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Surface characteristics of human articular cartilage—a scanning electron microscope study

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INTRODUCTION

Significant advances have been made in the study of articular cartilage since the introduction of the scanning electron microscope.

McCall (1968 *c*) described three zones in the fibrous structure of the superior aspect of the femoral head. These comprised a superficial layer of large parallel fibres, an intermediate zone of unorientated fibres, and a radial zone of tightly packed fibres. Two types of articular surface were described. Immature specimens had a fine fibrous surface mesh overlying 'cell nests', and mature specimens had a surface composed of large parallel ridges attributed to the presence of fibre bundles on or immediately below the surface layer. Walker (1969) and colleagues also assumed that the asperities on articular cartilage surfaces were due to large parallel fibre bundles underlying the surface. Localized deposits, thought to be derived from synovial fluid, were found on articular surfaces which had been loaded in contact with synovial fluid. These 'trapped pools' of synovial fluid may have a role in the lubrication of articular cartilage. J. Graham (1969, personal communication) compared the structures of loaded and unloaded articular cartilage and found that the first visible sign of failure under load was a splitting of the surface layer of the specimen.

Gardner & Woodward (1969) studied the surfaces and surface replicas of guinea-pig articular cartilage using light microscopy, scanning and transmission electron microscopy techniques. The surface characteristics appeared to vary from region to region. Arrangements of orientated ridges were observed in some regions and were thought to be due to an orderly array of fibres underlying the articular surfaces. Other regions contained hollows 20–30 μm in diameter superimposed on larger hollows 200–400 μm in diameter. It was suggested that the smaller hollows could be the site of subarticular chondrocytes but there was no definite evidence put forward for this. There also appeared to be a background of fine orientated fibres on the surface of specimens which had been treated with hyaluronidase.

The present study of articular cartilage is part of a continuing research programme in progress at the University of Strathclyde and has been undertaken to determine whether the cartilage structures described to date are typical of the hip joint cartilages. The features presented here describe the surface characteristics of both the femoral and acetabular components of the human hip joint.

MATERIALS AND METHODS

Post-mortem material, 32–64 years of age, which showed no obvious signs of degeneration was used. The material was stored at −29 °C prior to fixation in 10% formol saline solution. The specimens were prepared for the scanning electron microscope using the techniques developed by McCall (1968*a*) and were subsequently coated with gold-palladium (60/40).

The regions which were systematically examined at this stage comprised the articular surfaces on the upper half of the femoral heads and the corresponding acetabular surfaces with which these femoral regions could possibly be in contact in the normal standing position. The structures underlying the articular surface were also studied. In preparing these specimens a pair of fine forceps was used to peel off a thin translucent layer from the articular surfaces of dehydrated specimens. The surface thus revealed on the specimen will be termed the 'exposed surface' and the layer torn off will be termed the 'peeled layer'.

The stub-mounted specimens were viewed at 45° inclination to the electron beam in a Cambridge Stereoscan IIA electron microscope and photographed on Ilford FP4 roll film.

RESULTS

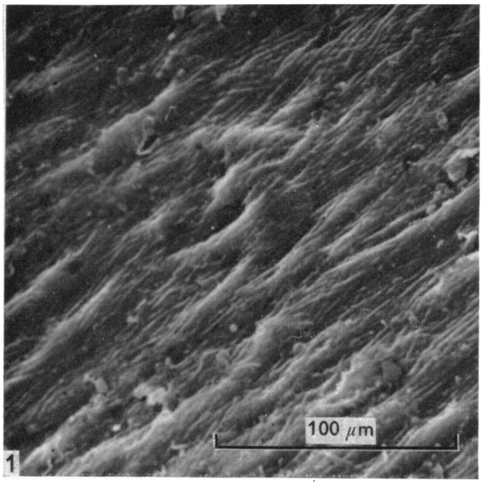
Ridges and depressions on the articular surfaces

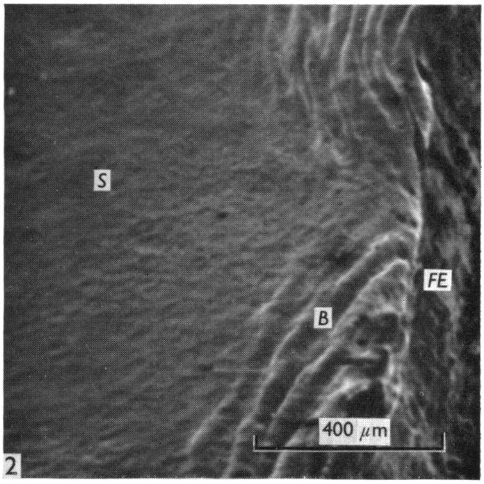
A pilot study by the author seemed to indicate that the articular surfaces contained an arrangement of ridges (Fig. 1). The local periodicity of these ridges was quite regular but varied between sites and specimens. The calculated periodicity from a number of specimens ranged from 1 to 100 μm. The wide variation in surface ridges suggested that the changes in surface contours were not due to normally occurring discrete fibre bundles in the underlying fibre network.

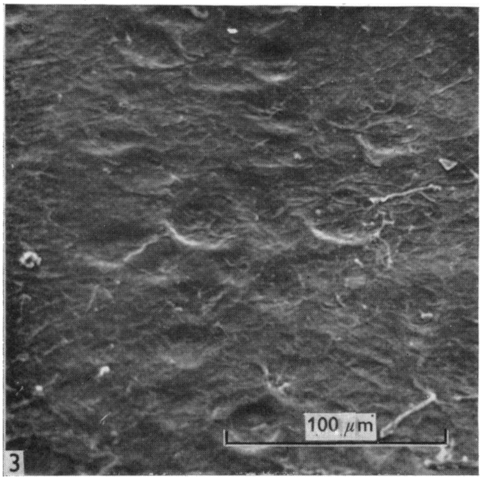
In this series of studies, ridges were more frequently found adjacent to fractured edges and were always parallel to these edges (Fig. 2). The ridges did not normally extend far from the fractured edges and rapidly gave way to a surface which contained only depressions. In some instances, surface depressions were apparent on top of these ridges. These bowl-shaped depressions were apparent on the surfaces of both acetabular (Fig. 3) and femoral (Fig. 4) cartilages at low magnifications. The surfaces were otherwise featureless, apart from small artefacts on the surface. Higher magnifications revealed that the depressions had regular contours—either a compact 'figure of eight' (Fig. 5) or an elongated 'figure of eight' (Fig. 6). The diameter of the depressions varied from 15 to 30 μm and their depth was estimated at from 1 to 6 μm. Shaded contour maps of these depressions derived from Figs. 5 and 6 are illustrated in Figs. 7 and 8 respectively.

'Exposed surface' lacunae

The fibre network of the 'exposed surface' was visible as a fine mesh with no apparent orientation. The fibres constituting this network had a mean diameter of 200 nm (Figs. 9, 10). Lacunae with diameters ranging from 10 to 40 μm were visible in the fibre network. The central area of each lacuna frequently contained a number of fibres but the general impression was one of a non-fibrous region. Occasionally these areas were occupied by apparently shrunken material which may have been derived from chondrocytes. The lacunae were shallow and lay in planes parallel to the original articular surface. They were generally arranged in pairs and the 'figures of eight' which they thus formed had a striking similarity to the depressions seen on the articular surface.







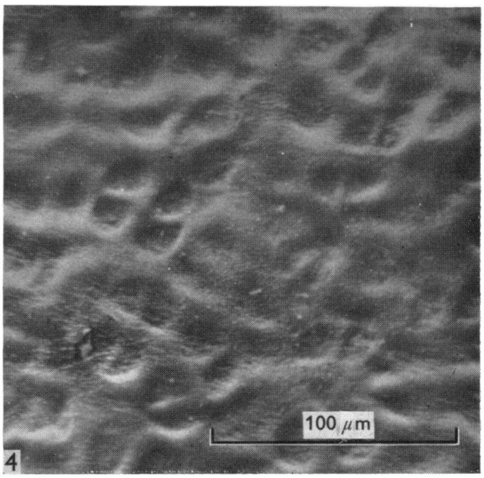


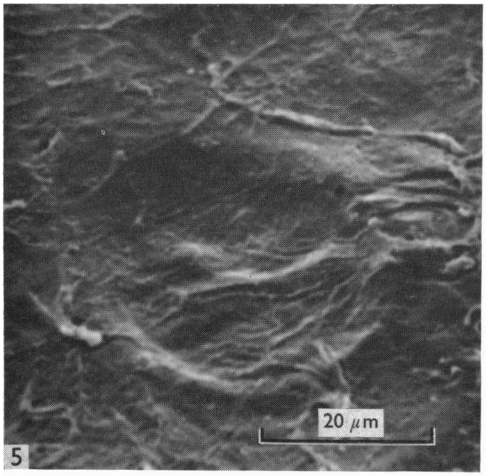
Fig. 1. Pattern of ridges or 'bundles' visible on the surface of femoral cartilage, 64 years of age.

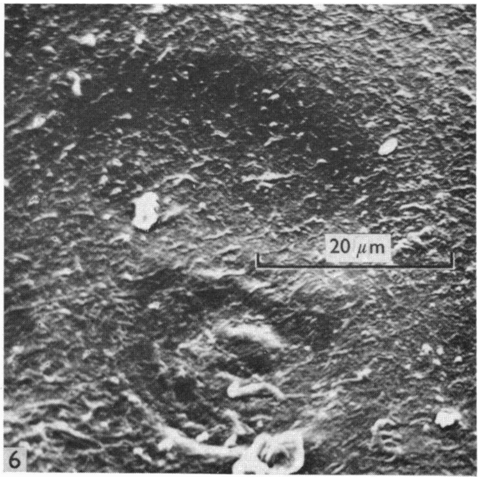
Fig. 2. View of 32-year-old acetabular specimen with depressions visible on the articular surface (*S*), and ridges (*B*) alongside and parallel to the fractured edge (*FE*).

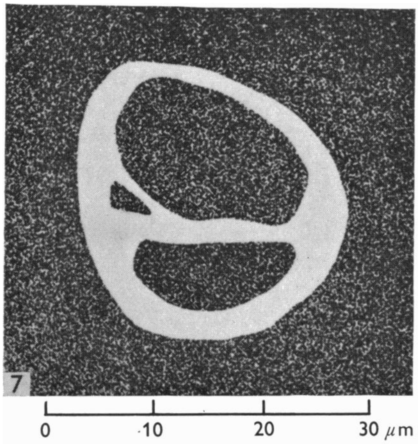
Fig. 3. Surface depressions observed in acetabular cartilage, 32 years of age.

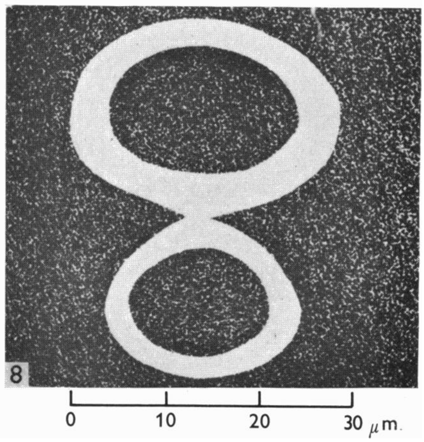
Fig. 4. Surface detail of cartilage from the femoral head, 32 years of age.

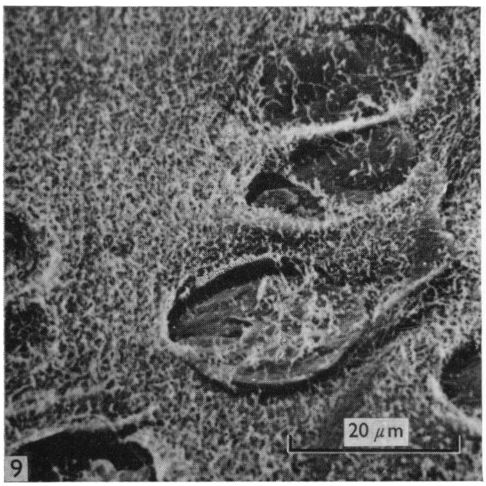
A layer-like appearance was visible on the 'exposed surfaces' of a few specimens (Fig. 11), but since these 'layers' followed the direction of the tear in the surface zone, it is thought that they were produced by the tearing action during specimen

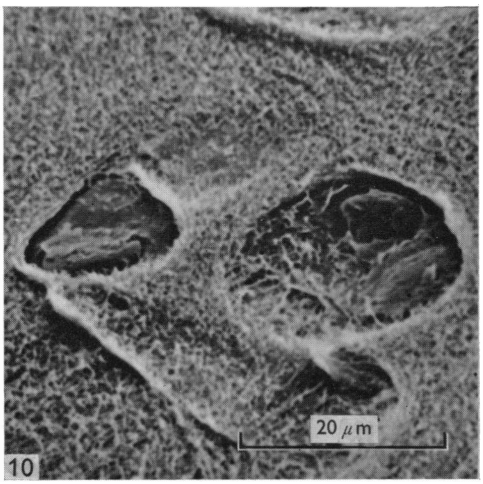












Figs. 5, 6. Surface depressions in acetabular cartilage (32 years of age) viewed at higher magnification.

Figs. 7, 8. Tracings made of the depression outlines in Figs. 3 and 4 respectively, showing the 'figure of eight' patterns.

Figs. 9, 10. Outlines of lacunae visible in the exposed superficial matrix of acetabular cartilage (32 years of age) after removal of the surface layer.

preparation. Areas which were apparently non-fibrous, but could be artefacts, were also visible in the fibrous networks.

A specimen which had been grossly torn appeared to have a multitude of 'bundles' on the 'exposed surface', but on closer examination the fibre network had clearly torn along the path of the 'peeled layer' and the resulting tendrils of torn fibre network had a bundle-like appearance (Fig. 12).

Underside of the 'peeled layer'

A fibre network similar to that seen on the 'exposed surface' of the specimen was observed on the underside of the 'peeled layer'. Lacunae were also present in this fibre network. Fibre 'bundles' occasionally appeared at the edges of the 'peeled layer' (Fig. 13) but at higher magnifications these bundles were seen to be folds in the fibre network.

Areas which were apparently non-fibrous were also visible in the network of the 'peeled layer' (Fig. 14). Lacunae-like forms could be seen in these areas and at higher magnifications (× 20 000), a ridging or corrugation was also visible. Whether these areas were artefacts created during preparation has not yet been resolved.

DISCUSSION

The results presented here show that articular cartilage specimens contained surface depressions due to the underlying lacunae and that surface ridges only occurred alongside the fractured edges of the specimens. The superficial layer was shown to consist of a fibrous network with no obvious signs of orientation.

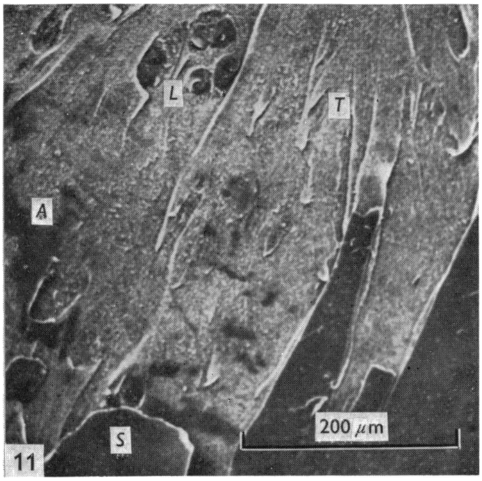
Depressions on the articular surfaces

The similarity in shape and magnitude of the superficial lacunae and the surface depressions suggests that the depressions can be directly attributed to the presence of the underlying lacunae. Meyer (1931) has described the appearance of degenerate nuclei lying parallel to and just below the surface of human articular cartilage and Davies and his colleagues (1962) have described in young adult rabbit cartilage the appearance of superficial chondrocytes separated from the articular surface by an acellular layer 2–3 μm thick. The evidence suggests that the lacunae are indeed those of the superficial layer chondrocytes and that the depressions on the surface are due to the acellular surface layer of cartilage following the outlines of these lacunae or indeed even collapsing into them.

The larger type of hollow, measuring 200–400 μm, described by Gardner & Woodward (1969) was not evident in this series of experiments.

Fibre bundles on the articular surfaces

Although previous studies have indicated that the ridges appear alongside and parallel to surface cracks or fractured edges and sometimes occur over the total specimen surface, the fact that they only occurred alongside fractured edges of the specimens in this investigation suggests that they are artefacts created by preparation techniques. Large fibre bundles have not been observed in cross-section at the fractured edge nor has the existence of such structures been reported in the literature.


L
T
A
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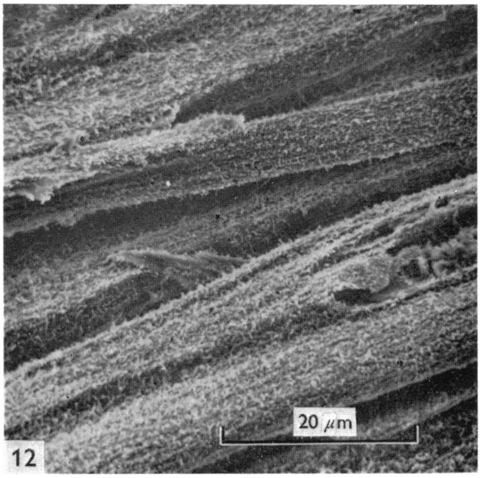
200 μm
11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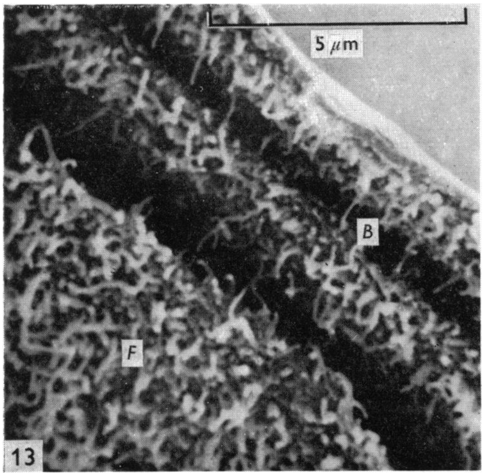
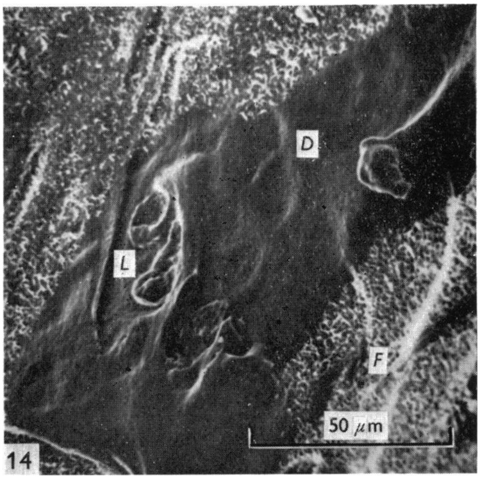

5 μm
B
F
13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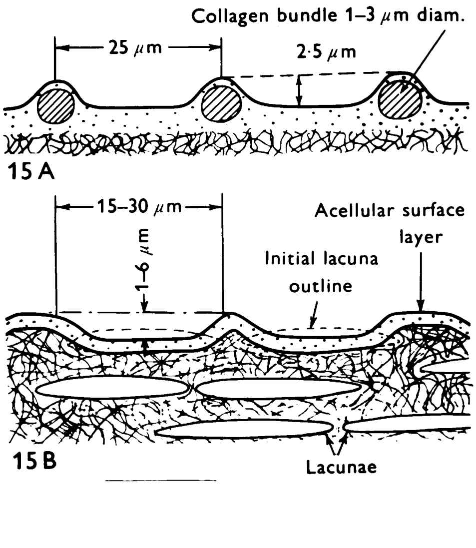
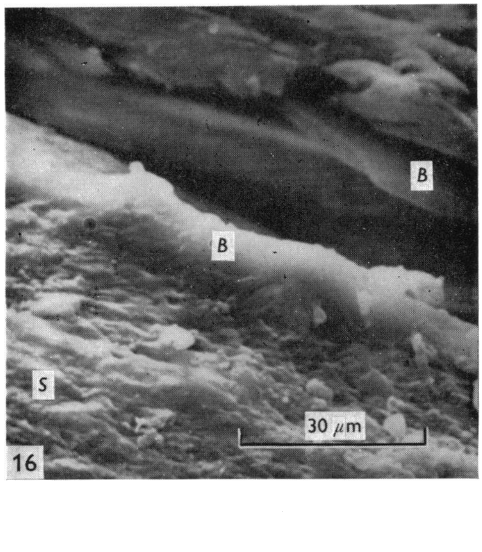

Collagen bundle 1–3 μm diam.
25 μm
2·5 μm
15 A
15–30 μm
1–6 μm
Acellular surface layer
Initial lacuna outline
Lacunae
15 B


B
B
S
30 μm
16

This confirms the suggestion that the bundles do not exist *in vivo*, but that specimen preparation produces pseudo-bundling due to buckling of the surface layer of cartilage. This effect could occur either during fracturing of the specimens or as a result of differential shrinkage during dehydration. It is possible that during the latter effect, if the surface layer does not shrink to the same extent as the rest of the cartilage structure, then it would buckle into ridges. Walker *et al.* (1969*a*, *b*) estimated that a 10 % decrease in volume occurred during preparation, accompanied by edge curling. It is conceivable that this shrinkage could buckle the surface layer. The substantial amount of subchondral bone attached to the specimens in the present series of experiments has reduced the amount of shrinkage and apparently eliminated curling. This may account for the almost total absence of surface ridges in these specimens.

Relation of surface depressions to Talysurf readings

The surface roughness of articular cartilage was formerly believed to be less than 0·1 μm (Davies, 1966). The Talysurf readings (Walker, Dowson, Longfield & Wright, 1968) indicated cartilage surface asperities with a mean periodicity of 25 μm and a mean peak to valley depth of 2·5 μm. The asperities were interpreted by Walker and his colleagues to be due to ridges 25 μm apart formed by large fibre bundles underlying the surface. Valleys with a depth of 2·5 μm appeared to be formed between ridges due to finer fibres separating the large fibre bundles (Fig. 15A). However, the surface depressions previously described (Figs. 5, 6) were of similar magnitude to the Talysurf results (Fig. 15B), and it has been demonstrated that such asperities can be attributed to the cellular structures underlying the surface of cartilage and do not indicate the existence of the fibre bundle formations as postulated by McCall (1968*a*) and by Walker *et al.* (1969*a*)

Surface characteristics in vivo

The question now arises whether these surface irregularities actually exist *in vivo*. Living surfaces may well be smooth as would be indicated by Davies's figures of surface roughness. Freezing, fixation, dehydration or vacuum techniques could result in irregularities in the articular surface, including collapse of the surface layer into the underlying lacunae. The fact that the depressions were more apparent in some specimens than others would be consistent with this view. However, Gardner &

Fig. 11. Superficial network of acetabular cartilage (32 years of age) revealed by peeling off the surface layer (*S*). Areas which appear to be non-fibrous (*A*) are visible in the 'exposed surface'. Lacunae (*L*) and rupture or tear lines (*T*) are also evident.

Fig. 12. Superficial network of a grossly torn acetabular specimen (32 years of age). The fibrous network has ruptured to form layers of 'bundles'.

Fig. 13. Underside of the 'peeled layer'. The superficial fibrous network (*F*) has buckled into folds or ridges (*B*) at the edge in this 32-year-old acetabular specimen.

Fig. 14. The underside of the 'peeled layer' of a 32-year-old acetabular specimen at lower magnification. The fibrous network (*F*) contains areas which do not appear to be fibrous and lacunae-like forms are evident in these areas (*L* and *D*).

Fig. 15. Models for a sectional view through the surface layer of cartilage: (A) as described by Walker *et al.* (1969 *b*); and (B) as interpreted by the author from the data discussed in this report and shown in Figs. 6 and 10.

Fig. 16. Prick pattern in 64-year-old femoral articular surface. Ridges and bundle-like structures (*B*) visible both in the fissure and on the articular surface (*S*).

Woodward (1970, personal communication) have been able to show by means of light microscopy that the hollows exist in fresh specimens a few minutes after death. This implies that unless there is a sudden change following death, the hollows must exist *in vivo*.

Orientation of the superficial layer

Although no large fibre bundles have been seen in the superficial layer of articular cartilage using the scanning electron microscope, there is undeniably some orientation in the structure as the well-known phenomenon of prick patterns indicates. Bullough & Goodfellow (1968) showed that the prick pattern or split-lines were parallel to the principal orientation of fibres in this zone. The fibrous network revealed by the scanning electron microscope has so far not shown any preferred fibre orientation. The appearance of 'bundles' or ridges parallel to some prick patterns is believed to be due to the combined effects of the probe and the split distorting the surface zone adjacent to the fissure (Fig. 16).

SUMMARY

The articular surfaces of human femoral and acetabular cartilages have been studied using the scanning electron microscope.

Surface depressions found on articular cartilage specimens are shown to be due to the collapse of the surface layer into the underlying lacunae. Published values of surface roughness correspond in magnitude to these surface depressions, but the existence of these depressions *in vivo* has not yet been established beyond dispute.

It is suggested that the surface ridges visible in some specimens are artefacts created by preparation techniques and that the structure of the superficial zone is a fine fibre network which appears orientated in some torn surfaces but which generally has a random appearance in the plane parallel to the articular surfaces.

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