Mode of formation of synovial villi

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SUMMARY Synovial tissue from 6 normal pigs and from 16 patients undergoing arthrotomy for joint disease was examined by dissecting microscopy. Scale models were constructed of 3 human synovial specimens from photographic magnifications of serial sections. Surface bridging and subintimal cavitation were observed, particularly in tissue from patients with rheumatoid arthritis. These features suggest that synovial surface projections (villi) do not form simply by outgrowth. Reference to original haematoxylin and eosin stained sections suggested that tissue splitting contributes to the formation of villi.

The mode of formation of synovial villi is obscure. It is not known whether they develop as outgrowths of the synovial membrane or by delamination and partial detachment of small strips of the surface tissue.¹ The distinction between these 2 possible mechanisms is essential to the understanding of the increase in tissue bulk which occurs in chronic synovitis and which contributes to joint deformity and loss of function.

The detailed structure of synovial villi could provide circumstantial evidence for their mode of formation. Certain structural patterns cannot arise by simple outgrowths. These include perforations, bridged projections (attached to the tissue base at more than one point), and secondary blind cavities in the deep tissue. These features can occur only by either splitting or fusion of preformed projections. The present study was designed to assess whether these structural patterns occur in normal or arthritic synovial membrane.

Materials and methods

Pig material. Fresh pigs' hind trotters were obtained from a commercial butcher. Skin, subcutaneous tissue, and tendons were dissected away from the dorsal surface of the ankle joint. Synovial membrane was removed for dissecting microscopy in pieces approximately 1 cm wide.

Human material. Synovial material was collected from 16 patients. They included patients with simple

traumatic lesions such as torn meniscus, osteoarthritis, and rheumatoid arthritis. A full description is given in Table 1. Tissue was cut into pieces 5-20 mm wide. All specimens were viewed under an Olympus dissecting microscope. Selected specimens with various surface appearances were embedded in paraffin for sectioning. Considerable care was taken to ensure that the specimens were embedded so that sections were cut at right-angles to the surface. Sections were stained with haematoxylin and eosin.

Two samples of synovial membrane from patients with rheumatoid arthritis and one from a patient with degenerative joint disease with different surface appearances were selected for more detailed structural analysis. The specimens were serially sectioned at 5

Table 1 *Specimens of human synovial membrane*

Case no., Diagnosis	Age	Sex
(1) Torn meniscus, knee	22	M
(2) Osteoarthritis, hip	66	M
(3) Osteoarthritis, knee	60	F
(4) Osteoarthritis, hip	60	M
(5) Osteoarthritis, knee	65	F
(6) Rheumatoid arthritis, knee	52	F
(7) Loose body, knee	23	M
(8) Rheumatoid arthritis, regenerated synovial membrane around shoulder prosthesis	59	M
(9) Osteoarthritis, hip	59	M
(10) Torn meniscus, knee	49	M
(11) Osteoarthritis, knee	65	M
(12) Osteoarthritis, knee	63	M
(13) Rheumatoid arthritis, elbow	65	F
(14) Osteoarthritis, knee	52	F
(15) Rheumatoid arthritis, regenerated synovial membrane around pseudoarthrosis	48	F
(16) Rheumatoid arthritis	62	F

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μm thickness, and sections at $50\ \mu\text{m}$ intervals were photographed under low-power magnification. The photographs were projected on to polystyrene sheets to produce an image with a total magnification of $\times 160$.

The polystyrene sheets were cut to the outline of the section and glued together to form a scale model. Each $50\ \mu\text{m}$ thickness of tissue was represented by one 8 mm sheet of polystyrene (magnification $\times 160$). Tissue surfaces were represented in the models only if they were coated with a recognisable layer of 'lining cells'. Unlined spaces were ignored as being possible artefacts. The models were then examined in combination with the original sections and the microstructure interpreted in the light of both.

Results

PIG MATERIAL

Pig synovial specimens had flat, laminated, and villous areas in different parts of each specimen. Villi were commonest adjacent to the junction with the cartilage. All 6 specimens had examples of bridged projections ('villi'). Characteristically, flange-like projections carried villous branches, some of which were joined together at the tip or part of the way up to form a loop or bridge. All joints appeared to be perfectly healthy, with no evidence of swine arthritis or inflammation of any sort. No fibrin was present in the joints.

HUMAN MATERIAL

Human specimens showed a wide range of surface appearances from smooth and flat to highly villous. Both flat and complex surfaces were seen in specimens taken from different areas of the same joint.

Specimens taken from patients with simple trauma (cases 1, 7, 10) had been taken from the anterior aspect of the knee cavity only. Further interference with the synovial membrane was not considered ethical in these cases. All specimens were uniformly smooth and flat, probably reflecting the anatomical site.

Forty-five of 56 specimens taken from patients with osteoarthritis had a flat synovial surface. Two of these specimens had clefts entering the subintima. Of 9 specimens with projections (villi) 3 had perforated laminar projections forming bridges (Fig. 1). Ten bridges were seen in total (specimens from cases 5, 9, and 14). Bridging occurred exclusively between projections derived from the same laminar base.

Twelve of 33 specimens taken from patients with rheumatoid arthritis had either flat or villous surfaces with no evidence of bridging or clefts entering the subintima. Fourteen specimens had clefts entering

Fig. 1 Villi from a patient with osteoarthritis as seen under the dissecting microscope. The longest projection (double arrows) is attached to the tissue base through at least 4 routes (single arrows). There was no evidence of fibrin deposition in the neighbourhood at removal. The bridging occurs between projections derived from the same fold of tissue. ($\times 12$).

the subintima in otherwise flat areas, and as such could not merely be considered as recesses between villous outgrowths. Eight specimens carried bridged projections.

As with the osteoarthritic specimens bridging was seen only between projections derived from the same laminar base, and did not occur between villi in contact with each other but derived from separate nearby laminae. In 12 specimens surface clefts were found to be in continuity with interconnecting spaces in the subintima parallel to the surface (cases 6, 13, 15, and 16). These spaces could not be considered as simple recesses, since they could be fully exposed from the surface only by cutting away superficial layers of tissue with a scalpel. In specimens showing many interconnecting spaces the subintima could be expanded by traction at right-angles to the surface to show a mass of wafer-like laminations. In 3 specimens (case 15) it was impossible to identify a surface plane because projections and subintimal laminations formed a continuous complex mesh. Interconnecting spaces ran the full thickness of the tissue (5 mm). The presence of completely blind cavities could not be assessed, but deeper cavities appeared almost totally enclosed.

POLYSTYRENE SCALE MODELS

Three specimens were chosen for modelling. One osteoarthritic specimen (case 5) was chosen because

it showed prominent projections (villi). One rheumatoid specimen (case 6) was chosen because it showed subintimal spaces, which were not manipulated during dissecting microscopy in this case. A second rheumatoid specimen was chosen because it showed adjacent areas of projections and lamination (case 16).

The first model included 10 projections which varied from finger-like to flange-like. Finger-like projections arose either directly from the tissue base or from laminae. The subintima was solid apart from one cavity which ran the width of the model and did not open on to the surface in the modelled area (Fig. 2).

Fig. 2 A section of the first model constructed from tissue from a patient with osteoarthritis. An arrow indicates the site of a subintimal space which is bordered on one side by a lamina corresponding to only 30 μm thickness in the original specimen (arrowhead).

Fig. 3 A surface view of the second model. A continuous tape has been passed through the various surface defects to show their subintimal communication.

Figs. 4, 5 Two sections of the second model shown at right-angles to the surface. Fig. 4 The 2 subintimal spaces described in the text are seen separated by a thin lamina (arrow).

of the specimen) and approximately 5 mm in diameter.

This cavity was separated from a similar deeper cavity by a layer of tissue 30–200 μm thick. The 2 cavities communicated directly through a channel 50 $\times$ 150 μm in size and indirectly through other nearby cavities (Figs. 4, 5).

The cavities described showed typical 'synovial lining cells' on the H and E sections. These cells were continuous with the lining cells on the tissue surface, although they tended to be flatter and tended to be limited to a monlayer (Fig. 6).

Fig. 5 A connection with surface is seen (arrow), as is a connection between the spaces (arrows). A third space is also present on the right.

The third model included both an area of upright flange-like projections and an area with subintimal spaces as described above. Clefts between projections and subintimal cavities differed only in the extent to which they could be reached from the surface (Fig. 7). Cavities were found deep in the fibrous subintima whose relationship with the surface could not be traced. There was no way of knowing whether these cavities were blind or whether they had distant connections with the main synovial cavity.

One apparently blind cavity was found. This was separated from the surface by tissue 30–50 μm thick. A communication with the surface through pores less than 50 μm in diameter could not be excluded, since this was the distance between sections used for modelling.

A large number of bridges appeared to be present in the more filamentous projections, but these were often difficult to demonstrate with certainty because of the limit of accuracy of alignment of polystyrene sheets (5 mm). Four well-defined bridges were seen in an area of thick flange-like projections.

Reviewing the H and E sections of rheumatoid specimens in the light of the models, we made some other observations of relevance to the mode of formation of the lining surface. In addition to the cavities described above, which were included in the polystyrene models because they carried an intact layer of lining cells, other cavities were found with every gradation between an unlined fibrous surface and a more or less complete lining cell layer. In some cases one side of a cavity was lined but the other was

Fig. 6 Section of tissue from the third model showing a series of subintimal spaces. The tissue surface is marked S. Some spaces are lined with cells (L), but some are only partially lined (P) and others appear to be simply lines of least resistance (LR) which have separated during fixation. There is no clear distinction between lined and unlined spaces. The cells lining the deeper spaces are flatter than the surface cells, but were found on examination of the model to be in continuity. The single blind cavity in this model was immediately deep to the surface, resembling the upper lined space marked L in this section. (H and E, $\times$ 76).

Fig. 7 A view of the third model at right-angles to the surface. Villi on the left blend in with subintimal laminations on the right.

Fig. 8 Section of tissue represented in the third model. Parts of two large projections (P) are seen, above and below. Alongside the upper projection is a strip of tissue coated on both sides with lining cells. Alongside the lower projection is a very similar strip whose inner surface, together with the apposed surface of the main projection, is disrupted. The lower strip may have separated either in vivo or during processing of the specimen. Either way the extreme similarity to the upper strip suggests that the upper strip may have also formed by separation with subsequent relining. (H and E, $\times 132$).

unlined or coated with loosely arranged inflammatory cells. This appearance suggests that the formation of unlined tissue spaces may precede generation of a lining layer of cells. Although some of these cavities may have been formed by mechanical separation dur-

ing surgery or processing, the presence of partial lining and of haemosiderin in many of the surface cells strongly suggest that tissue separation occurred during life.

Although fibrin (as judged by red staining with

Martius scarlet blue (MSB) and brown staining with van Giesen) was seen in some specimens, it was absent from sites of bridging, and nothing was found to support the suggestion that bridges had formed by organisation of fibrin. Avascular strips of tissue, 30 μ m or less in thickness and coated on one or both sides with typical lining cells, were frequently seen lying parallel to a larger projection (Fig. 8).

These strips were attached by three-quarters or more of their margin to the larger projection, as judged by serial sections. Although the outer surface of the strip always carried an intact lining layer, the apposed surfaces of both the strip and the large projection were often only partially lined with evidence of cell damage. The appearance was consistent with splitting away of the avascular strip from the larger projection. It seems unlikely that such strips could develop by any other means, since they had no intrinsic blood supply.

Discussion

These observations indicate that synovial villi do not form solely by outgrowth. The presence of bridging indicates that either splitting or outgrowth, followed by subsequent fusion, occurs.

Various factors indicate that splitting is more likely than outgrowth with fusion. Bridging was found only between projections arising from the same lamina or fold of tissue. There was no cross-bridging between adjacent villi derived from separate folds of tissue. The absence of fibrin in the bridged areas, and the occurrence of bridging in normal pig tissue, where fibrin would not be expected to occur, suggest that bridging cannot be ascribed to fusion via fibrinous adhesions. The presence of avascular strips of lined tissue and the similarity between lined and unlined subintimal spaces also support the occurrence of splitting.

Although one apparently blind secondary cavity was found, it seems that subintimal spaces generally communicate with the main joint cavity. This may be

because synovial fluid tracks into such spaces and keeps them open.

Rheumatoid synovial cells produce more collagenase in culture than normal synovial cells.² If this reflects higher production of collagenase in vivo, rheumatoid synovial membrane might be expected to disintegrate more easily than normal synovial membrane, having been weakened by autolysis. This is in keeping with the suggestion that 'rice bodies' are, at least in part, made up of fragments of detached synovial surface.³ It also makes increased splitting seem a logical explanation for the increase in complexity of villi seen in rheumatoid tissue.

Bywaters⁴ has suggested that the propagation of rheumatoid synovitis is dependent on movement. Movement may initiate changes in the tissue in a number of ways. The observations made in the present study suggest that splitting of an internal tissue surface may be one way in which movement could be linked to local inflammatory change. In rheumatoid arthritis the basic immunological abnormality may lead to an excessive inflammatory response, with overproduction of collagenase.

This could act as a predisposing factor for further splitting at the same site, leading to foci of chronic inflammation. In this way collagenase, and perhaps other autolytic enzymes, could be implicated in the propagation of the chronic synovitis itself and not merely in the subsequent damage to cartilage and bone.

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