The Endothelial Cytoskeleton: Organization in Normal and Regenerating Endothelium*1

AVRUM I. GOTTIEB

The Toronto Hospital–General Division, Vascular Research Laboratory, 200 Elizabeth Street, CCRW 1-857, Toronto, Ontario, Canada M5G 2C4

ABSTRACT

The components of the endothelial cell cytoskeleton that have been shown to be important in maintaining endothelial structural integrity and in regulating endothelial repair include F-actin microfilament bundles, including stress fibers, and microtubules, and centrosomes. Endothelial cells contain peripheral and central actin microfilaments. The dense peripheral band (DPB) consists of peripheral actin microfilament bundles which are associated with vinculin adhesion plaques and are most prominent in low or no hemodynamic shear stress conditions. The central microfilaments are very prominent in areas of elevated hemodynamic shear stress. There is a redistribution of actin microfilaments characterized by a decrease of peripheral actin and an increase in central microfilaments under a variety of conditions, including exposure to thrombin, phorbol-esters, and hemodynamic shear stress. During reendothelialization, there is a sequential series of cytoskeletal changes. The DPB remains intact during the rapid lamellipodia mediated repair of very small wounds except at the base of the lamellipodia where it is splayed. The DPB is reduced or absent when cell locomotion occurs to repair a wound. In addition, when cell locomotion is required, the centrosome, in the presence of intact microtubules, redistributes to the front of the cell to establish cell polarity and acts as a modulator of the directionality of migration. This occurs prior to the loss of the DPB but does not occur in very small wounds that close without migration. Thus, the cytoskeleton is a dynamic intracellular system which regulates endothelial integrity and repair and is modulated by external stimuli that are present at the vessel wall–blood interface.

Keywords. Endothelium; cytoskeleton; actin; tubulin; centrosomes; microfilaments

INTRODUCTION

The pathogenesis of atherosclerotic lesions is closely associated with the structure and function of endothelial cells (106). Based on a solid foundation of traditional morphology, modern cell and molecular biology are providing information on structure function relationships, especially as they relate to thrombo-resistance and the macromolecular barrier function of endothelium. In addition to acting as a thrombo-resistant surface and macromolecular barrier, endothelial cells are also very active metabolically during the initiation and subsequent growth of the fibro-fatty plaques. Endothelial functions have been shown to be inducible (17) and important physiological and pathological interactions occur at the vessel wall–blood interface. Regulation of these interactions involves the constituents of the subendothelium (55), hemodynamic forces (18, 72), and many components of the blood, including platelets (65, 140), lipoproteins (36), coagulation factors (119, 120), fibrinolytic substances (80, 81), leukocytes (6, 21, 33) and the smooth muscle cells of the wall as well. The endothelial cells are active participants since they synthesize and secrete coagulation (5, 60) and anticoagulation factors (120), profibrinolytic factors (16), platelet antiaggregation factors (129), cytokines (79, 90), growth factors (24, 38), and many other important agents.

ENDOTHELIAL INTEGRITY

Although the sequence of events that lead to the initiation and growth of the fibro-fatty atherosclerotic plaque is not fully understood, the disruption of endothelial integrity is important in atherogenesis (106). Loss of integrity occurs due to overt structural changes and/or due to functional changes. Denudation of the endothelium may occur due to frank...
loss of endothelial cells, due to retraction of adjacent cells leaving endothelial gaps, or due to subtle changes in intercellular adhesion. Much less is known about the last category. The ability of the endothelial cells to adapt to these changes and to repair them once they occur is very important in the maintenance of endothelial integrity. The endothelial cytoskeleton consists of intracellular systems which are likely to regulate both the structural integrity of the endothelium and important repair processes.

**Endothelial Denudation**

Denuded endothelium, even only several cells wide, is not found in the normal endothelium (46). Previous reports of denudation are considered to be artefacts due to fixation and tissue processing. There is a need to control pressure, temperature, and osmolality during fixation; to avoid heparin; and to handle the specimens carefully post fixation to reduce artefacts (124). The endothelium which covers the surface of early atherosclerotic lesions in hyperlipidemic cynomolgous monkeys did not reveal degeneration, disruption, or loss of endothelial cells. Instead, structural adaptations were observed consisting of increased cellular organelles, attenuation and reshaping of cells and a decrease in the extent and complexity of lateral contact regions between adjacent endothelial cells (124). In other studies, however, the surface of prominent fatty streak lesions showed gaps which formed between endothelial cells covering the surface of the lesion (21). Denudation also occurs on the surface of well developed fibro-fatty plaques, especially when they are complicated by surface erosion and ulceration. When focal denudation does occur, it is most likely due to severe cell injury or cell death (47). Hemodynamic forces are unlikely by themselves to cause frank denudation under acute conditions (71), however, chronic hemodynamic abnormalities will result in denudation and slow repair (73). In a model of endotoxie endothelial injury in rats, it has been postulated that endothelial cells which die were lifted off from the monolayer by lamellipodia from neighboring cells which undermined the dead cells (98). This method of endothelial repair, thus, limits the area of frank denudation, minimizing the exposure of the subendothelium to the blood stream and is, thus, a very important method of rapidly restoring structural integrity.

**Endothelial Regeneration**

*In Vivo Models*

The endothelium is a single layer of flat, very slowly replicating cells (111), which show a high degree of density inhibition of growth. Orientation and cell shape are strongly influenced by blood flow (23, 72). Studies have been carried out *in vivo* to describe the events which occur following disruption of the contact-inhibited confluent endothelial cell monolayer. Since large areas of endothelial denudation may be present on the surface of well developed atherosclerotic plaques, many *in vivo* models consisted of denudation of large areas of aortic endothelium produced by an inflated balloon catheter (50, 110, 118), by air-drying (22), or other means. The media, however, was also inadvertently injured in many of these model systems with death of smooth muscle cells (100). These models did provide information on how the endothelium regenerated. Endothelial cells along the edge of the wound extended prominent lamellipodia, elongated, and began to translocate into the area of injury. The cells migrated as a continuous sheet of cells with very few cells at the leading edge migrating as single cells (110). This suggested that some cell-to-cell contacts were maintained during the process of reendothelialization. There was subsequent endothelial cell proliferation associated with this migration which provided new cells to replace those that were removed (109).

Once it became known that large areas of endothelial denudation do not occur *in vivo* in the normal endothelium, model systems were developed to produce very small denuding injuries. Controlled injury of the endothelium by a small fine surface, such as the tip of a thin nylon filament, was used to make small linear and circular wounds. Single wounds or multiple wounds could be made. These very small injuries are thought to closely mimic naturally occurring *in vivo* injury (97, 99). Within 8–10 hr following a denudation of 3–5 cells wide, the spreading and migration of endothelial cells adjacent to the wound resulted in a fully repaired wound. Proliferation was not required initially, although cell proliferation may have occurred later in order to restore the original number of cells (97). The rate of rapid repair of small injuries appeared to be independent of the presence of hypertension or hyperlipidemia (95). The effect of hemodynamic shear stress on small wound repair has also been examined using a co-artercation model and small *in vivo* wounds (73).

*In Vitro Models*

Tissue culture systems have been developed to study endothelial structure and function *in vitro* (34, 61). We have used the large *in vitro* wound model system to provide important information on the cellular processes involved in the repair processes. We utilize porcine aortic endothelial cells since the pig develops spontaneous atherosclerosis (45) and has a cardiovascular system similar to that of man (112). In the large experimental wound model (114),
porcine thoracic aortic endothelial cells behave in a manner similar to that seen in the in vivo large wound models. Once a wound is made in the confluent monolayer using a teflon spatula, the cells migrate as a sheet of cells, although each individual cell migrates on its own within the advancing monolayer. The endothelial cells at the leading edge extrude lamellipodia, elongate, and then migrate into the denuded area. This was similar to the finding of other in vitro (125, 127) and in vivo studies (50). By carrying out the wounding on confluent monolayers grown on glass coverslip, we have been able to study the cytoskeleton during repair. Quantitative measurements of rate of migration and cell proliferation can be easily made, live cell migration can be observed by time lapse cinemicrophotography (40), and cytoskeletal proteins can be localized using light- and electron-microscopic immunocytochemical and fluorescent methods. In addition, Madri (82) has carefully studied the role of extracellular matrix components including type I and III and type IV collagen, fibronectin and laminin on endothelial cell proliferation and sheet migration in similar models.

We have also developed and characterized an aortic organ culture model to study reendothelialization (39). Rectangular pieces of full thickness porcine aorta are cut out of the thoracic aorta with a scalpel blade away from any branch sites. Half of the endothelium is denuded lengthwise using a single gentle stroke of the scalpel blade in the direction of blood flow. The extent of denudation and the mode of repair is documented by scanning and transmission electron microscopy (39). Pederson and Boyer (88) also have reported on a rabbit aortic organ culture system to create small intimal wounds of defined width. The advantages of organ culture over cell culture include the presence of a substratum similar to that found in vivo, which is known to play an important role in endothelial cell migration (10), as well as the omission of a cell dispersion step which may lead to selection of particular cell phenotypes.

We have developed a unique single cell wound model of endothelial injury (137) (Fig. 1). To make the wound, a confluent monolayer grown on a glass coverslip is visualized under an inverted-phase microscope and a single endothelial cell is removed using gentle suction delivered by a micropipette attached to a micromanipulation system. All the endothelial cells around the periphery of the wound extrude lamellipodia into the area of denudation to close the wound within 30–40 min (Fig. 1). Once the wound closes it does not open. There is no cell translocation nor is there cell proliferation in this single cell wound model system.

A variety of soluble factors have been shown to effect reendothelialization in these model systems. TGFβ has been shown to delay endothelial repair within the first 24 hr following wounding (51). Fibroblast growth factor β is required for the migration of bovine aortic endothelial cells. Endogenously released βFGF not only promotes migration but also regulates the basal levels of plasminogen activator release and DNA synthesis (108). This autocrine role for βFGF may be mediated by release of βFGF from endothelial cells through mechanically induced cell membrane disruption (86). Whether these factors have direct or indirect effects on the cytoskeleton is not known. It is of interest that platelet derived growth factor, which is secreted by endothelial cells (19), does induce actin and vinculin reorganization and membrane ruffling in fibroblasts (52).

The Endothelial Cell Cytoskeleton

The 3 major cytoskeletal systems of the endothelial cell include microfilaments, microtubules, and intermediate filaments (8, 26, 62). The microfilaments and microtubules undergo rapid filament assembly and disassembly. They are regulated with respect to cross-linking and polymerization by many associated proteins (123). Although these systems are distinct with respect to structure, biochemical and immunological properties, and function, there are likely to be important interactions between them (20, 91, 116). Since the intermediate filaments are not as dynamic as the microfilaments and microtubules, and since they do not appear to be associated with the regulation of endothelial shape or motility, they will not be considered further.

Microtubules and Centrosomes Organization

The centrosomes of endothelial cells consist of the paracentral paired centrioles and the amorphous material around them. The centrosome is a microtubule organizing center, a site capable of initiating the polymerization of microtubules from tubulin both in vivo and in vitro (66, 93, 96). Microtubules emanate from the centrosomal area and are very prominent toward the center of the cell and less so at the periphery (62). The distribution of microtubules is similar in both low density and confluent cultures. Several studies have also explored the role of microtubules (2, 126) in cell migration. Badley et al (2), comparing the cytoskeleton in single migrating and stationary chick fibroblasts using immunofluorescence microscopy, concluded that the distribution of microtubules does not alter significantly during the conversion from the migratory to the stationary state. There are, however, studies that show that coordinated movement in one direction requires the presence of microtubules and that movement is either reduced (126), inhibited (35),
or can occur only randomly (7, 15, 31) when microtubules are disrupted as in colchicine-treated cells. Malech et al (83) showed that colchicine had no effect on random migration of human neutrophils; however, activated random migration was minimally decreased and directed migration was markedly inhibited. They also showed that the position of the centriole and its associated microtubules appear to be important in establishing the direction of migration of neutrophils. In other systems, the centrosome does not appear to direct migration (107).

**Actin Organization**

Actin, a contractile protein, is present in endothelial cells, as in most eucaryotic cells, in the filamentous form, F-actin, and the monomeric form, G-actin (67, 92). It is likely that the shift in equilibrium between the monomeric and the polymeric forms of actin is associated with many actin-mediated cell functions (29, 56). For example, studies comparing nonmigrating and migrating endothelial cells show that, in the latter, there is a shift in the ratio of G- to F-actin favoring G-actin while total actin remains unchanged (131). Filamentous actin is organized into a diffuse network of short microfilaments (94) and into prominent microfilament bundles (59, 78, 117). The diffuse network is present mainly in the cell cortex as well as in cellular processes such as lamellipodia (122). The microfilament bundles include several different types (11), including the stress fibers. The stress fiber bundles are composed of actin filaments in parallel alignment with nonuniform polarity (14, 37), and were first identified and studied in vitro. In addition to actin, the stress fibers contain myosin, tropomyosin, and alpha actinin (76, 77, 128), and are thought to be contractile and under mechanical stress (12, 13, 48, 122).

Microfilaments bundles have been considered important in providing the force of contraction for cell migration (68). In addition, it is likely that at least some of these bundles function as long substrate adhesion complexes, especially in well spread nonmigrating fibroblasts (115). The term stress fibers has been applied to these ventral microfilament

---

**Fig. 1.**—Phase contrast (A, B) and immunofluorescence (C) photomicrographs of closure of a single cell wound made in a confluent monolayer of porcine thoracic aortic endothelial cell. (A) Following wounding; (B, C) Closure of wound. Note that endothelial cells have not translocated; instead closure is by lamellipodia extrusion from adjacent cells. Note localization of tubulin to show that there is no redistribution of centrosomes. (Arrows mark same spot in an endothelial cell adjacent to wound.) × 740.
bundles since they are thought to be contractile and under mechanical stress, as the cell attempts to pull against a site of adhesion to the substratum (49). Studies have also shown, however, that in some systems microfilament bundles did not appear necessary for cell motility. In fact, when the bundles became more prominent cells became extremely flattened on the substratum and were non motile (9, 54). It is likely that there is an optimum number of stress fibers in a given cell to provide optimum conditions for migration.

**Endothelial Microfilament Bundles In Vivo**

The actin microfilament bundles have been identified in vessel wall endothelial cells from a variety of locations using transmission electron microscopy (27, 28) and more recently by in situ localization using immunofluorescence microscopy (103, 130, 132) (Fig. 2). The actin microfilaments can be localized by using antibodies to actin as well as by derivatives of phallacidin and phalloidin such as 7-nitrobenz-2-oxa-1,1-dia-zole (NBD) phallacidin or rhodamine phalloidin, a phallotoxin isolated from the amanita family of mushrooms that has a very high affinity for F-actin (4). Actin microfilaments bundles are located at the periphery of the cell as well as within the cell (Fig. 2a). In situ staining has shown that these microfilaments contain myosin and alpha actinin. Recently, we have developed a technique in which perfusion fixed aortas were stained in situ for F-actin by infusing rhodamine phalloidin via a peristaltic pump into the aortas at a slow flow rate. This new technique resulted in excellent visualization of branch points and allowed for a precise description of the actin microfilament bundles in endothelial cells along flow dividers (64). The actin microfilaments reorganize in response to changes in hemodynamic shear stress (63) (Fig. 2b) and show modifications in regions of atherosclerotic susceptible arteries prone to lesion formation (139).

**Endothelial Microfilament Bundles In Vitro**

We have shown that when endothelial cells form a confluent contact inhibited monolayer, the periphery of the cell contains prominent peripheral circumferential microfilament bundles which we have termed the dense peripheral band (DPB) as well as shorter central microfilament bundles (41) (Fig. 3A). In low density culture, even in islands of endothelial cells where there is cell to cell contact, a DPB was not formed (133). We have shown, using double immunofluorescence microscopy, that within this DPB there was colocalization of actin with myosin, with tropomyosin, with alpha actinin and with vinculin (Fig. 3A, B) (133). Although there were microtubules extending toward and into the DPB, there did not seem to be any preferential localization of microtubules within the band. Occasionally, microtubules were seen running parallel to the band along the inner aspect of the band. As noted above, in vivo actin microfilaments are also distributed as peripheral and central bundles. In the thoracic and abdominal aorta, the peripheral actin is less prominent than that observed in vitro in confluent cultures. The central bundles are similar being more prominent in the abdominal aorta and being oriented in the long axis of the cell. At areas where there is very low net shear stress, the cells are cobblestone and the morphology of the peripheral bands is similar to that seen in vitro.

Transmission electron microscopic examination of the DPB has shown that there were microfilaments which emanated from the band and extended into junctions which had cytoplasmic plaques. Often, the junctions of adjacent cells had microfilaments extending into their respective plaques and the microfilaments appeared to be in alignment with each other (133). Since they had actin microfilaments extending into them and since vinculin was present at the periphery of the endothelial cells associated with the DPBs, we postulate that these plaques are similar to an adherens junction (32). In addition, gap and tight junctions have been described in ultrastructural studies of aortic endothelium (58). Recently, monoclonal antibodies have been described which show a staining pattern on endothelium which suggests that the antigen is part of a junctional component (87).

**In Vitro Compared to In Vivo Microfilaments**

A comparison between the microfilament bundles that we observed in in vitro endothelial cells with those described in recent in vivo studies indicates many similarities. Prominent microfilament bundles were present at the cell periphery of aortic endothelial cells from several species (30, 132). Central microfilament bundles were present in those endothelial cells that were located in areas which were believed to be under increased hemodynamic stress. In vivo, actin-containing microfilaments were reduced at the periphery of aortic rabbit endothelial cells following balloon denudation, while central microfilament bundles (termed stress fibers by the authors) increased in the cells migrating into the wound (30, 105). Since central bundles persisted long after reendothelialization had ceased, and since they were also normally present in areas of high shear force, the authors concluded that these fibers were not directly related to cell movement but instead were involved in adherence of the endothelial cell to the substratum. Endothelial cells both in vivo (18, 72) and in vitro (25, 53) are indeed responsive...
to hemodynamic shear stress and undergo changes in shape and orientation (18, 72) and in cytoskeleton (18, 25, 53) in response to altered flow. In an in vivo ultrastructural study (57) it has been shown that there is an increase of microfilament bundles (stress fibers) in the cytoplasm of regenerating endothelial cells as compared to normal resting endothelial cells. These microfilament bundles are connected to...

Fig. 3.—Immunofluorescence and fluorescence localization of endothelial cytoskeletal proteins in a confluent monolayer (A, B) and in a closing wound (C, D). (A, C) Actin microfilaments localized with rhodamine phalloidin and photographed...
by focusing on vinculin. Note dense peripheral band (large arrowhead) and central microfilaments (small arrowhead, some out of focus). (B, D) Vinculin plaques localized by antibody to vinculin (large arrow). Note reduction in peripheral actin (small arrow) and associated vinculin (small arrow) during the translocation of cells to close a wound. \( \times 540 \).
membrane domains located exclusively at the abluminal aspect of endothelial cells. There are subplasmalemmal microfilament condensations at the lateral endothelial cell membrane in relation to tight junctions, but microfilament bundles were not shown to occur in association with these lateral membrane condensations. Actin stress fiber modification \textit{in vivo} is associated with compromised endothelial barrier function and coincides with the accumulation of low density lipoproteins in areas of intimal thickening (3).

The Regulation of the DPB

The DPB can also be regulated by external factors including thrombin, phorbol 12-myristate 13-acetate (TPA) (135), ethchlorvynol (138) and histamine (137). These agents promote the reversible breakdown of the DPB in the intact confluent monolayer. We have reported that incubation of confluent monolayers of endothelial cells with thrombin resulted in the reversible loss of the DPB and was associated with a change in the shape of the cells from cobblestone to elongated. Associated with the DPB disruption, peripheral vinculin plaques were also lost only to reappear in association with the return of the DPB following wash out of thrombin. It is possible that thrombin may enhance the rate of repair by promoting rapid reversible reorganization of microfilaments similar to the pattern present in migrating cells. Wysolmerski et al (138) showed that the cause of reversible pulmonary edema by ethchlorvynol may be related to the loss of the DPB which results in loss of endothelial integrity. Shasby et al have proposed that the cytoskeleton is important in the regulation of endothelial permeability (113). Recently, recombinant tumor necrosis factor and immune interferon caused human endothelial cells to rearrange their actin cytoskeleton so that the DPB was lost and central microfilament bundles became prominent (121). Hyperoxia is associated with alterations of actin distribution in endothelial cells (89). DPB becomes disrupted or lost and stress fibers are increased in number and thickness. Total actin remained the same while F-actin was increased. Thus, the DPB is sensitive to soluble factors which may in some cases, such as thrombin, be present at the site of endothelial injury. The nature of this effect and whether it is direct or indirect await further study.

Reendothelialization

Microfilaments

The large wound experimental model system was used to study the changes in the F-actin cytoskeleton during reendothelialization. We found that 4 zones were present at the wound edge each with characteristic features (41). Cells in the first 2 zones, the leading edge and elongated zone, showed absent or markedly reduced DPBs and prominent migration while the other 2 zones had intact DPBs and the cells did not migrate. Thus, the presence of the DPB was associated with a marked reduction or inhibition of cell migration.

Studies on the single cell wound model showed that following removal of the cell the part of the DPB facing the wound showed some splaying and became more prominent (136). Microfilaments were seen to emanate from the band into the lamellipodia. Wounds incubated with cytochalasin B at concentrations which caused loss of the DPB with sparing of the central microfilament showed very little closure after a period of 6 hr. Complete closure occurred normally between 30-40 min. However, when the cytochalasin was washed out, the microfilaments of the DPB began to reappear immediately, and were associated with lamellipodia formation. Thus, reendothelialization, occurring by spreading, requires an intact DPB; however, the centrosome was not involved since centrosomal redistribution did not occur.

Centrosomes and Microtubules

The function of the centrosome (1) during endothelial regeneration has been described using the large wound experimental \textit{in vitro} model system (42, 44). We have shown that endothelial cells migrating into the wound rapidly redistributed their centrosomes to the front of the cell between the nucleus and the leading lamellipodia. If the wound was treated first with colcemid to breakdown microtubules, redistribution did not occur. We have also shown the redistribution occurred independent of cell migration since wounds treated with cytochalasin B at

---

**Fig. 4.**—Phase contrast photomicrograph of porcine aortic endothelial cells observed sequentially during closure of a small in vitro wound. (A) zero time, (B) 30, (C) 60, (D) 90, (E) 120 and (F) 150 min. Small arrows in (C) and (D) demarcate the margins of the wound and large arrows in (B, F) identify the location of the centrosome, an organelle associated with maintaining directionality of endothelial cell migration. Bar = 20 μm. (Modified from Wong and Gotlieb: \textit{J. Cell Biol.} 107: 1777, 1988 (135).)
concentrations which just inhibited migration still showed centrosomal redistribution. This redistribution, however, occurred more slowly than under normal conditions suggesting that interactions between microtubules and microfilaments may play some role in enhancing centrosome redistribution (42).

Since the distribution of various cytoskeletal components may change when cells are removed from their in situ environment and grown in vitro (104), we verified our in vitro observations with in vivo experiments. We showed that centrosome redistribution occurred not only in tissue culture but also in organ culture (102) as well as in vivo following wounding (101). The orientation occurred most rapidly in the tissue culture model suggesting that the subendothelial matrix and hemodynamic factors may act to regulate centrosomal distribution. Using bovine aortic endothelial cells, centrosomal redistribution was shown to be enhanced by several factors including serum, multiplication stimulating factor, and insulin (84, 85). Although platelet derived growth factor had no effect on its own, it had a stimulatory synergistic effect with sub-effective doses of serum, insulin, and multiplication stimulating factor. These studies are important since they show that cytoskeletal events occurring during endothelial regeneration can be regulated by soluble external factors.

Centrosomal redistribution has been shown to occur in a variety of other cell systems under a variety of conditions (69, 70). In endothelial cells, it appears that reorientation is important in the initiation and regulation of migration and may, thus, act as a pre-programmed internal control. It has been suggested that since the Golgi apparatus redistributes along with the centrosome, the function of centrosomal redistribution is to provide new membrane for extruding lamellipodia during cell migration (69).

What induces centrosomal redistribution? It appears to be triggered when the cell receives an external signal that migration is eminent. Within the confluent endothelial monolayer, we have seen centrosomal redistribution occurring well before the break down of the DPB in cells away from the leading edge (43). Although these cells were not migrating, they received some type of signal to prepare for directed migration. Two likely possibilities are that the signal is a physical stimulus which might occur when the monolayer becomes less tightly packed, or a chemical signal passed through gap junctions (74, 75).

The mechanism by which the centrosome moves toward the front of the cell is also not known. As noted previously, centrosomal redistribution requires intact microtubules and appears to be delayed somewhat if the microfilaments are disrupted (42). One possibility is that there are direct or indirect connections between the microtubules and the DPB with the band acting to anchor the microtubules as the centrosome redistributes. Using time lapse cine-microphotography, we have noted that in some cases the centrosome redistributed independently of any detectable nuclear movement. In other instances, however, it appeared that centrosomal redistribution was associated with rotation of the nucleus, so
that they appeared to rotate together in the same direction.

The Sequential Steps in Endothelial Monolayer Repair

To characterize the full spectrum of cytoskeletal events occurring during small wound repair, the in vitro model was used to compare wounds of 1–4 cells with those of 6–12 cells (134). The former closed rapidly within an hour, as described above, and the latter closed from 3–6 hr (Fig. 4). The repair process was observed by time-lapse cinemicrophotography. Using fluorescence and immunofluorescence microscopy, the cellular morphological events were correlated with the localization and distribution of actin microfilament bundles and vinculin plaques, and centrosomes and their associated microtubules. Single to 4-cell wounds underwent closure by cell spreading while wounds 7–9 cells in size closed by initially spreading which was then followed at ~1 hr after wounding by cell migration. These 2 processes showed different cytoskeletal patterns. Cell spreading occurred independent of centrosome location. However, centrosome redistribution to the front of the cell occurred as the cell began to elongate and migrate (Fig. 5A). While the DPB persisted during cell spreading, it broke down during migration and was associated with a reduction in peripheral vinculin plaque staining (Figs. 3C, D, and 5B). Thus, the major events characterizing the closure of endothelial wounds were precise in nature, followed by a specific sequence, and were associated with specific cytoskeletal patterns which most likely were important in maintaining directionality of migration and reducing the adhesion of cells to their neighbors within the monolayer.

ACKNOWLEDGMENTS

This work was supported in part by Medical Research Council Grant MT-6485 and Heart and Stroke Foundation of Ontario Grant T1259. Photography was carried out by MKK Wong and DW Kim. Secretarial work was carried out by Sursattie Sarju.

REFERENCES

18. Dewey CF, Bussolari SR, Gimbrone MA, and Davies PF (1981). The dynamic response of vascular...


50. Haudenschild CC and Schwartz SM (1979). Endo-
enthelial regeneration. II. Reconstitution of endothelial continuity. Lab. Invest. 41: 407-418.


115. Singer II (1982). Association of fibronectin and vin-


