

**Fig. 4.** Low-magnified SEM image of Descemet's membrane (DM) exfoliated from the stroma (ST) by the surface tension. Bar = 1  $\mu$ m.



Fig. 5. SEM images of the endothelial surface of Descemet's membrane with platinum coating in a surface tension specimen (a) and without coating in a heating specimen (b). The metal-coated Descemet's membrane is composed of fine granular substances, whereas the uncoated Descemet's membrane shows a felt-like appearance with fine fibrous structures. Bar = 100 nm.

eter ( $30.0 \pm 8.0$  nm: mean  $\pm$  SD, calculated on all prepared specimens) (Fig. 5a), whereas in the uncoated specimen, it showed a felt-like appearance with fibrous structures of approximately 15 to 20 nm in diameter (Fig. 5b).

On the other hand, the stromal surface of the membrane with platinum coating in a surface tension specimen was composed of relatively illbalanced, larger-sized granular substances of approximately 26 to 78 nm in diameter ( $50.0 \pm 12.8$  nm: mean  $\pm$  SD, calculated on all prepared specimens) (Fig. 6a), whereas in the uncoated specimen, it showed a felt-like appearance with fibrous structures of approximately 30 to 50 nm in diameter, which were thicker than that on the endothelial side (Fig. 6b).

## Discussion

The undulation of the basal surface of the endothelium observed by TEM (Fig. 1) is supposed to correspond to small wrinkles on the basal surface of the endothelium observed by SEM (Fig. 3a). Small openings on the basal surface of the endothelium observed by SEM (Fig. 3a, arrows) are thought to correspond to orifices of pinocytotic vesicles beneath the basal cell membrane of the endothelium observed by TEM (Fig. 1). Pinocytotic vesicles are supposed to be concerned with the active pumping action of the endothelium, by which intracorneal fluid is pumped out into the anterior chamber when the fluid volume is increased.

In TEM images, the border between the stroma and Descemet's membrane is less distinct than that between the endothelium and the membrane, because of the presence of collagen fibrils within the stromal side of Descemet's membrane (Hogan et al., 1971; Komai et al., 1990). The present TEM study demonstrated the binding between the stroma and the membrane by the collagen fibrils (Fig. 1).

According to the three-dimensional observation of the basal lamina in pancreatic acinar cells, cardiac muscles and blood capillaries, each basal lamina consisted of globular materials of various sizes which were attached to or buried in a flat meshwork composed of fine filaments and amorphous substances (Sawada, 1981). In this study, Descemet's membrane with platinum coating in the surface tension specimen consisted of fine granular substances as observed by Sawada (1981) (Figs. 5a and 6a). However, the size of the granular substances obviously differed between the endothelial and stromal sides. These results suggest that their native sizes also differ between the two surfaces.

Descemet's membrane was mainly composed of type VIII collagen, partly by type IV and barely by type VI collagens (van der Rest and Garrone, 1991). Marshall et al. (1993) demonstrated, in an



Fig. 6. SEM images of the stromal surface of Descemet's membrane with platinum coating in a surface tension specimen (a) and without coating in a heating specimen (b). The metal-coated Descemet's membrane is composed of fine granular substances of larger size, whereas the uncoated Descemet's membrane shows a felt-like appearance with thicker fibrous structures than on the endothelial side. Bar = 100 nm.

immunoelectron study, that type VIII collagen was abundant in the stromal side of Descemet's membrane and type IV collagen was rich in the endothelial side. They also showed that collagen types V and VI were present in the corneal stroma and its transitional zone to Descemet's membrane, for adhering the stroma to the membrane.

Our observation of Descemet's membrane without metal coating clarified that its endothelial surface was composed of felt-like fine filamentous substances (Fig. 5b), whereas the stromal surface was composed of thicker fibrous structures (Fig. 6b). These results may reflect a difference of the collagen types between the two surfaces of the membrane.

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Corresponding author: Akihiko Tamai, MD