Collagen Fibrils in the Peripheral Nerves

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Abstract: Peripheral nerves have three layers of connective tissue sheaths, i.e., endoneurium, perineurium and epineurium. Each of them has some proper cells and contains different types of collagen fibrils. Endoneurium consists of thin collagen fibrils whose diameters are uniform. These thin collagen fibrils are made of collagen type III which are produced by Schwann cells. The quantity of the endoneurial collagen fibrils depends on the sorts of peripheral nerves. Generally there is some space without collagen fibrils in the endoneurium, but the endoneurial space of facial nerve is filled up with abundant thin collagen fibrils. It is thought that these collagen fibrils protect the nerve fibers against external forces. On the other hand, the optic nerve has a central nervous system-like morphology and no collagen fibrils are seen between axons. At the beginning of the development, thin collagen fibrils appear not only in the endoneural space but also in peri- and epineurial spaces. During development endoneurial collagen fibrils do not change their diameter, but peri- and epineurial collagen fibrils increase in their diameter. Finally, epineurium consists of the typical thick collagen fibrils which are the same as the collagen fibrils in the skin. Epineurial collagen fibrils are mainly made of type I collagen. The perineurium consists of both thin and thick collagen fibrils, displaying a wide spectrum of the fibril diameter. It is thought that they contain both type I and III collagens. They show characteristics intermediate between endo- and epineurium.

Key words: endoneurium, perineurium, epineurium, development, electron microscopy

INTRODUCTION

The axons in the peripheral nerves are surrounded by three layers of connective tissue sheaths, i.e., endoneurium, perineurium and epineurium. It is evident by transmission electron microscopy (TEM) that each of these sheaths contains different sizes of collagen fibrils. The endoneurium consists of bundles of thin collagen fibrils which run in parallel with the axons. A small number of fibroblasts are present in the endoneurium. The perineurium consists of several layers of flat perineurial cells and some collagen fibrils in the narrow spaces between perineurial cells. The epineurium consists of thick collagen fibrils in the same fashion as in the other organs, such as dermis. These three kinds of collagen fibrils which cover the nerves have distinctive typical morphologies, and have different constitutions. In this review we concisely summarize the electron microscopic observations of the three kinds of collagen fibrils in the peripheral nerves, comparing the different kinds of peripheral nerves, paying attention to the changes during development, and making clear the differences among them (Fig. 1a, b).

Collagen Fibrils in Endoneurium

The peripheral nerves consist of many axons running in parallel. These axons do not contact each other, but there are some interspace among them, i.e., the endoneurial space (Fig. 1a, b). The major part of this space is occupied by abundant endoneurial collagen fibrils.

Being different from the other connective tissues, there are only a small number of fibroblasts in the endoneurium, therefore it is thought that Schwann cells produce endoneurial collagen fibrils.

It is known the collagen fibrils in the endoneurium have uniform and small diameter, Thomas (1963) mentioned that the endoneurial collagen fibrils were of smaller diameter (500-600 Å) than those of the epineurium (700-850 Å), using rabbit as material. Gamble (1964) reported 250-650 Å as the diameter of rat endoneurial collagen fibrils, which was smaller than the 250-1000 Å diameter of epineurial collagen fibrils, and Gamble and Eames (1964) reported a 300-650 Å diameter of human endoneurial collagen fibrils, which was also smaller than the 600-1100 Å diameter of epineurial collagen fibrils. Osaka and Ide (1986) and Osaka and Nozaka (1993) measured the thickness of the endoneurial collagen fibrils of mouse sciatic nerve during development. According to their studies, endoneurial collagen fibrils were thin and had a narrow spectrum in thickness (200-450 Å) with most of the endoneurial collagen fibrils in the range of 250-300 Å in...
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Fig. 1a. Schematic diagram showing the basic constitution of the peripheral nerve.
Nerve fibers are surrounded by three connective tissue sheathes, i.e., endoneurium (EN), perineurium (P) and epineurium (EP). Both Schwann cells (S) and the perineurial cells have basement membranes (BM). A, axons, M, myelin sheaths.

Fig. 1b. Transmission electron micrograph showing the endo- (EN), peri- (P) and epineurium (EP) of an adult Wistar rat mental nerve.
The perineurium consists of several layers of flattened cells. They have thick lamina densa on both surfaces, except the outermost cell, which lacks basement membrane on the outer surface (arrow heads). Lamina densa often fuse and fill up the interspace between perineurial cells (★). The difference in the thickness of collagen fibrils in the endoneurium and epineurium is clear. In the wide outermost layer of the perineurial space, both the thin and the thick collagen fibrils are seen, being mixed. The inner layers of the perineurial space contain only thin collagen fibrils which are densely packed (arrows). bar=1 μm.×20,000. (Osawa T. and Nozaka Y. Connective Tissue 24 (1993), with the permission of the publisher)

diameter. These collagen fibrils do not change their diameter during development. At the beginning of the development thin collagen fibrils appear not only in the endoneurial space, but also in the peri- and epineurial spaces. The collagen fibrils in peri- and epineurium increase their diameters during development, displaying a wide spectrum of diameters. However, the collagen fibrils in the endoneurium remain thin, maintaining the uniformity of the diameter even in the adult animals (Fig. 2, 3)(9,10).

Endoneurial collagen fibrils tend to gather to make bundles. However, neighboring collagen fibrils do not contact each other. There are certain interfibrillar spaces between collagen fibrils, and there are some thin or amorphous materials between collagen fibrils. After ruthenium red staining, the fibrous or amorphous substances in the space between endoneurial collagen fibrils showed the positive reactions. Ruthenium red-positive substances are also seen at the banding of the collagen fibrils which are longitudinally cut and on the basement membrane of Schwann cells (Fig. 4). This fact indicates that proteoglycans or glycoproteins are on the endoneurial collagen fibrils, especially at the bandings, and these substances connect collagen fibrils to form bundles.

It is known there are smaller collagen fibrils on the Schwann cell basement membrane. They do not make large bundles, but run irregularly. Thomas (1963)(1) already pointed out that the collagen fibrils aggregating around the nerve fibers were separable into two layers, i.e., the outer layer consisting of fibrils running mainly longitudinally and densely packed, and the fibrils of the inner layer having a slightly lesser diameter and not oriented in any uniform direction (Fig. 5). Friede and Bischhausen (1978)(11) observed the endoneurial collagen fibrils of rat sciatic nerve by scanning electron microscopy (SEM), and showed the longitudinal aggregates of collagen fibrils. Later, Ushiki and Ide (1986)(12) demonstrated the three-dimensional architecture of this inner layer of thin collagen fibrils by SEM, using mouse sciatic nerve as material. According to their study, endoneurial collagen fibrils of the outer layer run in a longitudinal direction along the axis of nerve fibers, forming bundles approximately 2–3 μm in width. Beneath these collagen bundles, the network of fine collagen fibrils lining the contour of nerve fibers in a continuous sheath are revealed by SEM. These fine fibrils are oriented in every direction, forming a delicate meshwork. It is thought that these observations by SEM coincide with those by TEM.

Usually the endoneurial space is almost filled up with the thin collagen fibrils, but some space has no collagen fibrils (Fig. 1a, b). However, the quantity of the collagen fibrils in the endoneurium depends on the type of nerve. For instance, the endoneurial space of facial nerve is filled up with abundant collagen fibrils, and it is hard to find any space without collagen fibrils (Fig. 6). It is thought that these collagen fibrils protect the nerve fibers against external forces.

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thickness of collagen fibrils in the endo- and epineurium (ddY mouse, sciatic nerve) during development.

In mice of 15 days of gestation, there is no difference in the diameter of collagen fibrils in the endo- and epineurium. Seven days after birth, epineurial collagen fibrils tend to become thicker in diameter. There is a clear difference in the thickness of collagen fibrils between the endo- and epineurium starting 2 weeks after birth. (Osawa T. and Ide C. Acta anat. 125 (1986), with the permission of the publisher)

On the other hand, optic nerve has almost no endoneurial space or endoneurial collagen fibrils. It has the central nervous system-like morphology (Fig. 7). It is known that in the central nervous system, there is only a small quantity of extracellular matrix which is stained with ruthenium red, and there are no collagen fibrils between myelinated nerve fibers.

It is ascertained that these thin endoneurial collagen fibrils do not consist of type I collagen, but they consist of type III collagen by the Picrosirius-polarizing method, and by immunohistochemistry.

It is known that reticular fibers react with anti-type III collagen antibodies, and are surrounded by a large quantity of ruthenium red-positive materials, which is digested by papain and creates the interfibrillar space. It can be said that endoneurial collagen fibrils resemble reticular fibers not only in their morphology, but also in their constitution.

There are many studies of the cross-links in collagen. It is thought that during development the cross-links in collagen increase and make collagen insoluble and stable. It is also supposed the cross-linking is related to the growth of fibrils. The endoneurial collagen fibrils remain in their immature form even in adult animals, therefore they may have a different form of cross-links from the epineurial collagen fibrils.

It is known that collagen fibrils similar to the endoneurial collagen fibrils are seen in the endoten- dinium. At the beginning of the development, the tendon fascicles and endoteninum both contain almost the same quantities of type I and III collagen and have the collagen fibrils with small diameters. During development the collagen fibrils in the tendon fascicles lose type III collagen and come to have mainly type I collagen, and they increase in diameter. On the other hand, in the collagen fibrils in endoteninum the ratio of type III/type I collagen increases and endoteninial collagen fibrils remain thin during development. Thus, it can be said that the endoneurial and the endoteninial collagen fibrils have similar characteristics.

**Collagen Fibrils in Perineurium**

The perineurium consists of several layers of flattened perineurial cells and collagen fibrils in the space between perineurial cells. It is thought that perineurial cells have the same origin as Schwann cells. Osawa and Ide (1986) reported that in embryonic mice of 12 days of gestation, the sciatic nerve contained densely packed fibrils...
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Fig. 4. A mental nerve of adult Wistar rat stained with ruthenium red.
Globular substances (arrow heads) are seen on the banding of the collagen fibrils which are longitudinally cut, and also on the Schwann cell basement membrane (BM). M, myelin sheath; EN, endoneurial collagen fibrils. bar=0.5 μm. × 37,000. inset: high magnified view of endoneurial collagen fibrils and Ruthenium Red-positive substance. The fibrous substances which connect endoneurial collagen fibrils are stained (arrows). × 96,000.

The perineurium consists of several layers of flattened perineurial cells, forming several layers of perineurial spaces. Sometimes the outermost perineurial space is more extensive than the other inner spaces and contains many collagen fibrils. The collagen fibrils in the outermost perineurial space increase in diameter during development, displaying a wide spectrum of diameters. The thinner fibrils are as thin as the endoneurial collagen fibrils, and the thicker fibrils are as thick as the epineurial collagen fibrils, having characteristics intermediate between endo- and epineurial collagen fibrils (Fig. 9)10).

It is possible to remove the cellular elements by the NaOH cell-digestion method. With this method all the cellular elements of peripheral nerves can be digested, leaving the mass of collagen fibrils. Using the specimens prepared as above, the three-dimensional observation of the perineurial collagen fibrils by SEM was performed by Ushiki and Ide (1990)25). According to their study, the perineurial spaces filled with collagen fibrils and the those occupied by the cellular elements were clearly distinguished by SEM. They revealed the fact that the perineurial collagen fibrils were interwoven to form a characteristic lacework structure.

By TEM it is evident that the inner layers of perineurial collagen fibrils form tightly condensed bundles in some places. The basement membranes of the adjacent

axons, which were surrounded by developing Schwann cells. In embryonic mice of the later developing stages, the sorting out of the axons by developing Schwann cells proceeded, forming a 1:1 relationship between axons and Schwann cells. The rest of the cells, which surrounded ed the axon bundles (but did not form the tight contacts with the axons), differentiated into perineurial cells. Considering the fact that the Schwann cells and the perineurial cells have the same origins, it is likely that the perineurial cells have basement membranes and produce thin collagen fibrils just as Schwann cells do. In fact, perineurial cells have basement membranes on both of their sides1−9,10). The outermost and the innermost layers of the perineurium also consist of flattened cells; however, they have no basement membranes and they are thought to be fibroblastic cells11).

Collagen fibrils in the perineurium are as thin as those in the endoneurium at the beginning of the development. The collagen fibrils in the inner spaces of the perineurium do not change their diameter during development. They are densely gathered and lose their interfibrous space. The lamina densa of the adjacent perineurial cells often fuse and fill up the perineurial space (Fig. 8)10).
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Fig. 6. A facial nerve of adult Wistar rat.
The endoneurial space is filled up with numerous endoneurial collagen fibrils (EN). The space between the perineurial cells (P) and the epineurial space (EP) is also filled up with collagen fibrils. a, axons; M, myelin sheaths. bar=1 μm. X 11,000.

perineurial cells fuse in some places and the amorphous materials of lamina densa fill up the interspaces between perineurial cells. The bundles of perineurial collagen fibrils are separated by the perineurial cells and the fused basement membranes, forming a lacework (Fig. 1a, b, 6). It is thought that the lacework of the perineurial collagen fibrils observed by SEM after the digestion of cell elements coincides with the TEM observation.

Collagen Fibrils in Epineurium

The collagen fibrils in epineurium increase in their diameter during development, finally showing as thick a diameter as the collagen fibrils, which are found in the other tissues, such as dermis, etc. (Fig. 1a, b, 9). Luque et al. (1983)\(^1\) reported that the diameter of the epineurial collagen fibrils were 1.5~1.8 times larger than those of endoneurial collagen fibrils in the various kinds of nerves of the domestic fowl. Junqueira et al. (1979b)\(^1\) reported the epi-/endo-ratios of 1.31~1.88 in fish, amphibian, reptiles and mammals, and those of 1.74~2.05 in the various nerves of rat. Gamble and Eames (1964)\(^2\) reported a 600~1100 Å diameter of the epineurial collagen fibrils in the human nerves; Gamble (1964)\(^3\)

Fig. 7. An optic nerve of adult Wistar rat.
There is almost no space between myelinated axons. No collagen fibrils are seen. a, axons; M, myelin sheaths. bar=1 μm. X 25,000.

Fig. 8. A mental nerve of adult Wistar rat.
Electron micrographs showing the endoneurial collagen fibrils (a) and collagen fibrils in the inner layer of the perineurium (b). The thickness is almost the same, but in (b) the space between collagen fibrils is reduced, forming tight bundles. bar=0.1 μm. X 80,000. (Osawa T. and Nozaka Y. Connective Tissue 24 (1993), with the permission of the publisher)
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Fig. 9. A mental nerve of adult Wistar rat.

a. Endoneurial collagen fibrils. b. Collagen fibrils in the outermost layer of the perineurium. c. Epineurial collagen fibrils. The differences among the collagen fibrils in the three connective sheaths are clear. Collagen fibrils in the endoneurium are thin and uniform in diameter. Almost all the epineurial collagen fibrils are thick. Perineurial collagen fibrils seem to be formed by the mixture of endo- and epineurial collagen fibrils. Both the thin and thick collagen fibrils are seen, showing wide spectrum in diameter. bar = 0.1 μm × 80,000. (Osawa T. and Nozaka Y. Connective Tissue 24 (1993), with the permission of the publisher)

reported a 250-1000 Å diameter of the rat epineurial collagen fibrils; and Thomas (1963)1 reported a 700-850 Å diameter of the epineurial collagen fibrils. All of these studies reported much larger diameters for the epineurial collagen fibrils than those for the peri- and endoneurial collagen fibrils.

Osawa and Ide (1986)9 reported the comparatively uniform diameter of the epineurial collagen fibrils of ddY mouse sciatic nerve in 15 days of gestation (150-400 Å), and a wide spectrum (200-650 Å) of diameter in adult (5 months old) ddY mouse (Fig. 2). It is evident epineurial collagen fibrils display not only a thick diameter, but also a wide spectrum of diameter in the adult animals. Collagen fibrils in epineurium are as thin as those in the endo- and perineurium, and show the narrow spectrum in diameter at the beginning of the development. The wide spectrum of the diameter is attained during development.

Junqueira et al. (1979b)10 and Luque et al. (1983)11 demonstrated that epineurium consisted of type I collagen by the Picrosirius-polarization method. Shellswell et al. (1979)12 demonstrated the type I collagen in the epineurium immunohistochemically. It is assumed that epineurial collagen fibrils are common collagen fibrils such as dermal collagen fibrils, not only in their morphology but also in their constitution.

CONCLUSIONS

In the present review, we clearly demonstrated the morphological and constitutional differences among the collagen fibrils in the endo-, peri- and epineurium. Among them, epineurial collagen fibrils seem common collagen fibrils which are seen in many kinds of tissue. They are thick and they consist of mainly type I collagen. On the other hand, the endoneurial collagen fibrils are distinct from the other collagen fibrils. They appear in the endoneurial space with a small diameter in the early stages of the development, and their diameter does not change during development. Even in the adult animals, the bundles of endoneurial collagen fibrils are seen with as small a diameter as when they appear in the endoneurial space at the beginning. They consist of type III collagen and are similar to reticular fibers. Perineurial collagen fibrils display the intermediate characteristics between endo- and epineurial collagen fibrils. Both the thick and the thin collagen fibrils are seen especially in the outermost perineurial space (Fig. 9). Immunohistochemical study indicated they consist of both type I and type III collagen.

Thus the peripheral nervous system has three kinds of connective sheathes of collagen fibrils, and it is thought that these collagen fibril arrangements serve to protect the nerve fibers against external forces.
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