A Corrosion Casting/Scanning Electron Microscope Method That Simultaneously Demonstrates Clear Outlines of Endothelial Cells and Three-Dimensional Vascular Organization

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Summary. This paper describes a method that can definitively demonstrate endothelial cell boundaries on corrosion casts of arteries, veins, and capillaries. After perfusion with silver nitrate, a casting medium was injected into the entire vascular bed. The injected tissues were either exposed to light or immersed in the photographic developer to develop the silver halide, and corroded in a 5% NaOH solution at 60°C overnight. Observations of the casts containing water in a low vacuum scanning electron microscope equipped with a cooling stage clearly showed endothelial cell boundaries on casts of every type of vessel as well as their three-dimensional architecture. The low vacuum scanning electron microscope images of wet casts were almost identical in quality to the back-scattered electron images of dried casts without any coating. Secondary electron images of the dried casts with metal coating clearly showed endothelial cell outlines and nuclear imprints. The secondary electron images at high magnification indicated that silver granules were precipitated in the grooves along endothelial cell boundaries on the casts. Since this method can demonstrate endothelial cell boundaries of every type of vessel in addition to their three-dimensional architecture, it will be a powerful tool for examining endothelial cell morphology and microvascular organization in pathological as well as normal tissues.

Since MURAKAMI (1971) introduced the corrosion casting/scanning electron microscope (SEM) technique, this method has been extensively used in research of microvascular architecture in various tissues and organs for a large variety of animal species, and has revolutionarily increased our knowledge of the morphological aspects of microcirculation. Although vessel types of casts may be identifiable by the imprints of endothelial cell nuclei on the casts, demonstration of endothelial cell boundaries on

the casts not only facilitates a distinction between arterial and venous casts but also permits morphological studies of endothelial cells undergoing pathological changes such as in inflammation in addition to the precise three-dimensional visualization of vascular organization. REIDY and LEVESQUE (1977) reported a method for demonstrating outlines of endothelial cells on corrosion casts that has long interested us. So far, however, this method seems to have been applied only to the aortae in rats and rabbits. In the present study, we systematically assess the applicability of the method and show that it is highly useful to clearly mark endothelial cell boundaries on casts of every type of vessel including capillaries.

MATERIALS AND METHODS

Five male Wistar rats weighing 400-450 g were used for this study. Under deep anesthesia with diethyl ether, a thoracotomy was performed and a 13-gauge needle was inserted into the thoracic aorta. Solutions were then infused at about 120 mmHg in the following sequence: approximately 150 ml of 1% paraformaldehyde in 0.075 M cacodylate buffer, about 100 ml of 0.9% NaCl, 50 ml of 5% glucose in water, 50 ml of 0.2% AgNO₃ in water, and 50 ml of 5% glucose in water. Three rats were then injected with about 50 ml of Mercor (Oken-shoji, Tokyo). Those parts of the animal bodies to be observed were either excised and exposed to 100-W bulb for 30 min or immersed in a photographic developer (Korectol, Fujifilm, Tokyo). The remaining two rats were perfused with about 50 ml of the photographic developer, which was immediately followed by an injection of Mercor. After the

Fig. 1. Corrosion cast of the muscular and submucosal vessels in the rat small intestine observed under a low-vacuum SEM equipped with a cooling stage at -10°C , 50 Pa and 15 kV of acceleration voltage. Outlines of endothelial cells can be seen on almost all of the vessels as white (bright) lines. In this micrograph, two capillaries (*) are devoid of the cell boundary marks, for unknown reasons. *A* artery, *a* arteriole, *C* capillary, *v* venule, *V* vein. Back-scattered electron image. $\times 280$

casting medium had completely polymerized, the tissues excised from the animal bodies were corroded in 5% NaOH at 60°C overnight, and gently rinsed in water. The corrosion casts containing water were mounted on sample holders with water-soluble wood-working paste (S-6040, Konishi Co., Osaka) and observed at -10°C , 50–60 Pa, and 15 kV accelerating voltage in a Hitachi S-3200 SEM equipped with a cooling stage (Hitachi, Tokyo). After observation of the wet casts, they were dried in the air and observed in the same SEM in a low vacuum. Then the dried casts were coated with gold, and again observed in the same SEM in a high vacuum and at 15 kV accelerating voltage to obtain secondary electron images.

RESULTS

Low vacuum SEM of the corrosion casts clearly depicted the endothelial cell borders of all types of vessels as bright lines on the cast surfaces in addition to the three-dimensional architecture of the vessels (Figs. 1, 2). Although the endothelial cell borders of venules and capillaries were not demonstrated at times, those of arteries, arterioles, and veins were regularly visualized by bright lines. At a low magnification, secondary electron images of dried casts coated with gold also showed endothelial cell borders, which were, however, less bright than back-

Fig. 2. Corrosion casts of an artery (**a**) and a vein (**b**) observed under a low-vacuum SEM equipped with a cooling stage at -10°C , 50 Pa and 15 kV of acceleration voltage. The arterial endothelial cells show elongated spindle shapes with undulating margins, while venous ones are broader and less undulating than the arterial ones. Back-scattered electron images. $\times 800$

scattered electron images obtained by low vacuum SEM (Fig. 3). A high magnification showed silver granules deposited in the grooves formed by endothelial cell borders (Fig. 3 inset).

Exposing the tissues injected with the casting medium to light served to develop only the silver halide near the surface of the tissues, while immersion in the photographic developer developed the silver halide deep in the tissues. Perfusion of the photographic developer before injecting Mercor produced large amounts of silver granules within the vessels, which hindered the filling of the casting medium.

Endothelial cells of arteries and arterioles showed an elongated spindle shape with undulating margins (Fig. 2). Capillary endothelial cells also revealed a spindle but more irregular shape (Fig. 1). Two or three endothelial cells usually formed the circumference of the capillaries (Fig. 1). Endothelial cells of

venules and veins had approximately the same length as those of arteries, but were larger in width than those of the latter. The endothelial cell outlines of the former were less undulated than those of the latter (Fig. 2).

DISCUSSION

The present study describes a method that can clearly mark endothelial cell boundaries on the corrosion casts of blood vessels. Since RANVIER (1875), it has been known that endothelial cell borders of blood vessels can be stained with silver nitrate (ROMEIS, 1948). Light microscopy of silver-stained vessels has been useful for studies of blood vessels (e. g., FLOREY et al., 1959; GOTTLÖB and HOFF, 1968; ZAND et al., 1982; McDONALD, 1994), though these could not be

Fig. 3. Secondary electron images of casts coated with gold. **a.** Outlines of endothelial cells are seen on the cast. *A* artery, *a* arteriole, *v* venule, *V* vein. $\times 410$. **b.** A higher magnification of a submucosal venous cast in the rat cecum showing both endothelial cell boundaries as bright (white) lines with undulations and round or oval nuclear imprints. $\times 830$. **c.** Silver granules are deposited in the grooves along endothelial cell boundaries on the cast and form bright (white) lines approximately $0.5 \mu\text{m}$ in width. $\times 4,000$

visualized in their three-dimensional organization. The advantage of the present method is that it allows simultaneous demonstration of the outlines of endothelial cell and three-dimensional extension of blood vessels.

REIDY and LEVESQUE (1977) and LEVESQUE et al. (1979) made corrosion casts of aortae in rats and rabbits after the infusion of silver nitrate and showed

outlines of endothelial cells on the casts by scanning electron microscopy. It is unknown whether they observed blood vessels other than the aorta with this method. There is no evidence that later researchers employed this method, either. In the present study, we successfully applied their method to clearly demonstrate endothelial cell boundaries on corrosion casts of all types of vessels stained with silver

nitrate. The reason why outlines of endothelial cells were occasionally not marked on the casts is not known at this time.

The present study has also shown that the low vacuum SEM is highly useful for simultaneously observing the outlines of endothelial cells formed by silver granules on corrosion casts and the three-dimensional organization of vessels. Furthermore, low vacuum SEM equipped with a cooling stage allows observation of wet samples without any further treatment such as drying and coating, which saves much time. However, secondary electron images of the metal-coated casts prepared by the present method are more informative because they can demonstrate both endothelial cell boundaries and nuclear imprints.

The size and shape of endothelial cells observed on the casts prepared by the present method are basically the same as those detected by light microscopy of silver-stained vessels themselves (MCDONALD, 1994). Thus, the present corrosion casting/SEM method can be used to examine both the morphology of endothelial cells and the three-dimensional organization of vessels in pathological conditions as well as in normal tissues and organs.

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