Ultrastructure of Endothelial Cells Under Flow Alteration

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ABSTRACT Endothelial cells are stable and quiet in normal animals. They arrange regularly and have a smooth lumen surface and thin endothelial wall. According to Thoma’s principle (1893) and Kamiya and Togawa’s principle (1980) on the relationship of the vascular diameter to flow alteration, blood flow is in equilibrium to the diameter and in a physiological state. That is to say, there is no fast flow or slow flow. To understand the nature of the endothelial cells, we should investigate endothelial cells under flow alteration to break the equilibrium state. Endothelial cells under increased flow were studied in arteries with an arteriovenous fistula or in the capillaries of myocardium with volume-overloaded hearts or of the skeletal muscle by electrical stimulation. Those under decreased flow were studied by the closure of the fistula or by ceasing the stimulation. Endothelial cells in the coarctation of the arteries were also observed. Endothelial cells were activated by increased flow in the arteries and capillaries, while they were inactivated by decreased flow. Endothelial activation is characterized as lumen protrusions, increase of cytoplasmic organelles, abluminal protrusions, basement membrane degradation, internal elastic lamina degradation in the arteries, and sproutings in the capillaries. These are ultrastructurally comparable to angiogenesis. Endothelial inactivation is characterized by the decrease of endothelial cell number with apoptosis, which is ultrastructurally comparable to angioregression. We assume that endothelial cells respond to increased flow by angiogenesis and to decreased flow by angioregression.


INTRODUCTION

Endothelial cells usually show stable and quiet faces with smooth lumen surface and scanty organelles with flat nucleus (Rhodin, 1968; Crawford et al., 1976; Reidy and Langille, 1980; Zarins et al., 1980; Rhodin, 1980; Langille and Adamson, 1981). On the contrary, we feel that endothelial cells are active, so as to maintain blood flow, which has high energy. Are they really quiet or temporarily in a quiet state?

Considering the cardiovascular system, blood flow is its principal element, purpose, and function and it is continuous through all channels. At the same time, endothelial cells form a thin sheet of a monolayer covering the whole lumen surface of the cardiovascular system. Therefore, endothelial cells are one of the principal elements of the cardiovascular system, but not its purpose or its function. When endothelial cells are observed from the blood flow, that is to say, from the lumen of the blood vessels, the endothelial layer is smooth, gradual, and not interrupted. Therefore, we can have a conceptual aspect that blood vessels consist of blood flow in its core and endothelial cells surrounding the core.

More than 100 years ago, Thoma (1893) showed that when blood flows fast, blood vessels enlarge, and they narrow when blood flows slowly. Kamiya and Togawa (1980) showed that the principal stress which influences arterial dilatation was wall shear stress. When increased flow induces the elevation of wall shear stress, blood vessels enlarge to reduce the wall shear stress to a physiologically determined level, which is considered about 1 Pascal. When decreased flow induces the reduction of wall shear stress, blood vessels narrow to elevate the wall shear stress to the physiologically determined level. This feedback mechanism keeps the flow profile in a physiologic equilibrium state. To consider that blood vessels consist of blood flow in the core and endothelial cells surrounding them, it is assumed that the endothelial cells control this flow and lumen diameter relationship. However, until now it is not proven how endothelial cells control lumen diameter, although many reports ascertain that the flow and lumen diameter relationship follows Kamiya and Togawa’s principle (Guyton and Hartley, 1985; Zarins et al., 1987; Masuda et al., 1993, 1989.

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1999, 2000; Tohda et al., 1992; Langille, 1993; Tronc et al., 1996, 1998; Wong and Langille, 1996; Sugiyama et al., 1997; Singh et al., 1997; Cho et al., 1997; Zhuang et al., 1998; Sho et al., 2001). Recently, research progress to understand how endothelial cells sense blood flow or wall shear stress used in vitro studies (Ando and Kamiya, 1993; Ando et al., 1993); however, here we restrict our review to the in vivo findings of the ultrastructure of endothelial cells rather than the mechanism.

It is therefore suggested that there is no special high-flow segment in the cardiovascular system in normal animals, if Kamiya and Togawa’s feedback mechanism actually works. This is to say, if we observe endothelial cells in normal animals, only one quiet and equilibrium profile of the endothelial cells is observed. Therefore, to observe other aspects of endothelial cells we should alter blood flow. In this review, we show the ultrastructure of endothelial cells in blood vessels in which flow is altered by variable methods, such as an arteriovenous fistula (AVF) method (Kamiya and Togawa, 1980; Zarins et al., 1981, 1983, 1985, 1986, 1987, 1988, 1989a, 1989b, 1993, 1999, 2000; Tohda et al., 1992; Tronc et al., 1996; Sugiyama et al., 1997; Singh et al., 1997; Zhuang et al., 1998; Negishi et al., 2001; Sho et al., 2001) or an arterial coarctation method (Bomberger et al., 1981; Zarins et al., 1981; Langille et al., 1986; Zand et al., 1991; Nanjo et al., 2001; Xu et al., 2001). Furthermore, to observe capillaries under flow alteration, we used volume-overloaded rat heart (Kawamura et al., 1990) and electrically stimulated skeletal muscle (Hudlicka, 1984, 1998; Hudlicka et al., 1992; Ebina et al., 1996; Hansen-Smith et al., 1996; Egginton and Hudlicka, 1999; Pearce et al., 2000). Decrease or normalization of blood flow was also achieved by closure of the AVF (Singh et al., 1997; Zhuang et al., 1998; Sho et al., 2001) or by ceasing the electrical stimulation (Hoshi et al., 2000).

RABBIT CAROTID IS SUSCEPTIBLE TO FLOW ALTERATION

Common carotid arteries of dogs (Kamiya and Togawa 1980; Masuda et al., 1982, 1985, 1986, 1987, 1989a), rats (Tohda et al., 1992; Sugiyama et al., 1997), and rabbits (Masuda et al., 1993, 1999, 2000; Singh et al., 1997; Tronc et al., 1996; Zhuang et al., 1998; Negishi et al., 2001; Sho et al., 2001) are commonly used for flow increase experiments induced by AVF because they are easily accessible and have no branching except

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Fig. 1. Scanning electron microscopy (SEM) micrographs of the lumen surface of the rabbit common carotid arteries proximal to the arteriovenous fistula (AVF) between carotid artery and jugular vein. Flow is from the left to right. Bars indicate magnification in microns. A: (Cont), nonoperated control. B: (1h), One hour after AVF. C: (6h), 6 hours after AVF. D: (1d), One day after AVF. E: (2d), 2 days after AVF. F: (3d), 3 days after AVF. G: (4d), 4 days after AVF. H: (1W), One week after AVF. I: (4W), 4 weeks after AVF. Endothelial cells arrange parallel to the flow. They become long at 1 day (D) to 2 days (E) and protrude at 3 days (F). There are circumferential fissure-like depressed areas, which are compatible with the gaps of internal elastic lamina (IEL) at 1 week (H) and 4 weeks (I (*)). At 4 days (G), there are small gaps of IEL (arrow).
the thyroid artery. Common iliac arteries of rabbits were also used (Masuda et al., 1989b); however, ischemic changes sometimes happened in the leg.

Increased flow induces dilatation in these common carotid arteries of dogs, rats, or rabbits; however, the degree of dilatation is different. For example in dogs, 3-fold high-flow induced nearly 1.2-fold enlargement of carotid diameter after 6–8 months (Kamiya and Togawa, 1980). In rats, 11-fold high-flow induced nearly 1.3-fold enlargement of carotid diameter after 4 weeks (Tohda et al., 1992). In rabbits, blood flow increased 3.5–4-fold immediately after AVF, increased further 6-fold at 3 days, 15-fold at 4 weeks, and 18-fold at 8 weeks (Masuda et al., 1999). Diameter of the carotid enlarged 1.7-fold at 4 weeks and 1.8-fold at 8 weeks. The susceptibility of the carotid artery dilatation induced by increased flow is high in the rabbit carotid as compared to that of rats or dogs. Therefore, we usually used rabbit common carotid arteries.

**ENDOTHELIAL CELLS UNDER INCREASED FLOW IN THE RABBIT CAROTID WITH AVF**

In this section, we describe the ultrastructure of endothelial cells in the rabbit common carotid arteries after AVF (Masuda et al., 1999). In controls, endothelial cells are flat and elongate (Fig. 1A). Nuclei portions are slightly elevated. The lumen surface is smooth (Fig. 2). Cytoplasmic organelles are scanty and the Golgi apparatus is small. There are small bundles of microfilaments, but not as distinct. Abluminal plasma membrane is smooth, with indistinct dense patches. Intercellular junctions are simple. Basement membrane is thick. The subendothelial space is very narrow. Internal elastic lamina (IEL) is constant with occasional fenestrae, but its lumen side is sometimes irregular.

One hour after AVF (Fig. 1B), endothelial cells are slightly elongated, forming a tadpole shape (Fig. 1B). Nuclei are in the downstream portions. Endothelial cells were edematous, but cytoplasmic organelles were not increased (Fig. 3A). The intercellular junction is simple, but slightly elongated. Six hours after AVF (Fig. 1C), endothelial cells are distinctly elongated. Cytoplasmic organelles slightly increase (Fig. 3B) and cytoplasmic vesicles appear. Twelve hours after AVF, endothelial cells are considerably elongated. Intercellular junctions are straight and elongated (Fig. 3C). Cytoplasmic organelles increase further. One day after AVF (Fig. 1D), endothelial cells are distinctly elongated. Endothelial cells become thick (Fig. 3D). Cytoplasmic organelles are increased distinctly. Cytoplasmic vesicles are increased. The intercellular junction becomes straight and distinctly elongated. Lumen plasma membrane shows many caveolae. Microfilaments and intermediate filaments are increased. Abluminal plasma membrane is dense. Subendothelial spaces are narrow and thin. The basement membrane is slightly thickened. IEL is regular.

Two days after AVF, endothelial cells remarkably elongated and narrowed (Fig. 1E). Lumen plasma membrane became irregular, with many caveolae (Fig. 4). There are many vesicles in the cytoplasm. Cytoplasmic organelles are increased. Rough endoplasmic reticulum is small.
ulun (RER) and smooth endoplasmic reticulum (SER) are increased. There are many mitochondria. Golgi apparatus is prominent. Abluminal plasma membrane becomes irregular and thick, with many small cytoplasmic protrusions. Lumen surface is rather irregular, with vesicles or caveolae. There are many intercellular junctions, because endothelial cells are elongated and narrow. Abluminal cytoplasmic protrusions are frequently observed. They originate either from single cells (A) or from the intercellular junctions (B, C, D). They penetrate into IEL (A, C) or in the irregularly degraded lumen side of the IEL (B). They are usually filled with microfilaments. In the fenestrae of IEL, endothelial cells are sometimes compacted with irregular cytoplasmic protrusions (D). Subendothelial space becomes irregular and occasionally wide and the lumen side of IEL is sometimes degraded. There are small globules in the subendothelial layer in B, but we interpret them as artifacts.

Three days after AVF, endothelial cells are narrow and protruded (Fig. 1F), the density of which is very high (nearly 2-fold higher than controls) (Masuda et al., 1999). Endothelial cells are filled with organelles (Fig. 5). RER and SEM are abundant. Golgi apparatus is well developed. Mitochondria are frequent. Lumen plasma membrane is markedly irregular, with many caveolae. There are many vesicles in the cytoplasm. Intercellular junctions become irregular and distinct. There are many small cytoplasmic protrusions and some large protrusions to the subendothelial space, while there are occasional cytoplasmic protrusions into the lumen. These abluminal cytoplasmic protrusions occur always in the enlarged fenestrae of the IEL. In these places endothelial cells occasionally contacted smooth muscle cells of the media. It is interesting that there are no smooth muscle cells, which migrate into the subendothelial space, although cytoplasmic protrusions of the smooth muscle cells occasionally reach into the subendothelial space, while cytoplasmic protrusions of the endothelial cells occasionally reach into the media through the fenestrae of the IEL. The space

![TEM photomicrographs of endothelial cells in the rabbit common carotid arteries at 2 days after AVF. Cross-section. Bars indicate magnification in microns. Many endothelial cells appear in a unit width. Arrows, intercellular junctions. Arrowheads, abluminal cytoplasmic protrusions. Endothelial cells contain many organelles, such as mitochondria, RER, SER, or vesicles. Lumen surface is rather irregular, with vesicles or caveolae. There are many intercellular junctions, because endothelial cells are elongated and narrow. Abluminal cytoplasmic protrusions are frequently observed. They originate either from single cells (A) or from the intercellular junctions (B, C, D). They penetrate into IEL (A, C) or in the irregularly degraded lumen side of the IEL (B). They are usually filled with microfilaments. In the fenestrae of IEL, endothelial cells are sometimes compacted with irregular cytoplasmic protrusions (D). Subendothelial space becomes irregular and occasionally wide and the lumen side of IEL is sometimes degraded. There are small globules in the subendothelial layer in B, but we interpret them as artifacts.](image-url)
between the endothelial cells and IEL becomes rather narrow as compared with at 2 days. Basement membrane is dense and irregular.

Four days after AVF, there appear small depressed areas, which are compatible with the gaps of IEL as described by Masuda et al. (1999) (Fig. 1G). These gaps of IEL are the disruptions of the IEL, which are different from the physiological fenestrae of the IEL. The mechanism of the morphogenesis of the gaps of IEL is not yet understood. It is interesting that endothelial cells are always preserved in the gaps. Endothelial cells are as active as at 3 days (Fig. 6A, B), especially in the gaps of IEL.

At 1 week after AVF, the gaps enlarged (Fig. 1H). Percentage of gaps to the lumen is around 10%. At the margin of the gaps (Fig. 6C), lumen was completely covered with endothelial cells. They are very active in the gaps (Fig. 6D), where smooth muscle cells close to the endothelial cells. Basement membrane is thick.

At 4 weeks after AVF, the gaps widened further (Fig. 1I). Percentage of gaps to the lumen is nearly 60%. In the gaps (Fig. 6E), endothelial cells proliferate and are increased as active as at 3 days. Endothelial cells are rather quiet in the IEL-preserved areas (Fig. 6F). Basement membrane is thin and regular. Subendothelial space is narrow. At 8 weeks after AVF, appearance of the endothelial cells is almost similar to that at 4 weeks.

**Fig. 5.** TEM photomicrographs of endothelial cells in the rabbit common carotid arteries at 3 days after AVF. Cross-section. Bars indicate magnification in microns. Many endothelial cells appear in a unit width. Arrows, intercellular junctions. Arrowheads, abluminal cytoplasmic protrusions. Endothelial cells swell remarkably with abundant organelles. Lumen plasma membrane is irregular and protruded with many vesicles. Intercellular junctions become irregular and elongated. Microfilaments are rich in the abluminal portion and intercellular junctions. Basement membrane is irregular and dense (A). Cytoplasmic protrusions are frequently observed and penetrate into IEL (B). IEL is usually thin, with degradation at the lumen side. Subendothelial space is irregular, but not so wide as at 2 days. In the fenestrae of IEL (C, D), endothelial cells protrude through IEL layer to media. The protrusions are larger than cytoplasmic protrusions observed at 2 days. Microfilaments are rich in these protrusions. Occasionally these protrusions contact with smooth muscle cells (D).

**Fig. 6.** TEM photomicrographs of endothelial cells in the rabbit common carotid arteries at chronic stage after AVF (4 days to 4 weeks). Cross-section. Bars indicate magnification in microns. Many endothelial cells appear in a unit width. Arrows, intercellular junctions. A (4d) and B (4d): 4 days after AVF. C (1W) and D (1W): One week after AVF. E (4W) and F (4W): 4 weeks after AVF. Endothelial cells in the IEL-gaps compact together at 4 days (A, B). They have rich organelles and distinct nuclei. Cytoplasmic protrusions (arrowheads) are observed. At 1 week (C), the margin of the IEL-gap is very steep, but endothelial cells cover all the surface. Endothelial cells in the IEL-gap are active with abundant organelles (D). At 4 weeks (E), the IEL-gap is sometimes indistinct, but endothelial cells pack markedly in the gaps (left half). In the IEL-preserved areas (F), endothelial cells are well-preserved and lumen surface is smooth, although cytoplasmic organelles are still rich.

**ENDOTHELIAL CELLS UNDER INCREASED FLOW IN RATS AND DOGS WITH AVF**

These ultrastructural changes of endothelial cells appeared in the common carotid arteries of dogs (Masuda et al., 1989a). In the endothelial cells of the canine carotid arteries, increased flow induced a distinct increase of stress fibers (Masuda et al., 1985, 1986) at 1 week after AVF. At 4 weeks after AVF, endothelial cells proliferated and filled in the lumen surface (Masuda et al., 1989a). However, appearance of the gaps of IEL was very rare, although subendothelial layer changed distinctly at 1 week after AVF (Masuda et al., 1987). In rats, a similar endothelial cell proliferation was evident at 1–2 weeks after AVF (Tohda et al., 1992). At 4 weeks after AVF, endothelial cells became flat again. Appearance of the gaps of IEL was not evident. Sugiyama et al. (1997) showed regenerating endothelial cells were not as susceptible to the flow increase in rats. These ultrastructural studies in dogs and rats revealed that endothelial cells really were
activated by increased flow; however, arterial wall structure, such as IEL, did not so easily remodel as in the rabbit carotid.

ENDOTHELIAL CELLS UNDER DECREASED FLOW WITH CLOSURE OF AVF

When AVF was kept for 4 weeks in the rabbit common carotid arteries, as stated above, the arteries dilated by the remodeling (Masuda et al., 1999). When the AVF in these dilated arteries was closed, blood flow rate soon reduced to the normal level and wall shear stress reduced remarkably, nearly 0.1–0.2 Pascal (Singh et al., 1997; Sho et al., 1998; Zhuang et al., 2001). As we revealed that intimal thickening occurred after closure by the proliferation and migration of the smooth muscle cells, endothelial cells very quickly change their shape and the gaps of IEL disappear (Zhuang et al., 1998; Sho et al., 2001). One day after closure of AVF, endothelial cells become short and the bottom of the gaps become shallow (Fig. 7A). Some endothelial cells are small, with irregular lumen protrusions (Fig. 7B). Endothelial cells are irregular with indistinct intercellular junction (Fig. 7E). Apoptotic endothelial cells sometimes appear beneath the endothelial cells or in the endothelial layer. Three day after closure of AVF, all endothelial cells become very short, forming hexagonal shape (Fig. 7C, D). Some endothelial cells are very small (Fig. 7F). Endothelial surface is rather flat, but there are large mononuclear cells beneath the endothelial layer. They sometimes show apoptotic figures. Sho et al. (2001) revealed there were apoptotic mononuclear cells in the subendothelial space and occasionally smooth muscle cells phagocyte them, although they could not confirm that they originated from endothelial cells. They considered that endothelial cells decrease in number by apoptosis, when wall shear stress decreased, which might migrate into the subendothelial layer and be processed by the smooth muscle cells.

FLOW ALTERATION BY OTHER METHODS

Langille and colleagues utilized various methods to alter flow (Langille and O’Donnell, 1986; Wong and Langille, 1996; Cho et al., 1997). They used flow increase by growth (Wong and Langille, 1996; Cho et al., 1997), flow decrease of the common carotid artery by the ligation of the external carotid artery to induce reduction of flow (Langille and O’Donnell, 1986), and flow increase in the contralateral common carotid artery, when a common carotid artery was closed (Wong and Langille, 1996). They revealed that flow-dependent remodeling was endothelial cell-dependent; however, ultrastructural changes of the endothelial cells were not conspicuous. It is considered that these flow alterations are mostly moderate changes as compared to the flow changes induced by AVF and AVF closure. Therefore, endothelial cell activation or inactivation by flow changes is not distinctive.

ENDOTHELIAL CELLS IN THE COARCTATION

Coarctation of the canine common carotid arteries was made by the application of silver clips, 1.0 mm round lumen and 3.0 mm in length (Figs. 8, 9). It is assumed that in the coarctation channel blood flows very fast, while blood flows very slowly or reversely immediate downstream of the coarctation channel (Legg and Gow, 1982). Therefore, in the coarctation channel, endothelial cells should perform as observed in the arteries with increased flow. On the contrary, in the downstream of the channel endothelial cells perform as observed in the arteries with decreased flow. Two weeks after coarctation (Fig. 8), endothelial cells are narrow and protrude in the coarctation channel (Fig. 8B), as observed in the rabbit carotid at 3 days after AVF. They are short and wide downstream (Fig. 8C). Four weeks after coarctation (Fig. 9), poststenotic dilatation occurs. As observed in the rabbit carotid at 4 weeks after AVF, there appear gaps of IEL downstream (Fig. 9C), while there are regular folds along the flow line in the coarctation channel. Similar observations on the endothelial cell integrity of coarctation of the artery was reported (Langille et al., 1986; Nanjo et al., 2001; Zand et al., 1991; Zarins et al., 1981) and
Xu et al. (2000, 2001) showed differences of molecular expressions in the proximal and distal segments.

ENDOTHELIAL CELLS MIGRATE INTO THE LUMEN WHEN FLOW IS EXTREME

When AVF was used in the common carotid arteries of rats, endothelial cells were usually desquamated in the segment near the AVF, where high-flow-induced dilatation did not occur (Tohda et al., 1992; Sugiyama et al., 1997). On the other hand, the carotid artery significantly dilated in the endothelial cell-preserved segment (Tohda et al., 1992). In the rabbit common carotid arteries, the endothelial cell desquamated segment occasionally appeared near the orifice of the AVF. This segment also did not dilate, while other endothelial cell-preserved segments dilated remarkably. When blood flow increased gradually after AVF, as described by Masuda et al. (1999), blood flow speed or wall shear stress became extremely high in the endothelial cell desquamated segment. The segment did not dilate. At the segment immediately proximal to the endothelial cell desquamated segment, where endothelial cells were preserved and extremely high wall shear stress existed, endothelial cells migrated into the lumen, forming strange tower-like monuments consisting only of endothelial cells (Fig. 10). Vortex-like endothelial cell arrangement was sometimes observed in these extreme flow segments in the rat carotid or iliac arteries. We consider that extremely high flow induces extreme activity of endothelial cells. However, in this case, extreme proliferation of endothelial cells grow over the extent of the dilatation induced by increased flow, which is usually in equilibrium with the proliferation of endothelial cells. Therefore, endothelial cells would protrude into the lumen.

CAPILLARIES IN THE VOLUME OVERLOADED RAT HEARTS

To observe capillaries in hearts, which may be overloaded by flow (we did not measure directly), we tried to make volume-overloaded rat hearts by using AVF in the common carotid arteries and external jugular veins (Kawamura et al., 1990). Because cardiac outputs increased almost 2-fold as large as the age-matched controls, we assumed myocardial capillary flow increased significantly. Capillary density and the capillary-tofiber ratio elevated only slightly after 2 weeks; however, endothelial cell number per cross section of capillaries increased (Fig. 11). Endothelial cells were thick, with many organelles in the overloaded hearts. We consider that endothelial cells in the capillaries of the volume-overloaded hearts may be stimulated by increased flow, but angiogenesis is not well attained, as

Fig. 8. SEM micrographs of the lumen surface of the canine common carotid arteries with coarctation for 2 weeks. Coarctation was made using a silver clip (lumen diameter 1.0 mm, lumen length 3.0 mm). Flow is from left to right (arrow). Bars indicate magnification in microns (A, 1,000 μm). In the coarctation channel, endothelial cells are narrow, elongated, and protruded (B). In the poststenotic area, endothelial cells are short and wide with many fine projections.

**CAPILLARIES IN THE ELECTRICALLY STIMULATED SKELETAL MUSCLE**

By electrical stimulation of the peroneal nerve (4 Hz, 24 hours per day), extensor digitorum longs (EDL) was chronically stimulated in adult rabbits (Salmons and Vrbova, 1969; Ebina et al., 1996; Egginton and Hudlicka, 1999; Hansen-Smith et al., 1996; Hudlicka, 1984, 1998; Hudlicka et al., 1992). Femoral artery flow was significantly elevated almost 2-fold and tissue-blood flow of the EDL increased about 2.5-fold after 2 weeks. Capillary density and capillary-to-fiber ratio increased about 2-fold at 1 week and 3-fold at 2 weeks. In the controls (Fig. 12A), most capillaries are located in the zones among three muscle fibers. Lumen is flat and endothelial cells are thin. At 1 week after stimulation, endothelial cells became thick with many organelles. Furthermore, there appeared many small capillaries, which had very narrow and small lumen (Fig. 12B). Sprouting-like endothelial cytoplasmic protrusions occasionally appeared. Occasionally there appeared capillaries with two lumens (Fig. 12C). It is known that these capillary changes are compatible with angiogenesis (Hudlicka, 1984, 1998; Hudlicka et al., 1992). Hudlika (1998) thought that increased blood flow and therefore increased wall shear stress induced angiogenesis together with tensile stress exerted by lumen pressure. So far as we observed, angiogenesis occurred in the electrically stimulated skeletal muscle and is a standard physiologic angiogenesis initialized by the sproutings. Intussusceptive vascular growth (Burri and Tarek, 1990; Patan et al., 1992) was not observed. It is known that new capillaries may also develop through the internal division of an individual capillary without abluminal invagination in the stimulated skeletal muscle (Egginton et al., 2001; Zhou et al., 1998a). Our capillary in Figure 12C resembled them, but when we followed it carefully, it was continuous to the regular sprouting or branching. There were no such types of angiogenesis.

**CONCLUDING COMMENTS**

In the arteries or the capillaries, it is assumed that under flow increase endothelial cells become activated. They become thick with many organelles and proliferate. On the contrary, flow decrease induces endothelial cell number decrease with apoptosis. Therefore, we consider that the activity of endothelial cells varies in a wide spectrum from the high-flow condition to the low-flow condition. In normal animals, in which vessel diameter is in equilibrium to the flow, the activity of the endothelial cells represents one spectrum of a physiological state.

All the ultrastructural changes observed in the activated endothelial cells in the arteries are nonspecific and are characterized as follows.
1) Irregular lumen plasma membrane with cytoplasmic protrusions.
2) Increase of organelles.
3) Increase of cytoplasmic vesicles.
4) Irregular abluminal plasma membrane with cytoplasmic protrusions.
5) Irregular and elongated intercellular junctions.
6) Increase in the chromatin density and distinct nucleoli with occasional mitosis.
7) Dense and irregular basement membrane.
8) Widening of the subendothelial layer with degradation of the IEL in its lumen side.
9) Formation of gaps of IEL with endothelial cell invasion into the media through the gaps.
10) Contact of endothelial cells and smooth muscle cells.

On the other hand, all the ultrastructural changes observed in the activated endothelial cells in the capillaries are also nonspecific and are characterized as follows.

1) Irregular lumen plasma membrane with cytoplasmic protrusions.
2) Increase of organelles.
3) Increase of cytoplasmic vesicles.
4) Irregular abluminal plasma membrane with cytoplasmic protrusions.
5) Irregular and elongated intercellular junctions.
6) Increase in the chromatin density and distinct nucleoli with occasional mitosis.
7) Dense and irregular basement membrane.
8) Sproutings with new channel formation.

It is interesting that principal changes 1) to 6) in the arteries and in the capillaries are common, although changes in the arteries include relationships to the IEL, 7), and to the smooth muscle cells, 8), and changes in the capillaries include angiogenesis with sproutings, 7). Because it is clear that these changes in the endothelial cells observed in the electrically stimulated skeletal are compatible with angiogenesis, those in the endothelial cells during dilatory remodeling in the increased flow-loaded arteries may characterized as angiogenesis in the arteries. If so, capillary angiogenesis is characterized by the formation of new capillary channels, while the latter did not form new capillaries; however, angiogenesis and dilatory remodeling are common on the point of lumen widening.

On the other hand, angioregression observed in the previously electrically stimulated skeletal muscle may be compatible with a decrease of endothelial cells observed in the previously increased flow-loaded arteries. Studies on capillary regression are insufficient, however, capillaries decreased in number when stimulation ceased. Therefore, as we consider that activation of endothelial cells induced by increased flow is consistent with angiogenesis, inactivation of endothelial cells induced by decreased flow may be consistent with angioregression. We have not performed thorough experiments on the
relationship of the blood vessels to flow. However, it may be simple and easy to understand what the endothelial response is to flow alteration, if we assume that angiogenesis is the answer to the increased flow and angioregression to the decreased flow.

REFERENCES


