ARTICULAR CARTILAGE: A REVIEW AND SCANNING ELECTRON MICROSCOPE STUDY

1. The Interterritorial Fibrillar Architecture

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The structure of articular cartilage has received considerable attention since Hunter (1743) suggested that the tissue contained a fibrous component orientated mainly vertical to the joint surface. This radial system of fibres was apparently anchored in the subchondral bone and further supported by an interlacing system of transverse fibres. Overlying these fibres was a well defined layer, or "skin", which formed the joint surface and could in fact be peeled off in strips.

This concept of a fibrous structure within articular cartilage was supported by the work of Ranvier (1875), Von Ebner (1882) and Van der Stricht (1885), although studies by Hassall (1849), Kölliker (1854) and Schäfer (1891) suggested that the tissue was homogeneous and did not therefore contain fibres. However, Hansen (1900, 1905) suggested that cartilage only appeared homogeneous because the fibres were masked by "hyaline", the interfibrillar component.

FIG. 1

Various zones described by light microscope (Policard 1936), transmission electron microscope (Davies *et al.* 1962) and scanning electron microscope (present study). The areas scanned at nominal magnifications in this study are indicated for comparison with the accompanying micrographs.

Policard	Davies	Present study
T - tangential zone	SZ = superficial zone	SL = surface layer
Tr - transitional zone	UM = upper middle zone	LS = lower superficial zone
R - radial zone	LM = lower middle zone	MZ = middle zone
C - calcified zone	DZ = deep zone	
B - subchondral bone		

Hultkrantz (1898) attempted to illustrate the fibre orientations in the surfaces of a number of joints. He used a round awl to puncture the articular surfaces and suggested that the splits which resulted were created by a predominantly parallel orientation of surface fibres. Benninghoff (1925) extended this work to include the deeper zones of articular cartilage and proposed his well known arcade concept of fibre structure. This modified the views of Hunter (1743) by suggesting that the calcified zone performed the dual role of anchoring the radially orientated cartilage fibres and of providing an interface between subchondral bone and

uncalcified cartilage. These radial fibres appeared to extend almost to the surface but they turned obliquely in the transition zone (Fig. 1) to run parallel to the articular surface for a short distance in the tangential or superficial zone.

The arcade concept of Benninghoff withstood the test of time, although Shipley (1928) restated the earlier view that cartilage was homogeneous. In 1951 MacConaill argued for a more oblique orientation of fibres rather than the radial system proposed by Benninghoff. However, studies by Ruth (1946) and Randall, Fraser, Jackson, Martin and North (1952)

TABLE I

VALUES OF FIBRIL DIAMETERS AND PERIODIC BANDING OBSERVED BY TRANSMISSION ELECTRON MICROSCOPE STUDIES IN RELATION TO SPECIES AND AGE GROUPS

The periodicities apparently separated into three groups, while the diameters were divided into three classes defined by Silberberg *et al.* (1961)

Authors	Species	Fibril diameter nm			Fibril periodicity nm		
		I	II	III	a	b	c
Randall <i>et al.</i> 1952	Adult, fowl, elephant, human			30-50		18-20	64
Martin 1954	Chick embryo to adult fowl			40	17	27	55
Scott and Pease 1956	Kitten, 2-8 weeks	10			No clear striations		
Cameron and Robinson 1958	Human neonates			25-50			64
Follis and Tousimis 1958	Young rats		18-22		10-13	18-20	
Durning 1958	Rats, 7-48 days			50			
Zelander 1959	Rodents, various ages			55			58
Godman and Porter 1960	Rats, embryo and newborn	7-12	10-18	15-50	7-9		
Takuma 1960	Mice, 1-8 days			20-50	No clear striations		
Silberberg <i>et al.</i> 1961	Mice, 1 day-2 years	10	30	70+		20-30	60-70
Davies <i>et al.</i> 1962 Barnett <i>et al.</i> 1963	Rabbits, 62-74 days 9 months-6½ years		8-25	40-90 280	10	20	40-65
Revel and Hay 1963	Amblystoma, larval	2	10	20-40	Large fibrils striated		
Matukas <i>et al.</i> 1967	Chick, embryo			50			64
Smith <i>et al.</i> 1967	Bullocks, 18 months			25-90			64
Rüttner and Spycher 1968 Spycher <i>et al.</i> 1969	Human, 64-76 years		9-30	15-170			65
Weiss <i>et al.</i> 1968	Human, 15-25 years	4-12		30-80			64
Muir <i>et al.</i> 1970	Human, 26-60 years	10	34	70-140			64

1 nm = 10^{-9} metres = 10 Ångstrom units.

demonstrated that articular cartilage from a variety of species contained a dense network of fibres. Randall described coarse fibres and fibre bundles which exhibited the 64 nm periodicity typical of collagen. These were intermeshed with finer 30 to 50 nm diameter fibres, described as fibrils, which exhibited indistinct periodicities of 18 to 20 nm (Table I).

Sokoloff (1969) has emphasised that the term fibre defines collagen units of width approximately 100 μ m thick, whereas fibrils have widths of the order of 0.1 μ m (100 nm). The term fibril will therefore be employed where appropriate.

Zbinden (1953) and Martin (1953, 1954) reported that the fibrillar network became coarser with age. In addition Martin (1954) described fibrils of 40 nm diameter and above with periodicities 17, 27 and 55 nm (Table I).

Cameron and Robinson (1958) described a fibrillar network in the superficial zone of neonate cartilage which was quite distinct from that elsewhere in the epiphysis. This zone contained 25 to 50 nm diameter fibrils, with the typical collagen periodicity, packed in bundles parallel to the articular surface. The fibrils below this zone were rarely bundled and did not exhibit any periodicity.

Little, Pimm and Trueta (1958) established that this distinct surface layer was present from the foetal stage of development to at least middle age. Below this zone the fibrillar network was random although radial orientations appeared to develop in the deep zones with ageing, especially in osteoarthritic material.

The orientated superficial zone described by Cameron and Robinson (1958) was also evident in the rodent cartilage described by Zelander (1959). However, below this zone, Zelander described a completely random fibrillar network with no observed orientations of either fibrils or fibril bundles. These fibrillar networks were termed interterritorial to distinguish them from the pericellular fibrils, which were referred to as territorial.

This concept of a completely random unbundled fibrillar network in cartilage overlaid by a distinct superficial zone of orientated fibrils or bundles has received further support from the work of Davies, Barnett, Cochrane and Palfrey (1962), Silberberg, Silberberg and Feir (1964), Silberberg, Hasler and Silberberg (1966), Silberberg (1968), Rüttner and Spycher (1968) and Weiss, Rosenberg and Helfet (1968). However, studies by Bullough and Goodfellow (1968) suggested that the fibrils below the superficial zone were radially orientated and furthermore were intermeshed with a system of bracing fibrils, similar to that proposed by Hunter (1743). In addition to the differing ideas on fibrillar orientations, studies with the transmission electron microscope (TEM) have reported variations in diameter and periodicity of the cartilage fibrils. The observed periodicities tend to fall into three categories: 7–17, 18–30 and 40–70 nm, while more recent studies have consistently found periodicities of 64–65 nm (Table I).

Two distinct but interrelated gradients of fibril diameters have been noted, one age-dependent and the other related to the depth of section from the articular surface. Silberberg, Silberberg, Vogel and Wettstein (1961) described three classes of fibrils which appeared at different stages of development in mice. Primitive 10 nm diameter fibrils with indistinct 20–30 nm periodicity (Class I) were present at birth but gradually became less numerous, till at the age of six months they were rarely seen. Young collagen fibres, 30 nm diameter with 60–70 nm periodicity (Class II), were first identified one week after birth and were still present in animals of two years and older. Mature collagen fibres, 70 nm and more in width with 60–70 nm periodicity (Class III), appeared three weeks after birth and were predominant in the older animals.

In addition to this age-related gradient, the transmission electron microscope studies have consistently described an increase in fibril diameter with depth from the articular surface. Thus in a recent study Muir, Bullough and Maroudas (1970) described fibril diameters of 34 nm, 70 to 100 nm and 140 nm in the superficial, middle and deep zones respectively. However, there were also fibrils of less than 10 nm diameter present in all zones, a feature also commented on by Weiss *et al.* (1968).

In complete contrast to the above transmission electron microscope studies, a scanning electron microscope (SEM) study by McCall (1969*a, b*) described very large unbanded fibrils in human articular cartilage. In the superficial zone 100–200 nm diameter fibrils were tightly packed in bundles which lay parallel to the surface. In the middle zone the fibrils were random and varied from 400–600 nm in diameter, while the deeper zone contained 300–1,400 nm diameter fibrils (0.3–1.4 μ m) tightly packed together in a radial manner.

TABLE II
SUMMARY OF FIBRIL ORIENTATIONS DESCRIBED BY VARIOUS AUTHORS, ILLUSTRATING THE DIVERSITY OF SPECIES AND JOINTS SELECTED FOR STUDY

Authors	Species	Site	Surface layer	Zones		
				Superficial	Middle	Deep
Robinson and Cameron 1956 Cameron and Robinson 1958	Human neonates	Distal femoral condyle	Fibrils end abruptly at surface	Bundles parallel to articular surface	Epiphyseal fibrils predominantly radial	Random fibrils but become radial with ageing
Little <i>et al.</i> 1958	Human, foetus to old age	Proximal femoral condyle		Fibrils parallel to articular surface	Random fibrils	Random fibrils but become radial with ageing
Zelander 1959	Rodents, various ages	Distal femoral condyle and patella		Bundles parallel to articular surface	Random fibrils	Random fibrils
Takuma 1960	Mice, 1-8 days	Phalangeal epiphysis		Random fibrils		
Davies <i>et al.</i> 1962 Barnett <i>et al.</i> 1963	Rabbit, 62-74 days 9 months-6½ years	Distal femoral condyle	Afibrillar	Bundles parallel to articular surface	Helicoidal, but may become radial with ageing	
Camosso and Marotti 1962	Cow, 2 months-18 years	Proximal epiphysis of metacarpal		Fibrils parallel to articular surface	Fibrils parallel to surface but become radial with ageing	
Balazs <i>et al.</i> 1966	Cow, embryo to adult (12 years)	Carpo-metacarpal and tarso-metatarsal	Random filaments, 6-9 nm diameter	Random fibrils		
Silberberg <i>et al.</i> 1961, 1964, 1968	Mice, birth to senility	Proximal femoral condyle	Fibrils end abruptly at surface	Sheets of fibrils parallel to articular surface	Random fibrils	Random fibrils
Weiss <i>et al.</i> 1968	Human, 15-25 years	Distal femoral condyle	Random filaments, 4-10 nm diameter	Bundles parallel to articular surface	Random fibrils	Random fibrils
Bullough and Goodfellow 1968	Human	Various		Fibrils parallel to articular surface	Predominantly radial fibrils	
McCall 1969a	Human, various ages	Proximal femoral condyle	Random fibrillar network	Bundles parallel to articular surface	Random fibrils	Radial fibrils
Rüttner and Spyker 1968	Human, 64-68 years	Proximal and distal femoral condyles	Afibrillar	Fibrils parallel to articular surface	Random fibrils	
Meachim and Roy 1969	Human, 37-83 years	Distal femoral condyle and patella	Membrane	Fibrils parallel to articular surface		
Present study	Human, 32-79 years	Proximal femoral condyle and acetabulum	Random fibrillar network, 80-100 nm diameter	Bundles parallel to articular surface	Random fibrils	Random fibrils

It seems likely that the above variations in cartilage architecture could be related to specific dissimilarities in differing species, age groups, joints (Tables I and II) and—equally important perhaps—to methods of tissue preparation.

The scanning electron microscope may provide a means of obtaining clearer answers to these problems. The magnification range of $\times 20$ to $\times 20,000$ is ideally suited to investigations of the fibrillar structure of articular cartilage (Fig. 1). In addition the high resolution and the great depth of focus combine to give a three-dimensional image of the areas under investigation.

The objective of this study was therefore to relate the fibrillar structure of articular cartilage as seen by the scanning electron microscope and the above transmission electron microscope studies, so as to establish an overall picture of the "normal" pattern which could then perhaps be used as a basis for further investigation.

MATERIAL AND METHODS

Human femoral heads and acetabula were obtained at post-mortem from cadavers of subjects aged thirty-two to seventy-nine years. Specimens were selected from areas which were either smooth or fibrillated, but which still retained all or part of the superficial zone of fibrils.

FIG. 2

Directions of applied fracturing force. *a*) From subchondral bone to articular surface; *b*) from articular surface to subchondral bone; *c*) parallel to the plane of the articular surface. *d*) Sketch of a cartilage block revealing fractured surface A fractured as in Figure 2*a*, and surface B fractured as in Figure 2*b*. Ridges or bundles are sometimes evident around the original edges of the specimen. Tufts of surface layer are evident in the articular surface which can be peeled back to reveal the underside of the peeled layer and the underlying exposed surfaces.

The internal fibrous structure of the specimens of articular cartilage was revealed by ripping open small blocks of tissue. This was generally done after fixation and dehydration, but specimens were also fractured at various stages of preparation for comparison. The rupturing force was applied in three alternate directions (Figs. 2*a*, *b*, *c*) and was reduced by partly undercutting the subchondral bone layer. This in turn determined the plane of fracture.

Blocks of cartilage approximately 12×6 millimetres with subchondral bone attached were sawn from the joints, sketched for identification and orientation, trimmed of excess bone, and subsequently washed in Ringer's solution to remove synovial fluid deposits and bone debris. The prepared blocks were fixed in 10 per cent buffered formol-saline solution for periods of

one to four weeks. After fixation, specimens were washed in water and stored in 70 per cent alcohol if not required immediately.

Dehydration was accomplished in ascending strengths of either acetone, or alcohol, or alcohol followed by amyl acetate or di-ethyl ether (Boyde and Wood 1969), with subsequent removal of solvents in vacuo. The specimens were generally fractured at this stage, although freeze-dried specimens were freeze-fractured in Arcton 12 refrigerant prior to dehydration.

The fractured blocks were usually mounted on stubs with Evostick and coated with gold palladium, while the freeze-dried material was mounted with Silver Dag metallic paint and coated with carbon and gold palladium. The specimens were studied in a Stereoscan 11A scanning electron microscope using gun voltages of 10,000, 20,000 and 30,000.

DEFINITION OF TERMS

The areas prepared and examined are illustrated in Figure 2*d* and are as follows: *surface layer*, intact articular cartilage surface normally exposed to the joint cavity; *peeled layer*, thin layer which can be stripped off the articular surface; *exposed surface*, area of the superficial zone lying parallel to the original articular surface and revealed when the peeled layer is stripped off; *fractured surface*, region extending from the subchondral bone to the articular surface, revealed by rupturing the specimen (Figs. 2*a*, *b*, *c*).

For comparison between specimens, six zones were defined in the fractured surfaces as indicated in Figure 1: 1) *superficial zone (SZ)*—10–40 μm deep region below the articular surface—generally appearing afibrous in the fractured surfaces; 2) *lower superficial zone (LS)*—region of fibrillar network immediately below superficial zone; 3) *deep zone (DZ)*—region immediately above the calcified zone; 4–6) *upper, lower and middle zones (UM, LM and MZ respectively)*—equidistant regions between the superficial and deep zones (Fig. 1).

The relationship between these zones and those defined by Benninghoff (1925) and Davies *et al.* (1962) are illustrated in Figure 1. The areas viewed with the SEM at nominal magnifications are also indicated for comparison with the accompanying micrographs.

RESULTS

The tissue blocks exhibited varying degrees of shrinkage after dehydration. The distortion which resulted was particularly evident in some specimens where the cartilage had contracted inwards from the edges of the blocks and was apparently accompanied by a compacting of the more central areas (Fig. 2*d*). The fractured surfaces studied in this report were from the relatively undisturbed central regions of the specimens rather than the peripheral areas, where it has been suggested earlier (Clarke 1971*a*) that ridge-like artefacts can occur.

Articular surface layer—A dense network of approximately 100 nm diameter fibrils was generally evident on intact articular surfaces (Fig. 3). In fibrillated areas this network became progressively disrupted until eventually it could not be identified. Instead, irregular layers of fibrillar appearance 1–50 μm wide were evident, lying parallel to the original articular surface (Fig. 4).

Exposed surfaces—As a result of fracturing the cartilage blocks upwards from the subchondral bone (Fig. 2*a*), specimens frequently had a portion of the surface layer peeled off adjacent to the fracture plane, thus revealing the fibrils on the exposed surfaces (Fig. 2*d*). These areas could generally be torn further by means of fine forceps.

The depth to which the tears extended below the surface as estimated from the thickness of the peeled surface layers was approximately 1–5 μm . Each peeled layer became progressively thinner as it tore across the surface until it finally ruptured free (Fig. 6). The fibrillar networks thus exposed were easily damaged by the electron beam and such areas appeared as darker patches in the network when re-examined at lower magnifications. To minimise this phenomenon, gun voltages of 10 kilovolts were used, although this could, depending on the

FIGS. 3 TO 6

Figure 3—Dense fibrillar network of the surface layer, with strands of thickness 80–100 nm, following the contours of a 30 μ m diameter depression on a femoral head articular surface, in a subject 43 years of age. ($\times 9,600$.) Figure 4—Random fibrous layers or bundles 2–20 μ m wide, running parallel to the fibrillated articular surface from the posterior aspect of a femoral head in a subject 71 years of age. ($\times 100$.) Figure 5—Fibrillar network evident on the underside of a peeled layer (UP) which is in the process of being torn off an acetabular specimen (Fig. 1*d*), in a subject 32 years of age. Layers 8–40 μ m wide ruptured from the network on both the underside of the peeled layer and the exposed surface (ES) are orientated parallel to the direction of tear. ($\times 185$.) Figure 6—Torn region of exposed surface (ES) lies parallel to direction of two split-lines and contains ruptured layers of network orientated parallel to tear and to split-lines. From the acetabulum of a subject 64 years of age. ($\times 90$.)

FIGS. 7 TO 10

Figure 7—Underside of peeled layer from acetabular specimen in a subject 58 years of age, illustrating the relationship between the split-line (dark region) and the torn layers of network. ($\times 280$.) Figure 8—Large bundles of fibrils varying from 150 nm to 3.7 μm wide, found adjacent and parallel to split-line on the underside of a peeled layer. From the acetabulum of a subject 58 years of age. ($\times 3,200$.) Figure 9a—Layered arrangement of fibrils on the exposed surface from the acetabulum of a subject 58 years of age. Only a few cellular structures are evident (*arrowed*) in the layers 10–60 μm wide which are orientated in the general direction of the tear. ($\times 390$.) Figure 9b—Inset of Figure 9a. Fronded fibrils are evident on the exposed surface. ($\times 1,600$.) Figure 10—Fronded fibrils on the exposed surface of a femoral specimen in a subject 68 years of age. The fibrils appear to anastomose as a result of their cross-over points being masked by a coating of mucin. ($\times 7,500$.)

FIGS. 11 TO 15

Figure 11—Fractured surface of a femoral specimen in a subject 71 years of age. Cellular structures are evident as dark spots in the radially orientated layers of the network. ($\times 34$.) Figure 12—Middle zone from a femoral specimen in a subject 68 years of age, illustrating the radial layers of network and empty cellular cavities. ($\times 145$.) Figure 13—In the middle zone of an acetabular specimen in a subject 32 years of age, the radial layers terminate at or near the cellular cavities and tend to spiral around them. ($\times 260$.) Figure 14—In the middle zone of an acetabular specimen from a subject of unknown age, the radial layers reveal well defined edges and a random network of fibrils. ($\times 965$.) Figure 15a—Oblique view of femoral fractured surface from a subject 71 years of age. At this acute angle the radial layers are seen as overlapping structures extending from the articular surface layer (SL) to the subchondral bone layer (B). ($\times 55$.) Figure 15b—Oblique view from the middle zone of the fractured surface illustrated in Figure 15a. The overlapping nature of the radial layers is evident. ($\times 1,700$.)

FIGS. 16 TO 19

Figure 16—Fractured surface (FS) and fibrillated articular surface (S) of the specimen illustrated in Figure 4. Numerous fibrillar tufts protrude from the surface and there is no sign of a distinct superficial zone. ($\times 90$.) Figure 17—Fractured surface of the superficial zone of an acetabular specimen from a subject of unknown age. The radial layers run almost to the superficial zone (SZ) before turning to run parallel to the surface. ($\times 315$.) Figure 18—Fractured surface of femoral specimen from a subject 71 years of age. The radial layers turn obliquely at or near the upper middle zone (UM) and run through the lower superficial zone (LS), terminating at or near the superficial zone (SZ). ($\times 105$.) Figure 19—The articular surface (SL) tears in an irregular manner forming a multilayered superficial zone (SZ). The underlying lower superficial zone (LS) contains the upper portion of a radial layer. Femoral specimen from a subject 73 years of age. ($\times 700$.)

FIGS. 20 TO 28

The micrographs shown in Figures 20 to 24 illustrate the various zones from the superior posterior aspects of a femoral head from a subject 71 years of age, prepared by freeze-fracturing and freeze-drying. Figure 20a—A fibrous appearance of the superficial zone (SZ) contrasted with the fibrillar network of the lower superficial zone (LS). The dark oval in the lower superficial zone may be the site of a cell. ($\times 765$.) Figure 20b—The lower superficial zone fibrils are 90 to 180 nm thick. ($\times 5,420$.) Figures 21a to 24a—Upper middle, middle, lower middle and deep zones (UM, MZ, LM and DZ respectively). The fibrillar networks exhibit little variation in either density or orientation. ($\times 1,750$.) Figures 21b to 24b—The corresponding zones at higher magnification contain fibrils averaging 100 to 200 nm in width with no apparent variation with depth. The individual fibrils have a matted appearance. ($\times 10,000$.) Figures 25 to 28—Figure 25—Adjacent areas in the lower middle zone of a femoral specimen from a subject 79 years of age, which exhibit differing predominant orientations: a) random, b) oblique, and c) radial. ($\times 4,500$.) Figure 26—The basal layer of cartilage from a freeze-fractured femoral specimen in a subject 71 years of age. The deep zone (DZ) fibrillar network merges with the distinct calcified zone (CZ) which overlies the layers of subchondral bone (B) enclosing the marrow cavities. ($\times 130$.) Figure 27—Basal layer of an acetabular specimen from a subject of unknown age. The calcified zone (CZ) is quite distinct and contains cellular remains (arrowed). The subchondral bone layer (B) contains many openings 4–7 μm in diameter, probably blood vessels. ($\times 150$.) Figures 28a and b—Calcified matrix from the specimen illustrated in Figures 20 to 24. ($\times 1,750$ and $\times 10,000$ respectively.)

microscope performance, result in some loss of resolution at the higher magnifications (Figs. 20*b* to 24*b*).

Orientated layers of fibrils were evident on both the exposed surfaces and the underside of the peeled layers (Fig. 5). These 5–40 μm wide layers were often parallel to each other and to the direction taken by the surface tears. It was also noted that these tears generally followed the orientation of the split-lines in the surface (Fig. 6). Indeed it was difficult to tear the surface layer in a direction normal to the splits but comparatively easy in a direction parallel to them. In the undersides of these peeled layers, the layers of fibrils generally followed the orientation of the split-lines (Fig. 7) while in a few specimens they appeared to have ruptured further into 1–5 μm thick "bundles" (Fig. 8).

On some exposed surfaces the layers of fibrils overlapped and intersected one another but still maintained a general orientation parallel to the direction of tear (Fig. 9*a*). Within these layers the individual fibrils appeared unorientated (Fig. 9*b*) and generally exhibited a fronded appearance (Fig. 10). However, in a few torn areas the fibrils within the layers were apparently orientated parallel to each other and to the direction of tear, although fronded fibrils were still evident.

Fractured surfaces: general features—To the naked eye, the fractured surfaces of articular cartilage apparently contained a series of parallel radially orientated ridges which ran from the subchondral bone to just below the articular surface, reminiscent perhaps of the radial "fibres" described by Hunter (1743). However, when studied by scanning electron microscope at low magnifications the ridges appeared as a series of 10–80 μm wide layers of fibrillar network in the fractured surfaces (Fig. 11). This was a consistent feature of the fractured surfaces, regardless of the method of fracture or dehydration. When viewed normal to the torn surfaces, the position of these layers was marked by bright radially orientated lines which were apparently created by the electron beam charging up the network at the discontinuities (Figs. 12 to 14). The layers generally terminated at or near cellular structures and varied in appearance from the irregular nature of Figure 12 to the more ordered arrangement of Figures 13 and 14.

The overlapping layered arrangement of the network was more evident when the fractured surfaces were studied at an acute angle (Figs. 15*a*, *b*) while the fibrillar network, evident at magnifications approaching $\times 1,000$, was generally of an unorientated nature (Figs. 14, 15*b*).

Fractured surfaces: zones—The radial layers of the fractured surfaces generally extended into the lower superficial zone with which they merged, although in a few fibrillated specimens this was not evident (Fig. 16). In some surfaces the radial layers ran almost to the superficial zone before they turned to run parallel to the surface (Fig. 17), whereas in other specimens the radial layers turned at or near the upper middle zone and then continued obliquely through the lower superficial zone to the superficial zone (Figs. 18 and 19). This arrangement of fractured surface layers was strikingly similar to the arcade structure described by Benninghoff (1925) (Fig. 1).

The superficial zone of the fractured surfaces was unique in that it seldom exhibited either fibrils or cellular structures. It varied from 10 to 40 μm in depth below the articular surface and gave the impression of being composed of several parallel layers (Figs. 19 and 20*a*).

There was an abrupt transition from this zone to the random fibrillar network of the lower superficial zone (Fig. 20*b*), which generally extended to the calcified region with little variation in density or orientation (Figs. 20 to 24). The fibrils generally varied from 40 to 460 nm, although strands of over 2,100 nm (2.1 μm) have been measured in the matted networks of specimens fractured prior to fixation and dehydration. In certain specimens local areas of orientated fibrils were observed (Fig. 25), but these were not generally representative of the interterritorial fibrillar network.

At low magnifications the calcified zone was seldom well defined (Figs. 11, 15*a* and 26), while at higher magnifications it appeared as an undulating afibrous region separating the deep

fibrillar network from the underlying subchondral bone and marrow cavities (Figs. 26 and 27). The calcified matrix had a tufted appearance in the fractured surfaces (Figs. 28 *a* and *b*).

The calcified zone and deep zone boundary were generally well defined whereas the calcified zone and subchondral bone boundary were relatively indistinct. In many of the fractured surfaces the subchondral bone layers and calcified matrix exhibited cracks or fissures (Fig. 11), which were attributed to the stresses imposed during the fracture of the tissue.

DISCUSSION

The varying amounts of distortion encountered in these specimens after dehydration must affect the fibrillar network to some degree, yet little can be done at this stage to control it. Walker, Sikorski, Dowson, Longfield, Wright and Buckley (1969) estimated this shrinkage as an overall linear contraction of 10 per cent accompanied by edge curling. Indeed in the experience of the author it can be much greater and would appear to vary with the thickness of the cartilage. Thus the fibrillar organisation of the specimens must be interpreted within these limitations.

In this study the fibrils generally varied from 40 to 640 nm in width, with average values of approximately 100 to 350 nm, although some specimens had a badly matted network with fibrils of width greater than 2 μm (2,000 nm). The latter were probably comparable with the 1.4 μm diameter fibrils described by McCall (1969 *a* and *b*). These values contrasted sharply with the coarser collagen fibrils described by transmission electron microscope, which varied from approximately 20 to 90 nm in diameter (Table I), although values of 280, 170 and 140 nm were observed by Barnett, Cochrane and Palfrey (1963), Rüttner and Spycher (1968) and Muir *et al.* (1970). In addition, Koizumi (1964) found aggregations of fibrils which were 200–300 nm thick.

However, the fibrils described in this present study, although of similar size, did not appear to be aggregates of smaller fibrils. Rather their appearance suggested that some form of coating obscured the fibrils. This would indicate that the fibrils in this study 40 nm and upwards of 200 nm in diameter represented the 10 and 80 nm in diameter fibrils described by Weiss and colleagues, and that they only appeared three to four times thicker due to an adherent coating of some substance. It would explain why the cross-over points in the fibrillar network were very often obscured, the lack of variations in fibril diameter with depth, and also the lack of striated fibrils at higher magnifications. That the scanning electron microscope can reveal such periodic structures has been conclusively demonstrated in our laboratories by Finlay and Hunter (1971).

The existence of a network structure at all in our surface-scanning studies was probably due to the fact that aldehyde fixation results in the loss of a large proportion of the amorphous ground substance (Szirmai 1963), and this would also explain the empty spaces around the fibrils. However, apparently not all the mucin content was washed out. This may have been a result of the protein polysaccharide complexes being physically entangled with the collagen fibrils (Campo and Dziewiatkowski 1962, Revel and Hay 1963) or due to an actual bond between collagen and protein polysaccharides (Myers, Highton and Rayns 1969; Campo 1970; Podrazky, Steven, Jackson, Weiss and Leibovich 1971), or both. It may be possible to remove this coating by enzymatic digestion or maceration by alkalis, but research along these lines has so far been unencouraging (Clarke 1971*c*).

The surface layer of articular cartilage has been described as hyaline by MacConaill (1951), as fibrillar by Cameron and Robinson (1958), as afibrillar by Davies *et al.* (1962), as an absorbed layer of hyaluronic acid by Balazs, Bloom and Swann (1966) and as a membrane-like layer by Meachim and Roy (1969). In the present study, the intact areas of the articular surfaces generally exhibited a dense unorientated fibrillar network, even in the very aged material. In addition this type of surface layer was apparent in the very young specimens described by

McCall (1968). It would therefore appear likely that this fibrillar network is a feature of intact human articular surfaces, regardless of age. This network was quite distinct from the fibrillar appearance of the superficial zone, a feature also commented on by Wolf (1969) in a replica study of articular surfaces. Wolf suggested that this surface layer was a "chondral membrane" as distinct from the joint cartilage and was present from birth to adulthood. However, while many of the features described by Wolf have been further confirmed by scanning electron microscope, the author feels that the existence of a non-cartilaginous membrane has neither been proved nor disproved. It would appear likely that differing techniques used by various investigators have resulted in varying appearances of the same type of surface layer. Thus the articular surface networks described by scanning electron microscope appeared similar to the random fibrillar surface layer described by Weiss *et al.* (1968).

The internal fibrillar structures described in this report were prepared by tearing selected areas of tissue apart. Conventional microtome sections of frozen material were of little help because the fibrillar structures were not recognisable on such surfaces (Clarke 1971*b*). However, the fracturing technique is not without disadvantages. Boyd and Wood (1969) commented that although ripping of tissue was the most satisfactory method of preparing material for the scanning electron microscope, the possibility of general damage and structural displacement must be ruled out before any novel interpretations could be made. Two obvious criticisms that can be levelled against this mode of sectioning are that the tearing process might orientate an otherwise random network in the direction of the tear (Clarke 1971*a*) and, conversely, that fibrils in an orientated structure might become disrupted and obscure the orientation.

In this study the torn surfaces generally exhibited both random and orientated structures. At low magnifications both the fractured surfaces and exposed surfaces contained overlapping layers, usually parallel. In the fractured surfaces the path followed by these layers was strikingly similar to the pattern of Benninghoff's arcades (Fig. 1), which suggested that these were attributable to some morphological arrangement and were not therefore artefacts produced by fracturing the tissue. Why the layers tore in this manner could not be satisfactorily explained from the scanning electron microscope micrographs. The fibrillar network was apparently unorientated and would therefore have had little influence on the plane of fracture. However, it was noted that the classical description of the cellular organisation frequently followed the arcade pattern with radial rows in the deeper zones; arranged in groups in the intermediate zone; and at the surface, lying in layers parallel to the joint cavity (Barnett, Davies and MacConaill 1961). In fact, before the advent of the transmission electron microscope, microscopy techniques were unable to resolve individual cartilage fibrils and thus their position and orientation were usually inferred from the cellular arrangements (Hamerman and Schubert 1962). Thus the arcade structural concept may be more related to cellular than to fibril orientations. One could therefore speculate that the planes of weakness which govern the fracture of the tissue were provided by either the cellular organisation, the disposition of the matrix by the cells, or both. In this respect it was interesting that the radial layers in the fractured surfaces frequently terminated at or near the cellular structures, much akin to the arrangement of Benninghoff's "chondrones" or cell baskets.

Nevertheless, the arcade-like layers defined the tangential, transitional and radial zones of light microscopists (Benninghoff 1925) while at higher magnifications only the orientated superficial zone and random deeper networks described by Weiss *et al.* (1968) were evident. Several authors have described a radial predominance of fibrils in the deeper zones with advancing age (Table II), a feature especially evident in osteoarthritic cartilage (Little *et al.* 1958). In the material examined above, which included fibrillated specimens from subjects up to seventy-nine years of age, there was little evidence of a predominantly radial orientation of fibrils. In some specimens there may have been some slight radial alignment of the network

as a whole, but this was so minimal that it would have taken a computer analysis of the micrographs to be certain. However, local regions of orientated fibrils were occasionally found, but these may have been part of the territorial (pericellular) fibrillar organisation of cells not evident in the plane of fracture.

On the exposed surfaces the interweaving layers may be comparable to the intersecting bundles of parallel fibrils described by various authors in the superficial zones of articular cartilage (Table II). These bundles apparently determined the direction of the surface tears and split-line orientations as described by Bullough and Goodfellow (1968). In a similar study of exposed surfaces Mital and Millington (1971) described a zone of cross-linked fibrils lying subjacent and parallel to an amorphous surface layer. Fronded fibrils, still orientated parallel to the surface, occurred below this zone while a true random layer was not found until much deeper. In the present study the precise arrangement of fibrils within these surface layers could not be satisfactorily determined, no doubt due to the disruption experienced within these layers during tearing.

Thus while the scanning electron microscope can reveal the internal architecture of articular cartilage to great advantage, the nature of the preparation techniques makes the interpretation somewhat speculative. However, it should be possible to combine both scanning electron microscope and transmission electron microscope techniques in a controlled study to overcome these difficulties.

Since the identification of fibrillar structures in articular cartilage there has been much speculation regarding their roles as load-carrying members. Benninghoff suggested that the load carried by the surface layer was transmitted to the calcified zone by means of radial fibrils in the deep zone (Fig. 1) while MacConaill (1951) argued that a system of fibrils oblique rather than radial would be more suited to resist the applied forces. However, transmission electron microscope and scanning electron microscope studies have demonstrated that while both radial and oblique fibrils exist to some extent, the overall appearance is virtually that of a random network except for the distinct region subjacent to the articular surface.

It is possible that this superficial zone of closely packed fibrils is the main structural component on which depends the entire load-carrying ability and ultimately the integrity of the tissue. Though permeable to tissue fluids to a certain degree, it is much less permeable than the underlying zones (Muir *et al.* 1970) and could therefore function as the limiting envelope or "skin" of a fluid-filled system comprised of the cartilage matrix. This system would permit joint loads to be transmitted through the matrix to the underlying bony skeleton, in the same manner that a balloon when stepped upon will transmit the load to the floor. However, as the load is applied, a balloon membrane can freely expand in these regions away from the loaded area; this expansion includes a displacement of the membrane outwards and a lateral expansion of the membrane itself.

One hypothesis which suggests itself is that the fibrillar structures of cartilage are adapted to withstand such derangements. Thus the interwoven fibril bundles parallel to and just below the articular surface may resist any lateral expansion of the cartilage, while the random underlying fibrillar network, anchored in the calcified zone, may act as a bracing system under tension for the restraint of the surface envelope, that is the superficial zone. This implies that the continued existence of the surface zone of articular cartilage is of prime importance in maintaining the load-carrying ability and integrity of cartilage, and one could speculate on how the onset of fibrillation could easily upset this "closed" system. It is therefore of interest that Kempson, Swanson and Freeman (1967) found that areas of visibly normal cartilage of femoral heads showing some degree of fibrillation were generally softer than corresponding areas on non-fibrillated heads. Based on the above load-carrying hypothesis this suggests that fibrillation may create a "leak" in the envelope of the "closed" system and allow excessive depletion of matrix, either continuously or as and when load is applied, with subsequent loss of stiffness.

SUMMARY

1. The fibrillar networks of adult human articular cartilage, taken from femoral and acetabular specimens, have been systematically examined by scanning electron microscopy. The internal structures revealed by rupturing the tissue were compared with published findings from transmission electron microscope studies.
2. Though this technique demonstrated the internal fibrillar appearance of cartilage to a remarkable degree, it had several attendant limitations. On final drying, specimens generally exhibited shrinkage which varied within wide limits; this could have altered the internal architecture to some extent. In addition, the rupturing technique, which at the time of this investigation was the only satisfactory method of revealing the fibrillar cartilage structure, may well have had a great influence on the fibril orientations.
3. The fibrils revealed no characteristic collagen periodicity and were considerably thicker than those observed by transmission electron microscopy. It is suggested that a coating of mucin on the collagen fibrils might account for this.
4. At low magnifications the torn layers in the fractured surfaces extended radially from the calcified zone and turned obliquely at or near the articular surface to merge with the distinctly layered superficial zone, thus forming arcade-like structures. That these were not artefacts produced by the fracturing technique was shown by their similarity to the classical arcade pattern of light microscopy. However, the factor which governed the direction of these planes of weakness, be it collagen, mucopolysaccharides or cells, could not be satisfactorily determined.
5. At higher magnifications only three regions of distinct fibrillar organisation could be identified: 1) a surface layer consisting of a random fibrillar network; 2) a superficial zone composed of layers of fibrillar network, intersecting and overlapping in planes parallel to the surface; and 3) elsewhere below the superficial zone a network of virtually random fibrils which extended to the calcified region with apparently little variation in thickness or density. There was little variation from this pattern even in aged fibrillated specimens.
6. At the lower magnification range the scanning electron microscope has revealed the arcade pattern described by light microscopy, while at the higher magnifications the fibrillar organisation as seen by scanning electron microscopy correlated well with the concepts developed by transmission electron microscopy, that is, a random network of fibrils overlaid at the articular surface by a membrane-like system of bundled fibrils.
7. A possible role in the transmission of joint forces is outlined for the above fibrillar organisation.

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