The Filamentous Meshwork in the Schwann Cell Basement Membrane as Revealed by Transmission and Scanning Electron Microscopy

Tatsuo Ushiki¹, Shuichiro Hayashi² and Chizuka Ide³

Department of Anatomy¹, Hokkaido University School of Medicine, Sapporo; Laboratory for Electron Microscopy², Iwate Medical University School of Medicine, Morioka; and Department of Anatomy³, Kobe University School of Medicine, Kobe, Japan

Received July 23, 1990

Summary. The three-dimensional architecture of filamentous components in the lamina densa of the Schwann cell basement membrane was studied in the mouse sciatic nerve by transmission and scanning electron microscopy after osmium-maceration treatment, and also by conventional electron microscopy. In conventionally prepared specimens, the lamina densa of the basement membrane was the most electron-dense, showing up as a felt-like layer 20-30 nm thickness. The interstitial surface of this layer had a spongy appearance with numerous shallow pits. Maceration of the specimens with 0.1% OsO₄ for 2-4 days effectively removed amorphous, non-filamentous components from the basement membrane, thus exposing fine filamentous structures embedded in the lamina densa; these were about 10-15 nm in diameter and elaborately interwoven and/or connected with each other to form the framework of the lamina densa. Occasionally, some of them appeared to twine around adjoining collagen fibrils. The nature of these filamentous structures is discussed in terms of the chemical components of the basement membrane.

Basement membranes are specialized extracellular matrices underlying the interstitial surface of various cell types such as epithelial, muscular, and nervous (i.e., glial and Schwann) cells. Conventional transmission electron microscopy (TEM) allows them to be generally subdivided into three layers: the lamina lucida, the lamina densa and the reticular lamina (see review by Kefalides et al., 1979; Reale, 1984; Loblond and Inoue, 1989). The lamina lucida is an electron-translucent layer adjacent to the cell membrane, and the lamina densa is the most electron-dense layer composed of felt-like materials. The reticular lamina is a very loose, fibrous layer consisting of anchoring fibrils, microfibrils and occasional thin collagen fibrils; this layer is continuous with neighboring connective tissue matrices.

Biochemical studies, on the other hand, have revealed basement membranes as containing various substances such as type IV collagen, laminin and heparan sulfate proteoglycan (see review by Timpl and Dziadek, 1986; Loblond and Inoue, 1989). Immunohistochemical studies by TEM have also disclosed the presence and localization of these chemical substances in basement membranes (Laure et al., 1982; Tohyama and Ide, 1984). Based on the findings of these studies, several investigators have proposed models for the molecular organization of basement membranes, especially of the sublayer of lamina densa (Timpl et al., 1981; Bailey et al., 1984; Yurchenko and Furthmayr, 1984; Loblond and Inoue, 1989). However, little is known about the three-dimensional ultrastructure of basement membranes because its morphological analysis is limited and insufficient in conventional TEM observations.

The present study was, therefore, designed to elucidate the three-dimensional ultrastructure of basement membranes by high resolution scanning electron microscopy (SEM) in combination with TEM. We applied the osmium-maceration method to demonstrate the Schwann cell basement membrane of murine sciatic nerve. This method was originally devised by Tanaka and his co-workers (Tanaka and Noguro, 1981; Tanaka and Mitsushima, 1984) to observe cell organelles by SEM. However, we have recently noticed that osmium-maceration selectively digests certain components of basement membrane matrices, thus enabling the analysis of the ultrastructure of basement membranes. Special attention
was focused on the filamentous meshwork of the basal lamina which was first visualized by this maceration method.

**MATERIALS AND METHODS**

Adult mice (ddY strain) of either sex were used in the present study. They were anesthetized by intraperitoneal injection of Nembutal (pentobarbital sodium, 50 mg/kg body weight) and perfused through the heart with either 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) or a fixative containing 0.5% glutaraldehyde and 4% paraformaldehyde in the buffer solution. After perfusion, the sciatic nerve was removed and cut into small segments. These were then stripped of their epineurium and perineurium, and teased longitudinally with forceps into thin bundles, under a dissecting microscope (USHIKI and IDE, 1986). These bundles were rinsed in the buffer solution for several minutes and fixed with 1% osmium tetroxide in 1/15M cacodylate buffer solution (pH 7.4) for 1-2 h. The specimens were then divided into two groups and processed as follows:

1) One group (for conventional preparation) was directly conductive-stained by MURAKAMI's tannin-osmium method (1973): this method entails treating the specimens with 2% tannic acid aqueous solution for 1 h, rinsing in distilled water for several hours and immersing them in 1% osmium aqueous solution for 1 h.

2) The other group (for osmium-maceration) was immersed in 0.1% osmium tetroxide in 1/15 M cacodylate buffer solution at 20°C for 3-5 days and then conductive-stained as above.

Specimens from each group were dehydrated through a graded alcohol series, transferred to isoamyl acetate and critical point-dried using liquid CO₂. The dried specimens were then affixed onto aluminum stubs with double-sided tape and coated thinly (5-10 nm) with platinum in an ion coater (Eiko IB-5, Eiko Engineering Co., Japan). They were then observed in a high resolution scanning electron microscope (S-800, Hitachi) with an accelerating voltage of 10 kV. Some specimens of both groups at the 100% ethanol step were embedded in Epon for TEM. Thin sections were stained with uranyl acetate and lead citrate and observed in a transmission electron microscope (H-600 or H-700, Hitachi).

**RESULTS**

**Observations of conventionally prepared specimens**

In a TEM survey of thin sections, the basement membrane of the Schwann cell was a continuous sheet with a thickness of about 50-70 nm (Fig. 1). This membrane was subdivided into the lamina lucida and densa, though the boundary between the two was not clearly defined. The reticular lamina as present in the epidermal basement membrane was less developed and almost lacking in the Schwann cell basement membrane. At high magnification, the lamina densa was observed as an electron dense, felt-like zone of 20-30 nm thickness, possessing fine fuzzy materials on both the outer and inner sides. Similar fuzzy materials were also found on the collagen fibrils of the endoneurium. Some of the fibrils seemed to be attached to the lamina densa with these fuzzy materials.

By SEM, the interstitial surface of the lamina densa of Schwann cells could be easily observed on myelinated nerve fibers of the teased nerve (Figs. 2, 3). This surface had a spongy appearance with small shallow pits of various sizes up to about 20 nm. Fine filamentous materials (10-20 nm in diameter) were also seen embedded in the spongy substances of the lamina densa. These filaments appeared to give off some branches, though they were not clearly out-

---

**Fig. 1.** Transmission electron micrograph of the conventionally prepared nerve. The Schwann cell basement membrane is found on the surface of a myelinated nerve fiber. It is subdivided into the lamina lucida (L) and lamina densa (D), though their boundary is obscure. Note the fuzzy materials surrounding individual collagen fibrils. ×125,000

**Fig. 2.** Scanning electron micrograph of myelinated nerve fibers of the teased nerve prepared by routine techniques. The interstitial aspect of the Schwann cell basement membrane (Bm) is exposed beneath the collagen fibril meshwork (M). ×6,000

**Fig. 3.** High magnification view of the interstitial aspect of the Schwann cell basement membrane on the myelinated fiber. The basement membrane is seen as a spongy sheet with various-sized shallow pits on the surface. Fine filamentous materials (arrowheads) appear embedded in the basement membrane. Some filaments appear to cross over collagen fibrils (arrows). ×100,000
Figs. 1-3. Legends on the opposite page.
Figs. 4 and 5. Legends on the opposite page.
lined. In places, endoneurial collagen fibrils measuring 30-40 nm in diameter were attached to the surface of the lamina densa. These collagen fibrils were often anchored to the lamina densa by the above-mentioned filaments overarching the fibrils.

**Observations of the specimens after osmium-maceration**

Observations by TEM showed that maceration of the fixed specimens with 0.1% OsO₄ for about three days preserved well the collagen fibrils and membranous structures of the cells (Figs. 4, 5a). This method, however, extracted fuzzy materials present on individual collagen fibrils (Figs. 4, 5a). Fuzzy materials in each layer of the basement membrane were also largely extracted, leaving only non-extractible components of the lamina densa; accordingly, the lamina densa was found as a less electron-dense, partially discontinuous layer, and the electron density of the lamina lucida was markedly reduced. Oblique sections revealed that the remaining lamina densa consisted of a meshwork of fine filamentous components (Fig. 5b). These filaments measured 3-5 nm in thickness, and the holes in the meshwork varied in width from about 4 to 20 nm.

The meshwork structure of the lamina densa was most clearly visualized by SEM in the osmium-macerated specimens (Fig. 6). This meshwork consisted of fine filamentous components ranging 10-15 nm in diameter. Although some of filaments appeared to bifurcate repeatedly, others ran rather longer (0.5-0.7 µm) without bifurcation. They were oriented in various (circular, longitudinal and oblique) directions, and then were elaborately interwoven or connected with each other forming a complex irregular meshwork. Collagen fibrils lying on the lamina densa were often crossed over by these filamentous components, thus being anchored to the lamina densa.

**DISCUSSION**

The present study has combined SEM and TEM to demonstrate in detail the three-dimensional ultrastructure of the Schwann cell basement membrane in the mouse sciatic nerve. Tanaka and his colleagues first showed that maceration of tissues with diluted osmium solution consistently extracts cytoplasmatic matrices, microtubules and microfilaments with no apparent damage to membranous structures of cells (Tanaka and Naguro, 1981; Tanaka and Mitsu-shima, 1983). Since then, this method has been proven to be useful for SEM demonstration of intercellular organelles of various tissues (Ogata and Yamasaki, 1985; Tanaka et al., 1986; Yoshikane et al., 1986; Lea and Hollenberg, 1989). The present study has revealed that osmium-maceration also causes the extraction of certain components of the basement membrane, thus exposing the filamentous meshwork of the lamina densa.

At present, we cannot exclude the possibility that the meshwork structure demonstrated might be one of artifacts, since extraction effects of the diluted osmium to the basement membrane are uncertain. However, similar meshworks of the basal lamina have been reported in thin-section studies using other chemical treatments such as enzyme digestion (Makino et al., 1980; Inoue et al., 1983; Shikata et al., 1990) and salt extraction (Yurchenco and Ruben, 1987). They have been also found in the quick-freeze deep-etch studies of tissues as muscles (Frank and Beyerl, 1965) and renal glomeruli (Kubosawa and Kondo, 1985). Furthermore, Sawada (1981) noticed in his SEM studies of the basement membrane of various tissues that the filamentous components with a diameter of 15 nm are present on and in the lamina densa; he considered the lamina densa to consist of the filamentous meshwork embedded in the amorphous substances. We are, therefore, reasonably confident that the meshwork structure demonstrated in the present study actually represents the frame-

**Fig. 4.** Transmission electron micrograph of the osmium-macerated nerve. No fuzzy materials are found on the surface of collagen fibrils. The lamina densa (D) of the Schwann cell basement membrane remains as a partially discontinuous sheet. ×125,000

**Fig. 5.** High magnification views of the Schwann cell basement membrane of the osmium-macerated nerve. a. Transverse section of the basement membrane. The lamina densa (D) is seen as a somewhat discontinuous layer with filamentous components. C collagen fibrils. b. Oblique section of the basement membrane. The lamina densa (D) is composed of a meshwork of thin filamentous components. Sm plasma membrane of the Schwann cell, C collagen fibril. ×250,000
Fig. 6. Scanning electron micrograph of the interstitial aspects of the Schwann cell basement membrane after osmium-maceration. The lamina densa consists of mainly thin (10–15 nm) filamentous components. These filaments are interwoven into a fine meshwork. Some of these filaments (arrows) cross over collagen fibrils (C). Arrowheads show some bifurcations of the filaments. ×100,000
work of the lamina densa.

The nature of the filamentous components remains unknown, leaving us to only speculate about it from the obtained findings. Previous studies have revealed that the Schwann cell basement membrane contains type IV collagen, laminin, fibronectin and proteoglycans (TOHYAMA and IDE, 1984; MCGARVEY et al., 1984; IDE et al., 1989; KUECHERER-EHRET et al., 1990). It should be noted that osmium-maceration extracted fuzzy substances on the endoneurial collagen fibrils. This finding indicates that proteoglycans can be extracted by the treatment. Therefore, the filamentous meshwork demonstrated in the lamina densa must be composed of materials other than proteoglycan. On the other hand, endoneurial collagen fibrils were well preserved after osmium-maceration. This finding suggests that the collagen molecules of the fibrils are resistant to osmium-maceration. Although the molecular structure of type IV collagen is indeed somewhat different from that of the interstitial collagen, it is reasonable to consider that type IV collagen in the basement membrane can withstand osmium-maceration. Thus, we presume that most, if not all, parts of the filamentous components are composed of type IV collagen.

Based on in vitro studies of type IV collagen molecules using the rotary shadowing technique, several investigators have proposed that the structural scaffolding of the basement membrane is formed by polymerized networks of type IV collagen; TIMPL et al. (1981) observed that type IV collagen molecules form tetramers through the association of their amino-terminal ends, and believed them to be arranged in a diamond-shaped pattern with sides of about 0.8 μm, forming a two-dimensional network in the lamina densa. On the other hand, YURCHENCO and his associates (YORCHENCO and FURTHMAYR, 1984; YORCHENCO et al., 1986) noticed lateral associations of the molecules and proposed another model of a regular polygonal network with sides about 0.2 μm long. Although the filamentous meshwork found in the present study resembles the polygonal model rather than the diamond-shaped one, some differences between the former two can be still appreciated as follows:

1) The meshwork observed after osmium-maceration is more irregular and denser than the YURCHENCO’s network. Actually, the irregularity of the type IV collagen network in situ has been admitted by YURCHENCO and RUBEN (1987) using the human amniotic basement membrane.

2) Filaments in the present study are larger in diameter than those of the YURCHENCO’s network; this difference, however, might depend on the metal coating of specimens for SEM.

Another remarkable finding may be the fact that the above mentioned filaments anchored collagen fibrils to the lamina densa. Similar findings were reported by ICHIMURA and HASHIMOTO (1984) in their SEM studies of the astrocytic basement membrane, though they regarded the filaments as microfilaments in nature. Studies using other digestion methods will give us further information.

REFERENCES


Dr. Tatsuo USHIKI
Department of Anatomy
Hokkaido University School of Medicine
Kita-15, Nishi-7, Kita-ku
Sapporo, 060 Japan

木本 辰男
060 札幌市北区北15条西7丁目
北海道大学医学部
解剖学第三講座