Collagen Fibers, Reticular Fibers and Elastic Fibers. A Comprehensive Understanding from a Morphological Viewpoint

Tatsuo Ushiki
Division of Microscopic Anatomy and Bio-imaging, Department of Cellular Function, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

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Summary. Fibrous components of the extracellular matrix are light-microscopically classified into three types of fibers: collagen, reticular and elastic. The present study reviews the ultrastructure of these fibrous components as based on our previous studies by light, electron, and atomic force microscopy.

Collagen fibers present a cord- or tape-shape 1-20 μm wide and run a wavy course in tissues. These fibers consist of closely packed thin collagen fibrils (30-100 nm thick in ordinary tissues of mammals), and exhibit splitting and joining in altering the number of the fibrils to form a three-dimensional network as a whole. Individual collagen fibrils (i.e., unit fibrils) in collagen fibers have a characteristic D-banding pattern whose length is ranges from 64 to 67 nm, depending on tissues and organs. During fibrogenesis, collagen fibrils are considered to be produced by fusing short and thin fibrils with tapered ends.

Reticular fibers are usually observed as a delicate meshwork of fine fibrils stained black by the silver impregnation method. They usually underlie the epithelium and cover the surface of such cells of muscle cells, adipose cells and Schwann cells. Electron-microscopically, reticular fibers are observed as individual collagen fibrils or a small bundle of the fibrils, although the diameter of the fibrils is thin (about 30 nm) and uniform. Reticular fibers are continuous with collagen fibers through the exchange of these collagen fibrils. In silver-impregnated specimens, individual fibrils in reticular fibers are densely coated with coarse metal particles, probably due to the high content of glycoproteins around the fibrils.

Elastic fibers and laminae are composed of microfibrils and elastin components. Observations of the extracted elastin have revealed that elastin components are comprised of elastin fibrils about 0.1-0.2 μm thick. Elastic fibers and laminae are continuous with networks and/or bundles of microfibrils (or oxytalan fibers), and form an elastic network specific to individual tissues.

The fibrous components of the extracellular matrix are thereby morphologically categorized into two systems: the collagen fibrillar system as a supporting framework of tissues and cells, and the microfibril-elastin system for uniformly distributing stress to maintain the resilience adapted to local tissue requirements.

Fibrous components of the extracellular matrix are classically divided into three types of fibers: collagen, reticular and elastic. This classification is based on the light microscopic findings (e.g., their shapes, staining properties and arrangements) and chemical properties of these fibers (e.g., MALL, 1896; FOOT, 1928; HAS, 1942); collagen fibers appear as thick and wavy strands stained pink with eosin, while reticular fibers are fine fibers stained dark with the silver impregnation method. Elastic fibers, on the other hand, are observed as a cord or sheet stained purple with resorcin-fuchs in or aldehyde-fuchs in staining, and are highly resistant to boiling water, in contrast with collagen fibers which are easily gelatinized in hot water.

Electron microscopy has also revealed the ultrastructures of these fibrous components, namely that the collagen and reticular fibers are composed of fibrils with a unique banding pattern (SCHMITT et al., 1942), and elastic fibers comprise both fibrous and amorphous elements (GREENLEE et al., 1966). Advances in biochemistry and immunohistochemistry have also provided detailed information on the nature of these fibrous components, and a number of reviews are available, especially in consideration of the biochemical properties of the fibrous components (e.g., ROSS, 1973; SANDBERG et al., 1981; KÜHN, 1987;
also see books edited by Hay, 1991; Yurchenko et al., 1994).

We have examined the three-dimensional ultrastructure and organization of these fibrous components mainly by scanning electron microscopy, paying attention to their functional roles specific to different tissues and organs. The present review thus highlights the three classical fibers, namely collagen, reticular and elastic fibers, especially based on findings by our group using various microscopic techniques including transmission electron microscopy (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM). The present paper will also present a current concepts on the fibrous components, with the intent that these might be useful for physiologically and pathologically understanding their functional roles in various tissues and organs.

I. COLLAGEN FIBERS

Basic structure of collagen fibers

Fresh collagen fibers are colorless strands 1 to 100 μm thick that usually follow a wavy course without branching in tissues. These fibers are stained pink with eosin and green with the Masson trichrome staining method (Fig. 1a). The birefringence of collagen fibers suggests the parallel arrangement of thinner fibrous components in collagen fibers. Electron microscopy shows collagen fibers to be a bundle of closely packed thin fibrils with periodical cross striations (Schmitt et al., 1942) (Fig. 1b, c); these unit fibrils are called “collagen fibrils”, although the term was earlier used as a very thin collagen fiber recognizable by light microscopy.

In specimens stained with a cationic dye such as Alcian blue and Cupromeronic blue, very thin filaments (less than 10 nm thick) are visible within the bundle of collagen fibrils (Fig. 1d) (Scott, 1980, 1988, 1995; Scott and Orford, 1981; Ruggeri and Benazzo, 1984, Raspanti et al., 2002). These filamentous structures have been considered proteoglycans, including large dermatan sulfate proteoglycans and such small molecules as decorin (Fleischmaier et al., 1991). The proteoglycan filaments appear to connect neighboring collagen fibrils by transversely and periodically attaching to a specific site of the fibrils. These findings indicate that proteoglycans play a role in synchronizing the position of bands in neighboring fibrils, and determine the distance of two neighboring fibrils to fasten themselves into a bundle.

Thus, the structure of collagen fibers in which parallel fibrils are bundled with flexible proteoglycans is in accordance with their mechanical properties, since collagen fibers are flexible but offer great resistance to a pulling force.

Arrangement of collagen fibers

The size and shape of collagen fibers (i.e., bundles of collagen fibrils) vary depending on tissues and organs, even within the same species. They are usually of a cord- or tape-shape with a width of 1-20 μm, and take a wavy course (Fig. 2a, b), even if they form dense fibrous connective tissues such as the tendon (Rowe, 1985). The wavy arrangement of these fibers probably provides resilience to the fibers themselves, which also serves as a cushion against the direct tension to collagen fibers.

In loose connective tissues, collagen bundles sometimes run parallel to each other to be twined into a larger bundle, while they come to split and join by changing the number of collagen fibrils, thus forming a three-dimensional meshwork throughout the tissues. Much thinner fibers also often participate in the collagen fiber network (Orberg et al., 1982; Ushiki and Ide, 1990); these fibers are composed of single or several collagen fibrils, which are produced by leaving the thicker fibers to rejoin them in another portion. This fibrillar network is similar to the network formed by reticular fibers, but does not have argyrophilic properties. This fibrillar network probably plays a role in maintaining a specific arrangement of collagen fibers in each tissues and organ.

Structure of collagen fibrils

As described above, collagen fibrils are unit fibrils which can be observed in individual collagen fibers by electron microscopy (Fig. 3a, b). These fibrils are cylindrical in shape with a diameter ranging from 10 to over 500 nm (mean diameter about 40-80 nm) in mammals (Parry and Craig, 1984). They show periodical striations (which are alphabetically named the A-E bands) in positively stained sections (Schmitt and Gross, 1948; Bruns and Gross, 1974), while the characteristic alternation of dark and light zones is found along the negatively stained fibrils by TEM (Tromans et al., 1963, Olsen, 1963). The periodicity of these structures is determined by the length of the two closest D-bands in positively stained fibrils, and called D-periodicity. The surface morphology of the collagen fibrils has also been studied by TEM of shadowed materials (Gross and Schmitt, 1948) and freeze-fractured replica (Marchini and Ruggeri, 1984; Raspanti et al., 1989), SEM (Raspanti et al., 1996) and AFM (Fig. 3c, d) (Baselet et al., 1993; Ushiki et al., 1996; Yamamoto et al., 1997). These studies revealed the presence of periodical grooves.
Fig. 1. Collagen fibers observed by light microscopy (a), TEM (b) and SEM (c, d).

a. Collagen fibers in the dermis in the human skin. These fibers are stained pink with hematoxylin-eosin and run in various directions. ×200.

b. Longitudinal section of a collagen fiber in the dermis of the human skin. The light-microscopically determined collagen fiber is a bundle of collagen fibrils. Note single fibrils (arrowheads) running independently from the bundle. ×21,000.

c. SEM image showing the three-dimensional structure of the collagen fiber in the mouse perilumineum. The collagen fiber is observed as a bundle of closely packed collagen fibrils with characteristic transverse bands. ×62,000 (Bar = 100 nm).

d. Closer view of a collagen bundle in the rat aortic adventitia, which has been treated with a 2% glutaraldehyde solution containing 0.05% Alcian blue and 0.3 M MgCl₂ (0.025 acetate buffer, pH 5.8). Thin filamnetous structures (arrowheads) appear to connect neighboring collagen fibrils by attaching transversely and periodically to a specific site of collagen fibrils. ×155,000 (Bar = 100 nm)
and ridges on the surface of collagen fibrils, which correspond to dark and light zones of negatively stained fibrils, respectively.

Numbers of studies have been devoted to the arrangement of collagen molecules in each fibril (e.g., see review of Chapman and Hulmes, 1984). They established that the periodic structure in collagen fibrils arises because the molecules about 300 nm long are assembled in parallel array and are mutually staggering by integral multiples of a D-period. The D periodicity has been estimated by electron microscopy and low-angle X-ray diffraction methods. Low-angle X-ray diffraction of collagen fibrils showed that D is close to 67 nm in wet samples, and around 64 nm in air-dried samples (Bear, 1944; Brodsky and Eikenberry, 1982). By electron microscopy, D varies from 64–70 nm in ultrathin sections, and the variability has been interpreted as the effect of various degree of shrinkage caused by the dehydration and embedment of samples. In contrast, some authors claim that the D-periodicity differs among collagen fibrils in different organs; for example, the D-periodicity of bovine corneal collagen fibrils was reported by X-ray diffraction to be shorter than that of rat tail tendon collagen fibrils (Marchini, et al., 1986).

On the other hand, the presence of subfibrils in collagen fibrils has been reported by previous investigators using TEM of such samples as glycerinated or denatured tissues (Fig. 4a) (e.g., Bouteille and Pease, 1971; Rayns, 1974; Lillie et al., 1977). These studies indicate that right-turning subfibrils are tightly packed in individual collagen fibrils. Ruggieri et al. (1979) also noticed that the subfibrils have a straight or helicoidal arrangement depending on the types of tissue located. We recently investigated collagen fibrils of the cornea and sclera by AFM, and found a difference in D-periodicity between corneal and scleral fibrils in relation to the inclination angle of the subfibrils (Yamamoto et al., 2000a). More precisely, the corneal collagen fibrils (with a D-periodicity of 63 nm) show a helicoidal arrangement of right-turning subfibrils with a 15° spiral angle, while subfibrils in the scleral collagen fibrils (with a periodicity of 67 nm) run almost longitudinally along the fibrillar axis. The relationship between these subfibrils and collagen molecules is still an open question (Chapman and Hulmes, 1984), although several authors consider the subfibrils to be aggregations of small numbers of collagen molecules (Veis, et al., 1967; Smith, 1968; Bouteille and Pease, 1971).

The diameter of collagen fibrils varies from 10–500 nm, depending on the locations of the tissues as well as the age and species of animal (Parry and Craig, 1984). For example, the cornea has collagen fibrils with a regular diameter of about 30 nm, while the diameter of scleral collagen fibrils variously ranges from 25–230 nm (Komai and Ushiki, 1991, Yamamoto et al., 1997). Collagen fibrils in tendons and ligaments show differing diameters with a peak one of 100–200.
Fig. 3. Collagen fibrils observed by TEM (a, b) and AFM (c, d). a. Collagen fibril (in the mouse tail tendon) stained positively with uranyl acetate and lead citrate. The fibril shows periodical striations with an identity period of about 67 nm. Arrowheads indicate d-bands. ×105,000. b. Collagen fibril (in the mouse tail tendon) stained negatively with phosphotungstic acid. The characteristic alternation of dark and light zones is found along the fibrils. ×105,000. c. AFM image of a bovine scleral collagen fibril. The fibril has periodical transvers grooves and ridges. Shallow longitudinal grooves are also found on the surface of collagen fibrils. d. The longitudinal section profile of the scleral collagen fibril between two asterisks in c. The height as well as the width of each portion can be measured from this profile. (Fig. 3a and b are from Mr. S. HAYASHI, Iwate Medical University. Fig. 3c and d are reproduced from YAMAMOTO et al., 2000a with permission).

nm (PARRY et al., 1978). Another example is the diameter of collagen fibrils in the peripheral nerves (USHIKI and IDE, 1990), where fibrils are thicker in the epineurium than in the endoneurium in various mammals (Fig. 4d). What determines the shape and size of collagen fibrils is an interesting question. Some investigators believe that the copolymerization of collagen molecules with other components of the extracellular matrix may influence the diameter of the fibrils formed (see review of CHAPMAN, 1989), while others have stated the importance of the copolymerization of different kinds of collagen molecules in one fibril (LAPIERE et al., 1977; FLEISCHMAJER et al., 1985, also see review of PROCKOP and HULMES, 1994).

Collagen molecule and its assembly

Chemical studies have revealed that the type I, II, and III collagen molecules self-assemble into banded fibrils. The shape of these collagen molecules has been studied previously by TEM using shadowing techniques (e.g., SILVER and BIRK, 1984; see also a book edited by MAYNE and BURGESON, 1987), and recently by AFM (SHATTUCK et al., 1994; LIN et al., 1999; YAMAMOTO et al., 2000b). As for the type I molecules, they are thin and flexible threads about 300 nm in length in contrast with type I procollagen molecules with a globular C-terminal propeptide and fuzzy N-terminal propeptide in either end (Fig. 4b, c). The individual collagen fibrils are generally consid-
Fig. 4a. SEM view of collagen fibrils (of the mouse tail tendon) treated with 8 M urea in a 0.2 M Tris-HCl buffer for 30 min. Longitudinal clefts are found in the individual fibrils, suggesting the dissociation of subfibrils. D-banding is still preserved in the denatured fibrils. ×49,500 (Bar = 100 nm). b. Collagen type I molecules observed by AFM. This molecule is observed as flexible thread 300 nm long. Globular bulges are present at both ends of the molecule. ×192,000 (Bar = 50 nm). c. Procollagen I molecules observed by AFM. Arrowhead indicates a globular C-terminal propeptide. (This sample was kindly provided by Dr. Fumio NAKAMURA, Hokkaido University) ×150,000 (Bar = 50 nm). d. TEM view of a transverse section of connective tissue sheath in the rat sciatic nerve. This section comprises the whole thickness from the epineurium (Ep), through the perineurium (P) to the endoneurium (En). The diameter of the collagen fibrils is 30–100 nm in the epineurium, 30–60 nm in the perineurium and 40–45 nm in the endoneurium. ×16,500 (Bar = 1 μm)

II. RETICULAR FIBERS

Basic structure of reticular fibers in relation to their staining properties

Reticular fibers are fine fibers forming an extensive network in certain organs. By light microscopy, these fibers are not visible in conventional stains such as hematoxylin and eosin, but are stained dark with a silver impregnation method (Fig. 5a) (MALLORY and PARKER, 1927; FOOT, 1928; NAGEOTTE and GUYON, 1930). Thus, reticular fibers are also called argyrophilic fibers. The distribution of reticular fibers is rather restricted: they are usually found mainly in the basement of epithelial tissues, the surface of adipose cells, muscle cells and Schwann cells, outside...
the endothelium of the hepatic sinusoid, and the fibrous reticulum of lymphoid tissues. These fibers have a diameter of less than 2 μm. Although there are several modifications of Bielschowsky's impregnation method (Maresch, 1905), a method reported by Ishii and Ishii (1965) yields specimens with suitable representation showing the fine structure of reticular fibers. In these specimens, reticular fibers are mesh-works of very fine, dark fibrils, and are continuous with thin and reddish collagen fibers (Fig. 5a).

Electron microscopy shows reticular fibers as individual collagen fibrils or a small bundle of collagen fibrils (Fig. 5b). These collagen fibrils have striations with a characteristic D-banding pattern similar to fibrils in collagen fibers, but their diameter is rather thin and uniform, ranging from 20-40 nm. Observations of silver-impregnated sections by TEM and SEM (using backscattered imaging) show that individual collagen fibrils in reticular fibers are densely coated with coarse metal particles, while fine granu-
lar particles are sparsely found on fibrils in collagen fibers (Fig. 5c–e) (SCHWARTZ, 1953; USHIKI, 1992b). This indicates that the size and density of metal-precipitation particles determine the difference in tone between reticular fibers and collagen fibrils light-microscopically.

Reticular fibers are also PAS-positive and have an affinity to cationic stains such as Ruthenium Red (IDE et al., 1989). These findings suggest that the surface of the individual fibrils in reticular fibers is embedded in an abundance of glycoproteins, which produce the stainability of fibers described above.

Chemical and immunohistochemical studies, on the other hand, have revealed that reticular fibers, in contrast to collagen fibers composed of collagen type I, comprise mainly collagen type III (FLEISCHMAIER et al., 1980; MONTES et al., 1980) in association with other types of collagen (e.g., collagen type V), glyco-

Fig. 6. SEM views of reticular fibers in specimens treated with an alkali-water maceration method by OHTANI (1987). In these specimens, cellular elements, elastic fibers and basal laminae are completely removed without any severe damage to the collagen fibrils of reticular fibers. a. Endomysial reticular fibers (R) of the dog lingual muscle. The space for accommodating the skeletal muscle fiber is demarcated by a cylindrical sheath of reticular fibers. Note the thicker collagen bundle (or collagen fibers, C) following a wavy course outside the endomysial sheath. ×1,200. b. Closer view of a part of Figure 6a. The sheath of reticular fibers consists of thin collagen fibrils, which are elaborately interwoven into delicate patterns of lacework. ×4,200. c. Reticular fibers in the deep cortex of the rat mesenteric lymph node. Because the cellular elements such as reticular cells and lymphocytes were removed by the maceration method, the three-dimensional network of reticular fibers can be directly observed by SEM. d. The reticular sheath for blood vessels. ×570. d. Closer view of the sheath of reticular fibers for blood vessels in the same specimen of Figure 6c. This sheath is observed as a lacework of thin collagen fibrils. Arrow indicates that portion where collagen fibrils in this sheath are continuous with thicker bundles of collagen fibrils as a reticular framework of the lymphoid tissue. ×1,400
proteins (Stenman and Vaheri, 1978), and proteoglycans/ glycosaminoglycans (Montes et al., 1980; Nishimura et al., 1996). The difference in collagen type between collagen fibers and reticular fibers might be related to the diameter of the fibrils in the two fibers, although further studies will be needed in this point.

Arrangement of reticular fibers

The arrangement of reticular fibers is important for understanding the functional role of the fibers in tissues and organs, and was first studied mainly by light microscopy (Plenk, 1927; Nageotte and Guyon, 1930). SEM further revealed the three-dimensional architecture of reticular fibers in relation to the surrounding components (e.g., Motta, 1975; Sawada 1981; Ushiki and Ide, 1986). The method introduced by Ohtani (1987) is also useful for visualizing the fibrillar arrangement more directly and precisely by SEM, since it successfully removes cellular elements, elastic fibers, and basal laminae without any severe damage to the collagen fibrils.

These findings show that reticular fibers form a delicate network of fine fibrils which underlie the basal lamina of such cells as epithelial, muscle and Schwann cells (Ohtani, 1988, Ohtani et al., 1988, 1991, Ushiki and Ide, 1990, Murakumo et al., 1993). The firm attachment of the individual fibrils with the basal lamina indicates that the collagen fibril meshwork and the basal lamina, as a whole, form a
Fig. 8. SEM images of elastic fibers (a, b) and elastin components (c, d). a, Elastic fibers (E) in the mouse aortic adventitia. Elastic fibers are observed as coiled or straight cords entangled in the net of microfibrils. C collagen fiber. ×9,100. b, Closer view of an elastic fiber (in the mouse subcutaneous tissue). The elastic fiber is covered densely with microfibrils which run in various directions. ×55,000. c, Elastin components of the rat aortic adventitia treated with the formic acid-digestion method (USHIKI, 1992a). This method removes cellular components, collagen components and microfibrils from tissues, while leaving elastin components unchanged at their original locations. Elastin fibers in this micrograph are observed as cords running in various directions. Note a connection between two crossed fibers (arrow). ×5,500. d, Internal elastic lamina of the rat aorta treated with the formic acid-digestion method. The lamina appears as a solid sheet with two large fenestrations. The surface of the lamina is somewhat fibrous. Fibrous structures are also found in the fenestrations where the fibrils extending the lamina to form a meshwork like a wire fence. ×3,000
III. ELASTIC FIBERS

Basic structure of elastic fibers

Elastic fibers are generally twisted or straight strands stained by a resorcin-fuchsin or aldehyde-fuchsin method (Fig. 7a); these fibers are about 0.2-1.5 μm and sometimes branch to form a coarse network in loose connective tissues. In dense elastic tissues such as the aorta, elastic fibers fuse to form flattened sheets, or elastic laminae. Biochemically, elastic fibers are highly resistant to boiling water, in contrast with collagen fibrils which are easily gelatinized in hot water (Richards and Gies, 1902).

By TEM of ultrathin sections stained with uranyl acetate and lead citrate, elastic fibers are seen to
consist of amorphous and fibrous components (Fig. 7b) (Greenlee et al., 1966; Ross and Bornstein, 1969). Amorphous components are densely stained with tannic acid treatment by TEM (Fig. 7c) (Mizuhira and Futae Saku, 1972) and are composed of substances which can be purified in boiling water and are recognized biochemically as the protein named elastin (e.g., see a review of Ross, 1973). Elastin endows elastic fibers with the characteristic property of elastic recoil. Fibrous components, on the other hand, correspond to the microfibrils which were recognized by TEM in various tissues and organs by Low (1962). Microfibrils are 10 nm in diameter and composed of various glycoproteins, including fibrillin (Sakai et al., 1986) and the amyloid P component (Inoue and Leblond, 1986; Inoue et al., 1986).

By conventional SEM, elastic fibers are observed as cobwebbed cords entangled with microfibrils (Figs. 8a, b, 11b) (Ushiki, 1992b).

**Arrangement of elastin fibers**

Since elastic fibers are intermingled with collagen fibrils and cellular elements in tissues, it is usually difficult to demonstrate their arrangement both extensively and three-dimensionally. For this reason, previous SEM investigators have attempted to extract elastic fibers by autoclaving tissues (Grun et al., 1977), or by utilizing treatments with chemical agents and enzymes: e.g., guaninim chloride, collagenase, sodium hydroxide, and formic acid (Kuhn, 1974; Kewley et al., 1977; Wasano and Yamamoto, 1983; Song and Roach, 1985; Crissman, 1987). These methods selectively remove non-elastin components including microfibrils, collagen fibrils and cellular elements, and are effective for observing the architecture of elastin components in tissues by SEM (Figs. 8c, d, 9c, d). On the other hand, the treatment of tissues with a KOH method is effective for observing the special relationship between elastin components and cellular elements by SEM, since this method removes collagen fibrils and basal laminae while leaving cellular and elastin elements unchanged at their original shapes and locations (Fig. 9a, b) (Ushiki and Murakumo, 1991).

Through these studies, several investigators have demonstrated that elastin components form a continuous network or sheet with a smooth surface (Kuhn, 1974; Wasano and Yamamoto, 1983), while others have considered them as a fibrous network or sheet composed of fibrils about 0.1–0.2 μm (Kewley et al., 1977; Hart et al., 1978). Our previous studies revealed that the surface structure and organization of elastin components are changeable depending on the procedures after extraction, and yielded evidence that the elastin component including aortic laminae are fibrous when extracted tissues are adequately treated (Ushiki and Murakumo, 1991; Ushiki, 1992a).

Elastin components show morphological features specific to individual tissues and organs (Ushiki and Murakumo, 1991). A typical organization of elastic fibers in the loose connective tissue is a loose network of elastic fibers about 0.2–1.5 μm thick (Fig. 8c). An elastin sheet lining the serosal covering of the mesothelium consists of fine fibers ranging from 0.1–1.0 μm thick, which run in various directions two-dimensionally and are elaborately interwoven, form-
Fig. 11a. Light micrograph of the mouse subcutaneous tissue stained with aldehyde-fuchs in after oxidation with peracetic acid. Oxytalan fibers run in various direction like a cobweb throughout the tissue. Arrowheads indicate elastin fibers. ×50. b. SEM view of a part of the rat aortic adventitia. Microfibrils leave the elastic fiber (E) to form a bundle of microfibrils (O), which corresponds light-microscopically to the oxytalan fiber. C collagen fiber. ×11,000

Fig. 12. SEM of a medial elastin laminae in the developing mouse aorta (at embryonic day 18.5) after KOH treatment. a. The elastic lamina is observed as a thin and fibrous sheet with numerous fenestrations. ×1,800. b. Closer view of the elastic lamina. The elastic lamina is composed of fine elastin fibrils, which run two-dimensionally in various directions to form a thin fibrous sheet. ×11,000
Fig. 13. Schematic representation of collagen fibrillar system and microfibril-elastin system. The collagen fibrillar system (green) composed of collagen fibrils which form thick bundles (i.e., collagen fibers, C) in the connective tissue and are arranged in a lace-like sheets or sheath (i.e., reticular fibers, R) attached to the basal laminae (yellow) of such cells as epithelial, endothelial or muscular ones. Thus, the collagen fibrillar system acts as a supporting framework of tissues and organs. The microfibril-elastin system is composed of microfibrils (fine solid lines) and elastin fibrils (violet), which usher in different proportions of the two components, to produce elastic (E), elaunin and oxytalan fibers (O). These three fibers of the microfibril-elastin system are continuous with one another, and form a three-dimensional network in tissues for maintaining the resilience adapted to local tissue requirements. Ep epithelium, V blood capillary, L initial lymphatic, F fibroblast

ing a delicate lacework-pattern (Fig. 9a, b). Elastic laminae in the aorta appear as a solid sheet about 2 \( \mu m \) thick with numerous oval fenestrations of varying diameters from 1–10 \( \mu m \) (Figs. 8d, 9d). These laminae appear to be composed of fibrous structures about 0.1–0.2 \( \mu m \) thick. It is therefore evident that extracted elastin components are basically composed of thinner fibrils about 0.1–0.2 \( \mu m \) thick (Fig. 9b), even though some investigators further recognized very thin (3–4 nm thick) elastin filaments by TEM of negative-stained or freeze-etched specimens (Gotte et al., 1974; Forneri et al., 1982). The elastin fibrils are present individually or in bundles, and so form elastin fibrils, fibers and/or laminae in individual tissues (Fig. 10) (Ushiki and Murakumo, 1991).

The organization of elastin fibers and laminae apparently influences the resilience of tissues suitable for their mechanical properties. Concentric elastic laminae with connecting interlaminar fibers are suitable for distributing blood pressure uniformly and effectively to the vascular wall. The elastic sheet lining the mesothelium is believed to give elasticity to the serous membrane and protect the mesothelium against any distention and contraction of such organs as the lung and urinary bladder.

**Microfibril-elastin network system**

Microfibrils are usually present in and around elastin fibers, where they appear to be arranged in random directions to the elastin fibers (Fig. 8b) (Ushiki, 1992b). In stretched fibers, the microfibrils change in their direction along the fibers, in response to stretch-
ing of the elastin fibers. Microfibrils often leave elastin fibers to form a bundle or cobwebby meshwork in various tissues (Figs. 8a, 11b).

Light-microscopically, characterized fibrous structures are observed when sections are treated with peracetic acid before aldehyde-fuchsin staining (Fig. 11a) (FULLMER and LILLIE, 1958). These fibrous structures are continuous with elastic fibers and are called oxytalan fibers. By TEM, the oxytalan fibers are observed as a bundle of microfibrils (Fig. 11b) (COTTA-PEREIRA et al., 1976). The oxytalan fibers can be also found in the zonule fibers of the eye and in the dermis where it connects the elastic fibers to the basal lamina. As far lymphatic vessels, oxytalan fibers act as anchoring fibers which connect the elastic fibers and lymphatic endothelium, thus preventing the collapse of initial lymphatics in tissues (GERLITZ et al., 1990). In addition, so-called elaunin fibers (GAWLICK, 1965) have intermediate characteristics between oxytalan fibers and elastic fibers by TEM (COTTA-PEREIRA et al., 1976). These findings indicate that microfibrils and elastin fibrils produce oxytalan fibers, elaunin fibers, and elastic fibers to form, as a whole, the microfibril-elastic fiber system which plays a role in maintaining the resilience adapted to local tissue requirements.

Elastogenesis

Previous TEM studies have demonstrated that bundles of microfibrils first appear during elastogenesis (ALBERT, 1972; SPICER et al., 1975). Elastin components are produced as the deposition of a small amount about 0.1 \( \mu m \) wide within the bundle. As the elastin components increase in number, they fuse together to become mature elastic fibers. According to our SEM studies on the extracted elastin components in the developing aorta, elastic laminae are first observed as a meshwork of fine elastin fibrils which increases in its density of elastin fibrils in the meshwork to become an elastic lamina with numerous fenestrations (Fig. 12a, b). These findings support the idea that microfibrils produce a fundamental framework of the microfibril-elastic system, which is added by the deposition of elastin fibrils about 0.1 \( \mu m \) thick, thus forming elastic fibers and laminae continuous with oxytalan fibers. Eiaunin fibers are considered a transition form between oxytalan fibers and elastic fibers.

IV. CONCLUSIONS

The present review describes the features of three major fibrous components: collagen, reticular and elastic fibers. For a comprehensive understanding of the fibrous components in connective tissues, we propose categorizing them into two systems (Fig. 13): the collagen fibrillar system and microfibril-elastic system. The collagen fibrillar system acts as a supporting framework of tissues and cells, where reticular fibers connect collagen fibers with the basal laminae of such cells as epithelial, muscle, adipose and Schwann cells. The microfibril-elastic system is composed of microfibrils and elastin fibrils, which use different proportions of the two components to produce elastic fibers, elaunin fibers and oxytalan fibers. The microfibril-elastic system thus plays a role in distributing stress forces uniformly in tissues.

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