Isolation and ultrastructural analysis of microfibrillar structures from foetal bovine elastic tissues

Relative abundance and supramolecular architecture of type VI collagen assemblies and fibrillin

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Summary

Extensive intact assemblies of matrix macromolecules have been solubilized from foetal calf skin, nuchal ligament and aorta by a new procedure that includes bacterial collagenase digestion under non-reducing, non-denaturing conditions and gel filtration chromatography. Type VI collagen was identified as the major microfibrillar element of these tissues by SDS–PAGE analysis and Western blotting. Rotary shadowing electron microscopy of these preparations revealed by far the most abundant and extensive arrays of intact collagen VI microfibrils isolated to date. The distinct microfibrillar species, fibrillin, which was identified on the basis of its periodicity and morphology, was also solubilized in abundance by this protocol. Analysis of these complex polymers has generated new information on their supramolecular architecture and relative abundance in these tissues. The protocol also demonstrates that the release of intact collagen VI microfibrils from these tissues is largely dependent on the removal of the major collagen fibrils.

Key words: microfibrils, collagen VI, fibrillin.

Introduction

The extracellular matrix of most connective tissues contains a variety of microfibrillar arrays (both collagenous and non-collagenous) of which the distinct macromolecular assemblies of type VI collagen and fibrillin represent major subclasses. Both of these species are structural elements of matrix with widespread distributions in elastic and non-elastic tissues (Sakai et al. 1986; Timpl and Engel, 1987; Trueb et al. 1987; Ayad et al. 1989; Gibson et al. 1989; Maddox et al. 1989; Peltonen et al. 1990; Keene et al. 1991), and they appear to be particularly important in maintaining the integrity of specific tissues such as blood vessel walls, lung and skin. However, there are few details available about their precise biological roles (co-operative or unrelated) or their complex structural organizations within tissues. The ultrastructural identification of filamentous aggregates of type VI collagen between collagen fibrils and near the surface of cells (Bruns, 1984; Bruns et al. 1986; von der Mark et al. 1984; Linsenmeyer et al. 1986; Keene et al. 1988; Bonaldo et al. 1990), together with their cell-adhesion and collagen-binding capacities demonstrated in vitro (Auville et al. 1989), has led to the proposal that collagen VI microfibrils perform a central role in cell–matrix interactions (Bonaldo and Colombatti, 1989). In contrast, virtually nothing is known about the function of fibrillin, although it is frequently found in association with elastic fibres (Kumaratilake et al. 1989; Maddox et al. 1989) and with basement membranes (Dahlback et al. 1990).

Type VI collagen is essentially a glycoprotein with a short triple-helical core and large N- and C-terminal globular domains, and its assembly into microfibrils has been demonstrated in biosynthetic systems (Engvall et al. 1986; Colombatti and Bonaldo, 1987; Colombatti et al. 1987). Higher-ordered collagen VI arrangements may also occur within the matrix. Details of the supramolecular architecture, interactions and potential dimensions of collagen VI have, however, proved difficult to obtain because of substantial difficulties in extracting intact, high-Mr, polymers (Timpl and Engel, 1987; Trueb et al. 1987). Indeed, procedures for the extraction of intact collagen VI chains have relied largely on the use of highly dissociative conditions, which disrupt the microfibrils into tetramers and invalidate attempts to elucidate their structure/function relationships. An alternative approach to the release of native intact collagen VI was developed by Wu et al. (1987) in their studies of intervertebral disc in which digestion with chondroitinase ABC lyase and purified hyaluronidase released native tetramers and short oligomers of collagen VI.

Fibrillin has recently been identified as a non-collagenous single-chain glycoprotein of Mr 350000 with intra-chain disulphide bonds, which assembles into periodic microfibrillar structures (Sakai et al. 1986; Wright and Mayne, 1988; Dahlback et al. 1990). A similar, if not identical, microfibrillar glycoprotein (MP340) has been described by Gibson and coworkers (Gibson et al. 1986; Kumaratilake et al. 1989; Gibson et al. 1989). Some structural details of this glycoprotein have emerged
recently from analysis of material released from nuchal ligament in reductive saline (Gibson et al. 1989) and from placenta after pepsinization (Maddox et al. 1989). Immunohistochemical analyses have identified fibrillin in a range of elastic and non-elastic tissues (Gibson et al. 1989; Inoue et al. 1989; Dahlback et al. 1990; Keene et al. 1991). However, the potential dimensions and higher-ordered in vivo arrangements of fibrillin polymers, their mechanism of assembly into microfibrils, and their repertoire of potential interactions within the matrix remain to be defined.

We here describe a method for the efficient release of abundant intact microfibrils of type VI collagen and fibrillin from foetal bovine skin, nuchal ligament and aorta by bacterial collagenase digestion under conditions where fibrillar collagens are digested but type VI collagen is not degraded. Analysis of this material has generated new data on the supramolecular form and relative abundance of these complex structures.

Materials and methods

Materials

Foetal calves were obtained from the local abattoir within 1 h of maternal death. Bacterial collagenase (type 1A), phenylmethanesulphonyl fluoride, N-ethylmaleimide, dithiothreitol and N-ethylmaleimide, dithiothreitol and prestained non-collagenous molecular weight markers were obtained from the Sigma Chemical Company, Poole, Dorset, UK. Tween-20 was obtained from BDH chemicals, Poole, Dorset, UK. Sepharose CL-2B and PD10 columns were supplied by Pharmacia-LKB, Milton Keynes, Bucks, UK. Peroxidase-conjugated swine IgGs to rabbit immunoglobulins were supplied by Dakopatt Ltd, High Wycombe, Bucks, UK. Mica sheets were obtained from TAAB Laboratory Equipment Ltd, Reading, Berks, UK.

Tissue digestion and solubilization

Samples of skin, nuchal ligament and aorta (approximately 2 g wet weight) were dissected and homogenized in 10 ml of 0.05 M Tris-HCl, pH 7.4, containing 0.4 M NaCl, 0.01 M CaCl₂, 2 mM phenylmethanesulphonyl fluoride (PMSF) and 10 mM N-ethylmaleimide (NEM). Bacterial collagenase (type 1A) was added to a final concentration of 0.2 mg ml⁻¹, and the digestion allowed to proceed at 4°C for 48 h with gentle stirring. The digestions were then terminated by addition of EDTA to a final concentration of 10 mM. The digested tissue homogenates were centrifuged at 10 000 g for 30 min. The supernatants containing solubilized material were designated the low salt extracts. The residues were resuspended in 10 ml of 0.05 M Tris-HCl, pH 7.4, containing 1 M NaCl, 10 mM EDTA, 2 mM PMSF and 10 mM NEM, and then extracted for 48 h at 4°C with gentle stirring, before being recentrifuged at 10 000 g for 30 min. The supernatants containing soluble residues were designated the high salt extracts. The final residues were resuspended in 0.6 M acetic acid at 4°C, and peptic was added at an enzyme-substrate ratio of 1:50. Samples from both low salt and high salt extracts were desalted by passing through PD-10 desalting columns equilibrated in distilled water, and freeze-dried prior to analysis by SDS–polycrylamide gel electrophoresis (SDS–PAGE).

Gel filtration chromatography

Low salt and high salt extracts were chromatographed directly without concentration under non-reducing, non-denaturing conditions on a column (1.5 cm × 200 cm) of Sepharose CL-2B. The column was equilibrated and eluted at room temperature with 0.05 M Tris-HCl, pH 7.4, containing 0.4 M NaCl. Column runs were constantly monitored at 230 nm and fractions corresponding to the different peaks pooled.

Electrophoresis and Western blotting

The low salt and high salt extracts and the column fractions were analysed by discontinuous SDS–PAGE on 6.5% gels (Laemmli, 1970) in the presence or absence of 10 mM dithiothreitol. Molecular weights were determined by reference to standards, which were collagenous (types I and V collagens) and non-collagenous. The prestained non-collagenous molecular weight markers obtained from Pharmacia-LKB, Milton Keynes, Bucks, UK were sandwiched between two sheets of freshly cleaved mica. The sandwiches were constantly monitored at 230 nm and fractions corresponding to the different peaks pooled.

Rotary shadowing electron microscopy

Samples from the excluded peaks were analysed by rotary shadowing electron microscopy for their content of intact high-M₅ aggregates from foetal bovine tissues

Results

Isolation of high-M₅ aggregates from foetal bovine tissues

The experiments presented in this section relate to tissue obtained from a single bovine foetus from the second trimester of gestation (140 days), but identical results were obtained with a number of animals of similar ages. Bacterial collagenase digestions of skin, nuchal ligament and aorta released complex mixtures of proteins and other components, the major components of which (61–73% wet weight of the tissues) were directly soluble in the digestion buffer, which contained 0.4 M NaCl. These digestes were designated 'low salt extracts'. Further extraction of the residues at 1.0 M NaCl yielded 'high salt' extracts containing additional solubilized material (approximately 21–32% wet weight of the tissues), and less than 10% remained as insoluble residues. The gross molecular compositions of these various extracts were compared by SDS–PAGE analysis (see below). The low salt extracts contained a higher proportion of low-M₅ components (including blood proteins) than the high salt extracts.

The low salt and high salt extracts were chromatoto-
Fig. 1. Isolation of high-$M_r$ aggregates by gel filtration chromatography. Low salt and high salt extracts of foetal calf aorta, nuchal ligament and skin were chromatographed at 20°C on a column of Sepharose CL-2B under non-reducing, non-denaturing conditions. The column buffer was 0.4 M NaCl, 0.05 M Tris-HCl, pH 7.5. The low salt profiles are represented as continuous lines, and the high salt profiles as broken lines. Fractions were pooled as indicated by the bars. (A) aorta; (B) nuchal ligament; (C) skin.

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Fig. 2. Electrophoretic analysis of pooled column fractions from low salt extracts. Samples isolated from skin (A) and aorta (B) were analysed by SDS–PAGE on 6.5% (w/v) gels under reducing conditions. Solubilized proteins were detected with Coomassie Brilliant Blue, and intact type VI collagen was identified by Western blotting using either/both of two specific polyclonal antisera. Tracks 1–4 (A) and 6–9 (B) represent protein-stained samples of fractions obtained after gel filtration chromatography. Tracks 5 (A) and 10 (B) are Western blots of fraction 1 from each column run. In A, the arrowhead indicates the α1(VI)/α2(VI) component.

consistent with our earlier work demonstrating reduced expression of the α3(VI) chain in early foetal skin (Kielty et al. 1990).

The skin-derived low salt excluded peak (fraction 1) was also electrophoresed on 2.5% SDS–PAGE gels under reducing and non-reducing conditions. In the absence of reducing agents, the fraction was resolved into a major (diffuse) component with a molecular mass of around \(2 \times 10^6\) and a small amount of very high \(M_r\) material that failed to penetrate the gel. After treatment with 10 mM cysteine, a lower \(M_r\) component of approximately \(1 \times 10^6\) was also observed. These molecular weights were determined by reference to cross-linked multimers of phosphorylase b. Since the \(M_r\) of a collagen VI monomer is approximately \(0.6 \times 10^6\), these results would be consistent with the presence of tetrameric aggregates in the unreduced samples, and tetramers and dimers in the reduced (cysteine-treated) material.

**Rotary shadowing electron microscopy**

The macromolecules present in the excluded peak of the low salt and high salt extracts of skin, nuchal ligament and aorta were visualized by rotary shadowing electron microscopy. This procedure provided direct confirmation that intact collagen VI molecules had been solubilized in abundance from these tissues by bacterial collagenase digestion. As expected, the samples were devoid of fibrillar collagen material, but the distinct non-collagenous entity, fibrillin, was identified in a number of fields on the basis of its morphology and dimensions (Wright and Mayne, 1988; Maddox et al. 1989). The high-\(M_r\) molecules present in each excluded peak were not, however, exclusively proteinaceous and both DNA and hyaluronan were identified by specific enzyme digestion analysis as components of moderate abundance (results not shown).

Type VI collagen, the most abundant high-\(M_r\) component solubilized from these tissues, was present predominantly in the form of long thin microfibrillar arrays of varying lengths up to 6000 Å (at least 50 tetramer units) and with a periodicity and internal dimensions consistent with a construction of tetramers arranged end-to-end (Fig. 3). Individual tetramers and double tetramers were occasionally observed. A wide distribution of microfibril lengths was observed at both salt concentrations, with assemblies composed of 9–10 tetramers particularly abundant in the high salt samples and longer sections predominating in the low salt material. A striking feature of these microfibrils was their high degree of flexibility and their ability to twist and loop around other macromolecules. There were no apparent structural differences between the collagen VI microfibrils solubilized from the low salt or high salt extracts of each tissue, or indeed between those extracted from the three tissues.

Analysis of the skin-derived collagen VI microfibrils at high magnifications (×50 000 to ×100 000) revealed little variation in the length or diameter of the central helical domain (54 nm and 4 nm, respectively). However, no such consistency was evident in the junction regions (N- and C-terminal non-helical sequences of two adjacent tetramers with short N-terminal helical regions) where a range of conformations from partially unwound to condensed and bead-like were observed (see particularly Fig. 3B,C). Consequently, deviations from the average length of each tetramer unit (102 nm) were recorded (see Fig. 5A). It is possible that the less-compact arrangements may rep-
Fig. 3. Electron micrographs after rotary shadowing of intact microfibrils of type VI collagen isolated from low salt tissue extracts. Samples were solubilized by bacterial collagenase digestion of foetal calf skin, aorta and nuchal ligament in conditions of low salt, isolated by chromatography on a column of Sepharose CL-2B and rotary shadowed. Extensive arrays of type VI collagen microfibrils were present in the excluded volume. Note their highly flexible nature, their capacity to twist and loop around each other, their potential for lateral alignment, and their frequent association with thin-stranded macromolecules (possibly hyaluronan). The micrographs illustrate the similar dimensions of intact collagen VI microfibrils isolated from skin (A,B), aorta (C) and nuchal ligament (D). Bar, 100 nm.
Fig. 4. Electron micrographs after rotary shadowing of intact collagen VI microfibrils demonstrating their propensity for lateral association into filamentous structures. High-$M_r$ aggregates from low salt extracts of foetal calf skin were rotary shadowed and the grids scanned for higher-ordered architectural arrangements. (A–E) Examples of the progressively complex supramicrofibrillar forms of collagen VI from simple lateral association of short aligned microfibrillar sections to long filaments apparently composed of several parallel microfibrils. (F) An example of the FLS-type collagen VI structures identified in a sample pre-incubated with the polyclonal antibody to the $\alpha 1$(VI)/$\alpha 2$(VI) component. Note that the overwhelming proportion of antibodies are directed to antigenic sites within the junctional regions. Bar, 100 nm.
Fibrillin was 51 nm (range 40-60 nm). Differences within the junction zones. (B) The periodicity of collagen VI microfibrils was 102 nm. The range of lengths recorded arose largely as a consequence of conformational differences within the junction zones. (B) The periodicity of collagen VI microfibrils and fibrillin. Samples were isolated by chromatography of low salt skin extracts. (A) The periodicity of collagen VI microfibrils and fibrillin. There were some clues to the possible mechanisms by which complex higher-order collagen VI structures might be constructed in vivo. The most frequently observed arrangement was the parallel alignment of two or more adjacent microfibrils by a mechanism apparently involving a strong lateral affinity between globular domains (Fig. 4). The presence of long thin strands extending both from the ends and from the internal globular domains of many of the microfibrils suggests an important structural association with a distinct matrix macromolecule such as hyaluronan (Fig. 3B-D). Broad, periodic FLS-type structures were observed only in antibody-treated samples (Fig. 4F). Fibrillin was present as intact microfibrils in moderate abundance in fraction 1 from both low salt and high salt extracts of skin and aorta, but significantly more fibrillin was detected in the corresponding nuchal ligament. The protocol involves an initial bacterial collagenase step under non-reducing conditions, which removes the fibrillar collagen framework and releases the microfibrils intact. The method relies in part on the well-documented resistance of type VI collagen to degradation by bacterial collagenase in the absence of a reducing agent (Timpl and Engel, 1987). The large structural assemblies solubilized in this way represented very significant proportions of the wet weight of each of the tissues and were predominantly composed of the two distinct microfibrillar species, collagen VI and fibrillin. Whilst the relative abundance of these components varied between these tissues, by far the major microfibrillar element of all three tissues was the type VI collagen microfibrils. These were identified biochemically on the basis of the electrophoretic mobilities of the component chains, immunologically by Western blotting, and ultrastructurally by their characteristic dimensions. The fibrillin microfibrils, present at highest levels in the nuchal ligament, were identified on the basis of their distinctive morphology and periodicity (Wright and Mayne, 1988; Maddox et al. 1989). Both collagen VI and fibrillin were clearly capable of existing as enormously long, thin polymers. Whilst an outstanding structural characteristic of the collagen VI microfibrils was their high degree of flexibility, the fibrillin microfibrils were, in

Discussion

A procedure has been developed for the selective isolation of intact microfibrillar arrays from the extracellular matrix of second trimester foetal calf skin, nuchal ligament and aorta. The protocol involves an initial bacterial collagenase step under non-reducing conditions, which removes the fibrillar collagen framework and releases the microfibrils intact. The method relies in part on the well-documented resistance of type VI collagen to degradation by bacterial collagenase in the absence of a reducing agent (Timpl and Engel, 1987). The large structural assemblies solubilized in this way represented very significant proportions of the wet weight of each of the tissues and were predominantly composed of the two distinct microfibrillar species, collagen VI and fibrillin. Whilst the relative abundance of these components varied between these tissues, by far the major microfibrillar element of all three tissues was the type VI collagen microfibrils. These were identified biochemically on the basis of the electrophoretic mobilities of the component chains, immunologically by Western blotting, and ultrastructurally by their characteristic dimensions. The fibrillin microfibrils, present at highest levels in the nuchal ligament, were identified on the basis of their distinctive morphology and periodicity (Wright and Mayne, 1988; Maddox et al. 1989). Both collagen VI and fibrillin were clearly capable of existing as enormously long, thin polymers. Whilst an outstanding structural characteristic of the collagen VI microfibrils was their high degree of flexibility, the fibrillin microfibrils were, in

Relative stabilities of microfibrils of collagen VI and fibrillin

The effects of reduction on the integrity of the collagen VI microfibrils and fibrillin were monitored by rotary-shadowing electron microscopy. The addition of 10 mM cysteine to the intact high-M₄ assemblies failed to disrupt the collagen VI microfibrils, and there was no increase in the proportion of individual tetramers present. It was noted, however, that these conditions induced a partial unwinding of the ultimate tetramer units of the microfibrils into component dimers (Fig. 8A,B). Cysteine had a considerably greater disruptive effect on the architecture of the fibrillin microfibrils, which disassembled and collapsed into irregular aggregates under these conditions (Fig. 8C).

The relative stabilities of the collagen VI and fibrillin microfibrillar arrays were assessed in high-M₄ fractions that had been stored for 4 weeks at 4°C in the presence of protease inhibitors. Under these conditions, virtually all the type VI collagen microfibrils had been disrupted into component tetramer units (Fig. 8D), in direct contrast to the fibrillin assemblies, which remained largely intact. It was noted, however, that after longer-term storage of up to 3 months, the integrity of the fibrillin microfibrils was also substantially disrupted.

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Fig. 6. Electron micrographs after rotary shadowing of intact microfibrils of fibrillin. Samples were solubilized by bacterial collagenase digestion of foetal calf skin (A), aorta (B) and nuchal ligament (C) in conditions of low salt, and isolated by chromatography on a column of Sepharose CL-2B. Extensive, branched arrays of fibrillin (diameter 10–14 nm) were observed in many fields, but there was no apparent association with collagen VI microfibrils. Bar, 100 nm.

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Fig. 7. Electron micrographs after rotary shadowing of intact fibrillin microfibrils illustrating examples of apparent branching.
High-Mr aggregates from low salt extracts of foetal calf skin and nuchal ligament were rotary shadowed and the grids scanned for higher-ordered fibrillin arrangements. A form of branching or possibly lateral association of short fibrillin sections was frequently observed in both extracts and is indicated by arrows. (A–C) skin; (D) nuchal ligament. Bars, 100 nm.

contrast, far more rigid, predominantly linear aggregates. These gross features presumably reflect the diverse structural roles of these macromolecules in vivo. The results presented here clearly demonstrate that neither collagen VI nor fibrillin arrays are structurally dependent on, or in covalent linkage with, the collagen fibrils. We have noticed that very little collagen VI is released from these tissues without prior bacterial collagenase digestion (C.M.K., unpublished observations). These data emphasize that the release of intact collagen VI microfibrils is largely dependent on the disruption and removal of the fibrillar collagen framework and strongly suggest that the primary location of the collagen VI microfibrils in these tissues is among the major collagen fibrils.

Despite the fact that type VI collagen has a ubiquitous tissue distribution and is now known to represent a significant component of many tissues, relatively little is known about its assembled macromolecular architecture in vivo, because it has proved an extremely difficult structure to extract in intact form. The procedure described in the present work has facilitated the extraction of the most extensive collagen VI assemblies described so far. These microfibrils may in turn be able to associate into higher-ordered structural arrays in vivo. Indeed, a strong lateral affinity between the microfibrils solubilized here was observed, which apparently involved association of the (mainly globular) junction domains of adjacent microfibrils. These interactions gave rise to filamentous structures reminiscent of the collagen VI-containing filaments identified in several tissues by immunohistochemistry (Bruns et al. 1986; Keene et al. 1988). Fibrous-long-spacing (FLS)-type collagen VI aggregates were only observed in antibody-treated fractions, and they might therefore represent non-physiological structures. Similar arrays

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Fig. 8. The relative stabilities of collagen VI microfibrils and fibrillin under reducing conditions and over time. The effects of
reduction and storage on the integrity of the high-M<sub>r</sub> collagen VI and fibrillin aggregates isolated from skin were assessed by
rotary-shadowing electron microscopy. (A,B) Treatment with 10 mM cysteine failed to disrupt the collagen VI microfibrils, but the
end tetramers were frequently partially unwound, and in some cases the ultimate subunits were dimeric (indicated by arrows).
(C) The macromolecular architecture of fibrillin was grossly disrupted by reduction. (D) After 4 weeks at 4°C in the presence of
protease inhibitors, the collagen VI was present virtually exclusively as single, double and triple tetramers, and there were no long
microfibrillar arrays present. Bar, 100 nm.

have been previously identified in (mainly pathological)
tissues and cell cultures (Timpl and Engel, 1987), but it has
never been clear whether they arise as a result of
prolonged incubation in buffer during staining.

The tetrameric end-to-end associations represent the
crucial extracellular step in collagen VI microfibril
formation, but there is astonishingly little information
available on the nature of these associations, despite the
fact that the entire amino acid sequences of the three
collagen VI chains are now known (Chu et al. 1988, 1989,
1990; Bonaldo and Colombatti, 1989; Bonaldo et al. 1989;
Koller et al. 1989; Trueb et al. 1989). Whilst the
involvement of aldehyde-mediated cross-linking residues
has been virtually excluded (Wu et al. 1987), the
significance of the numerous cysteine residues in both N-
and C-terminal sequences remains to be established. It has
been suggested that disulphide bonds between the globu-
lar domains of adjacent tetramers might be the basis of the
tetrameric aggregation phenomenon (Jander et al. 1984;
Timpl and Engel, 1987; Chu et al. 1989), although other
workers have demonstrated that collagen VI can be fully
extracted from some tissues with 4 M guanidinium
chloride in the absence of reducing agents (Wu et al. 1987;
Ayad et al. 1989). The demonstration in the present work
that reducing agents fail to disrupt the integrity of the
microfibrils provides confirmatory evidence that disul-
phide bonds do not play a primary role in these
interactions.

The isolation of abundant fibrillin microfibrils from
foetal calf tissues suggests that these macromolecules
must play a key role in the development of these tissues.
Indeed, it has been previously suggested on the basis of
immunohistochemical studies on human skin that fibrillin
may form a network that acts to anchor dermal elastic
fibres in the extracellular matrix and to the lamina densa
(Dahlback et al. 1990). The demonstration here that
fibrillin is already present in abundance in second
trimester pre-elastic tissues in the form of relatively
inflexible, long, branching arrays is consistent with the
possibility that they exist in vivo as coarse networks able
to direct and support the subsequent laying down of
elastin. The chemical basis of the substantial confor-
national variations observed in the fibrillin microfibrils is
not known, but it is clear that these structures are highly
dependent on disulphide bridges for maintenance of form.
Interestingly, there was no evidence for an association
between the microfibrils composed of fibrillin and type VI
collagen, which suggests quite separate structural roles
for these assemblies in the matrix.

Although many structural and functional aspects of
tissue microfibrils remain to be established, there is no
doubt that those composed of type VI collagen and of
fibrillin are major co-existing elements of developing
elastic tissues, with diverse structures and biological
roles. The solubilization and structural analysis of intact
collagen VI microfibrillar arrays described here has
revealed a macromolecule ideally suited to a central role
in cell–matrix communications, with a capacity to enmesh
and possibly interact directly with a range of matrix
components including collagen fibrils, hyaluronan and
proteoglycans, as well as with cells themselves. The gross
appearance of fibrillin microfibrils, however, provides few
clues as to its biological significance, but a potential role in
providing a supporting framework for the deposition of
elastin would fit its observed structural characteristics. It will be of particular interest to determine its role in non-elastic tissues as well as its capacity for cell–matrix interactions. The experimental system for isolating intact microfibrils described here may be further exploited as a means of defining the structure–function relationships of these molecular entities in a wider range of tissues and cell culture systems.

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