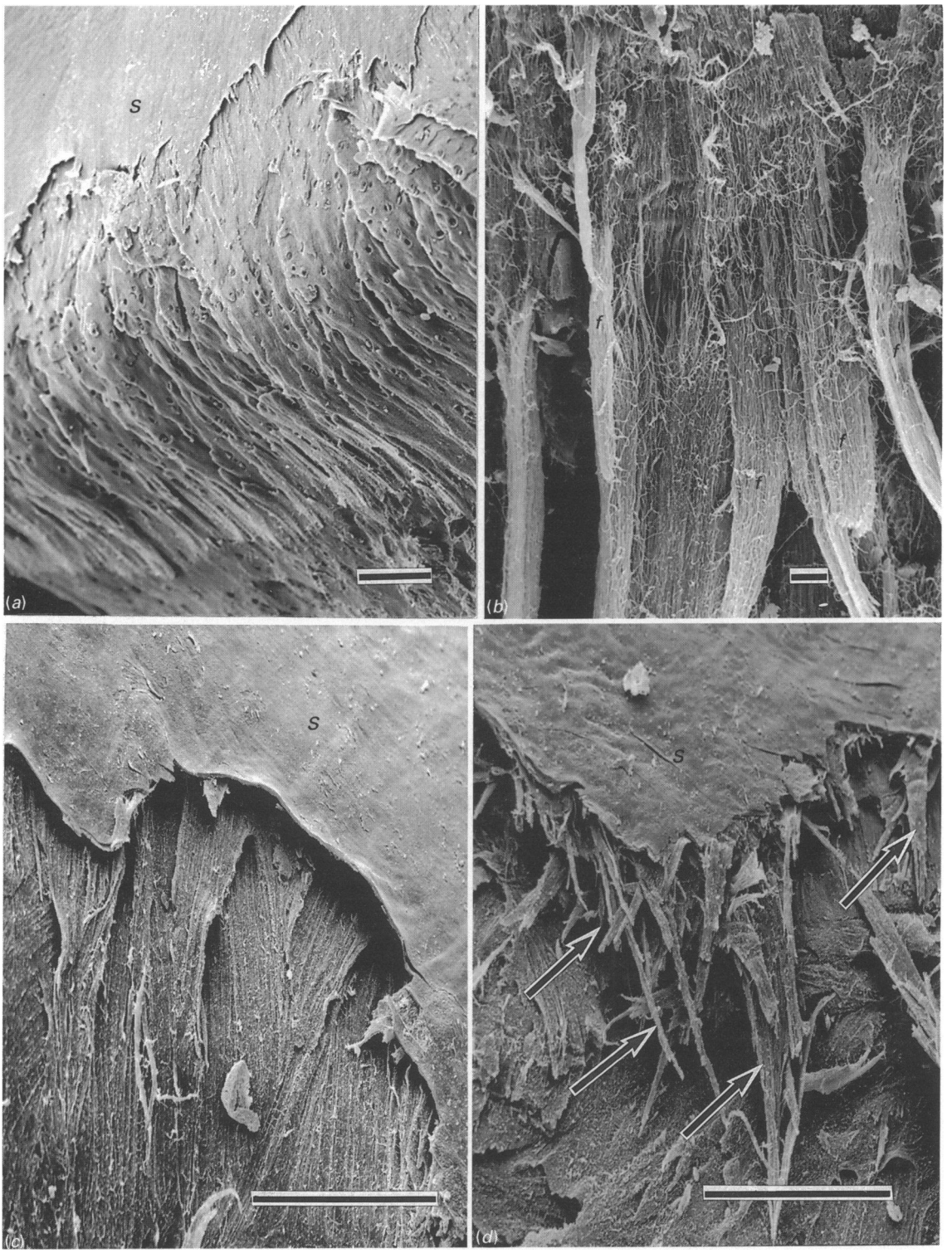
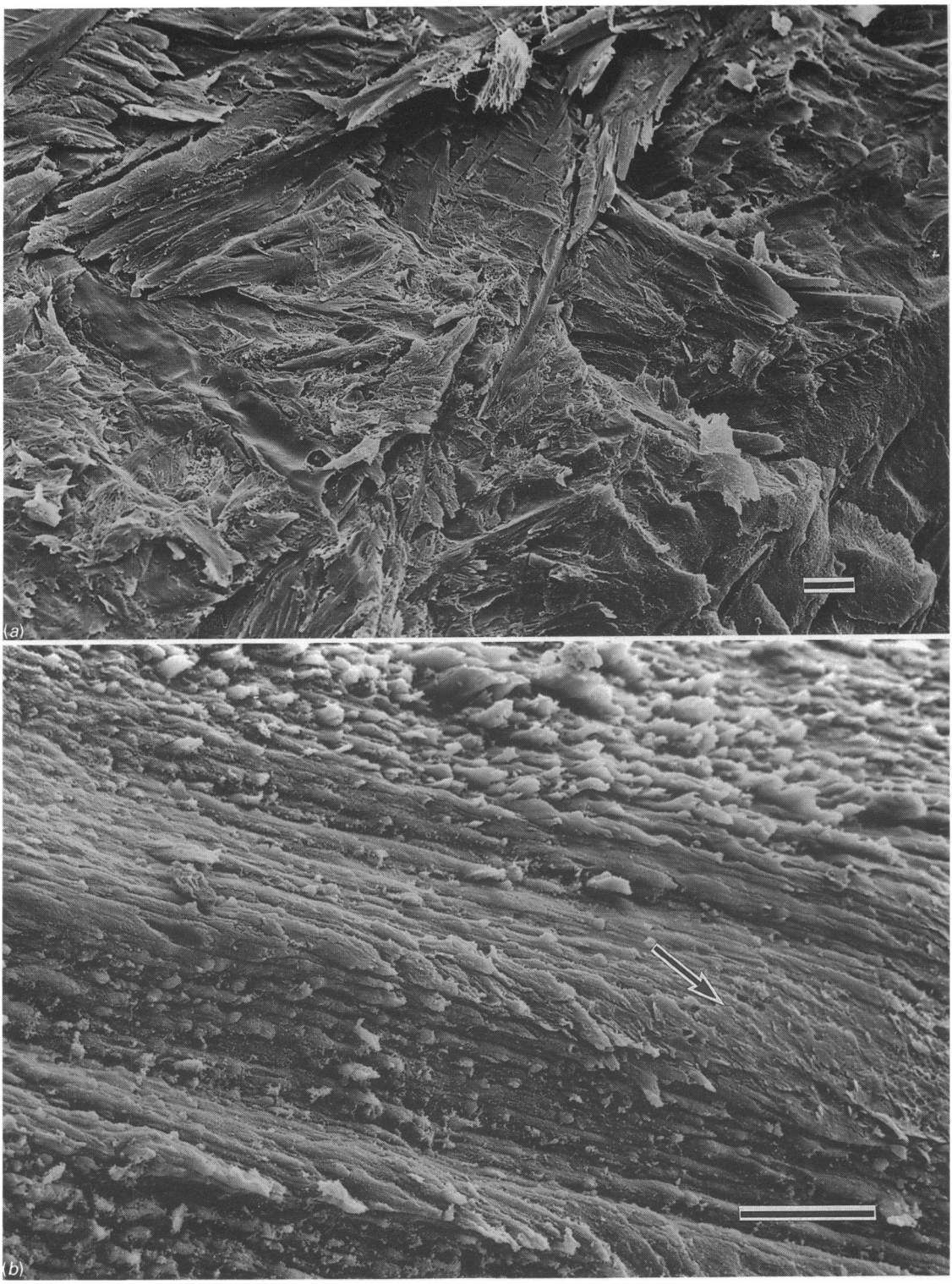


Fig. 4. For legend see p. 125.



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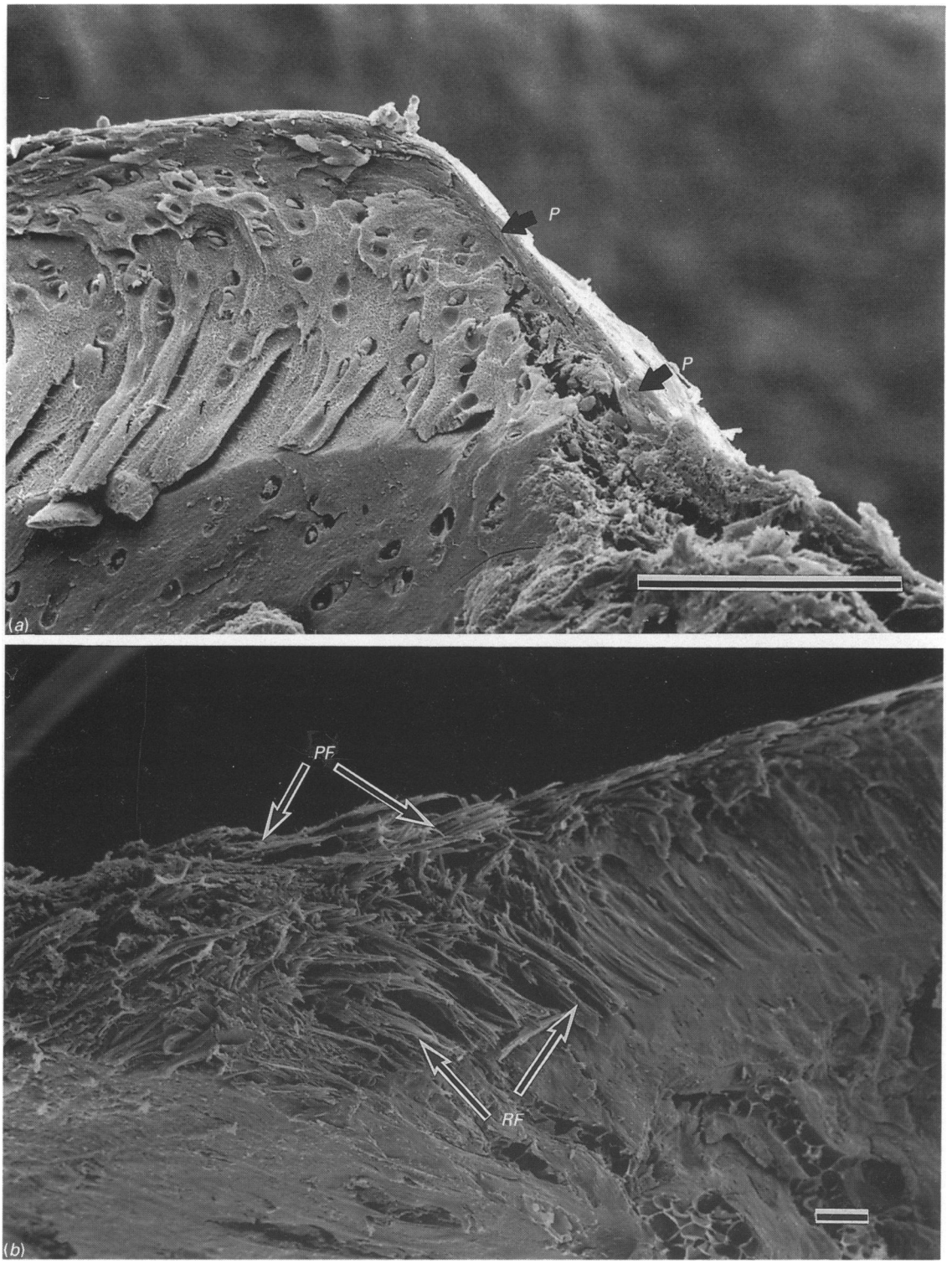

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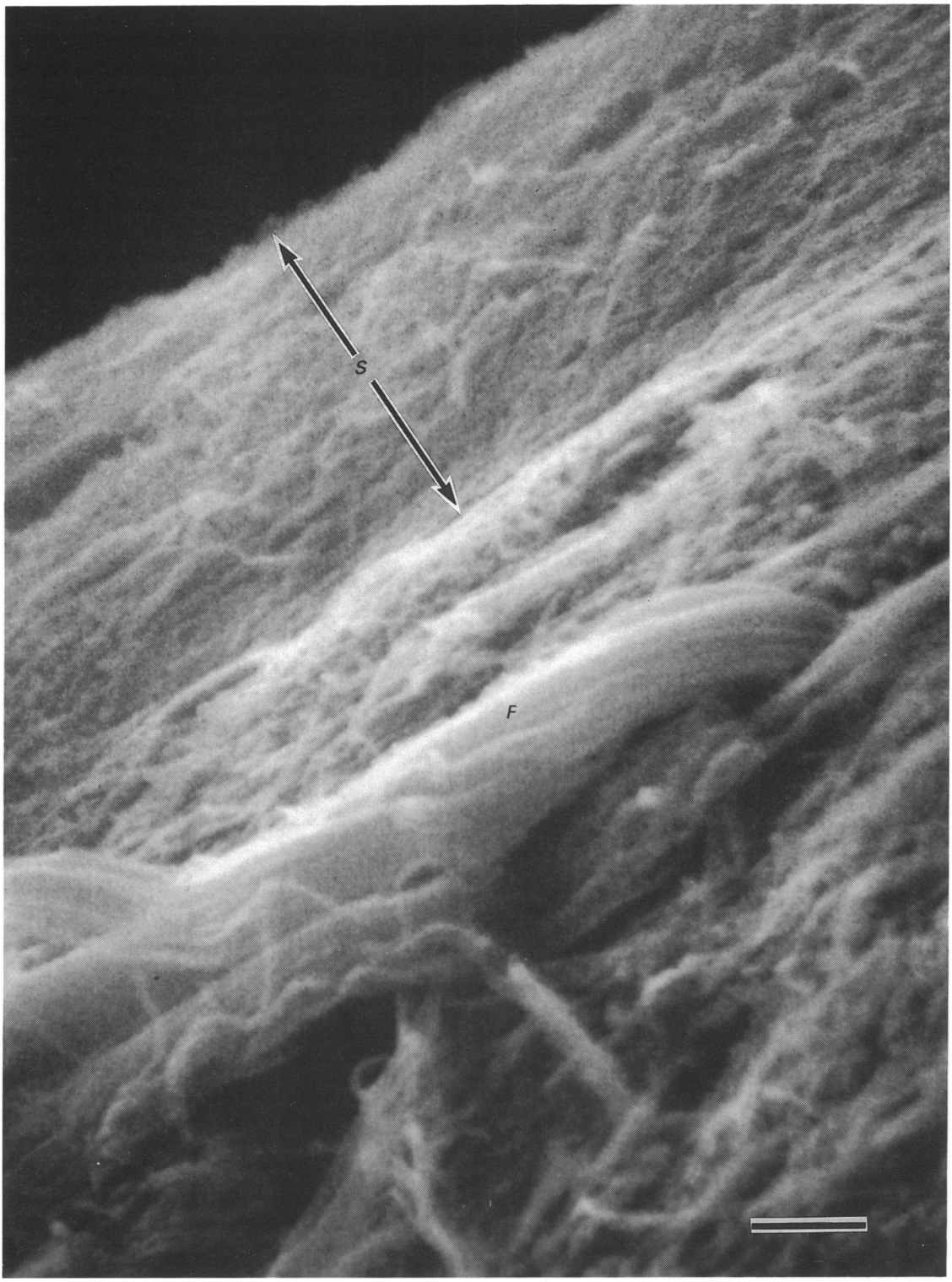

Fig. 7. For legend see opposite.

Generally, the structure of the radial collagen fibres was identical to that described previously (Clark, 1985). The fibre patterns were similar in all three species studied. The radial zone was composed of groups of fibrils (fibres), separated by vertical rows of cells in fibrillar capsules. Vertical fractures which met at right angles provided the most useful information about the organisation of the fibres in the radial and calcified zones. Comparison of the edges where two vertical planes met revealed that the radial fibres tended to run in rows or sheets separated by cell-rich layers. For this reason, the vertical collagen fibres and columns of cells were usually more apparent in one of the two intersecting planes. In the periphery of the tibial plateau, the fibre rows and cell-rich planes were parallel to the nearest edge of the joint surface. In the patella, the rows followed the sagittal plane. This aspect of radial fibre alignment was not mapped in other joint surfaces.

When a vertical fracture passed cleanly through the transitional zone, the surface labelled '*V*' in Fig. 1(*b*) was exposed and it was possible to trace radial fibres into the tangential zone. In all regions, the fibres turned from their vertical orientation as they approached the surface (Fig. 3). In most areas they overlapped and assumed a constant, tangential attitude. In the specimens studied here, the fibres in one region always turned in the same general direction, either towards or away from the edge of the articular surface. In a small number of specimens, an interface was observed where the direction of curvature in the transitional zone changed.

Occasionally, a vertically-directed fracture would turn and follow the radial fibres through the transitional zone into the surface, creating the plane designated by an '*X*' in Figure 1(*b*). These fractures clearly demonstrated the structure of the fibres in the transitional zone because an *en face* view of intact fibres was exposed (Fig. 4). At the periphery of the tibial plateau, the vertical fibres in each row appeared to coalesce into a broad sheet as they turned into the surface (Fig. 4*a*, *b*). In other areas, the individual vertical fibres remained separate and formed relatively narrow strips which remained distinct because they overlapped (Fig. 4*c*).

Fig. 4 (*a–d*). Surfaces exposed by fractures which curve and follow fibrous sheets through the transitional zone (surface '*X*' in Fig. 1*b*). (*a*) Rabbit patella. The fracture curves because it runs among fibres which curve in a common direction through the transitional zone. Intact surface (*S*) is seen at top. The fibres flatten, overlap and turn until they reach a tangential orientation. Bar, 100 μm. (*b*) Rabbit patella. This is a direct *en face* view of the transitional zone. In this section, the individual radial fibres (*f*) join and form broad, thin sheets as they turn into the surface. Bar, 10 μm. (*c*) Canine patella. This *en face* view is near to the centre of the joint surface. Here, the fibres tend to overlap and form narrow sheets. Intact surface (*S*) is visible at top. Bar, 100 μm. (*d*) Canine patella. This *en face* view is of the junction of the periosteum and articular surface. Round fibres (arrows) from the periosteum run out into the surface in a superficial plane. Intact surface (*S*) is seen at top and fibrous lamellae are visible under the periosteal fibres. Bar, 100 μm.

Fig. 5 (*a–b*). Tangential fractures (surface '*T*' in Fig. 1*b*). (*a*) Rabbit femoral condyle, fracture through the tangential zone. Here, the multiple lamellae formed by fibrous sheets are clearly exposed. In this location (the junction of the patellar groove and lateral condyle), the orientation of fibrils in the layers is highly divergent. Bar, 10 μm. (*b*) Canine tibial plateau, tangential fracture through the upper radial zone (detail of Fig. 2*b*). The tendency of radial collagen fibres to lie in rows is apparent. In some areas, (arrow) the fibres have begun to turn and overlap indicating that the fracture crosses the junction between the radial and transitional zones. Bar, 100 μm.

Fig. 6 (*a–b*). The relationship of periosteum to the articular surface. (*a*) Rabbit femoral head. The periosteum (*P*) extends over the joint surface without apparent interruption. In this location, the radial fibres (*f*) turn away (to left) from the joint edge. Bar, 100 μm. (*b*) Dog femoral condyle. Periosteal fibres (*PF*) run into the superficial layer of the cartilage. In this site, a few radial cartilage collagen fibres (*RF*) turn towards the periphery (to left) and appear to interdigitate with the periosteum. Bar, 100 μm.

Fig. 7. Rabbit femoral condyle, high magnification view of the most superficial layer. This layer (*S*) was less than 5 μm thick, relatively dense and contained fine fibrils less than 40 nm in diameter. The fractured end of a tangential fibre (*F*) runs just below the surface. Bar, 1 μm.

Tangential fractures through the superficial zones showed that, where the overlapping layers were tangential, they had flattened into thin lamellae (Figs. 3*b*, 5). These lamellae were one to three fibrils thick and were tightly apposed. Flattened superficial cells lay in the planes among these lamellae. Where several consecutive layers could be seen, it was apparent that the fibril direction in each was not consistent. The fibrils of adjacent lamellae crossed one another at angles which ranged from 0°–45°. The greatest variation in superficial tangential fibre direction was observed in the joint centres and where complex changes in joint shape occurred (Fig. 5*a*). The horizontal dimensions of individual sheets were difficult to ascertain, because the layers usually ran out of the plane of the fracture. Generally, the flattened fibres forming the surface lamellae in the central weight-bearing regions were shorter and narrower than those near the joint edge.

At the edges of the articular surfaces, heavy collagen fibres ran from the periosteum into the tangential layer (Figs. 4*d*, 6). Because these periosteal fibres were distinctively thick and round, they could be traced for about one millimetre. Over the extent to which they were visible, the periosteal fibres did not appear to flatten or leave the plane of the surface. Therefore, no continuity between the radial and periosteal fibre systems was demonstrated. In all specimens, the periosteally-based fibres ran in the most superficial layers (30 μm) of the articular surface.

At the joint edge, fibres rising from the radial zone either turned directly away from or towards the periosteum (Fig. 6). In the latter case, the radial fibres clearly interdigitated with the group arising from the periosteum. In our specimens this communication with the periosteum appeared to involve fibres in only the most peripheral areas of the cartilage. In the centre of the femoral head, the superficial fibres blended into the edge of the ligamentum teres.

Every joint surface except the medial tibial plateau was covered by a layer approximately 5 μm in thickness which rested directly upon, but was not continuous with, the deeper fibre systems (Fig. 7). This layer was also present on the medial plateau, but was interrupted by fine fissures in this region. Elsewhere, the layer was continuous and obscured the tangential fibres of the intact articular surface viewed *en face*. At high magnifications, small fibrils less than 40 nm in diameter and slightly thinner than those forming the underlying collagen fibres were found in the layer. On the vertical and tangential fracture surfaces examined here, no fibres or fibrils from deeper zones appeared to cross into this layer.

DISCUSSION

A detailed three-dimensional model of the collagen matrix organisation in articular cartilage is essential for interpreting the observed biomechanical and biochemical properties of the tissue. This study shows that the fibrous tangential layer of the articular surface is continuous with fibres of the radial zone (Fig. 8). This finding is compatible with most aspects of the classical Benninghoff model. It differs in two important ways: firstly, the fibres do not interdigitate in the transitional zone but, instead, overlap as thin lamellae. Secondly, the fibrous lamellae on the surface are not anchored in the periosteum (Fig. 8). We cannot explain why the collagen fibres appear to interweave in the transitional zone by polarised light microscopy (Speer & Dahners, 1979; Zambrano *et al.* 1982; Dunham *et al.* 1988). In the process of establishing that the radial fibres extend into the tangential zone, we discovered several other features of their structure (Fig. 9). The fibres tend to form rows in the radial zone which are more distinct towards the periphery of the joint surface. While the fibres always flatten

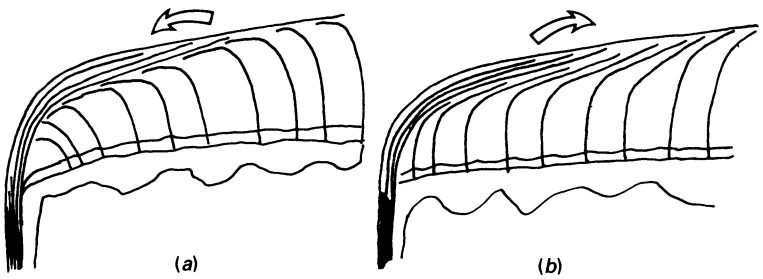

Fig. 8 (*a–b*). Scheme of fibre orientation in articular cartilage. The vertical fibres in the radial zone turn in one direction as they reach the transitional zone, where they flatten and overlap to a variable extent. These fibres turn either toward (*a*) or away (*b*) from the edge. At the joint edge, fibres from the periosteum extend into the articular surface in the most superficial plane.

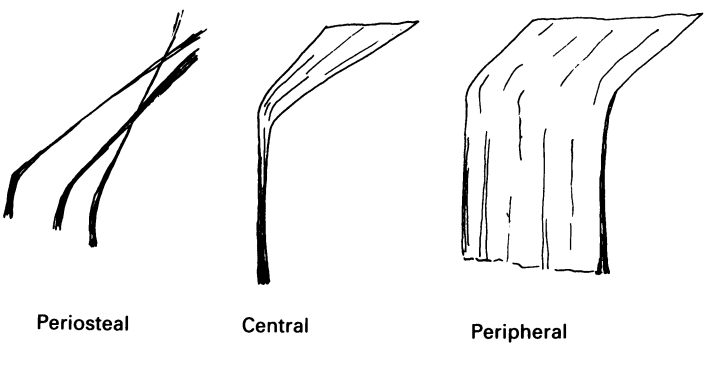

Fig. 9 (*a–b*). The structure of fibres in articular cartilage. (*a*) Illustration of the three types of fibre formations which enter the surface. Fibres from the periosteum are thick, widely spaced and do not flatten significantly as they run over the joint surface. In central areas of the joint surface, single vertical fibres in the radial zone turn and flatten individually, overlapping like scales. At the joint periphery, rows of vertical fibres form sheets in the radial zone which then continue into the surface as broad lamellae. (*b*) The relationship of collagen fibres in the radial zone to the lamellae observed in the surface. The vertical fibres tend to form rows which are separated by cells. As they turn, the fibres flatten, so that at the surface, they are thin. In the terminology used here, the transitional zone is the region where the fibres are bent, the radial zone where they are straight and vertical and the tangential zone where they are parallel to the surface.

at the surface, they form both broad and narrow lamellae which overlap at variable angles. These variations create complex configurations within the tangential layer.

Dunham *et al.* (1988) stressed the importance of light microscope sections made parallel to the collagen fibrils. Our results show that in the scanning microscopy of cartilage, this principle is also critical. Fractures which gave *en face* views of the fibrous layers are key factors in understanding the orientation of fibrils in the surface. Because these layers are thin, overlapping and curved, they are not apparent on flat sections cut for transmission microscopy or SEM. The tendency of the radial collagen fibres to form sheets separated by cells can be understood only through the comparison of vertical sections which intersected at right angles. Recent SEM studies which have reported that the collagen in the radial zone of articular cartilage is randomly organised (DeBont *et al.* 1986; Inoue, 1981) did not utilise freeze-fractures for exposure and did not methodically control the plane of section.

Generally, our findings correlate well with previous descriptions of joint surfaces. Lamellae or overlapping layers of broad fibres in the tangential zone have been described in SEM studies by Draenert & Draenert (1977) and DeBont, Boering, Havinga & Liem (1984). Broom & Myers (1980), who used Nomarski interference light microscopy, found that the lamellae were more distinct at the 'fibrous margin.' The transmission electron microscopic work of Meachim & Roy (1969), Muir, Bullough & Maroudas (1970), Bullough & Goodfellow (1971), Schenk, Eggli & Hunziker (1986) and Weiss, Rosenberg & Helfet (1968) all confirm the presence of interwoven collagen fibre bundles which are parallel to the surface but vary in orientation from layer to layer. With polarised light microscopy, Ortmann (1975) measured angles as large as 45° between crossing surface fibrils. Weiss *et al.* (1968) noted that the superficial layer was thicker peripherally and apparently continuous with the 'perichondrium' there. None of these studies demonstrated the origin of the surface lamellae however.

Light, scanning and transmission electron microscopy have been employed to investigate the relationship of fibre alignment in the tangential zone to the 'split line' patterns formed by pricking the articular surface with a pin (Meachim, Denham, Emery & Wilkinson, 1974; Ortmann, 1975; O'Connor, Bland & Gardner, 1980). Most studies have concluded that superficial collagen fibres play a role in directing the splits, but the relationship is not simple. O'Connor *et al.* (1980) classified the splits into three patterns: unidirectional layered, multidirectional layered and nonlayered. This classification fits well with the types of surfaces found here. In regions where the fibrils in lamellae are generally parallel, a pin should create a straight, 'unidirectional' split. Where they intersect at angles, a pin reasonably might produce stellate multidirectional splits. 'Nonlayered' splits probably form where the layers overlap over such a short distance that the lamellae will not propagate a split. There is some evidence that the rows formed by deeper, radial collagen fibres may also influence split line patterns. Speer & Dahners (1979) and Minns & Steven (1977) noted that the orientation of fibres in the radial zone was more evident in surfaces cut parallel to the surface split lines. The reason for this was obvious when we learned that the radial fibres were delineated by the cell columns in sections made in that plane (Fig. 9*b*). Kempson, Muir, Pollard & Tuke (1973) showed that tensile anisotropy related to split lines was most evident superficially but continued into the deep zones. Our model would not satisfactorily explain this observation, unless the tested specimens contained fibres from the transitional zone.

In every specimen studied here, we found evidence of a thin, but continuous layer covering the articular surface. In position and depth, this region corresponds to the most superficial zone described by Weiss *et al.* (1968) in a transmission electron

microscopic study and by Dunham *et al.* (1988) using polarised light. The existence of a distinct layer equivalent to the 'lamina splendens' is still debated. We concur with Weiss *et al.* (1968) in their observation that this layer contains small fibrils and is distinct from the underlying, collagen-rich tangential zone (I–S in their system). The layer is significant in any SEM study, because it effectively covers the surface of normal joints.

The general features of collagen matrix structure were remarkably similar among the species studied here. Variations in morphology were found when different areas of one joint surface or different joints were compared. The most apparent variation was in the proportion of the cartilage thickness devoted to the tangential layer. This work provides a basis for study of such specific points of anatomical variation and their relation to observed properties of the cartilage.

SUMMARY

The origin and structure of collagen fibres in the surface of articular cartilage were studied using SEM. Cryofracture was used to create orthogonal fracture surfaces in three planes. Fibres which originated in the radial zone could be traced into the surface where they flattened and overlapped in a common direction. Thick fibres from the periosteum ran into the surface as well, but apparently ended there and did not enter the radial zone. The tangential fibres were covered by a dense, separate layer of small fibrils. The fundamental aspects of the model proposed by Benninghoff are supported by these findings.

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