Scanning Microscopy

Volume 1 | Number 4 Article 22

8-21-1987

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Gotlieb, Avrum I.; Wong, Michael K. K.; Boden, Patricia; and Fone, Alan Choo (1987) "The Role of the Cytoskeleton in Endothelial Repair," *Scanning Microscopy*: Vol. 1 : No. 4 , Article 22.
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Scanning Microscopy, Vol. 1, No. 4, 1987 (Pages 1715-1726) 0891-7035/87$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

THE ROLE OF THE CYTOSKELETON IN ENDOTHELIAL REPAIR

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(Received for publication March 19, 1987, and in revised form August 21, 1987)

Abstract

The injured endothelium undergoes rapid repair of areas of cell desquamation in order to maintain the structural integrity of the endothelial surface. Endothelial repair involves a series of processes which include endothelial cell spreading, translocation, and proliferation. These processes are well defined events which occur sequentially in time. Spreading and translocation are mediated by the cell cytoskeleton - F-actin microfilaments and microtubules and associated centrosomes. The regulation of these processes is complex and is likely due to soluble factors present at the site of injury which are released from activated endothelial cells, platelets, the subendothelial substratum, activated serum factors, and hemodynamic shear stress. Cell replication occurs in order to replace lost cells and maintain the appropriate cell density of the endothelial surface. The factors which regulate endothelial cell proliferation are currently under study.

Key Words: Atherosclerosis, microfilaments, endothelium, microtubules, centrosomes, cytoskeleton, cell locomotion, wound repair, actin.

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Introduction

An understanding of the pathogenesis of the fibrofatty atherosclerotic lesion requires knowledge about the function of the vessel wall cells in health and disease. Due to the ability to harvest and grow large vessel endothelial cells in culture, studies carried out in the last 15 years have begun to show the mechanisms by which endothelial cells play a very important role in atherogenesis (Thorgeirsson and Robertson 1978; Ross 1986). It has become apparent that in addition to acting as a thromboresistant surface and macromolecular barrier, these cells are very active metabolically during the initiation and subsequent growth of the plaques. In addition, many endothelial functions have been shown to be inducible (Cotran et al 1986) and important interactions occur at the vessel wall-blood interface that regulate endothelial cell function. Involved in these processes are the subendothelium (Madri et al 1980), hemodynamic forces (Nerem and Cornhill 1980; Dewy et al 1981; Langille and Adamson 1981), and many constituents of the blood, including platelets (Kinlough-Rathbone et al 1983), lipoproteins (Levy 1981), coagulation factors (Engelberg 1985), fibrinolytic substances (Loskutoff and Edgington 1977) and leukocytes (Gerrity 1981; Faggiotto et al 1984; Bevilacqua et al 1985). In addition, the endothelial cells are active participants in many of the above processes since they synthesize and secrete coagulation (Jaffe 1977, Bevilacqua et al 1984) and anticoagulation factors (Stenflo 1984), profibrinolytic factors (Comp and Esmon 1981), platelet antiaggregation factors (Weksler et al 1977) and many other important agents.

Endothelial Integrity

Although the sequence of events that lead to the development and growth of the fibrofatty atherosclerotic plaque are not yet fully known, the role of the breakdown in endothelial integrity has been considered to be very important in the initiation of the lesion (Ross et al 1977; Ross 1986). Loss of integrity may

occur due to denudation of the endothelium 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 resist these changes and to repair them once they occur is very important in the maintenance of endothelial integrity. Since the components of the endothelial cytoskeleton are very good candidates for intracellular structures which regulate the structural integrity of the endothelium emphasis will be placed on the role of actin microfilaments, microtubules and centrosomes in repair. Since endothelial proliferation is also a very important component of the reendothelialization process, endothelial cell replication will also be discussed.

Endothelial Denudation

Current data suggests that denuded endothelium, even only several cells wide, is not found in the normal endothelium (Hansson and Schwartz 1983a). Previous findings which showed areas of denudation are considered to be artefacts due to fixation without perfusion at physiological pressures and due to tissue processing. The surface of prominent fatty streak lesions, however shows both gaps formed between endothelial cells covering the lesion as well as focal prominent denudation (Faggiotto and Ross 1984). There is also denudation on the surface of well developed fibrofatty plaques, especially when they are complicated by surface erosions and ulceration. When focal denudation does occur (Ross et al 1977), it is most likely due to severe injury or cell death (Hansson and Schwartz 1983b). Hemodynamic forces are unlikely by themselves to cause frank denudation under acute conditions (Langille 1984) however, chronic hemodynamic abnormalities will result in denudation and slowed repair (Langille et al 1986). In a model of endotoxic endothelial injury in rats, it has been postulated that endothelial cells which die were lifted off from the monolayer by lamellipodia from adjacent cells which undermined the dead cells (Reidy and Schwartz 1983). 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 endothelial integrity.

Endothelial Regeneration

The endothelium is a single layer of very slowly replicating cells (Schwartz and Benditt 1973), which show a high degree of density inhibition of growth. Cell shape and orientation are strongly influenced by flow (Flaherty et al 1972; Langille and Adamson 1981). Studies have been carried out in vivo to describe the events occurring following disruption of the contact-inhibited confluent endothelial cell monolayer. Since large areas of endothelial denudation are present over atherosclerotic plaques, many in vivo models

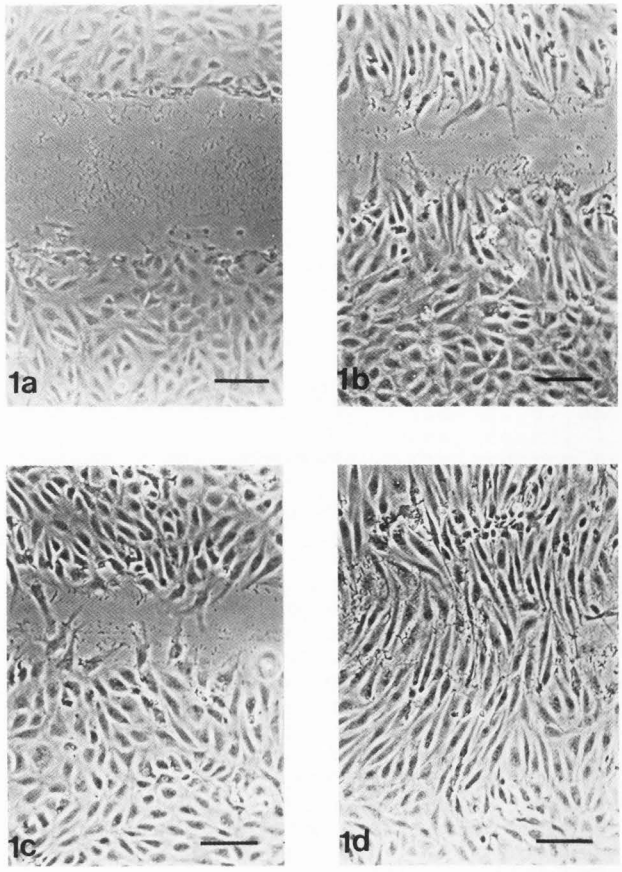


Figure 1 - Phase contrast photomicrograph of a linear 150 μm wound made in a confluent monolayer of porcine aortic endothelial cells. Wound closes by spreading and migration of cells at the wound edge. a) 0 time b) 8 h c) 12 h d) 18 h. Bar (a-d) 100μm.

consisted of large areas of aortic endothelial denudation produced by an inflated balloon catheter (Baumgartner 1963; Stemerman et al 1977; Schwartz et al 1978; Haudenschild and Schwartz 1979), by air-drying (Fishman et al 1975), or other means. The media was also inadvertently injured in these models systems (Reidy and Silver 1985). Scanning electron microscopic studies have shown that the endothelial cells along the edge of the wound extended prominent lamellipodia, elongated and began to migrate into the area of injury. The endothelium migrated as a continuous sheet of cells with very few cells at the leading edge migrating as single cells (Schwartz et al 1978). This suggested that 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 (Schwartz et al 1980).

We have used large in vitro wound model systems to provide important information on the cellular processes involved in repair and the

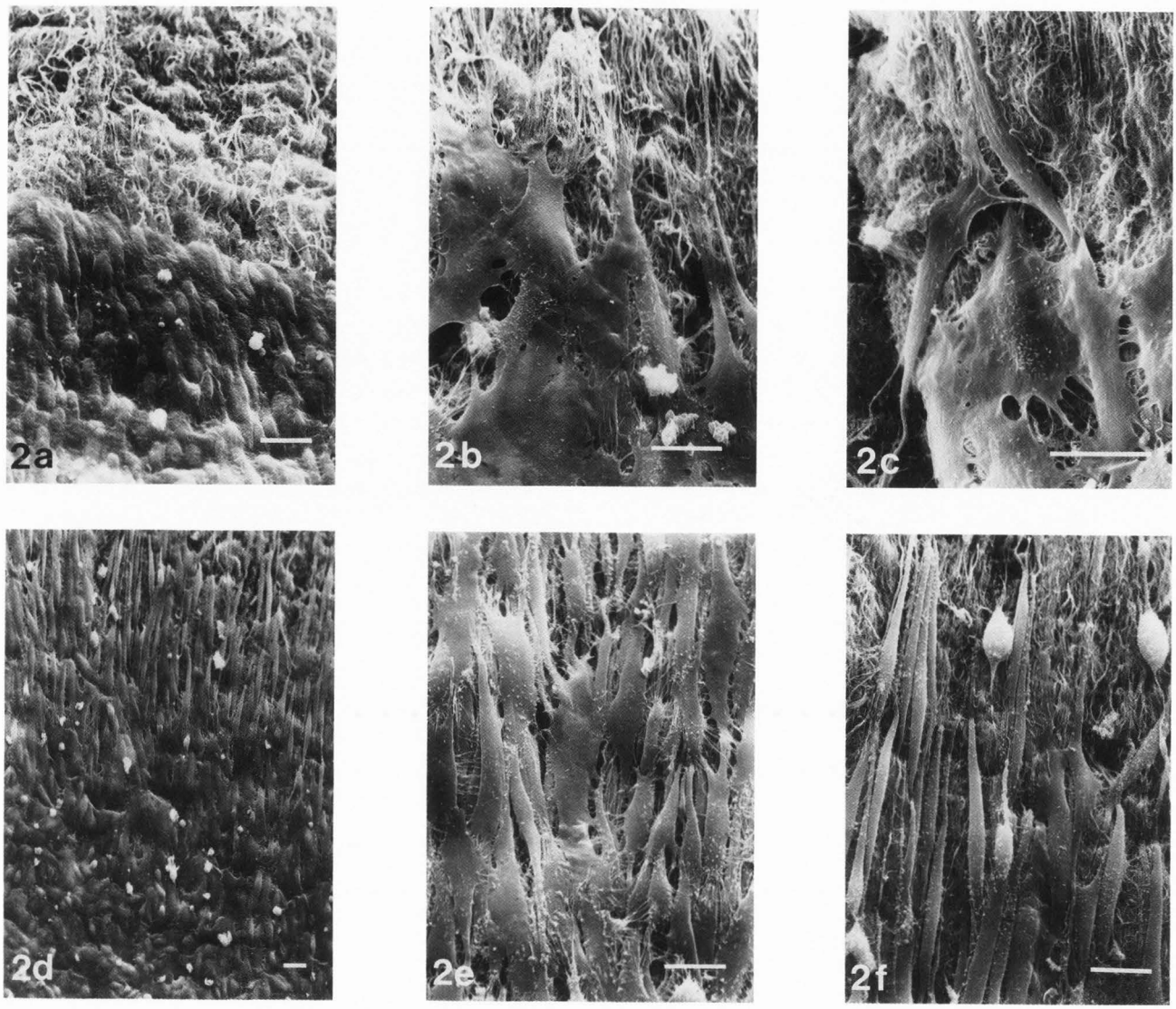


Figure 2 - Scanning electron microscopic photomicrograph of porcine thoracic aortic organ culture following endothelial denudation. a) Time 0 at the wound edge; b) 1 day at wound edge. Note retraction of cells and lamellipodia formation (l); c) 3 days at wound edge. Note elongation of cells as they move into wound; 7 days d) at original wound edge; e) middle of covered wound; f) the leading edge showing cells advancing into the wound. Bar (a-f) 10μm.

regulation of these processes. We have used porcine aortic endothelial cells since the pig develops spontaneous atherosclerosis (Gottlieb and Lalich 1954) and has a cardiovascular system similar to that of man (Scott et al 1979). In the large experimental wound model (Sholley et al 1977), porcine thoracic aortic endothelial cells migrated as a sheet of cells, although each individual cell migrates on its own within the advancing monolayer. The endothelial cells at the leading edge extruded lamellipodia, elongated and then migrated into the denuded area (Figure 1). This was similar to the findings of other in vitro (Wall et al 1978; Thorgeirsson et al 1979) and in vivo studies (Haudenschild and Schwartz 1979). By carrying out the wounding on confluent monolayers grown on glass coverslips 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 (Gotlieb and Spector 1981), and cytoskeletal proteins can be localized using light and electron-microscopic immunocytochemical and fluorescent methods.

We have also characterized an aortic organ culture model to study reendothelialization (Gotlieb and Boden 1984). Rectangular pieces of full thickness porcine aorta were cut out of the thoracic aorta with a scalpel blade away from any branch sites. Half of the endothelium was denuded lengthwise using a single gentle stroke of a scalpel blade in the direction of blood flow. The mode of repair was documented by scanning and transmission electron microscopy (Gotlieb and Boden 1984) (Figures 2,3). Pederson and Boyer (1985) 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 (Buck 1979), as well as the omission of a cell dispersion step which may lead to selection of particular cell types.

Since large areas of endothelial denudation have not been shown to occur in vivo, model systems using controlled injury of the endothelium by a small fine surface, such as the tip of a thin nylon filament, have been employed to make very small denuding injuries which closely mimic naturally occurring in vivo injury (Reidy and Schwartz 1981; Ramsay et al 1982). These studies have shown that within 8-10 h areas of denudation of 3-5 cells wide were repaired by the spreading and migration of endothelial cells adjacent to the wound. Proliferation was not required initially, although cell proliferation may have occurred later in order to restore the same number of cells as previously (Ramsay et al 1982). The rate of rapid repair of small injuries appeared to be independent of the presence of hypertension or hyperlipiemia (Prescott and Muller 1983). In culture the rate of repair of small linear wounds made in confluent monolayers was independent of serum concentration and did not depend on the presence of platelet releasate (Choo Fone and Gotlieb unpublished).

We have developed a unique single cell wound model of endothelial injury (Wong and Gotlieb 1984). To make the wound, a confluent monolayer grown on a glass coverslip was visualized under an inverted-phase microscope and a single endothelial cell was removed using gentle suction delivered by a micropipette attached to a micromanipulation system. All the endothelial cells around the periphery of the wound extruded lamellipodia into the area of denudation to close the wound within 30-40 minutes. There was no cell translocation nor was there cell proliferation in this model system. However, if more than 5 cells were removed then cell translocation was also required to close the wound.

The Endothelial Cell Cytoskeleton

The three major fibrous protein systems of the endothelial cell include microfilaments, microtubules and intermediate filaments (Kalnins et al 1981) (Figure 4). These systems are dynamic and microfilaments and microtubules undergo rapid filament assembly and disassembly. They are regulated with respect to cross-linking and polymerization by many associated proteins (Pollard et al 1984, Stossel et al 1985). Although referred to as the cell cytoskeleton these systems are distinct with respect to biochemical structure, immunological properties and function. There is however likely to be important interactions between these cytoskeletal systems (Singer et al 1981, Pollard et al 1984, Euteneuer and Schliwa 1985).

Actin microfilaments

Microfilament bundles (Stossel 1984) have been considered important in providing the force of contraction for cell migration (Kreis and Birchmeier 1980). In addition, it is likely that at least some of these bundles function as long substrate adhesion complexes, especially in well spread non migrating fibroblasts (Singer 1982). The term stress fibers has been applied to these ventral microfilament 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 (Burridge 1981). 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 nonmotile (Bradley et al 1980; Herman et al 1981). It is likely that there is an optimum number of stress fibers in a given cell to provide optimum conditions for migration.

The actin microfilament bundles have been identified in vessel wall endothelial cells from a variety of locations using transmission electron microscopy (Gabbiani et al 1975; 1979) and more recently by in situ localization using immunofluorescence microscopy (Wong et al 1983; White et al 1983; Rogers and Kalnins 1983a) (Figure 5). 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 (Barak et al 1980). Actin microfilaments bundles are located at the periphery of the cell as well as within the cell. In situ staining has shown that these microfilaments contain myosin and alpha actinin.

We have shown that when the 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 (Gotlieb et al 1984; Wong and Gotlieb 1984) (Figure 6). In low density culture, even in islands of endothelial cells where there is cell to cell contact, a DPB was not formed (Wong and Gotlieb, 1986). We have shown using double immunofluorescence microscopy that within this DPB there was a colocalization of actin with myosin, with tropomyosin, with alpha actinin and with vinculin (Wong and Gotlieb, 1986). We have also shown that 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. 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 (Figure 7A). Often the junctions of adjacent cells had microfilaments extending into their respective

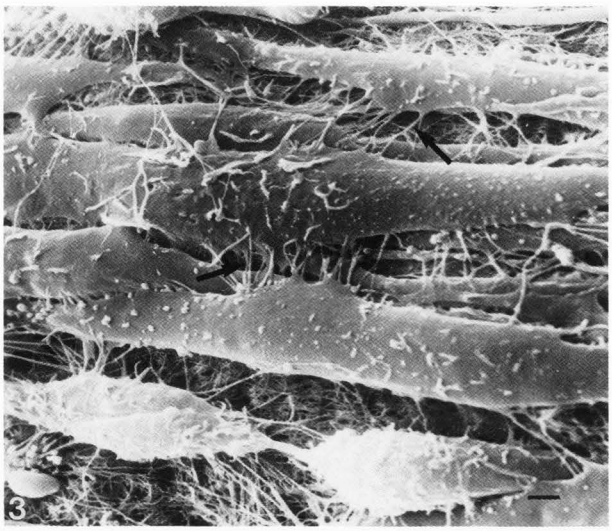


Figure 3 - Scanning electron microscopic photomicrograph of porcine aortic organ culture within covered area of wound at 4 days after wounding; Note numerous filopodia (arrow) forming contacts between adjacent cells. Bar 1 μm.

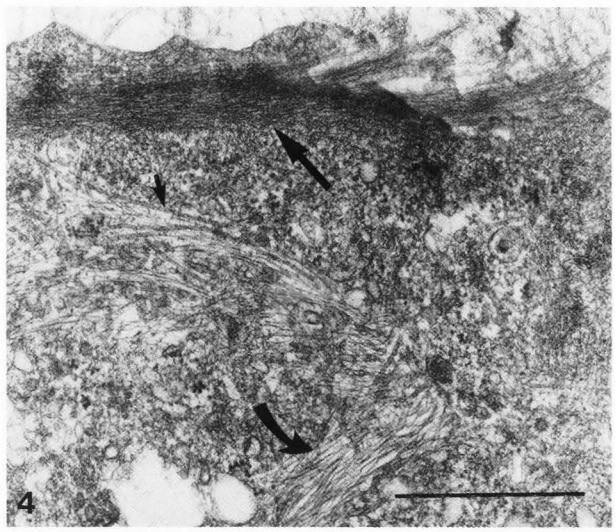


Figure 4 - Electron photomicrograph of a porcine aortic endothelial cell in monolayer culture. Note microfilaments (arrow), microtubules (small arrow) and intermediate filaments (curved arrow). Bar 1 μm.

plaques and the microfilaments appeared to be in alignment with each other (Figure 7B) (Wong and Gotlieb, 1986). At the present time the nature of these junctions is not fully known. Since they had actin microfilaments extending into them and since vinculin was present at the periphery of the endothelial cells associated with the DPB's (Wong and Gotlieb 1986), we postulate that these plaques may be similar to an adherens junction (Geiger et al 1983). In his ultrastructural study on aortic endothelium, Huttner et al. (1973) showed that the endothelial cells are interconnected by gap and tight junctions. Adherens junctions were not described to occur between these cells. This will have to be verified using cytohistochemical markers for the different types of junctions.

Microtubules and centrosomes

The centrosomes of endothelial cells consist of the paracentral paired centrioles and the amorphous material around them. The centrosome is a microtubule organizing center. Microtubules emanate from the centrosomal area and are very prominent toward the center of the cell becoming thinner in density toward the cell periphery (Kalnins et al 1981). The distribution of microtubules is similar in both low density and confluent cultures.

Intermediate Filaments

Endothelial cells contain intermediate filaments and these have been well described elsewhere (Franke et al 1979; Blose and Meltzer 1981). Their physiological role in endothelial cell function is not known. It is believed that intermediate filaments have a mechanical role in cell function (Lazarides 1980). They are not however as dynamic as the microfilaments and microtubules. At the present time, it is not clear that these filaments have a role in endothelial motility. For instance, antibodies to intermediate filaments injected into

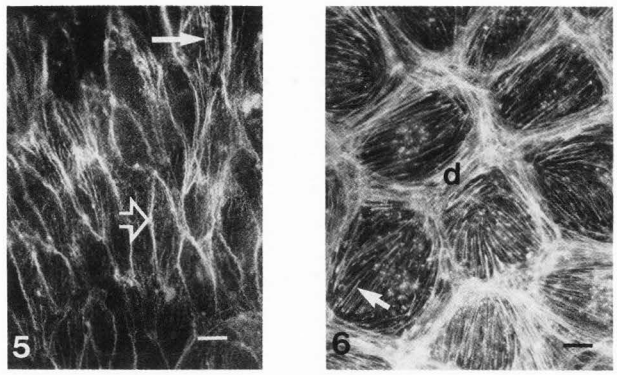


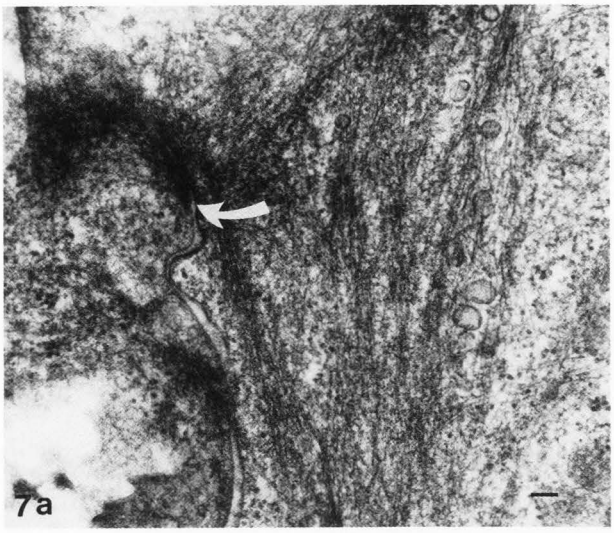
Figure 5 - Photomicrograph of in-situ localization of F-actin microfilaments in the endothelial cells of the rabbit thoracic aorta using rhodamine-phalloidin. Note both peripheral (open arrow) and central (arrow) microfilament bundles. Bar 10 μm.

Figure 6 - Photomicrograph of a confluent porcine aortic endothelial cell monolayer stained with rhodamine-phalloidin to localize F-actin. Note the presence of the DPB (d) and central microfilament bundles (arrow).Bar 10 μm.

fibroblasts induce the filaments to coil around the nucleus, however, locomotion and cell morphology are unaltered (Lin and Feramisco 1981; Gawlitta et al 1981).

Centrosomes and Microtubules in Rendothelialization

The function of the centrosome during endothelial regeneration has been described using the large wound experimental in-vitro model system (Gotlieb et al 1981; 1983). We have shown that endothelial cells migrating into



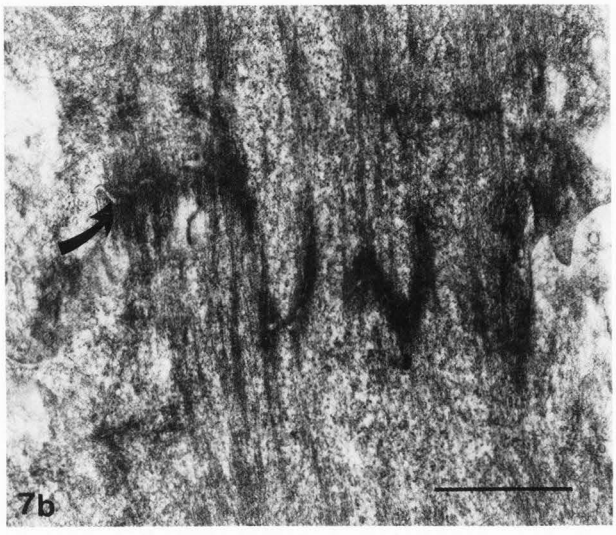
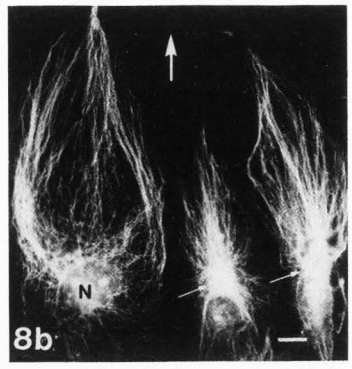


Figure 7 - Electron photomicrograph of a monolayer of porcine aortic endothelial cells showing the periphery of two adjacent cells (a,b). (a) Note the microfilaments of the DPB and the associated intercellular junctions (curved arrow). (b) Note that many of the microfilament bundles inserting into the junctions of the adjacent cells are in alignment with each other. Bar (a) 100 nm. Bar (b) 1 μm.



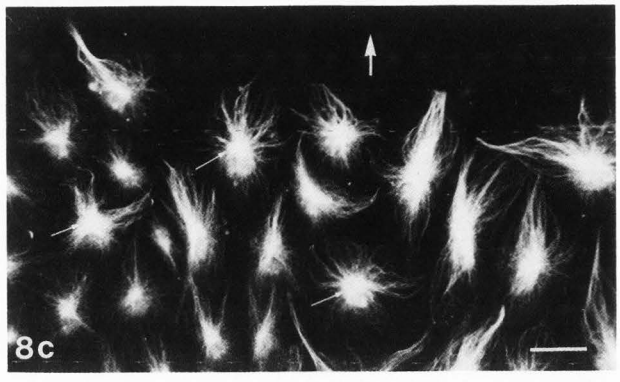


Figure 8 - Immunofluorescent photomicrograph of endothelial cells at the wound edge stained with antitubulin serum immediately after wounding before migration begins (a) and 44 h after wound (b and c). Note that the centrosomes (small arrows) which initially are randomly distributed relative to the wound edge and the position of the nuclei (a) become oriented so that they face the wounded area towards which the endothelial sheet is migrating (b and c). Large arrow is perpendicular to the wound edge and indicates direction of movement of endothelial cell sheet. Bar (a,b) 10 μm. Bar (c) 100 μm.Courtesy of the Journal of Cell Biology. The Rockefeller University Press. (Gotlieb et al, (1981) 91:589-594).

the wound rapidly redistributed their centrosomes to the front of the cell between the nucleus and the leading lamellipodia (Figure 8) (Gotlieb et al 1983). If the wound was treated first with colcemid to break down microtubules redistribution did not occur. We have also shown that redistribution occurred independent of cell migration since wounds treated with cytochalasin B at concentrations which just inhibited migration still showed centrosomal redistribution (Gotlieb et al 1983). 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 (Griffith and Pollard 1982).

Since the distribution of various cytoskeletal components may change when cells are removed from their in situ environment and grown in vitro, (Rogers and Kalnins 1983b) 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 (Rogers et al 1986) as well as in-vivo following wounding (Rogers et al 1985). The reorientation 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 (Mascardo and Sherline 1984). 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 (Malech et al 1977; Albrecht-Buehler and Bushnell 1979; Kupfer et al 1982; 1983). In endothelial cells it appears that reorientation is important in the initiation and regulation of migration and may thus act as a preprogrammed 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 (Kupfer et al 1982).

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 (Wong and Gotlieb 1985b). These cells were not migrating, however, they received some type of a signal from the cells in front of them which were migrating. The signal may be a physical stimulus as might occur when the monolayer becomes less tightly packed, or a chemical signal passed through gap junctions.

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 broken down (Gotlieb et al 1983). 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 centrosomes redistribute. Using time lapse cinemicrophotography, 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 (Akkor and Gotlieb 1984).

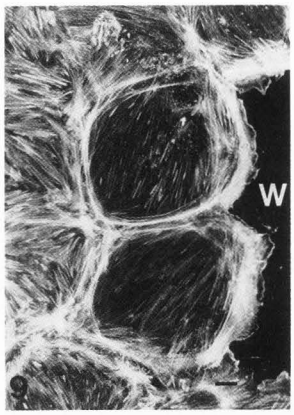
Microfilaments in Reendothelialization

The large wound experimental model system was used to study the changes in the F-actin cytoskeleton during reendothelialization. We found that four zones were present at the wound edge each with characteristic features (Gotlieb et al 1984). Cells in the first two zones, the leading edge and elongated zone, showed absent or markedly reduced DPB's and prominent migration while the other two zones had intact DPB's and the cells did not migrate. Thus the presence of the DPB was associated with a marked reduction or inhibition of cell migration (Figure 9).

A comparison between the microfilament bundles that we observed in in vitro endothelial cells with those described in recent in vivo studies indicate many similarities. Prominent microfilament bundles were present at the cell periphery of aortic endothelial cells from several species (Wong et al 1983; Gabbiani et al 1983). 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(Gabbiani et al 1983). 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 and in vitro are indeed responsive to hemodynamic shear stress and undergo changes in shape and orientation in response to altered flow (Langille and Adamson 1981). In an in vivo ultrastructural study, Huttner et al. (1985) have 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 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.

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 (Figure 10). 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 h. Complete closure occurred normally between 30-40 minutes. However, when the cytochalasin was washed out, the microfilaments of the DPB began to reappear immediately, associated with lamellipodia formation. Thus, reendothelialization occurring by spreading requires an intact DPB, while repair involving cell translocation requires the breakdown of the DPB.

The DPB can also be regulated by external factors including thrombin phorbal 12-myristate 13-acetate (TPA) (Wong and Gotlieb 1985a) and ethchlorvynol (Wysolmerski et al 1984). These



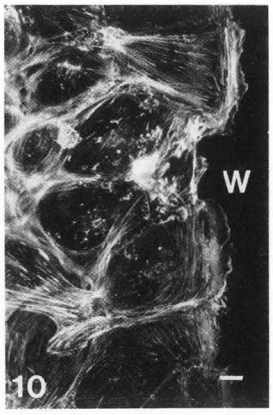


Figure 9 - Photomicrograph of endothelial cells stained with rhodamine-phalloidin at the edge of a large linear wound (W) 2 h after wounding. Note the reduction in the DPB in the first row of cells along the wound edge. Bar 10 μm.
Figure 10 - Photomicrograph of endothelial cells at the edge of a small in vitro wound (W) (6 cells wide) a half hour after wounding. The cells are stained with rhodamine-phalloidin to localize F-actin microfilament bundles. Note the extrusion of actin containing lamellipodia and the presence of the DPB. Bar 10 μm.

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 (Wong and Gotlieb 1985a). Wysolmerski et al (1984) 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. 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 (Stolpen et al 1986). 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.

Endothelial Cell Proliferation in Reendothelialization

An important component of endothelial regeneration is the proliferation of endothelial cells to replace lost cells. There are focal areas of increased turnover in areas of the aorta which are prone to atherosclerotic lesions suggesting that there is cell loss in these areas. Studies have been done using both small and large in vivo wounds as well as large cell culture in vitro wounds to study cell proliferation. In cell culture, proliferation occurred about 16-20 hours following wounding and was most pronounced at the wound edge (Schwartz et al 1979). The dividing cells remained attached to the monolayer and there were few free endothelial cells in the wound edge. Proliferation was markedly reduced in the monolayer away from the wound edge. In in vivo wounds proliferation began at 24 h after injury at the wound edge and was present in the monolayer well away from the edge as well. Thus, cells well away from the edge were receiving a signal to initiate DNA synthesis and cell mitosis. This may be associated with information transmitted via gap junctions which are well developed in these cells (Larson and Sheridan 1982). In small wounds, cell proliferation occurs after cell spreading and cell translocation. A wound may close prior to focal replication to replace the lost cells. In areas distal to coarctations, there is no visible denudation, however increased cell turnover occurs after a few weeks indicating that there must be cell loss and subsequent proliferation (Langille et al 1986).

Endothelial cells in culture do not respond very well to growth factors (Schwartz et al 1979). Although endothelial cells in monolayer culture or at the wound edge require high concentrations of serum for optimum growth many growth factors, including platelet derived growth factor, fibroblast growth factor, insulin and epidermal growth factor did not enhance the effect of low concentrations of serum on cell proliferation. Endothelial cells form platelet derived growth factor in culture (DiCorleto and Bowen-Pope 1983) although in vivo non-injured endothelial cells contain very low levels of mRNA for platelet derived growth factor (Barrett et al 1984). This may be important in the regulation of smooth muscle cell proliferation. Thus the factors governing the initiation of endothelial cell replication are not known. Work however has been done to define the mechanisms which inhibit endothelial cell growth once the confluent monolayer is formed. Surface proteins have been identified in confluent monolayers which are capable of inhibiting endothelial cell proliferation in culture (Vlodavsky et al 1979; Heimark and Schwartz 1985). Under some circumstances the outgrowth of endothelial cells to cover large areas of denudation is limited (Reidy et al 1982, 1983). The reason for the cessation of proliferation is not known, however it has been shown that repeat injury to the endothelial cells in the area of cessation of replication resulted in replication. Thus, the cells are capable of proliferating but for some reason perhaps related to a change in the underlying subendothelial matrix they stop replicating.

Summary

Endothelial regeneration, a process important in maintaining the integrity of the endothelium, involves a sequence of events characterized by cell spreading, cell translocation and cell replication. The F-actin microfilaments and the centrosome and microtubules are important cytoskeletal systems which regulate these processes and can in turn be influenced by factors which are likely to be located at sites of vascular injury and repair. Cell replication occurs to replace lost cells and the multiple factors that are likely to regulate proliferation have not yet been characterized.

Acknowledgements

We acknowledge Mrs. Brigitte Granton for technical assistance and Ms. Sue Sarju for secretarial work. Grant T432 Heart and Stroke Foundation of Ontario and MT 6485 MRC.

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Discussion with Reviewers

M. Richardson: Is there any evidence that the endothelium covering atherosclerotic lesions reacts differently from normal endothelium in terms of DPB formation or centriole orientation?
Authors: We have not studied this interesting question.

C.C. Haudenschild: What is the remaining material in the scratch in Fig. 1? Does it influence the migration?
Authors: We assume this to be matrix secreted by the endothelial cells. We have not studied the influence of matrix components on endothelial migration in our system. Others have shown that the matrix does modulate migration and the cytoskeleton. (Madri JA, Stenn KS. Aortic endothelial cell migration. I. Matrix requirements and composition (1982). Am. J. Pathol. 106:180-186; Pratt BM, Harris AS, Morrow JS, Madri JA (1984). Mechanisms of cytoskeletal regulation: Modulation of aortic endothelial cell spectrin by the extracellular matrix. Am. J. Pathol. 117:349-354).

C.C. Haudenschild: The regenerating cells in the organ cultures (Figs. 3,4) differ from regenerating endothelium, both in vivo and in vitro. What is the evidence that they are endothelium? Do they ever form a coherent monolayer of flat cells? What is the structure of the cytoskeleton?
Authors: The cells are in continuity with the endothelial monolayer and there are no free cells as would be expected in migrating smooth muscle cells. Ultrastructural examination of the cells did not reveal smooth muscle cell characteristics. Coherent monolayers of flat cells began to appear at the original wound edge after a number of days following wounding. The actin and microtubule/centrosome pattern was that of migrating endothelial cells.

E.A. Sprague: Two features of endothelial repair, cell migration and cell proliferation, appear to occur more rapidly in vitro compared to in vivo. One of the major differences between these conditions is hemodynamic shear forces. How do factors such as centrosome redistribution and cell proliferation related to endothelial repair respond to injuries induced in arterial areas of differing arterial wall shear stress?
Authors: This has not yet been studied.

E.A. Sprague: Is de novo synthesis of actin and/or tubulin stimulated in endothelial cells responding to repair an injured area?
Authors: Gabbiani et al. (Gabbiani G, Gabbiani F, Heimark RL, Schwartz (1984). Organization of actin cytoskeleton during early endothelial regeneration in vitro. J. Cell Sci. 66:39-50) showed that in early time periods following in vitro wounding of a confluent endothelial monolayer, there was no significant change in total actin however the ratio of G-actin to F-actin increased. This overall decrease in cytoplasmic F-actin was thought to be related with cell movement. We are not aware of similar studies on tubulin.