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The morphology of the surface of articular cartilage in adult rats

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INTRODUCTION

The low coefficient of friction exhibited by healthy synovial joints (Swanson, 1979), together with the durability of their articulating surfaces, has evoked many morphological studies in the past decade. Despite this, precise structural characteristics of the articulating surface remain controversial, particularly with regard to smoothness or roughness. The relative roughness observed by the earlier scanning electron microscopists (Walker, Dowson, Longfield & Wright, 1968; Gardner & Woodward, 1969; Walker *et al.* 1969; Redler & Zimny, 1970; Gardner, 1972; Mow, Lai & Redler, 1974) was reconciled with theories of lubrication which are themselves subject to serious criticism on experimental grounds (Maroudas, 1979). The apparent compatibility of structure with theory led, at first, to general acceptance of morphological findings and methods by which they were derived. More recently, however, morphologists have revealed possible discrepancies. Thus, Clarke (1972, 1973*a*) and Wilson & Bloebaum (1978) have cast doubt on the general reliability of observations made on acrylic replicas (Dowson, Longfield, Walker & Wright, 1968; Wright, Dowson & Kerr, 1973; Wright & Dowson, 1976) of the surface of articular cartilage. Scanning electron microscopy (SEM) of the articular surface has also given rise to controversy regarding the nature of surface ridges, undulations and other irregularities (McCall, 1968; Inoue, Kodama, & Fujita, 1969; Clarke, 1971*a*, *b*, 1973*b*; Longmore & Gardner, 1975; Ghadially, Ghadially, Oryschak & Young, 1976; Ghadially, Moshurchak & Thomas, 1977).

Some attempt must be made to integrate the consistent finding of smooth articular surfaces seen in transmission electron microscopy (TEM) (Davies, Barnett, Cochrane & Palfrey, 1962; Weiss, Rosenberg & Helfet, 1968; Meachim & Roy, 1969) with observations made using other forms of investigation. The present authors agree with Boyde (1978) who stated that results obtained using TEM methods – plastic embedding and ultramicrotomy – should be regarded as the standard against which to judge the virtues of all other tissue preparation techniques including those used for SEM.

The primary objective of this investigation was to substantiate previous *in vivo* replication findings in which the articulating surface was shown to be smooth (Bloebaum & Wilson, 1978). The thiocarbohydrazide (TCH) method described by Malick & Wilson (1975) for TEM and SEM has been applied to tissues for examination by SEM. At the same time, other preparation procedures have been used along with TCH so that the latter might be evaluated for versatility and general efficiency in the specific study of articular surfaces.

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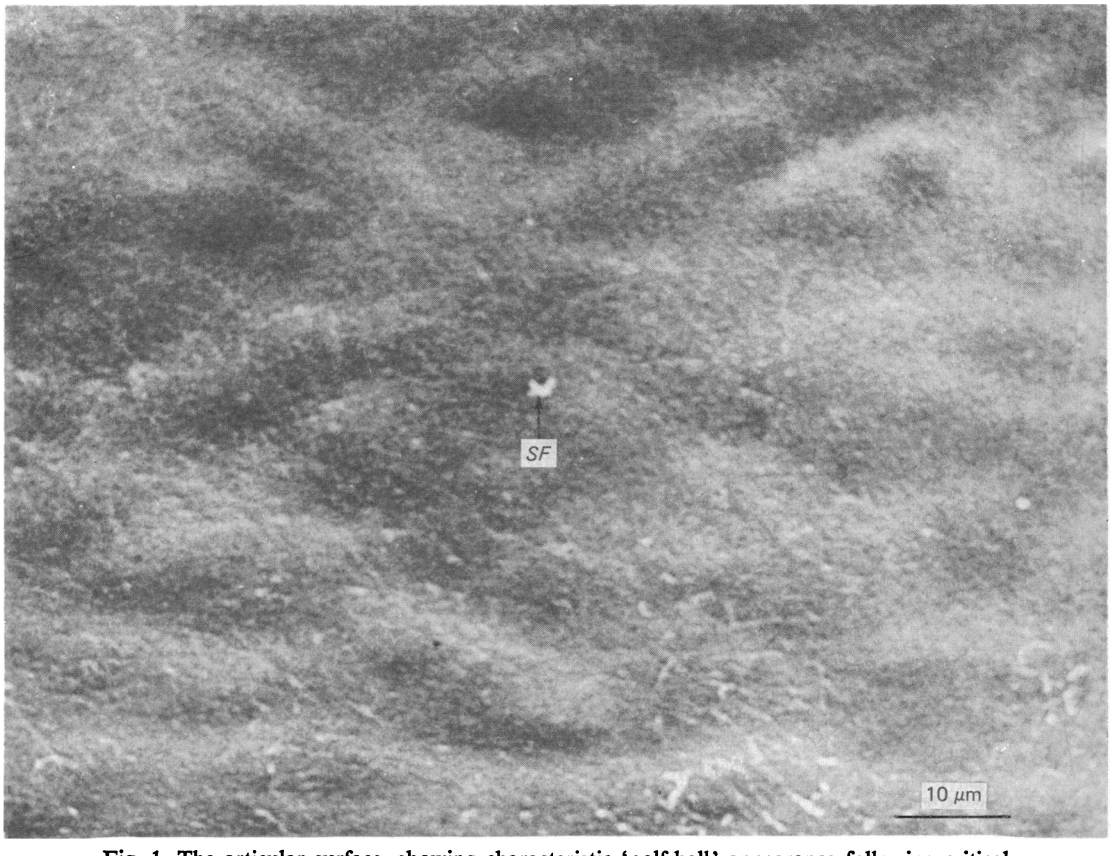

Fig. 1. The articular surface, showing characteristic 'golf-ball' appearance following critical point drying. Small white flecks of synovial fluid residue (*SF*) are present. From Group 1.

MATERIALS AND METHODS

Femoral condyles from five 3½–5 months old Wistar rats were used in each of the seven groups of specimens undergoing different methods of tissue preparation. The animals were anaesthetised with intraperitoneal Nembutal. The knee joint was opened anteriorly by cutting through the ligamentum patellae and the articular cartilage surfaces were immediately irrigated with a continuous jet of normal saline, using a hand-held wash bottle. Irrigation continued while the knee was disarticulated, the soft tissue surrounding the femur reflected proximally to the level of the third trochanter, and the limb amputated at the middle of the femoral shaft. Specimens thus consisted of the distal half of the femur.

Treatment of cartilage not previously exposed to the air (Groups 1–5)

In Groups 1–3, fixation was by immersion in 2·5 % aqueous glutaraldehyde in 0·1 M cacodylate buffer at pH 7·4 for 72–96 hours. The specimens were then washed three times in 0·1 M cacodylate buffer pH 7·4, post-fixed with 2 % osmium tetroxide in double-distilled water for 1½–2 hours, and washed six times in double-distilled water before processing as outlined below.

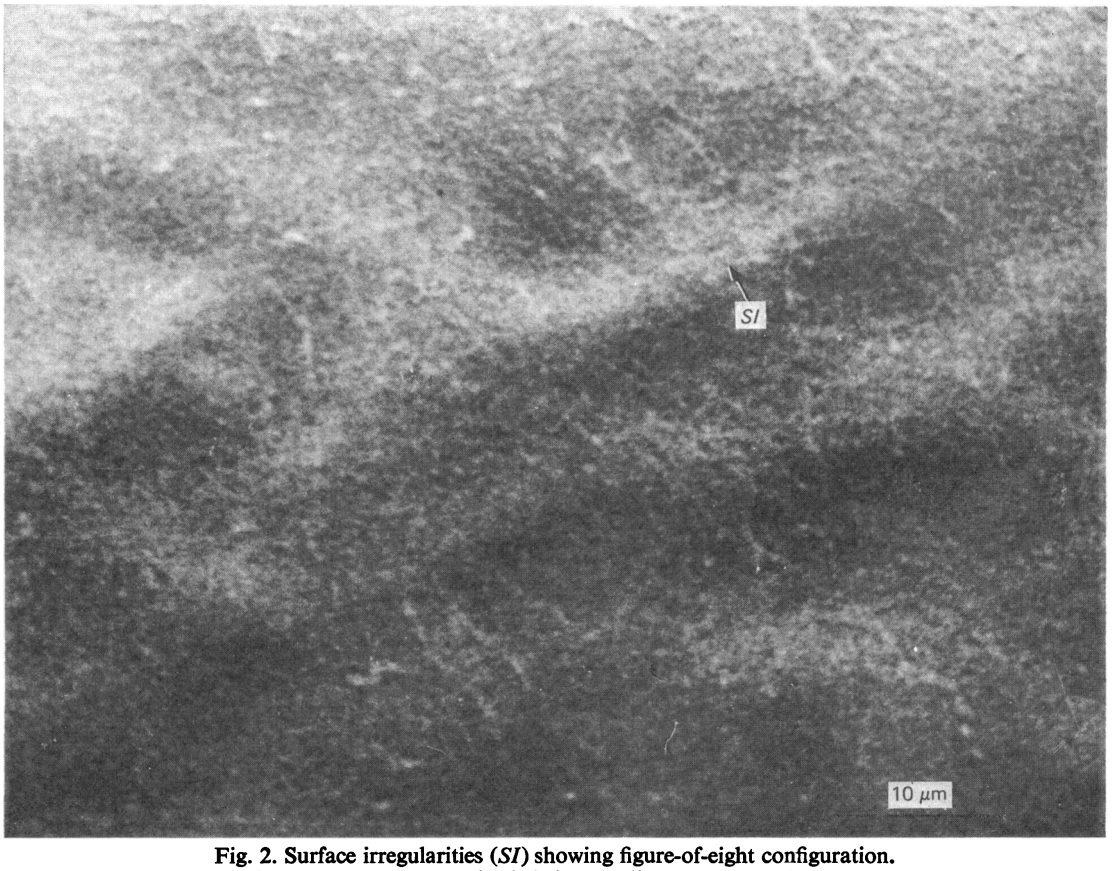

Fig. 2. Surface irregularities (*SI*) showing figure-of-eight configuration. These measure 8–15 μm in their largest diameter. From Group 1.

Group 1

Specimens were taken through ascending grades of ethanol (70–80–90–95–100 %) and subjected to critical point drying (CPD) in carbon dioxide.

Group 2

The modified thiocarbohydrazide (TCH) technique of Malick & Wilson (1975) was employed. Specimens were placed in freshly filtered 1 % TCH (Eastman Kodak) for 15–20 minutes. They were then washed six times in double-distilled water, placed in 1 % osmium tetroxide for 1½–2 hours and re-washed six times in double-distilled water. They were again placed in freshly filtered 1 % TCH for 15–20 minutes and washed six times in double-distilled water. Following this, they were placed in 1 % osmium tetroxide for 1½–2 hours and re-washed six times in double-distilled water. The specimens were then processed as in Group 1 for dehydration and critical point drying.

Group 3

Specimens were treated as in Group 2, except that the 1 % TCH steps were omitted. Hence this method involved multiple immersion in osmium tetroxide solution.

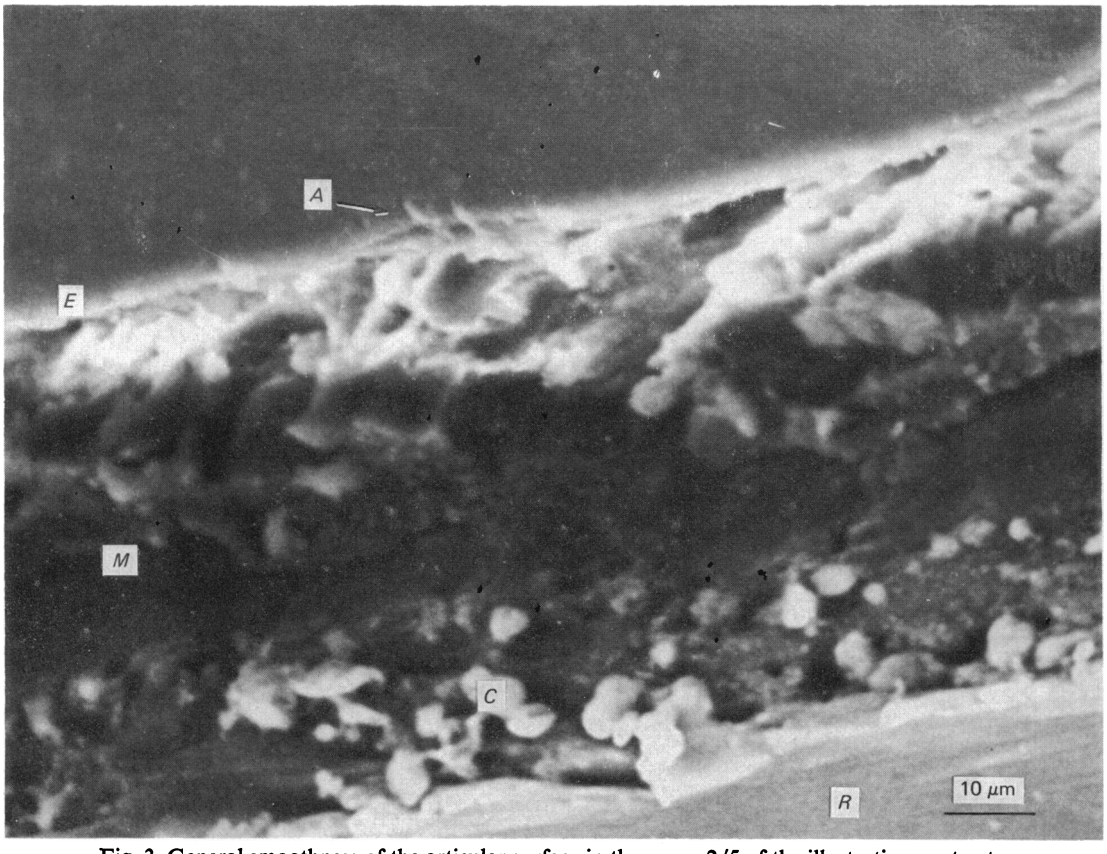

Fig. 3. General smoothness of the articular surface in the upper 2/5 of the illustration contrasts with a coincidental surgical lesion which extends from the abrasions (*A*) in the upper edge (*E*) to a lower edge in which some parallel ridges (*R*) can be seen. Some chondrocytes (*C*) are evident in the subjacent matrix (*M*). From Group 2.

Group 4

The steps involving prior fixation in aqueous glutaraldehyde and osmium tetroxide solutions were omitted in this method of tissue preparation. The unfixed specimens were placed on a screen-wire platform just above the level of a cottonwool pad soaked in 2 % osmium tetroxide in double-distilled water, in a closed vessel at room temperature. Thus, the tissue was fixed in osmium tetroxide vapour for 2 hours, before being passed through the TCH technique as described for Group 2, followed by critical point drying.

Group 5

Specimens prepared as described for Group 2 were gold-coated in a Dynavac CS300 vacuum-coating apparatus.

Treatment of cartilage previously exposed to the air (Groups 6 and 7)

Following irrigation and before fixation, the femoral condyles were placed on their sides and exposed to ambient atmospheric conditions for $1\frac{1}{2}$ hours. Certain structural features of the joint surface, such as depressions, undulations and ridging,

Fig. 4. General smoothness in upper ¾ of illustration contrasts with the roughened appearance of a small abrasion (*A*) at the edge of an accidental surgical lesion of the surface. In the depth of this defect the underlying matrix (*M*) is visualised. From Group 2.

are known to be present after such a procedure (Wilson, Rees & Bloebaum, 1980). After exposure to the air, two groups (6 and 7) of specimens were treated as described below.

Group 6

Specimens were treated exactly as described for Group 2, i.e. prior fixation with aqueous glutaraldehyde and osmium tetroxide solutions, followed by TCH processing and critical point drying.

Group 7

Specimens were treated as described for Group 4, i.e. prior fixation in osmium tetroxide vapour before processing by the TCH technique, and critical point drying.

Within 10 days of critical point drying, the femoral condyles were separated from the shaft of the bone, mounted on stubs for scanning electron microscopy and viewed in a Philips SEM 501 at 12 kV and 25 kV; photographic data were recorded on Ilford FP4. Quantitative photogrammetric analysis and qualitative stereoscopic analysis of SEM images were conducted according to Boyde (1973).

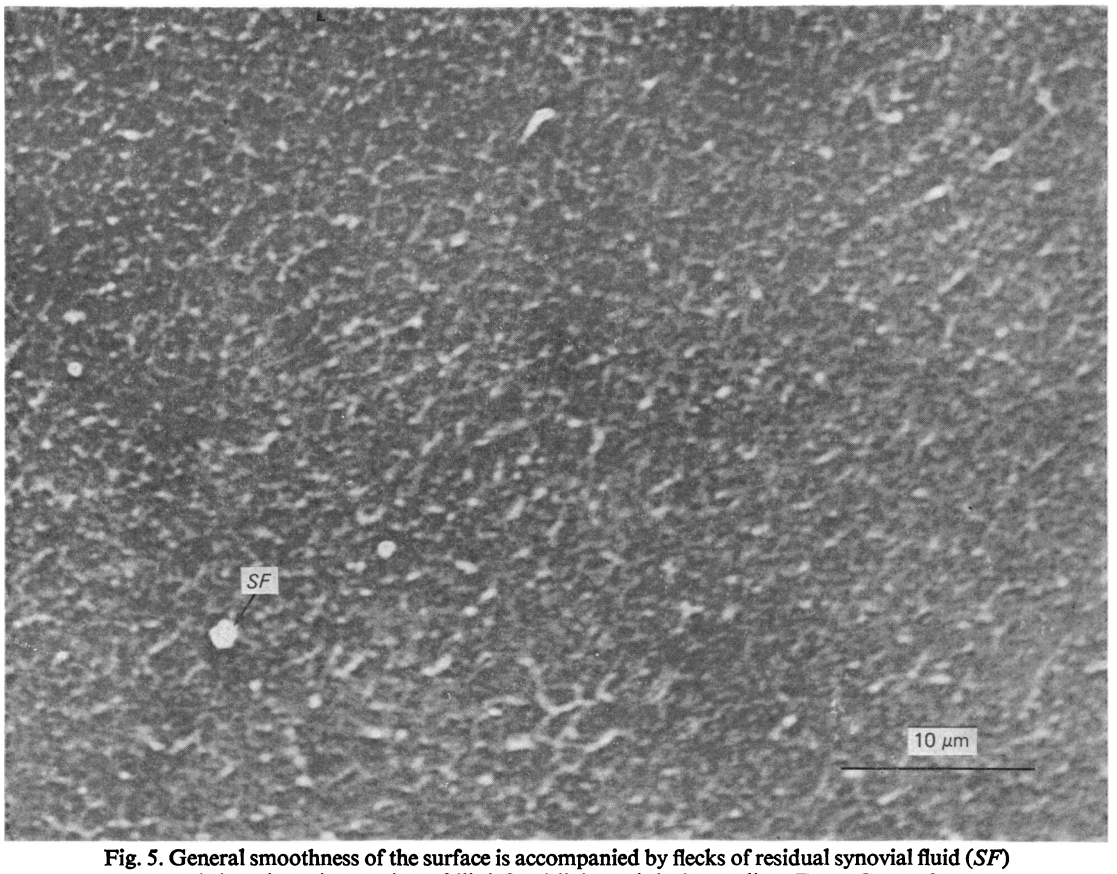

Fig. 5. General smoothness of the surface is accompanied by flecks of residual synovial fluid (*SF*) and there is an impression of ill-defined light and dark mottling. From Group 3.

OBSERVATIONS

In specimens prepared as described for Group 1, the articular surface showed ubiquitous superficial irregularities numbering approximately 3000/mm^2; each one measured 8–15 μm in its largest diameter. The surface had the general appearance of a golf ball (Fig. 1). Small white particles covered the surface but were not uniform in size or distribution. At higher magnification the surface irregularities were more conspicuous and the surface had a 'woven' texture (Fig. 2). Specimens prepared according to the Group 2 regime showed a smooth articular surface with randomly distributed lighter areas on a dark background. The light areas numbered approximately 2500/mm^2. When the surface was damaged accidentally by a relatively straight cut, distinct superficial ridging was seen only close to the edge of the laceration and parallel to its margin; elsewhere the surface was entirely smooth (Fig. 3). The overall smoothness of the surface contrasted clearly with the roughness of a coincidental surgical abrasion when this was present, exposing the underlying matrix (Fig. 4). Treated by the method described for Group 3, the general appearance of the surface was smooth, with a superimposed mosaic of white particles which were randomly distributed over the entire surface. A 'spotting effect' was recognised consisting of alternating dark and light zones, resembling Group 2 material. In this

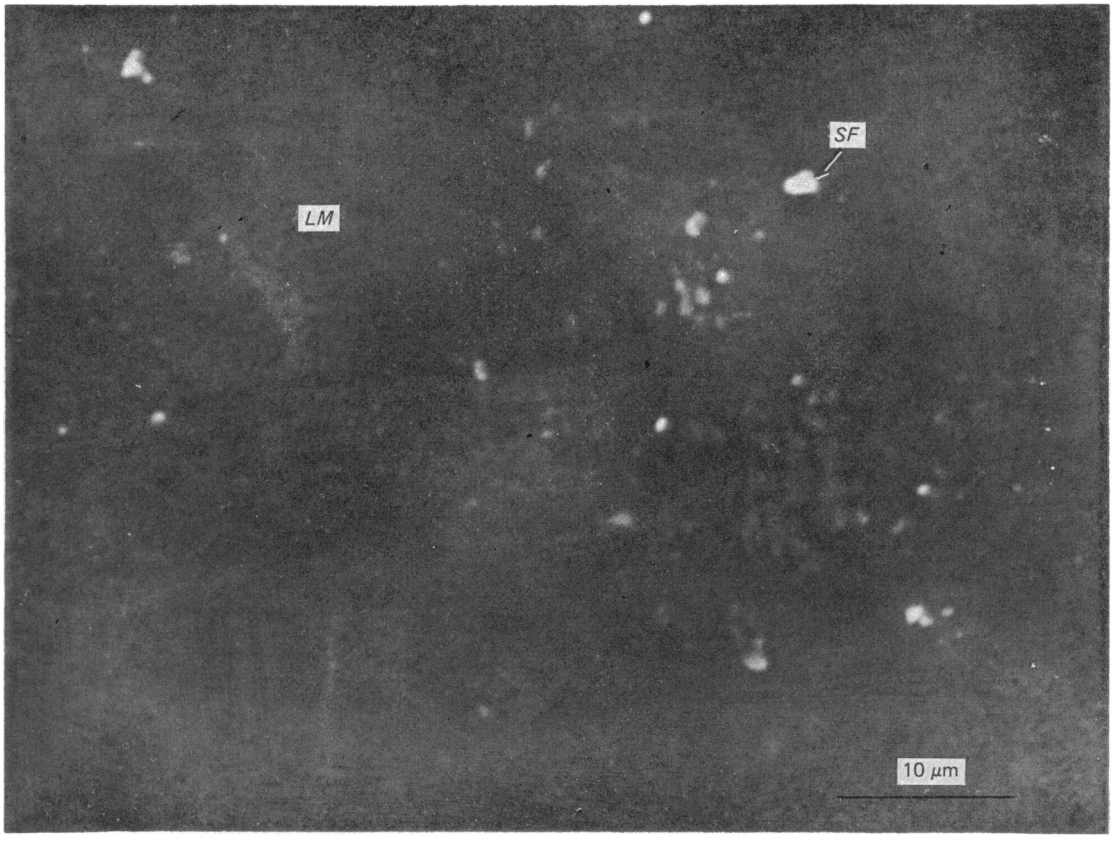

Fig. 6. General smoothness of the surface with distinct mottling effect. Lightly mottled areas (*LM*) are reasonably well defined. The label (*SF*) has the same meaning as in the previous legends. From Group 4.

case, however, the boundaries of the light areas were not sufficiently clear for accurate measurement or enumeration (Fig. 5). The surface of specimens prepared as described for Group 4 was also generally smooth, with irregular dark and light mottling. In this instance, the light areas measured approximately 8–15 μm in their largest diameter and numbered approximately 3500/mm^2 (Fig. 6). In Group 5 tissues the surface was, once more, featureless at low magnification (Fig. 7). At high magnification, however, the fine texture of the cartilage surface became apparent. General smoothness was still the dominant feature, surface irregularities like those in Group 1 being entirely absent (Fig. 8). Preparations in Group 6 showed general smoothness, but the surface features included fine wrinkles measuring 0·95–1 μm, randomly distributed. No evidence was obtained for larger surface irregularities (Fig. 9). In material treated as in Group 7, there were, again, surface irregularities of size and numbers (3500/mm^2) comparable to those described for Group 1 (Fig. 10). The surface showed gentle undulations and wrinkles (0·5–1 μm in width) at higher magnification (Fig. 11).

In all these studies, wherever large surface irregularities were seen (Groups 1 and 7), they measured approximately 8–15 μm in their largest diameter and corresponded closely to the size range observed for the light areas in TCH and osmium

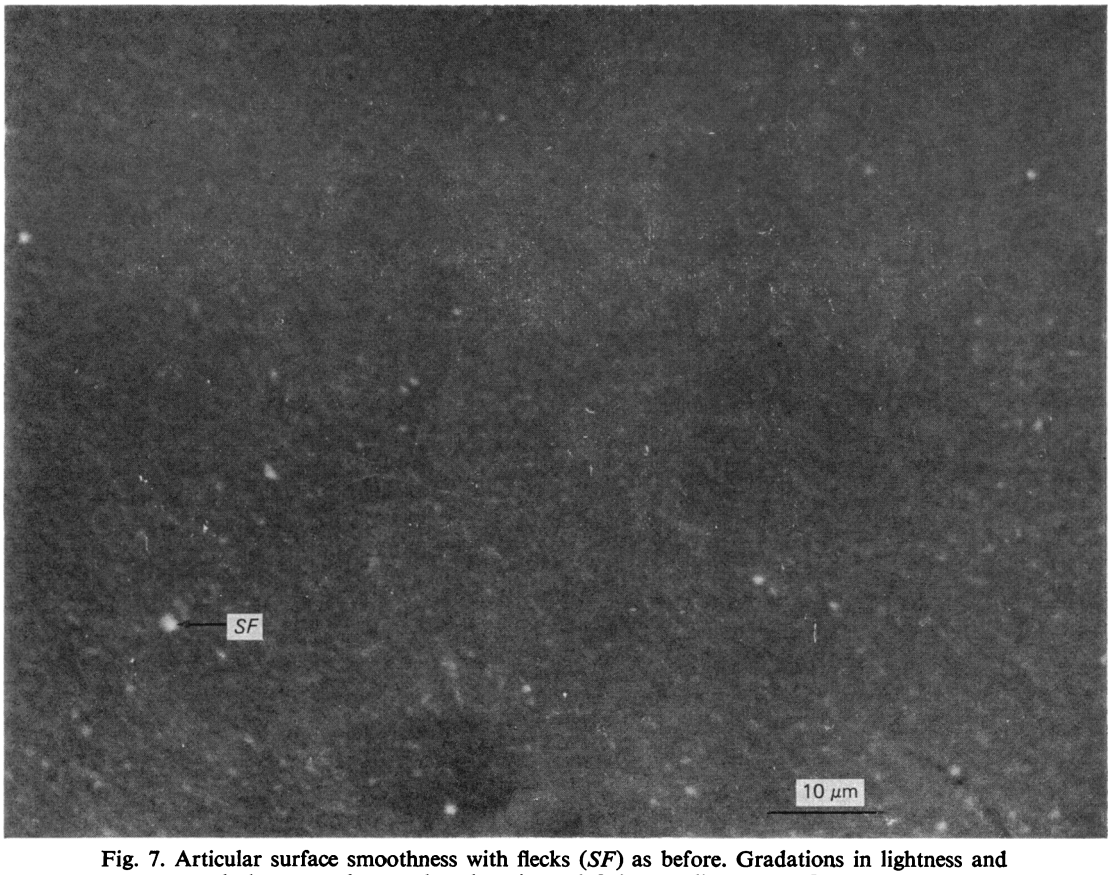

Fig. 7. Articular surface smoothness with flecks (*SF*) as before. Gradations in lightness and darkness can be seen but there is no definite mottling. From Group 5.

vapour mottled preparations (Groups 2 and 4). These light areas also corresponded approximately in frequency to the numbers of surface irregularities in Groups 1 and 7. Special attention is drawn to the fact that light areas observed in Groups 2 and 4 were relatively easy to quantitate and corresponded to Groups 1 and 7 reasonably well. In Group 3, however, the mottling was indistinct and the light areas did not lend themselves to accurate quantitation. It is important to note that these observations were made exclusively in the weight-bearing areas of the femoral condyles and that the specimens were in the non-weight-bearing state when examined.

DISCUSSION

The scanning electron microscope has been used to advantage in the elucidation of the morphology of articular cartilage by workers such as McCall (1968), Inoue, Kodama & Fujita (1969), Gardner & McGillivray (1971), Gardner (1972), Clarke (1971*a*, *b*; 1972; 1973*a*, *b*), Ghadially, Moshurchak & Thomas (1977) and Ghadially, Moshurchak & Ghadially (1978) but, until the present time, no SEM study has demonstrated that the articulating surface is smooth. Workers using the transmission electron microscope have consistently found that the surface of the articular cartilage is smooth (Davies *et al.* 1962; Weiss *et al.* 1968; Meachim & Roy, 1969).

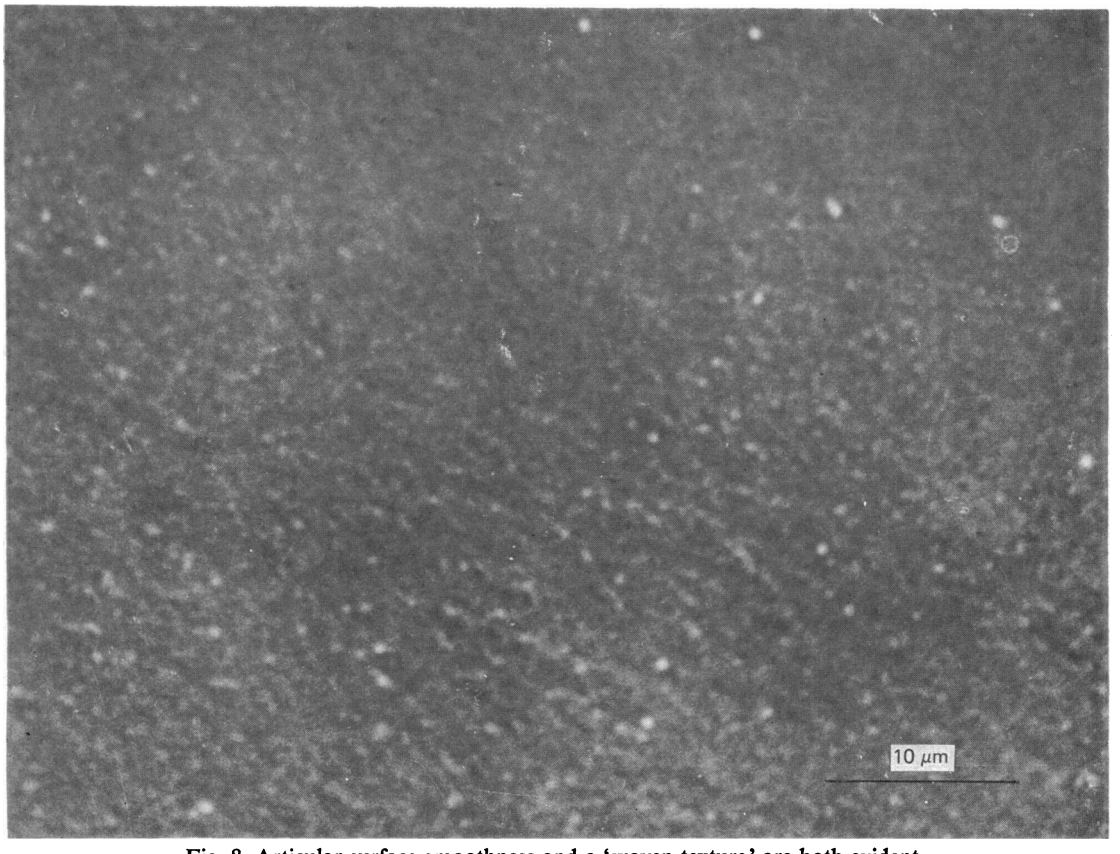

Fig. 8. Articular surface smoothness and a 'woven texture' are both evident. From Group 5.

In this investigation, surface smoothness has been demonstrated in tissues prepared as described for specimens in Groups 2, 3, 4 and 5 and examined under the SEM. The controversy regarding roughness or smoothness of the surface has frequently been attributed to the multiplicity of methods used and to the resulting futility of attempts to compare the findings of different investigators. Thus, several workers such as Cameron, Gardner & Longmore (1976) have advocated greater uniformity in methodology, calling for "two or more SEM preparative techniques... controlled by reflected light microscopy". The more recent statement of Boyde (1978), however, that techniques for TEM involving impregnation and embedding of tissues, should be regarded as the standard against which to judge all other techniques, had much greater influence on the selection of methods for this study. It is interesting to consider the recommendations of Cameron *et al.* (1976), quoted above, in the light of the findings in this investigation. Treated in the manner described for Groups 2, 3, 4 and 5, the surface smoothness of the specimens resembled that observed by Bloebaum & Wilson (1978) and contrasted markedly with Groups 1 and 7 in which the surface irregularities (8–15 μm) resembled those described by all scanning electron microscopists to date. It is clear from these comparisons that the mere discipline of applying two or more methods cannot be relied upon to give evidence which is "indicative of the true state of affairs" (Cameron *et al.* 1976). It would

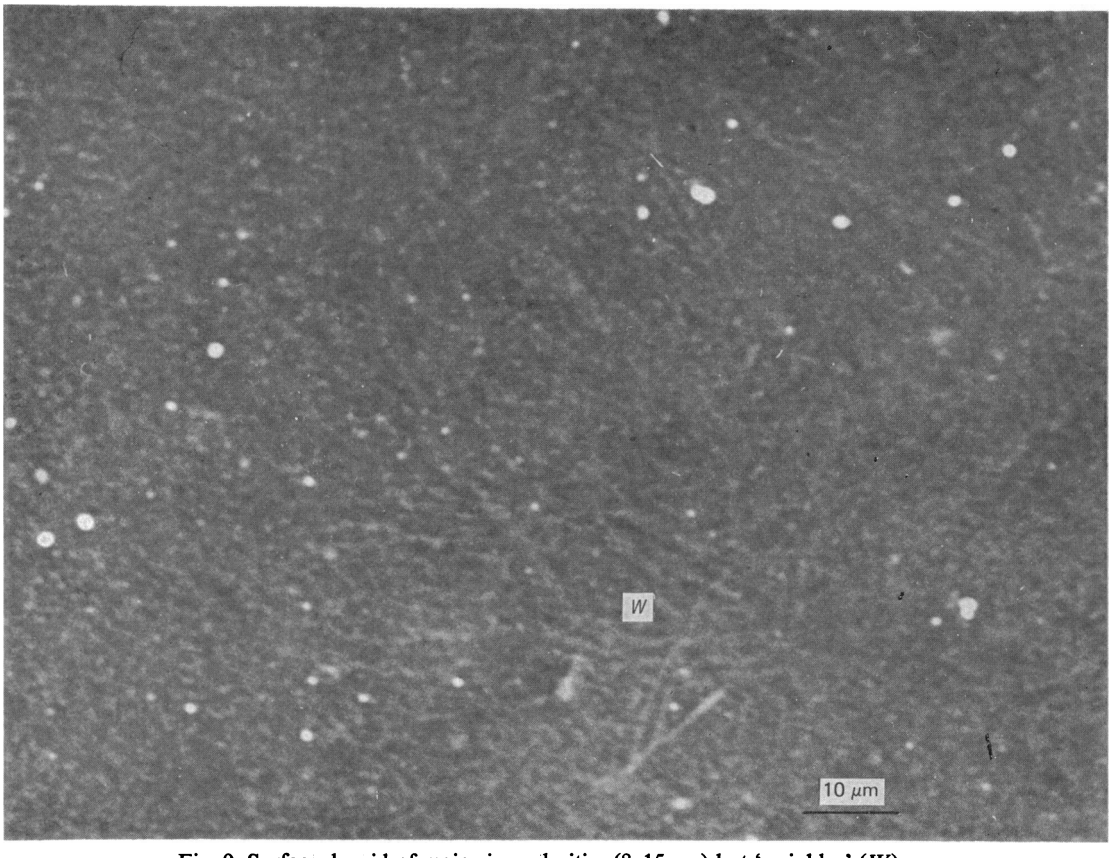

Fig. 9. Surface devoid of major irregularities (8–15 μm) but 'wrinkles' (*W*) are evident, giving a regional appearance of 'finger print'. From Group 6.

seem infinitely more important, in selecting a method, to adopt a rational, analytical approach to the entire problem. The TCH method of Malick & Wilson (1975) was selected here because of its applicability to both SEM and TEM studies. Designed initially to avoid metal-coating procedures, the method has the additional advantage of enabling both SEM surface study and subsequent examination of deeper layers using the TEM. Its mode of action as a bifunctional ligand (Murphy, 1978) makes it very suitable for multiple osmication in ultrastructural studies. The tissue support provided by the method gives an effect which resembles the results of a plastic impregnation and embedding procedure. In this study the supportive effect was evident in Groups 2, 4, 6 and 7, in which there was also an even distribution of osmium in the surface layers. In Group 3, where TCH was omitted, there appeared to be an uneven uptake of osmium and, in Group 1, where neither TCH nor multiple osmication was used, surface irregularities (8–15 μm) were in evidence.

Clarke (1971*a*), Ghadially, Ghadially, Oryschak & Young (1977) and Stockwell (1979) have suggested that some of the surface irregularities in articular cartilage may arise because of the dehydration processes associated with SEM tissue preparation. Boyde (1978) has quantitated the shrinkage of tissue following critical point drying and found a value of 60 % (vol.). In this study, by comparison of the smooth surface characteristic of Groups 2, 3, 4 and 5 with the rough irregular profiles in

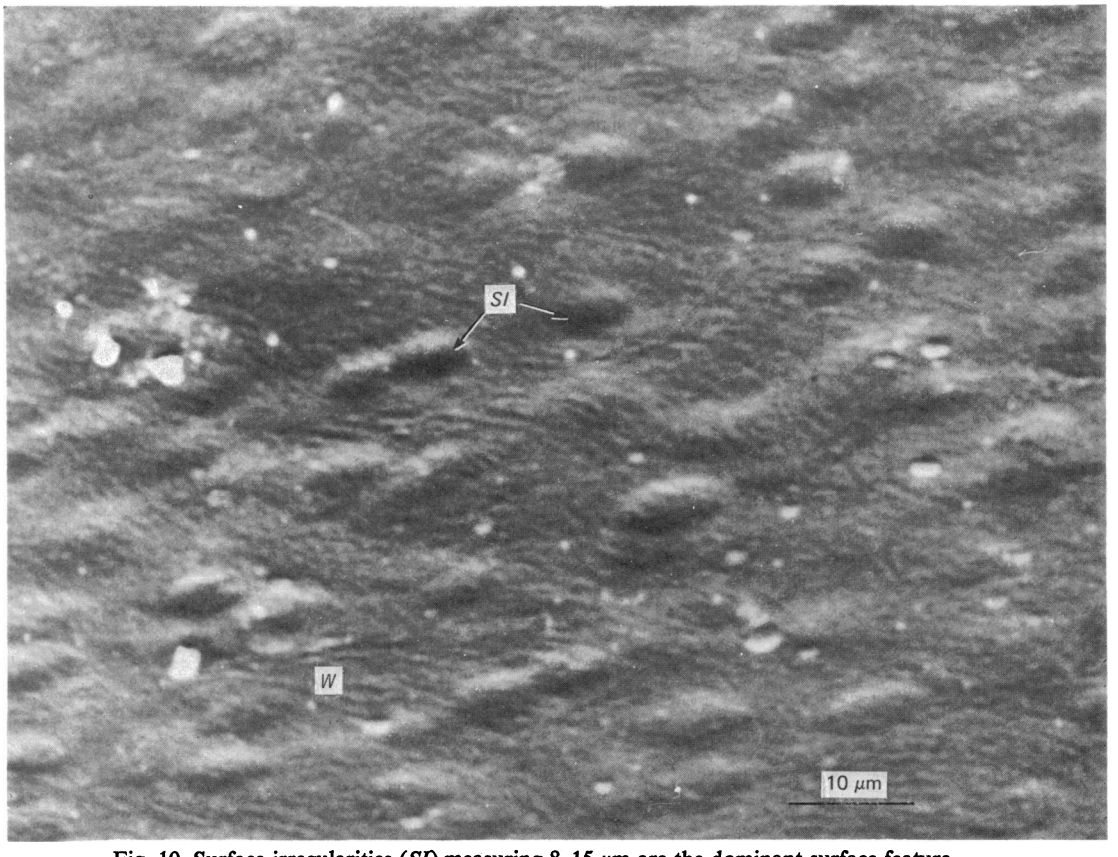

Fig. 10. Surface irregularities (*SI*) measuring 8–15 μm are the dominant surface feature. 'Wrinkles' (*W*) are also present. From Group 7.

Group 1, it is clear that critical point drying of inadequately supported tissue may be isolated as the cause of surface irregularities. Similarly, in Groups 6 and 7 the factor giving rise to surface changes is exposure to ambient atmospheric conditions.

To recommend a light microscope method as a 'control' is to disregard the possible inadequacies of its resolving power. Again, however, the factor of most significance is the tissue being studied, not the instrument employed. Work carried out in this laboratory by Wilson *et al.* (1980) has confirmed the views of Gardner & McGillivray (1971) that changes occur in articular cartilage within five minutes of exposure to ambient atmospheric conditions. This important information must cast doubt on all comparable findings where the articular surface was exposed for times in excess of five minutes in the absence of efficient means of maintaining surface moisture. From the preceding discussion, it is evident that one of the factors emerging as a key to understanding the precise morphology of the articular surface is water content, about which there is an urgent need for more information (Gardner, 1977; personal communication).

The strong possibility that the lability of water in articular cartilage may greatly influence morphological studies was thrown into sharp relief by an unexpected finding in Group 6 specimens. In accordance with previous findings (Wilson *et al.* 1980) it was expected that tissues exposed to ambient atmospheric conditions for

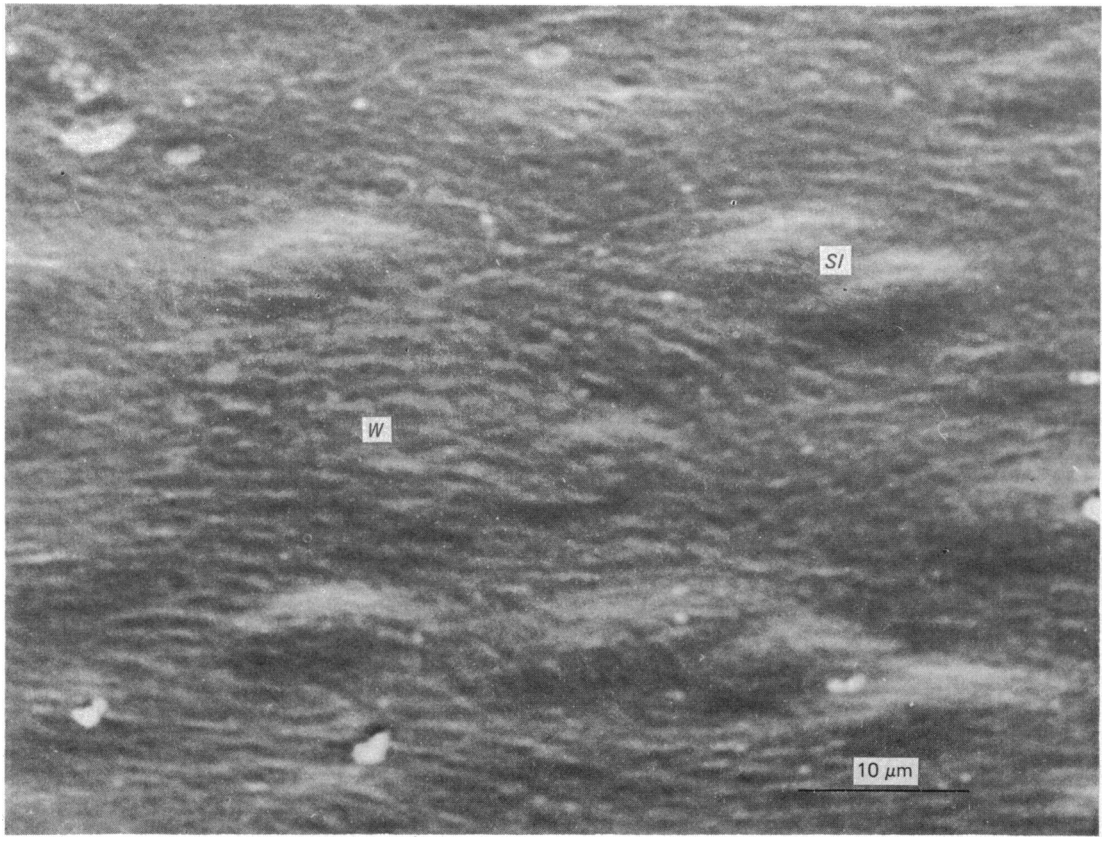

Fig. 11. In addition to the features described in Fig. 10, 'wrinkles' (*W*) and surface irregularities (*SI*), there is an impression of gentle undulation of the surface. From Group 7.

1½ hours would be certain to show surface irregularities. When such tissues were subsequently fixed by immersion in aqueous fixative (TCH method as in Group 2) the absence of surface irregularities (8–15 μm) could be explained only by invoking a mechanism whereby the water reached and rehydrated the tissue before the fixative could act. To test this hypothesis, specimens of Group 7, similarly exposed for 1½ hours, were fixed in osmium vapour under conditions in which the immediate action of the fixative would preclude any reasonable possibility of effective rehydration. In this case, surface irregularities resembling those described by Clarke (1971*a*) were a prominent feature. The possibility that osmium vapour *per se* could have produced the irregularities was eliminated by the findings in Group 4 studies.

The precise nature of these superficial irregularities is still the subject of speculation, but Clarke (1971*b*) considered that they might be attributable to cellular collapse immediately subjacent to the surface which, in turn, 'caves in', resulting in figure-of-eight depressions. This view was supported by the quantitative observations of Stockwell & Meachim (1979) on cells of the superficial layer and, in particular, the numerical data for rats correspond closely to numbers of surface irregularities estimated in this investigation. Stereoscopic analysis was unhelpful here in determining whether surface irregularities were indeed elevations or depressions. It was, however, a useful adjunct to determining the presence or absence

of surface irregularities. The findings in this study are compatible with Clarke's (1971*b*) viewpoint but do not concur with the finding of these irregularities in the non-weight-bearing physiological state. Furthermore, the 'mottling effect' studied quantitatively in Groups 2 and 4 is considered to indicate increased conductivity of osmium-impregnated cell domains (Murphy, 1978). The sizes and numbers of these high-conductivity areas correspond closely to the data applicable to surface irregularities. Gold coating of specimens in Group 5 was carried out to confirm that the mottling effect did not hinder surface analysis.

One final surface feature is worthy of discussion – the wrinkling effect observed in Groups 6 and 7. The individual wrinkles are in approximately the same size range as the 'quaternary ridges' which Longmore & Gardner (1975, 1978) described in ageing studies. These authors have attributed this observation to a gradual loss of surface matrix – probably proteoglycans – and have stated that the numbers of ridges increased as age advanced. It is interesting to speculate that the wrinkles observed in this study, considered to be attributable to air drying, appear to have analogues in the ageing process.

SUMMARY

Articular cartilage from the weight-bearing area of the femoral condyles in adult rats is smooth in the normal, non-weight-bearing condition, showing none of the surface irregularities (8–15 μm) reported by previous authors.

Demonstrable changes in the surface characteristics of articular cartilage may be attributable to preparation procedures.

The normal smoothness observed in TEM studies has been confirmed by an SEM method.

'Quaternary ridges' described as evidence of ageing may be analogous to 'wrinkles' observed on the surface cartilage exposed to ambient atmospheric conditions.

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