Microfibrillar elements in the synovial joint: presence of type VI collagen and fibrillin-containing microfibrils

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Abstract

Objectives—The aims were to isolate and positively identify the microfibrillar elements which have been observed in the synovial lining. In addition, synovial fluid was examined for these elements to improve the understanding of the role of these structures in health and disease.

Methods—Bacterial collagenase digestion of bovine synovial linings and human and bovine synovial fluids was used to release intact, non-denatured microfibrillar elements. The microfibrils were isolated by Sepharose CL-2B chromatography and viewed by rotary shadowing. They were characterised by immunogold labelling with specific antibodies.

Results—Intact type VI collagen microfibrils and fibrillin-containing microfibrils were isolated and positively identified in the synovial lining from bovine ankle joints by immunogold labelling. Type VI collagen microfibrils were also present in the synovial fluid.

Conclusions—The role of the microfibrillar elements in vivo is not fully understood, but their distribution in the synovial lining suggests they have an important role in the mechanical and physical properties of this tissue. The presence of type VI collagen microfibrils in synovial fluid poses the intriguing possibility that it may represent a product of microfibril turnover and a potential early marker for rheumatoid arthritis. Alternatively, type VI collagen may be specifically secreted into the synovial fluid to interact with hyaluronan and form part of the structure of synovial fluid.

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The synovial joint allows movement with a minimum of friction and wear. It contains a number of different tissues, each with its own specific function. Contained within the joint space is the synovial fluid which is responsible for lubricating the joint. Lining the inner surface of the joint capsule is the synovial lining and this is in direct contact with the synovial fluid. The synovial lining is a unique connective tissue which contributes to maintaining the structural and functional integrity of the joint. It is responsible for synthesising and secreting hyaluronan and other molecules important for lubrication into the synovial

fluid, and provides a blood supply for chondrocytes in the avascular cartilage.

The synovial lining has been relatively poorly characterised at the biochemical level despite the key role of this tissue in the functioning of the joint. It consists of a loose network of cells embedded in a collagenous interstitium through which solutes pass into the synovial fluid.¹ Histochemical analyses have shown the synovial lining to contain a number of structural macromolecules including collagens,^{2–3} proteoglycans,^{4–6} fibronectin,^{3–7} hyaluronan,^{8–9} and microfibrils.^{10–11}

The distribution of collagens in the synovial lining appears to be unique. Type IV collagen is found in the vascular basement membranes³ and cellular basement membranes¹² of the synovial lining. Type V collagen is present throughout the matrix of the synovial lining.¹³ Types I and III collagens, which form 67 nm banded fibres, predominate in the deeper regions of the synovial lining. In contrast with other tissues, where type VI microfibrils and type I collagen fibres interact, in the region of the synovial lining directly adjoining the synovial cavity the fibrillar collagens are largely absent, but this region is particularly rich in microfibrils.

There are two distinct and unrelated classes of connective tissue microfibrils. These are the type VI collagen microfibrils and the fibrillin-containing microfibrils, both of which have a widespread tissue distribution. Type VI collagen microfibrils are formed from triple helical monomers of type VI collagen which assemble laterally to form dimers and then tetramers. These associate in an end to end fashion to form extensive double beaded microfibrils with a periodicity of 100 nm and a diameter of 3–5 nm.¹⁴ The glycoprotein fibrillin is the principal structural component of a second distinct class of microfibrils which have a diameter of 10–13 nm and an average periodicity of 50 nm.¹⁵ In elastic tissues the fibrillin-containing microfibrils play a key part in elastogenesis¹⁶ and in vitro studies have shown that fibrillin-containing microfibrils can support cell adhesion.¹⁷ No specific role for these microfibrils has yet been demonstrated in non-elastic tissues, however.

Histochemical studies have shown the presence of microfibrils in the synovial lining.¹⁰ In addition, broad, banded aggregates (possibly consisting of microfibrils) called fibrous long spacing material have been reported in the synovial lining.^{6–18} These microfibrillar

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elements and the fibrous long spacing structures have been tentatively identified as type VI collagen.¹¹

We describe here the presence of type VI collagen microfibrils and fibrillin-containing microfibrils in synovial lining and type VI collagen microfibrils in synovial fluid.

Materials and methods

TISSUE EXTRACTION AND DISSOLUTION

Microfibrillar preparations from the synovial lining of adult bovine ankle joints were prepared as previously reported.¹⁹ The dissolved material was clarified by centrifugation at 10 000 *g* for 30 minutes and applied directly to a Sepharose CL-2B column (Pharmacia-LKB).

Samples of synovial fluid were obtained from fetal bovine knee joints and human osteoarthritic joints. For the synovial fluid an equal volume of 50 mM TRIS-HCl, pH 7.4, containing 0.4 M NaCl, 0.01 M CaCl₂, 2 mM phenylmethanesulphonyl fluoride (Sigma Chemical), 10 mM N-ethylmaleimide (Sigma Chemical), which also contained 0.5 mg bacterial collagenase (type 1A, Sigma Chemical) was added before incubation at 4°C for 18 hours. The reaction was terminated by the addition of 10 mM EDTA, clarified by centrifugation, and applied directly to a Sepharose CL-2B column for gel filtration chromatography.

The synovial lining samples were chromatographed directly without concentration under non-reducing, non-denaturing conditions on a column (3×75 cm) of Sepharose CL-2B equilibrated with 50 mM TRIS-HCl, pH 7.4, containing 0.4 M NaCl at a flow rate of 12 ml/hour. The synovial fluid solution was chromatographed on a column (1.2×20 cm) of Sepharose CL-2B equilibrated in the same buffer at a flow rate of 8 ml/hour. Column effluents were monitored at 230 nm. In each instance fractions were collected and pooled as appropriate. The pooled fractions which contained high molecular weight material eluting at or near the void volume were used for rotary shadowing electron microscopy and immunogold labelling.

ROTARY SHADOWING ELECTRON MICROSCOPY

The contents of each of the high molecular weight pooled fractions from the Sepharose CL-2B column were determined by rotary shadowing electron microscopy using the mica sandwich technique.^{19 20}

IMMUNOGOLD ELECTRON MICROSCOPY

Immunogold-protein complexes were prepared as previously described.²¹ Solutions were routinely filtered using 0.2 µm filters. Void volume samples (10 ml) were extensively dialysed against 20 mM magnesium acetate and placed in a PTFE dish. A small amount (<1 µl) of a 1 mg/ml solution of benzyl-dimethylalkylammonium chloride was taken up into a pipette tip and touched onto the

sample to form a thin film on the surface of the sample. After 20 minutes molecules were picked up onto the nickel grids by touching the surface of a grid onto the surface of the benzyl-dimethylalkylammonium chloride.

Grids were dried by dipping in absolute ethanol, blotting onto filter paper and blocked in 5 mM magnesium acetate/0.1% Tween 20 before being incubated in primary antibody (diluted 1:500 in 5 mM magnesium acetate/0.1% Tween 20) for one hour at room temperature. The antibodies to type VI collagen and fibrillin were as described previously.²² After washing four times for one minute each in 5 mM magnesium acetate/0.1% Tween 20 they were incubated in a 5 nm gold-protein A conjugate solution (diluted tenfold in 5 mM magnesium acetate/0.1% Tween 20; TAAB Laboratory Equipment) for one hour at room temperature. Grids were washed in 5 mM magnesium acetate/0.1% Tween 20 and finally in 5 mM magnesium acetate, dried by dipping into absolute ethanol, rotary shadowed using a platinum wire on a tungsten filament at an angle of 8°, and viewed under a JEOL 1200 EX electron microscope at 120 kV.

Results

Bacterial collagenase digestion of synovial lining homogenates removes the fibrillar collagens and effectively dissolves the microfibrillar components under non-reducing and non-denaturing conditions.¹⁹ Large macromolecular microfibrillar assemblies could be separated from plasma proteins and collagenous fragments on Sepharose CL-2B columns.

Rotary shadowing of the high molecular weight material obtained from the synovial lining from the adult bovine ankle joints showed the presence of type VI collagen microfibrils and fibrillin-containing microfibrils (fig 1A). These microfibrillar elements could also be isolated from the synovial lining of second and third trimester bovine fetal knee joints and porcine knee joints (results not shown).

Fibrillin and type VI collagen were present as extensive microfibrillar structures up to several micrometres in length. Type VI collagen microfibrils were consistently observed in more abundant amounts than the fibrillin-containing microfibrils in all the preparations. The type VI collagen microfibrils were flexible, double beaded microfibrils with a periodicity of 100 nm, consistent with previous observations.¹⁹ In some instances lateral alignment of these microfibrils was apparent (fig 1B). The fibrillin-containing microfibrils had a distinctive morphology and were clearly distinct from the type VI collagen microfibrils. These assemblies were characterised by a single beaded periodicity of approximately 50 nm and a diameter of 10–12 nm. (fig 1A).

The morphological characteristics were clearly indicative of type VI collagen microfibrils and fibrillin-containing microfibrils and these were positively identified by

Figure 1 Electron micrographs after rotary shadowing of type VI collagen and fibrillin-containing microfibrils. (A, B) Microfibrillar preparation isolated from synovial lining as described in the text. (C, D) Immunogold localisation of type VI collagen antibody (see Shuttleworth *et al*²² for details) as described in the text. (E, F) Immunogold localisation of fibrillin antibody (see Shuttleworth *et al*²² for details) as described in the text. Bars=100 nm.

immunogold labelling. The antibodies against type VI collagen microfibrils recognised a number of epitopes on the N- and C-terminal globular domains of the molecule as shown by the periodic distribution of gold particles (fig 1C). In a few instances two to three gold particles could be seen bound at the globular domains of these type VI collagen microfibrils. No staining was apparent on the fibrillin-containing microfibrils (fig 1D). The polyclonal antibodies to fibrillin bound predominantly to one side of the globular domains of these microfibrils (fig 1E). In general, antibody localisation disrupted the morphology of the fibrillin-containing microfibrils and was associated with one gold particle. The apparent lateral association of the microfibrils may be a consequence of antibody interactions, but further work will be required to resolve this possibility. No immunogold particles could be seen on juxtaposed type VI collagen microfibrils (fig 1F).

Fractionation of bacterial collagenase digests

of fetal bovine and human osteoarthritic synovial fluids by Sepharose CL-2B chromatography showed that, compared with synovial lining digests, only a small amount of high molecular weight protein could be recovered in the void volume fractions. For bovine tissue, however, rotary shadowing electron microscopic analysis showed that this fraction contained type VI collagen microfibrils with characteristic periodicity and dimensions (fig 2A). A number of microfibrillar aggregates could be seen in adult human synovial fluid, some of which had an apparent periodicity similar to type VI collagen (fig 2B).

Discussion

Histochemical studies of synovial lining tissue showed the presence of microfibrillar elements.^{10 11} We isolated and characterised microfibrils in their native, non-denatured state. Using this technique we have shown that type VI collagen microfibrils and fibrillin-

Figure 2 Electron micrographs after rotary shadowing of type VI collagen microfibrils isolated from synovial fluid as described in the text. (A) Bovine; (B) human. Bars=100 nm.

containing microfibrils are present in the synovial lining.

The distribution of microfibrils in the synovial lining is specific.¹¹ They are located predominantly at the inner surface of the synovial lining directly abutting onto the synovial fluid and are therefore in a key position to regulate the flow of material between the synovial lining and the synovial fluid. In rheumatoid arthritis there is an increased amount of plasma proteins in the synovial fluid,²³ indicative of an increased flux through the synovial lining. This may in part be due to the disruption of the normal organisation of this microfibrillar layer.

Interestingly, we have shown that small amounts of type VI collagen microfibrils are present in synovial fluid. These microfibrils have been shown to bind to hyaluronan²⁴ and may be related to the organisation of hyaluronan, which is found in great abundance in the synovial fluid. At this stage it is unclear as to the origin of type VI collagen microfibrils in the synovial fluid. They may be present in the synovial fluid merely as a result of type VI collagen turnover in the synovial lining. Alternatively, these type VI collagen microfibrils may be secreted specifically into the synovial fluid from cells present in the synovial fluid or from cells in the neighbouring synovial lining. It is already known that cells in the synovial lining are responsible for the secretion of hyaluronan and other molecules into synovial fluid and it seems equally feasible that these cells may secrete microfibrils into the synovial fluid.

The presence and location of microfibrillar elements in the synovial lining suggests that these may be critical determinants of the function of this tissue. In addition, the presence of type VI collagen in the synovial

fluid holds the tantalising possibility that this molecule represents an early marker in the disease process. It is clear that further work will be required to define the nature and role of the microfibrillar elements, but also to indicate any relevance of these molecules to the disease process.

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