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Abstract

We demonstrated the ultrastructure of rat glomerular basement membrane (GBM) by ultra-high resolution scanning electron microscopy. GBM prepared by sonication methods and conductive-staining could be observed without metal coating at magnifications as high as 400,000 times. The GBM showed an irregular meshwork structure composed of various strands and pores. The width of the strands ranged from 6 to 15 nm, and the diameter of pores ranged from 6 to 50 nm. The present study confirmed our molecular sieve theory of the basement membrane.

KEYWORDS: glomerular basement membrane, ultrastructure, ultra-high resolution scanning electron microscopy, conductive staining

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Three-Dimensional Architecture of Rat Glomerular Basement Membrane by Ultra-High Resolution Scanning Electron Microscopy

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We demonstrated the ultrastructure of rat glomerular basement membrane (GBM) by ultra-high resolution scanning electron microscopy. GBM prepared by sonication methods and conductive-staining could be observed without metal coating at magnifications as high as 400,000 times. The GBM showed an irregular meshwork structure composed of various strands and pores. The width of the strands ranged from 6 to 15 nm, and the diameter of pores ranged from 6 to 50 nm. The present study confirmed our molecular sieve theory of the basement membrane.

Key words: glomerular basement membrane, ultrastructure, ultra-high resolution scanning electron microscopy, conductive staining

The glomerular basement membrane (GBM) is an extracellular matrix, which lies between epithelial and endothelial cells, and plays an important role in glomerular filtration. In 1977, Ota *et al.* demonstrated by transmission electron microscopy (TEM) that negatively stained bovine GBM was composed of a porous meshwork (1). After that several authors described the meshwork structure of the basement membrane (2, 3). Recently, we have demonstrated a meshwork structure in the GBM after chemical treatment of ultrathin sections (4). However, the three-dimensional ultrastructure of the meshwork in the GBM has not been examined closely. Our previous study by conventional scanning electron microscopy (SEM) failed to demonstrate the meshwork structure in the GBM because of metal coating during the preparation of specimens and

the limited resolution of SEM (5). In the present study, we demonstrated the three-dimensional ultrastructure of rat GBM by high resolution SEM after conductive staining. This method allowed the observation of structures as small as 0.5 nm in diameter (6).

The GBM was isolated from Wistar rats by Spiro's method (7). The isolated GBM was fixed in 2.5 % glutaraldehyde in cacodylate buffer (pH 7.2) for 2 h, and was then underwent conductive staining (8). In brief, the specimens were fixed in 1.0 % osmium tetroxide solution for 2 h, and treated with 2.0 % tannic acid in distilled water for 2 h. The same treatment procedure was repeated twice, and then the specimens were fixed again in 1.0 % osmium tetroxide solution. Between each step the specimens were rinsed extensively with 0.1 M cacodylate buffer. The samples were dehydrated through a series of graded concentrations of ethanol, and dried in a

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critical point dryer (HCP-2, Hitachi Ltd, Japan). Then they were observed without any coating under an ultra-high resolution scanning electron microscope (S-900, Hitachi Ltd, Japan).

Conductive staining, which is a metal impregnating method using tannic acid and osmium tetroxide, enables the direct observation of biological specimens by the SEM (8). In this study, we observed GBM at magnifications as high as 400,000 times without metal coating.

Under low magnification, the GBM was observed as a convoluted sheet. The surface was covered with numerous microprocesses, which were presumably fragments of cell membrane (Fig. 1). Under high magnification, the GBM appeared as a network of anastomosing and branching strands (Fig. 2). The width of the strands was variable and ranged from 6 to 15 nm. The spaces or pores separating the strands were round or oval. Their diameters ranged from 6 to 50 nm. There were two patterns of meshwork: One of rough linkages as shown in the left side of Fig. 2, and the other of cross linkages as shown in the right side.

Makino *et al.*, by TEM after negative-

staining, demonstrated a regular meshwork of rat GBM prepared by the same methods (9). In their study, the strands were almost equal in width and measured 3 ± 1 nm and the pores were round or oval and measured 4 ± 1 nm. Although the dimensions of pores and strands in their study were smaller than those in this study, the minimum size of pores and strands was nearly equal. Preparation procedures such as fixation and critical point drying might contribute to the variability in the size of strands and pores. However, the fundamental meshwork structures were considered to be preserved. Thus, our present study further supports our previous findings that the GBM is a three-dimensional molecular sieve composed of pores and strands.

As epithelial cell debris remained on the GBM surface, the sonication procedure for isolation of GBM did not seem to remove cell components satisfactorily. We were able to observe only the surface of the GBM, which was probably the lamina lucida. Further study is necessary to observe deeper into the GBM.

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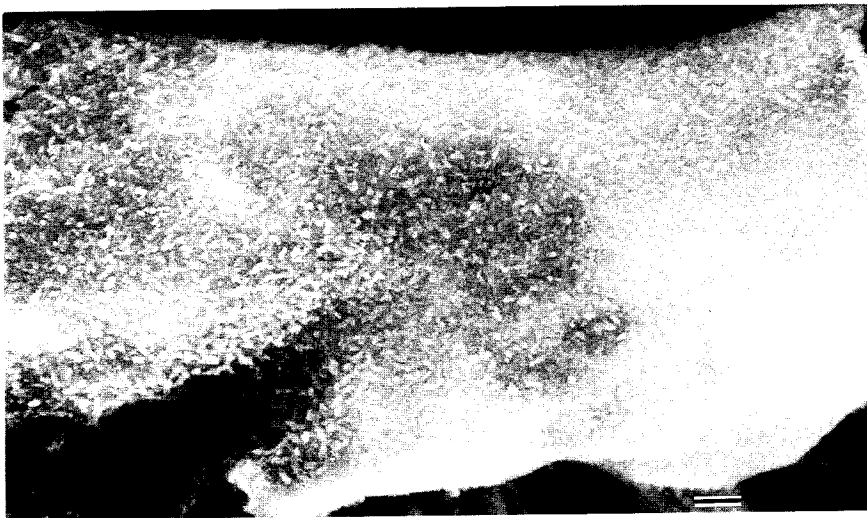


Fig. 1 Rat glomerular basement membrane (GBM) at low magnification. The GBM was covered with numerous microprocesses. Bar = 1 μ m.

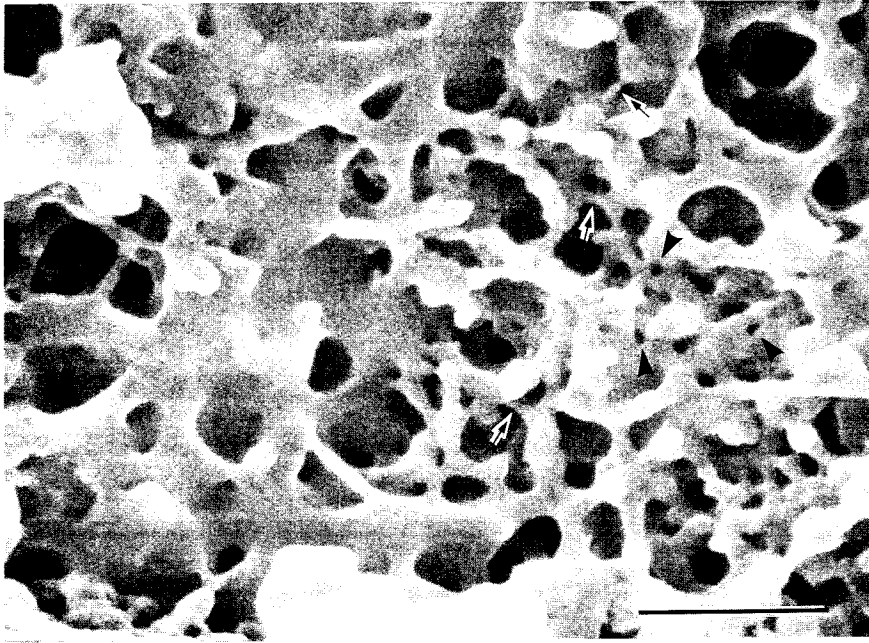


Fig. 2 Meshwork structure of the rat glomerular basement membrane at high magnification. Variable strands form a meshwork structure. Narrower strands anastomose with each other in the right side of the picture. Note the smallest pores (arrowheads) and the narrowest strands (arrows). Bar = 100 nm.

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