Morphology of surface synoviocytes in situ at normal and raised joint pressure, studied by scanning electron microscopy

J N McDONALD AND J R LEVICK

From the Department of Physiology, St George's Hospital Medical School, London

SUMMARY The synovial surface in rabbit knees was examined by scanning electron microscopy (SEM) to define normal surface contour, cell shape, and interstitial exposure. Comparison was made between specimens excised before immersion fixation (I), fixed in situ by vascular perfusion (V) before excision, or fixed in situ under an effusion pressure of 5–25 cmH₂O (E). The deeply convoluted appearance of rabbit areolar-muscular synovium fixed after excision (I) was found to be an artefact; areolar-muscular synovium fixed in situ (V) was much smoother. The well documented cobblestone contour of immersion fixed adipose synovium was present after fixation in situ, but may be exaggerated by the SEM preparative process. At higher magnification the synoviocytes showed evidence of considerable surface activity (smooth granules, large cauliflower-like excrescences, thin lamelliform filopodia). Cell shape was variable but many synoviocytes extended long cytoplasmic processes along the surface, producing fibroblastoid and even stellate outlines. At an intra-articular pressure of 25 cmH₂O (E) the cytoplasmic processes were elongated and branched, creating a highly dendritic outline. Also, the exposure of interstitium increased markedly at the higher pressure. It is concluded that extension of length of cytoplasmic processes is a feature of normal healthy synoviocytes, and that an increase in interstitial area with joint pressure contributes to the increased hydraulic permeability of synovium at raised pressure.

Key words: synovial surface structure, dendritic morphology.

The surface morphology of the synovial intima is of interest in relation to two areas of current research. Firstly, the hydraulic permeability of the intima has been shown to increase at raised intra-articular pressures and fluid volumes,¹ and intimal structure could provide clues to the cause. Secondly, a subpopulation of dendritic (stellate) synovial cells in rheumatoid synovium has recently excited considerable attention,^{2–7} rendering it a matter of some urgency to define the shape of normal synovial cells in situ for comparison.

The synovial lining consists of a discontinuous layer of cells (synoviocytes), which mostly lack intercellular junctions and are separated by a fibrous interstitium.^{8–9} The shape of normal synovial lining

cells is evidently complex and not easy to visualise from histological and transmission electron micrographs (TEMs). Cytoplasmic processes ~1 µm wide, apparently separated from cell bodies, are often caught in transverse or oblique section at the synovial surface, e.g., in man,^{8–10} pig,¹¹ and rabbit,¹² indicating that at least some normal intimal cells have long, finger-like processes extending over the surface. Such processes are quite distinct from intraluminal projections such as the leaf-like filopodia of 'A' cells. In contrast, however, lining cells reconstructed from serial TEM sections showed simpler morphology, without extensive cytoplasmic processes.¹³

Scanning electron microscopy (SEM) offers a more direct picture of synovial surface morphology. Many early low power studies of human, rabbit, dog, pig, and rat synovium concentrated mainly on surface contour,^{14–21} though Wysocki and Brinkhouse

briefly noted 'dendritic cytoplasmic processes' over canine fibrous synovium in addition to projections into the joint space.¹⁹ The surface of human and canine areolar synovium appeared folded and furrowed,^{16 17 19 21} but as the tissue was not fixed *in situ* the significance of this is not clear. Adipose synovium by contrast had a regular cobblestone appearance.^{14 17 19} Date studied the surface at sufficient magnification to reveal detailed cell morphology,²² and in an excellent but little quoted paper he described numerous flat interdigitating cell processes, cauliflower-like excrescences, and exposed interstitium in human and rabbit synovium. Cell surface protrusions and intercellular spaces were earlier reported in human synovium^{21 23} and tenosynovium.²⁴ All these tissues were excised before fixation.

There seem to have been no scanning electron microscopic studies of synovium fixed *in situ*. The present SEM study aims to clarify (a) the effect of excision on synovial surface contour; (b) the normal morphology of surface synoviocytes *in situ*; and (c) the effect of intra-articular distension by fluid on surface morphology. Morphometric investigations by light and transmission electron microscopy are in progress to quantify further the structural consequences of distensions.

Materials and methods

New Zealand rabbits (2–3 kg) were anaesthetised with intravenous pentobarbitone and urethane. Three kinds of synovium^{8 25} were sampled in each of six knees from three animals, namely areolar-muscular synovium from the medial aspect of the suprapatellar pouch, adipose synovium from the infrapatellar fat pad, and mixed synovium from the posteromedial pouch.

TECHNIQUES OF FIXATION

Synovial samples of surface area 2 mm×3 mm and thickness 2 mm were excised by scalpel under various conditions.

Immersion fixation

In two joints soft unfixed tissue was excised, then immersed at once in fixative at room temperature for two hours ('post-fixed' control). No intra-articular injection was made into these joints.

Vascular perfusion

In two joints the synovial lining was first fixed *in situ*, then excised and reimmersed in fixative at room temperature for two hours ('pre-fixed' control). No intra-articular infusion was made into these joints. Fixation *in situ* was achieved by

vascular perfusion. The abdominal aorta and vena cava were cannulated. After an initial flushing of the knee vasculature with buffer solution (0.05 M sodium cacodylate, 50 g/l sucrose) fixative was infused through the aorta from a reservoir 163 cm above heart level (120 mmHg infusion pressure) and allowed to drain away through the caval cannula. The joint very quickly became stiff. After seven minutes of vascular perfusion the knee was opened and samples taken of the firm, fixed tissue.

Articular perfusion

In two, volume expanded joints the synovial lining was fixed *in situ* for one hour at a raised intra-articular fluid pressure (5 cmH₂O and 25 cmH₂O respectively) before sample excision, then immersion fixed at room temperature for a further one hour. For these experiments the joint cavity was cannulated by two 21G cannulae. Intra-articular pressure was recorded through one cannula and Krebs' solution ion infused through the other from a reservoir whose height governed the intra-articular pressure, as described previously.²⁶ Krebs' solution,²⁷ which contains 0.9 g glucose/l as energy source, was pre-equilibrated with 95% O₂/5% CO₂ at pH 7.4. Intra-articular temperature was 35°C, as is normal in the rabbit knee.

The desired intra-articular pressure was approached in three or four pressure steps, allowing 15 minutes a step to reproduce the conditions of an earlier study of trans-synovial fluid exchange.²⁶ The infusate was then switched to fixative and the infused fluid allowed to drain out continuously through a third cannula connected to a collection reservoir with an overflow. By appropriate setting of the heights of the infusion reservoir and collection reservoir, and adjustment of the resistance of the overflow line by a screw clamp, the intra-articular pressure was held constant at 5 or 25 cmH₂O during continuous perfusion of the synovial cavity with fixative at 1 ml/min. At this flow the rate constant for the rise in intra-articular fixative concentration was 0.5–1.43 min⁻¹. A longer period (one hour) was required for intra-articular fixation than for per-vascular fixation because of the slower permeation of fixative into the periarticular tissues (e.g., muscle), which had to be fixed too in order to prevent recoil on opening the joint cavity.

The fixative in all cases was half strength Karnovsky solution—namely, 2% paraformaldehyde, 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.4) with 50 g/l sucrose as osmotic buffer.²⁸

PROCESSING OF FIXED SAMPLES FOR SEM

Samples were washed with three changes of buffer

(0.05 M sodium cacodylate with 50 g/l sucrose, osmolarity 250 mmol/l), diced finely and post-fixed in 1% osmium tetroxide in buffer for three hours at room temperature. After double rinsing in buffer the samples were dehydrated in an ethanol series, substituted with fluorocarbon, dried at the critical point with liquid CO₂ (E300, Polaron Ltd), and mounted on stubs with Araldite. Specimens were sputter coated with gold (E5100 series II, Polaron Ltd) and examined in a Coates and Welter field emission scanning electron microscope at 16.5 kV.

Results

GENERAL SURFACE CONTOUR

The cobblestone profile of adipose synovium

surface^{14 19} was observed in tissue fixed in situ as well as in post-fixed samples (Fig. 1), i.e., it was independent of the fixation method. Examination of the cut edge of the specimen showed that each surface 'cobble' (diameter 50 µm) was due to a fat cell some distance below the intima. The surface conformation of non-adipose synovium depended however, on the fixation method. Tissue excised before fixation had a wrinkled, convoluted surface with roughly parallel furrows 0.01 to 0.15 mm apart (Fig. 1A), very like immersion fixed human and canine areolar synovium.^{16 17 19 20} In contrast, the surface of samples fixed in situ was much smoother, though a few, wider spaced furrows were still seen in pre-fixed control and 5 cmH₂O samples. In samples fixed at 25 cmH₂O, surface furrows were eliminated.

Fig. 1 Lower power scanning electron micrographs of rabbit synovial surface to illustrate effect of fixation method and substructure on surface contour. Scale bar=50 µm. Red cells liberated during excision of sample have settled on the surface in places. (A) Areolar synovium from medial suprapatellar pouch excised before fixation then immersion fixed (post-fixed control). Surface is deeply furrowed. (B) Areolar synovium from medial suprapatellar pouch fixed in situ by vascular perfusion before excision (pre-fixed control). Surface is much smoother. (C) Adipose synovium over infrapatellar fat pads fixed in situ by vascular perfusion. The cobblestone surface persists and is due to underlying fat cells. (D) Areolar synovium from suprapatellar pouch fixed in situ in a joint distended to 25 cmH₂O intra-articular pressure. The long branched hummocks are probably due to blood vessels below the synovial surface.

ated, but long branching cylindrical ridges were conspicuous (Fig. 1D).

CELLULAR MORPHOLOGY AT NORMAL JOINT PRESSURE

The following description applies to synovium fixed *in situ* by vascular perfusion. At magnifications of $>1000\times$ the remarkably complex nature of the synovial surface became apparent. The surface consisted of cell bodies, cell processes, and, in between, interstitium exposed directly to the joint cavity. The general appearance varied considerably, some regions resembling a patchwork quilt (corresponding to relatively regular, non-dissected cell outlines) and others resembling a mass of worms (corresponding to irregular cells with extensive

processes), with intermediate gradations. Higher magnifications showed the cell outline was very variable, and a progressive, continuous gradation in complexity could be discerned (Fig. 2).

(i) The simplest cells were oval plates about $12\text{--}24\text{ }\mu\text{m}$ wide, with only moderately scalloped margins (Fig. 3); scalloping was due to very fine cytoplasmic strands attaching the cell to adjacent collagen bundles (Figs 3 and 4). This form was most common in adipose synovium but not unique to it.

Fig. 2 Tracings of intimal cell shape from the surface of synovium fixed *in situ* at normal joint pressure (cells a-f; pre-fixed controls) and at $25\text{ cmH}_2\text{O}$ joint pressure (cells g-i). Scale bars = $5\text{ }\mu\text{m}$. Dashed lines indicate obscuration by adjacent overlying cells. Cells a-f were selected to illustrate the diversity and gradation of normal cell morphology from (a) ovoid plate through (c, d) fibroblastoid and (e) stag head fern to (f) stellate. Intra-articular distension caused a great increase in dendritic (stellate) forms (e.g. g-i).

Fig. 3 Scanning electron micrograph at medium magnification to show spectrum of cell shapes constituting the synovial surface. Parts (A) and (B) are both from the medial suprapatellar region of the same knee, fixed *in situ* by vascular perfusion. (A) Cells with highly dissected shapes and much surface activity (filopodia, granules, and cauliflower excrescences) anchored by fine cytoplasmic filaments to the collagenous interstitium. Exposed interstitium accounts for 26% of the surface area in this field (pre-fixed control). Scale bar = $10\text{ }\mu\text{m}$. (B) Nearby region from same sample. Cells of less dissected, ovoid outline but similar surface activity. Interstitium forms 18% of the surface. Scale bar = $10\text{ }\mu\text{m}$.

Fig. 4 High power scanning electron micrograph to show exposed synovial interstitium between two cells. An irregular collagen meshwork spans the intercellular gaps. The cells are attached to the collagen network by fine cytoplasmic strands (arrows) which produce the scalloped margin. The cell on the left shows two surface features—a cusp-like leaf of cytoplasm (lamelliform filopodium, lf) and a smooth surface granule 0.5 μm in diameter (g). Posteromedial synovium fixed in situ at 25 cmH₂O. Scale bar = 2 μm . The insert shows a third kind of surface feature, the large cauliflower-like excrescence.

(ii) Cells of 'fibroblastoid' outline were more irregular, with two to six short, flat, broad cytoplasmic extensions: they strongly resembled monolayer cultured fibroblasts as illustrated in a standard SEM atlas.²⁹

(iii) Cells with greater elongation of the flattened, cytoplasmic processes were termed stag head fern cells (Fig. 2) owing to their strong resemblance to that plant. Such processes interdigitated with others and sometimes ran over or under adjacent cells.

(iv) The most dissected cell outlines (stellate or dendritic) comprised two to six cytoplasmic processes 1–2 μm wide and typically 6–12 μm long (max 25 μm). The processes entwined and overlapped, sometimes bifurcated, and often ended as flattened expansions with fine attachment filaments producing scalloped margins.

Because cell shape comprised a continuous spectrum, the relative prevalence was difficult to measure, but forms (ii) and (iii) seemed most common. The whole cell outline was not always visible—some bodies emerged from beneath an interstitial layer or had a thin skein of fibrils partially covering them. The shape of the cell below the surface plane was not of course revealed by SEM; nor were nuclear regions detected as these are usually 5 μm below the cell surface (TEM). Distinctive forms which might correspond to classical 'A' and 'B' cells of TEM³⁰ were not recognised by SEM, as noted by others.²³

CELL SURFACE STRUCTURES

Cell surface structures abounded and were of three kinds (Figs 3 and 4).

(a) Fine fingers and flaps of cytoplasm (thickness 0.1–0.2 μm) protruded into the joint cavity (Fig. 4), corresponding to the lamelliform filopodia seen in TEMs.¹¹

(b) Most cells had one or more larger (1.0–4.5 μm wide), knobby excrescences protruding into the joint cavity ('cauliflower-like protrusion' as described by Date²²). At high magnification these resolved into complicated folds of cytoplasm, fingers and clusters of small smooth granules of 0.2–0.3 μm diameter.

(c) Small smooth round granules were also common in isolation on cell and cell process surfaces and, being 0.2–0.5 μm wide, were probably the same structures as those occurring in clusters on the cauliflower excrescences.

SYNOVIAL INTERSTITIUM

Cells and processes were not in contact but separated by gaps filled with interstitium, visible as a random meshwork of collagen (Fig. 3—low pressure; Fig. 4—high pressure). A thin lacework of fibrils extended partly over some cell surfaces. The area between cells, estimated by a standard morphometric point counting method,³¹ averaged 26 (relative standard error (RSE) 3)% over $7 \times 10^3 \mu\text{m}^2$ areolar synovium. The range was from 18% to 29% of the surface for prefixed control tissue, the lower

Fig. 5 Scanning electron micrograph of posteromedial synovial surface fixed in situ at a joint distended to 25 cmH₂O intra-articular pressure. Cell morphology is more dendritic and many processes have one or more bifurcations. Interstitium, seen as a collagen meshwork, constitutes a greater proportion of the surface area (50.3%). Many surface excrescences are present, appearing as white structures protruding into the joint cavity. Scale bar = 10 μ m.

value corresponding to a region of plate-like cells (cell type (a) of Fig. 2) and the higher value to a region of more dissected cells (types (d), (e), and (f) of Fig. 2). Adipose and posteromedial synovia had interstitial areas of 24 (3)% and 29 (3)% respectively.

EFFECT OF INTRA-ARTICULAR DISTENSION

The general appearance of the surface did not differ markedly between tissue fixed in situ at normal joint pressure and tissue fixed in situ at 5 cmH₂O. The latter is well below the 'yield point' at which hydraulic permeability begins to increase.^{1 26} Distension by 25 cmH₂O intra-articular pressure (above yield point), however, produced dramatic changes in surface shape. Although some cells still retained scalloped oval outlines (especially, posteromedial samples), most became much more dendritic (Fig. 5). The cytoplasmic processes extending along the synovial surface (typically three to four) were much elongated (20–25 μ m) and thinned (0.4–0.8 μ m wide), resembling short unmyelinated axons. Many were branched and possessed small smooth granules or cauliflower-like excrescences, or both, on their surfaces.

Between the cell processes the area of exposed interstitium was very obviously increased at 25 cmH₂O. The point counting method applied over $3.8 \times 10^3 \mu\text{m}^2$ areolar synovium gave an interstitial area of 53 (RSE 2)%. Preliminary results from a morphometric study of TEMs also gave a high value (45%). For adipose and posteromedial synovium the interstitial areas were 43 (3)% and 35 (3)% respectively.

Structures just beneath the surface created long cylindrical hummocks along the surface (Fig. 1), and inspection of the cut edge of the sample indicated that these ridges were raised by microvessels very close to the surface.

Discussion

SURFACE CONTOURS

Comparison of tissue excised before fixation with tissue fixed in situ showed considerable deformation of areolar-muscular synovium in post-fixed samples, due presumably to elastic retraction of the soft tissue as it is excised. Inspection of the unfixed, exposed areolar synovial surface in vitro through a dissecting microscope in the course of previous studies²⁷ indicated that the normal hydrated areolar surface in situ resembled Fig. 1B rather than Fig. 1A. We conclude that the highly convoluted surface of rabbit areolar-muscular synovium excised before fixation is to a large extent an artefact. It is important to note, however, that synovium fixed in situ is not completely flat—some furrows persist, and may be functionally important in permitting synovium to stretch with minimal stress during joint movement.

For adipose synovium the fixation method did not influence surface contour (cobblestone pattern), presumably owing to the incompressible nature of packed fat globules. The cobblestone appearance may have been greatly exaggerated, however, by the SEM preparative technique. Rabbit infrapatellar adipose synovium in conventional paraffin embedded histological sections (excised tissue or

whole knee) has a predominantly smooth convex surface with only an occasional cobblestone corresponding to a fat cell (unpublished observation). Similarly, resin embedded adipose synovium has a mainly smooth surface not conforming to underlying fat cells, both on light microscopy of semithin sections (see Fig. 1 of reference 25) and on TEM (unpublished observation, Fig. 1 of reference 32, and Fig. 2 of reference 19), even when the fat cells are very superficial.¹⁹ The exaggerated cobblestone pattern of SEM may be due to collapse of synovium into the dehydrated angles between adjacent fat cells.

INTERCELLULAR SPACES

The substantial area of synovial surface composed simply of interstitium at normal joint pressure agrees with previous SEM studies.²¹⁻²³ The possibility of artefact must be considered as some tissue shrinkage occurs during preparation for SEM, though the critical point drying method is designed to minimise postdehydration changes. The linear shrinkage of synovial samples after ethanol dehydration, measured with a Vernier travelling microscope, averaged 7%. We consider it unlikely, however, that the interstitial exposure was primarily an artefact of differential tissue shrinkage, for several reasons. Firstly, the observations agree with the findings by light microscopy that the intimal cells form a discontinuous layer embedded in a fibrous interstitium.^{8-11 30} The interstitial exposure in Fig. 3B bears a close resemblance to that shown by silver nitrate staining of hydrated synovium (e.g., Fig. 12 of reference 8, Fig. 7 of reference 9). Secondly, the observation is supported by TEM; 17-21% of the surface of unstretched rabbit knee synovium was reported to consist of interstitium in a morphometric TEM study.¹² Thirdly, the observations are compatible with physiological properties—namely, permeability of the intima to quite large extracellular tracers like colloidal carbon.³³ Fourthly, cell bodies were not infrequently partially overlapped by interstitium, a situation unlikely to arise from cell retraction.

Specimens prepared by the alternative method of freezing and freeze drying do not seem to have differed markedly from those prepared by critical point drying as here.^{19 20} A low temperature SEM study of unfixed hydrated synovium might, however, be of interest.

CELL SHAPE

We must consider whether the observed cell shapes truly represent those *in vivo*. Opportunities for cell shape to change might arise (a) during exposure to intra-articular fluid for one hour, (b) in the course of

fixation, if slow, and (c) owing to shrinkage during processing.

(a) The intra-articular fluid was of physiological ionic composition (Krebs' solution), of normal pH, temperature, and osmolality, with glucose as energy source, and pre-equilibrated with an oxygen/CO₂ mixture. Moreover, intimal gas tensions and nutrition *in vivo* were maintained by the synovial capillary network, which exceeds that of skeletal muscle in density.²⁵ Quantitative physiological studies show that the permeability of the intima is unaltered by several hours of intra-articular Krebs' infusion.¹ In addition, TEM studies (unpublished) show a similar synovial structure after per-vascular fixation with no intra-articular infusion and after one hour of intra-articular Krebs' infusion below yield pressure followed by intra-articular fixation. For these various reasons it seems unlikely that cell shape was changed by the 'stress' of intra-articular infusion of Krebs' solution under low pressure.

(b) It seems unlikely that the novel synovial fixation methods changed cell shape. Fixation by vascular perfusion was very rapid because the synovial capillaries deliver fixative in seconds to within 6-11 μ m of the synovial surface,²⁵ across which distances diffusion times are very short. Although the half life for fixative concentration growth was slower with the intra-articular fixation method (30 seconds at 5 cmH₂O), the SEM results at 5 cmH₂O were similar to those after per-vascular fixation. Synoviocytes in culture are capable of reversibly transforming between fibroblastoid and more stellate morphologies, but such changes take more than three to four hours (typically 16-18 hours),^{4 6} a time scale far longer than allowed here.

(c) The demonstration of extensive cell processes fits with deductions from histology.⁸⁻¹¹ As long ago as 1930 Sigurdson, using the silver nitrate method on unfixed hydrated tissue, noted that some synovial cell surface profiles were relatively regular while others were 'large and irregular with numerous processes'.⁹

The observed cell shapes, with intermeshing cytoplasmic process of variable length and width, agree with studies of immersed, fixed tissue.^{8 22 34} Many cells strongly resembled fibroblasts in monolayer culture,³⁵ and some were more dendritic. It seems clear that even *normal* synovial intimal cells extend many cytoplasmic processes, and that these are made longer and thinner by intra-articular distension. Moreover, living surface synoviocytes freshly detached from normal pig synovium also have a dendritic or stellate outline.³⁶ The demonstration of a multiplicity of cytoplasmic processes in normal intimal cells *in situ* raises an interesting

question—Does the dendritic subpopulation of cells cultured from rheumatoid synovium²⁻⁷ represent a special class of cells involved specifically in the disease process or are such cells relatively normal, deformable intimal cells expressing their inherent phenotypic tendency to extend cytoplasmic processes? Reversible transformation of cultured rheumatoid dendritic cells into fibroblastoid morphology,⁴⁻⁶ collagenase and prostaglandin E₂ synthetic ability,⁴⁻⁶ and surface antigenicity⁷ have led to suggestions that such cells are closely related to fibroblasts—as are normal intimal 'B' cells—rather than to dendritic antigen presenting macrophages. Our observations of the synoviocyte in situ support that view. It appears that diverse cell types can adopt a stellate morphology given the appropriate conditions (e.g., tissue macrophages, blood monocytes in culture, synovial intimal cells, astrocytes).

CELL SURFACE FEATURES

The marked surface activity of a high proportion of the cells was suggestive of endocytosis or exocytosis, or both. The structure of filopodia, such as that in Fig. 4, seems compatible with endocytosis. The smooth granules of diameter 0.2–0.5 µm have been reported also in tenosynovium²³ and rheumatoid synovium.²⁰⁻²³ They may be secretory vesicles. The larger complex cauliflower protruberances, described by Date²² and confirmed here, could be either phagocytic or secretory in function. As they contain clusters of smaller granules a secretory function is perhaps more likely.

EFFECT OF INTRA-ARTICULAR VOLUME EXPANSION ON STRUCTURE

The structural changes at 25 cmH₂O fluid pressure help account for the increase in hydraulic permeability of synovium above yield pressure (9.5 cmH₂O). Several possible explanations have been advanced for the latter, including increase in intercellular area available for fluid exchange,¹ but direct evidence has until now been lacking. Here a marked increase in interstitial area was visualised directly. The increased area for fluid exchange must contribute to the reported increases in intimal hydraulic permeability at raised pressure; but interstitial area changes alone (~2×) do not seem quantitatively great enough to account fully for the far greater permeability changes.¹⁻²⁶ Studies of structural changes by TEM are in progress to assess whether path length as well as area is modified by intra-articular distension. Wide intercellular spaces are also prominent in scanning electron micrographs of the rheumatoid synovial surface.²³⁻³⁷

This research was supported by the Arthritis and Rheumatism Council.

References

- 1 Knight A D, Levick J R. Effects of fluid pressure on the hydraulic conductance of interstitium and fenestrated endothelium in the rabbit knee. *J Physiol (Lond)* 1985; **360**: 311–32.
- 2 Woolley D E, Brinckerhoff C E, Mainardi C L, Vater C A, Evanson J M, Harris E D. Collagenase production by rheumatoid synovial cells: morphological and immunohistochemical studies of the dendritic cell. *Ann Rheum Dis* 1979; **38**: 262–70.
- 3 Winchester R J, Burmester G R. Demonstration of Ia antigens on certain dendritic cells and a novel elongate cell found in human synovial tissue. *Scand J Immunol* 1981; **14**: 439–44.
- 4 Baker D G, Dayer J M, Roelke M, Schumacher H R, Krane S M. Rheumatoid synovial cell morphologic changes induced by a mononuclear cell factor in culture. *Arthritis Rheum* 1983; **26**: 8–14.
- 5 Hendler P L, Lavoie P E, Werb Z, Chan J, Seaman W E. Human synovial dendritic cells. Direct observation of transition to fibroblasts. *J Rheumatol* 1985; **12**: 660–4.
- 6 Gadher S J, Woolley D E. Comparative studies of adherent rheumatoid synovial cells in primary culture: characterisation of the dendritic (stellate) cell. *Rheumatol Int* 1987; **7**: 13–22.
- 7 Heino J, Viander M, Peltonen J, Kouri T. Adherent cells from rheumatoid synovia; identity of HLA-DR positive stellate cells. *Ann Rheum Dis* 1987; **46**: 114–20.
- 8 Key J A. The synovial membrane of joints and bursae. In: Cowdry E V, ed. *Special cytology*. Vol 2. New York: P B Hoeber, 1928: 1054–74.
- 9 Sigurdson L A. The structure and function of articular synovial membrane. *Journal of Bone and Joint Surgery* 1930; **12**: 603–39.
- 10 Hamerman D, Stephens M, Barland P. Comparative histology and metabolism of synovial tissue in normal and arthritic joints. In: Mills L C, ed. *Inflammation and disease of connective tissue*. Philadelphia: Saunders, 1961: 158–68.
- 11 Fell H B, Glauert A M, Barratt M E J, Green R. The pig synovium. I. The intact synovium in vivo and in organ culture. *J Anat* 1976; **122**: 663–80.
- 12 Knight A D, Levick J R. Morphometry of the ultrastructure of the blood-joint barrier in the rabbit knee. *Q J Exp Physiol* 1984; **69**: 271–88.
- 13 Graaback P M. Ultrastructural evidence for two distinct types of synoviocytes in rat synovial membranes. *J Ultrastruct Res* 1982; **78**: 321–39.
- 14 Woodward D H, Gryfe A, Gardner D L. Comparative study by scanning electron microscopy of synovial surfaces of four mammalian species. *Experientia* 1969; **25**: 1301–3.
- 15 Gryffe A, Gardner D L, Woodward D H. Scanning electron microscopy of normal and inflamed synovial tissue from a rheumatoid patient. *Lancet* 1969; **ii**: 156–7.
- 16 Redler I, Zimny M L. Scanning electron microscopy of normal and abnormal articular cartilage and synovium. *J Bone Joint Surg [Am]* 1970; **52A**: 1395–404.
- 17 Highton T C, Donaldson L A. Scanning electron microscopy of synovial joints with preservation of mucopolysaccharides. *Proceedings of the University of Otago Medical School* 1970; **48**: 64–5.
- 18 Highton T C. A scanning electron microscopic study of synovial joints. *J R Coll Physicians Lond* 1971; **6**: 25–32.
- 19 Wysocki G P, Brinkhaus K M. Scanning electron microscopy of synovial membranes. *Archives of Pathology* 1972; **93**: 172–7.
- 20 Cameron H U, MacNab I. Scanning electron microscopic studies of the hip joint capsule and synovial membrane. *Can J Surg* 1973; **16**: 388–92.
- 21 Gaucher A, Faure G, Netter P, Pouvel J, Duheille J. Apport de la microscopie électronique à balayage à l'étude de la synoviale humaine normale et pathologique. *Rev Rhum Mal Osteoartic* 1976; **43**: 51–60.

- 22 Date K. Scanning electron microscope studies on the synovial membrane. *Arch Histol Jpn* 1979; **42**: 517-31.
- 23 Fujita T, Inoue H, Kodama T. Scanning electron microscopy of the normal and rheumatoid synovial membranes. *Arch Histol Jpn* 1968; **29**: 511-22.
- 24 Inoue H, Takasugi H, Akahori O. Surface study of tenosynovium in hens and humans by electron microscopy. *The Hand* 1976; **8**: 222-7.
- 25 Knight A D, Levick J R. The density and distribution of capillaries around a synovial cavity. *Q J Exp Physiol* 1983; **68**: 629-44.
- 26 Levick J R. The influence of hydrostatic pressure on trans-synovial fluid movement and on capsular expansion in the rabbit knee. *J Physiol (Lond)* 1979; **289**: 69-82.
- 27 Knight A D, Levick J R, McDonald J N. Relation between trans-synovial flow and plasma osmotic pressure, with an estimation of the albumin reflection coefficient in the rabbit knee. *Q J Exp Physiol* (in press).
- 28 Karnovsky M J. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J Cell Biol* 1965; **27**: 137A.
- 29 Kessel R G, Kardon R H. *Tissues and organs: a text-atlas of scanning electron microscopy*. San Francisco: Freeman & Co, 1979: 12.
- 30 Ghadially F N. Fine structure of joints. In: Sokoloff L, ed. *The joints and synovial fluid*. Vol I. New York: Academic Press, 1978: 105-76.
- 31 Aherne W A, Dunnill M S. *Morphometry*. London: Arnold, 1982.
- 32 Ghadially F N, Roy S. Ultrastructure of rabbit synovial membrane. *Ann Rheum Dis* 1966; **25**: 318-25.
- 33 Levick J R. Blood flow and mass transport in synovial joints. In: Renkin E M, Michel C C, eds. *Handbook of physiology*. Vol IV. *The microcirculation*. Bethesda M D: American Physiological Society, 1984: 917-47.
- 34 Elliott S, Cooke T D V. Scanning electron microscopy of antigen induced arthritic joints. I. Inflammatory cell interaction at synovial-meniscal surfaces during the Arthus response. *J Rheumatol* 1986; **13**: 401-7.
- 35 Pratt R M, Yamada K M, Olden K, Ohanian S H, Hascall V C. Tunamycin-induced alteration in synthesis of sulphated proteoglycans and cell surface morphology in the chick embryo fibroblast. *Exp Cell Res* 1978; **118**: 245-52.
- 36 Barratt M E J, Fell H B, Coombs R R A, Glauert A M. The pig synovium. II. Some properties of isolated intimal cells. *J Anat* 1977; **123**: 47-66.
- 37 Hayashi T. Three-dimensional observations of rheumatoid synovial membrane. *Ryumachi* 1976; **16**: 35-56. (English abstract.)