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Decreased Blood Flow Rate Disrupts Endothelial Repair *in Vivo*

Sergey Vyalov, B. Lowell Langille, and Avrum I. Gotlieb

From the Vascular Research Laboratory, Banting and Best Diabetes Centre, The Toronto Hospital Research Institute, and the Department of Pathology and the Centre for Cardiovascular Research, University of Toronto, Toronto, Canada

Both local hemodynamics and endothelial injury have been implicated in vascular disorders including bypass graft failure and atherogenesis, but little is known about the effect of local blood flow conditions on repair of endothelial injury. We decreased blood flow rates and shear stresses in common carotid arteries of rabbits by ligating the ipsilateral external carotid artery. After 24 hours, endothelial cells were less elongated, contained fewer central microfilament bundles, and showed less polarity of the centrosome toward the heart than endothelial cells in unmanipulated carotid arteries. To examine wound repair, we made narrow longitudinal intimal wounds at the time of flow reduction using a nylon monofilament device. In arteries with normal blood flows, endothelial cells at the edge of the wound initially spread and elongated in the direction of the wound. The dense peripheral band of actin was attenuated and central microfilaments became more prominent. Endothelial cells remained in close contact with their neighbors in the monolayer. The centrosome of cells adjacent to the wound was redistributed toward the wound side of the nucleus at 6 and 12 hours. Complete closure occurred by 24 hours, at which time the elongated endothelial cells covering the wound were organized in a herringbone pattern with their downstream ends at the center of the wound. With decreased flow and shear stress, the cells at the wound edge spread less than those in normal vessels at 12 hours after wounding and were randomly oriented and polygonal in shape. Also, re-endothelialization proceeded more slowly and there was a marked reduction of central microfilaments in cells at the wound edge. At 24 hours, the wounds were still open, the endothelial cells covering the central portion of the wound did not maintain intimate contact with their neighbors, and orientation of the centrosome toward the wound was reduced. We hypothesize that loss of cell-cell contact during repair at low flow rates and low shear stress disrupts intercellular communication and results in disruption of cytoskeletal reorganization during repair, thereby slowing the repair process. (Am J Pathol 1996, 149:2107–2118)

Both mechanical forces associated with blood flow and endothelial injury are thought to be important in the development of atherosclerotic lesions[1] and in the restenosis after angioplasty, atherectomy, and vascular bypass surgery.[2,3] Fluid shear stress has direct effects on endothelial cell structure and function *in vitro*[4,5] and *in vivo.*[6,7] Furthermore, changes in shear stress can indirectly affect endothelial cell biology because they induce remodeling of the arterial wall, including deletion or proliferation of endothelial cells,[8] and they regulate endothelial-leukocyte interactions[9–12] as well as the local release of vasomotor substances, enzymes, cytokines, and growth factors (reviewed by Davies[13]).

Human atherosclerotic plaques develop most frequently at sites of low shear stress and low shears may occur near sites of vessel obstruction or vascular graft implantation.[14] However, the effects of low shear have been difficult to define because sites of low shear very often coincide with sites of flow disturbance and rapidly fluctuating shear. There is reason to believe that shear influences vascular pathologies through effects on injury and repair, as

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Address reprint requests to Dr. Avrum I. Gotlieb, Vascular Research Laboratory, The Toronto Hospital Research Institute, 200 Elizabeth Street, CCRW 1-857, Toronto, Ontario M5G 2C4 Canada.

complex local hemodynamics were shown to affect repair of endothelial injury at experimental aortic coarctations[15]; however, the influence of low blood flow rates and low shear on endothelial repair *in vivo* is unknown.

We developed a model that involves reducing blood flow and shear stress in the common carotid artery of the rabbit and we examined the effects of this flow perturbation on the endothelium in the absence of flow disturbances or fluctuating shear.[8,9] We reported that, at 5 days after the reduction of blood flow, cell shape and F-actin microfilament distribution were changed, some endothelial cells were lost, and monocytes adhered to an endothelial monolayer that expressed much increased levels of VCAM-1.[9,11] The alterations in the cytoskeleton suggested that other cellular processes regulated by microfilaments and associated cytoskeletal systems may also be altered. Data from *in vitro* studies on endothelial cell migration during wound repair implicate microfilaments in force generation[16] and in cell-substratum adhesion,[17–19] whereas the centrosome and its associated microtubules have an essential role in directional migration of endothelial cells.[20–23] Consequently, examination of cytoskeleton is critical to understanding factors that alter endothelial wound repair.

In the present study, we used this *in vivo* model to study the effect of decreased blood flow and shear stress on the repair of narrow longitudinal endothelial wounds. Both the effects of the low flow on the intact endothelium and on the cells at the wound edge that are involved in repair were considered. Repair over 24 hours, ie, before significant cell replication, was observed and the morphology of the cells was compared with endothelium exposed to normal flow rates. As cytoskeletal systems are important in regulating endothelial cell shape and movement during repair,[24–26] we characterized the sequence of structural cytoskeletal changes occurring in endothelial cells participating in the repair. Both F-actin microfilaments and centrosomes and microtubules were studied. The data from these studies suggest possible mechanisms to explain a reduction in the rate of endothelial repair that we observed with low blood flow rates and low shear stress.

Materials and Methods

Endothelial Injury and Alteration of Blood Flow

Full surgical anesthesia was induced in 34 adult male New Zealand White rabbits weighing 3.5 to 4.0 kg with intramuscular injection of 0.25 ml of xylazine (20 mg/ml) and 2.25 ml of ketamine (100 mg/ml). Anesthesia was maintained with continuous infusion of the 1:9 xylazine/ketamine mixture (0.153 ml/minute). The left facial artery and the external carotid were exposed via a midline incision of the neck. Narrow, denuding injuries to endothelium were made as described previously.[15] Briefly, two strands of 3-0 nylon filament were bonded together with cyanoacrylate glue 5 mm from one end, and the ends were then bent back, crimped, and advanced through a length of PE 90 tubing. The facial artery was exposed and the linear wound device was introduced and advanced retrogradely. The tip of the catheter was guided through the branch site of the external carotid artery. The monofilament nylon was advanced from the PE 90 catheter into the left common carotid artery so that the tips of the nylon splayed apart and contacted endothelium on opposite sides of the artery. The nylon filaments were advanced to the aortic arch, rotated through 90°, and withdrawn. This procedure resulted in four narrow, longitudinal wounds approximately 100 μm wide. In 17 of these rabbits, the facial artery was ligated and the neck incision was then closed (normal flow group). In 17 other rabbits, the ipsilateral external carotid artery was ligated to reduce blood flow in the left common carotid artery (low flow group). This ligation causes a 70% reduction of blood flow.[8] Blood flow rates are positive (directed downstream) throughout the cardiac cycle in both the normal-flow and low-flow group.[8] The neck incision was closed. The animals were sacrificed at 6, 12, and 24 hours after surgery. In 8 additional rabbits, the tip of the filament was enlarged to make larger wounds of 150 μm width. The external carotid arteries were ligated in 4 of these rabbits, and the 8 animals were killed 24 hours after surgery.

In twelve rabbits, no wound was made. Nine of these were low flow and were sacrificed at 6, 12, and 24 hours, and three were normal flow and were sacrificed at 24 hours after sham surgeries. One milliliter of Penlong XL (Benzathine penicillin G, 150,000 U/ml; procaine penicillin G, 150,000 U/ml) was given intramuscularly on the day of surgery to all rabbits.

Tissue Preparation for Fluorescence Microscopy

Arteries from six rabbits (three from the normal-flow group and three from the low-flow group) with 100-μm injuries were examined by fluorescence microscopy at 6, 12, and 24 hours after injury. Also, all

carotid arteries with 150-μm injuries were examined by fluorescence microscopy at 24 hours after injury. The rabbits were administered 1000 U of heparin in a volume of 1 ml *via* an ear vein catheter, and 1 minute later they were killed by intravenous infusion of 1.0 ml of euthanasia solution (T-61, Hoechst Canada, Montreal, Canada) into the same ear vein catheter. A laparotomy and thoracotomy were performed, and the descending thoracic aorta was retrogradely cannulated. After briefly flushing the aorta and carotid arteries with 60 ml of phosphate-buffered saline (PBS), the left subclavian artery was cannulated and the cannula was connected to a manometer. The carotid arteries were perfusion fixed with 3% paraformaldehyde in 0.1 mol/L phosphate buffer, 0.1 mmol/L $CaCl_2$ (pH 7.4) under a perfusion pressure of 100 mm Hg. They were then perfused with PBS for 10 minutes, permeabilized by flushing 30 ml of 0.2% Triton X-100 (Sigma Chemical Co., St. Louis, MO) over 30 seconds through the aortic cannula, and then washed with PBS for 5 minutes at 100 mm Hg. The left common carotid artery was carefully excised, cut transversely into three pieces, and opened longitudinally along the ventral surface.

Immunostaining

Immunofluorescence staining of tubulin was performed using a monoclonal anti-α-tubulin antibody (ICN, Costa Mesa, CA), diluted 1:500 in PBS, followed by staining with Texas-Red-conjugated goat anti-mouse IgG (Jackson Laboratories, West Grove, PA) 1:100 in PBS. Fluorescein phalloidin (Molecular Probes, Eugene, OR), diluted 1:10 in PBS, was used to stain F-actin. Twenty-six arteries carrying normal ($n = 13$) or low ($n = 13$) blood flow were stained at 6, 12, or 24 hours after surgery. Tissue pieces were incubated for 30 minutes, followed by three rinses of 5 minutes each in PBS. Tissue was mounted on glass slides under glass coverslips with glycerol/PBS (1:9) with the lumen side facing up. The specimens were examined using a laser scanning confocal microscope with a krypton/argon laser (BioRad MRC 600).

Analysis of the centrosome position was carried out as previously described[19] using the nucleus, the heart, and the wound edge as reference points.[27] A centrosome classified as toward the heart was located between the nucleus and the cell periphery on the heart side of the cell; away from the heart was located between the nucleus and the cell periphery. In wounded arteries, a centrosome classified as toward the wound was located between the nucleus and the wound edge, and away from the wound was between the nucleus and the side of the cell facing away from the longitudinal wound. In nonwounded arteries, the centrosomes were classified as being toward the heart, away from the heart, or lateral to the nucleus. An analysis of variance was carried out and a Newman-Keuls test was carried out to determine which groups exhibited significantly different centrosomal locations.

Silver Staining

The lumen of the left common carotid artery from 16 rabbits (4 normal flow and 4 low flow at each time point) with 100-μm wounds was stained with silver nitrate immediately after sacrifice at 12 and 24 hours after injury.[15] After flushing the artery with PBS, the vessel was perfused with silver nitrate (50 ml, 0.25%) for 30 seconds, followed by a second PBS flush. The arteries were then perfusion fixed using 2% glutaraldehyde in phosphate buffer (pH 7.4) for 25 minutes at a pressure of 100 mm Hg. The left common carotid artery was excised, cleared of adventitia, and cut into four pieces. The artery was opened longitudinally and pinned, endothelium side up, on cork. Arteries were dehydrated in an alcohol series, incubated in xylene twice for 10 minutes, and mounted on glass slides under glass coverslips with Permount (Fisher Scientific, Nepean, Ontario).

Cell Area Measurements

The surface area covered by individual endothelial cells of eight arteries was measured directly from whole-mount preparations of silver-stained arteries using a Bio Quant System IV image analysis system under a total magnification of ×400. Areas of 100 to 125 cells in the row of cells immediately adjacent to the wound edge were determined at 12 hours after injury in four control arteries and four arteries exposed to low flow rates. An unpaired Student's *t*-test was used to determine the statistical significance of differences in cell areas.

Results

Morphology and Cell Kinetics

At 6 hours, the longitudinal injuries were 8 to 10 cells wide and parallel to the direction of blood flow. In both normal- and low-blood-flow-rate groups, endothelial cells at the edge of the injury were slightly enlarged and formed an approximately straight wound edge.

At 12 hours, cells at the edges of the wound exhibited lateral spreading (Figure 1, A and B). With

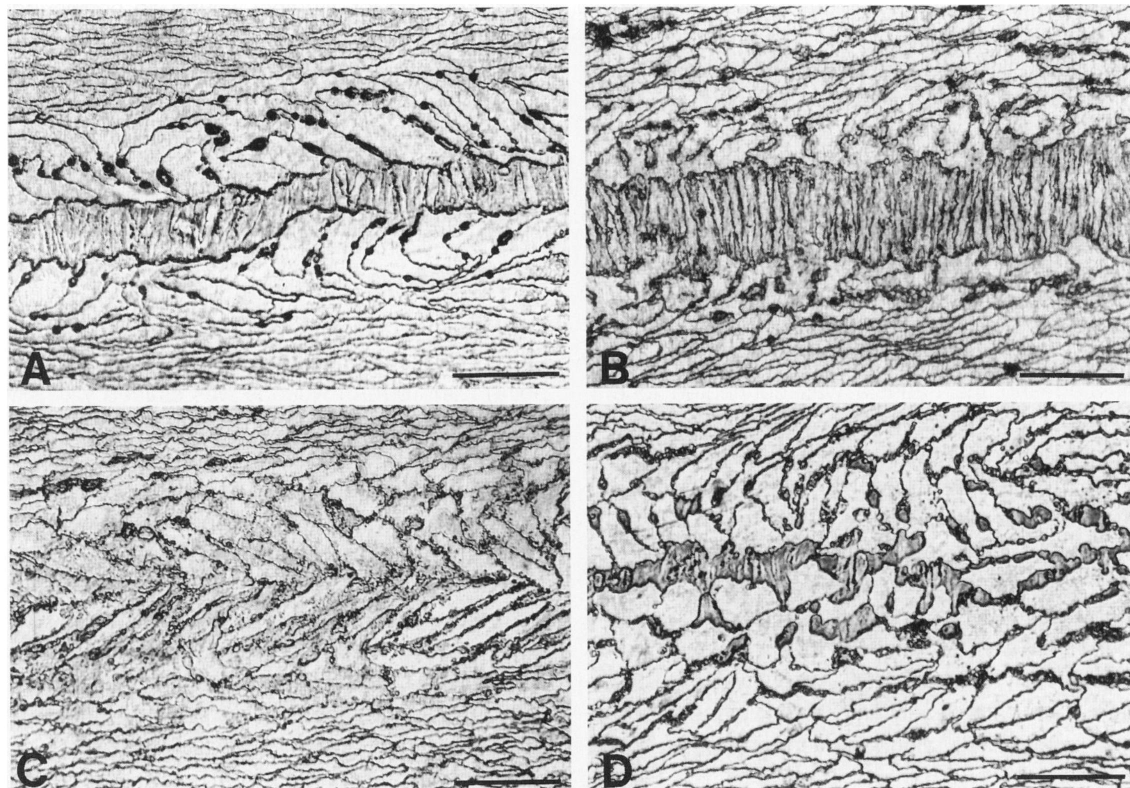

Figure 1. *The left common carotid artery 12* (A *and* B) *and 24* (C *and* D) *hours after narrow longitudinal injury stained with silver.* A *and* C: *Normal flow.* B *and* D: *Low flow. Blood flow is from left to right.* A: *In arteries with normal blood flows, cells at the wound edge are spread and are generally oriented at a slight angle to the direction of blood flow at 12 hours.* B: *In arteries with low flow and shear stress, many cells of the first row are polygonal, randomly oriented, and smaller than under normal flow.* C: *By 24 hours in arteries with normal flow, a herringbone pattern of endothelial cell orientation occurred in cells closing the wound.* D: *Re-endothelialization was not complete by 24 hours with low shear. The endothelial cells are separated from each other by gaps and are randomly oriented. Scale bar, 50 μm.*

normal blood flows, cells at the wound edge were elongated and generally were oriented at a slight angle to the direction of blood flow (Figure 1A). However, in arteries with low flow rates, the cells of the first row were randomly oriented and many cells were polygonal in shape. Less spreading into the wound occurred (Figure 1B). The mean area of the cells in the first row (819.04 ± 23.23 μm^2) was significantly less ($P < 0.05$) than that of first-row cells in arteries with normal flow (1126.34 ± 40.15 μm^2).

With normal blood flow rates, the wound was completely closed by 24 hours; however, the cells did not return to their normal morphology and orientation (Figure 1C). Instead, endothelial cells that had spread into the wound from opposite sides were oriented in a herringbone pattern with cells oriented at approximately 45° to the direction of flow. The downstream ends of the cells pointed toward the wound midline. Occasionally, a cell located in the mid-portion of the wound had an axial orientation. The adjacent one or two rows of cells were transitional in orientation between the herringbone pattern and the normal morphology of the undisturbed artery.

In contrast, re-endothelialization was not complete by 24 hours in arteries subjected to low flow rates. Many of the endothelial cells covering the central portion of the wound were polygonal in shape and were randomly oriented. These cells were separated from each other by many large gaps (Figure 1D). Cells far from the wound became less elongated over the 24 hours of low flow, as reported previously for 5 days of low blood flow.[9]

Microfilament Distribution in Nonwounded Arteries with Normal and Low Blood Flow Rates

With normal blood flow conditions, the distribution of actin microfilaments in endothelial cells in nonwounded rabbits was unchanged at 24 hours and was similar to that previously reported in normal arteries (Figure 2A). In arteries with low flow, actin microfilament distribution

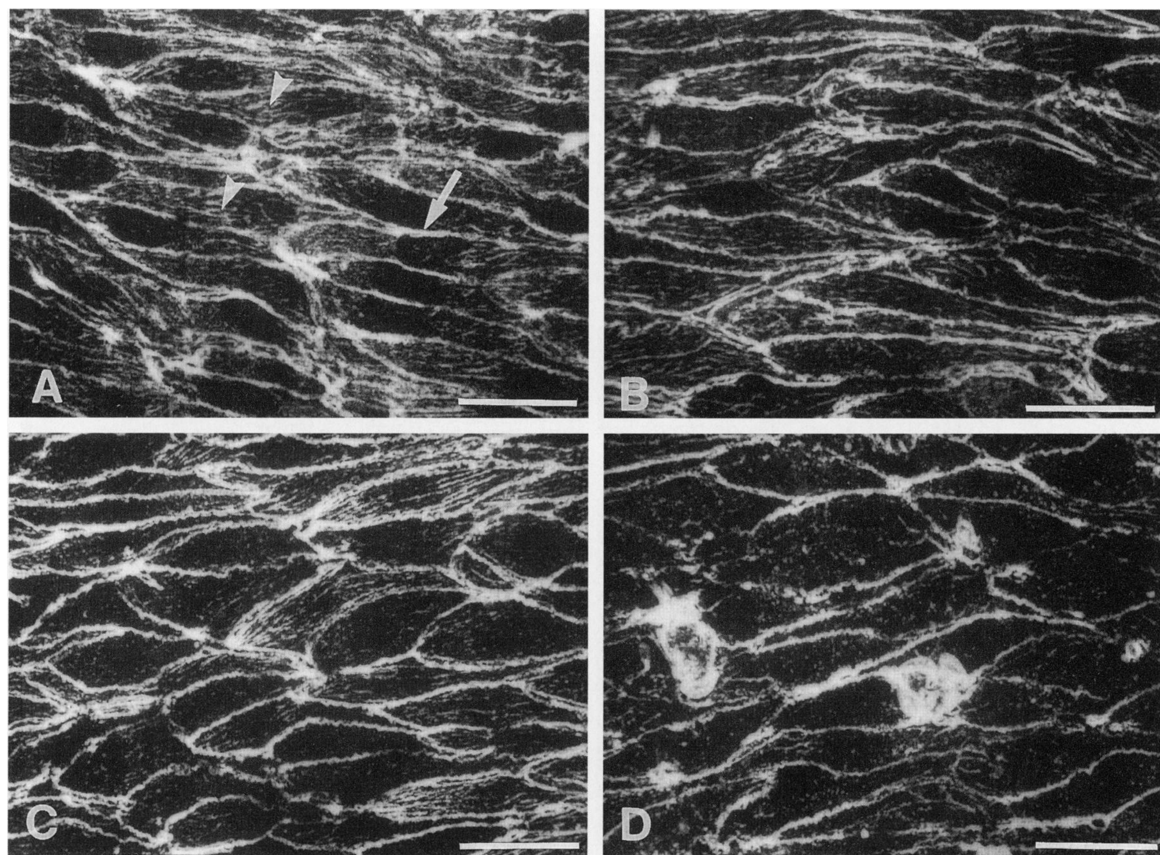

Figure 2. *Photomicrograph of actin microfilaments stained with rhodamine phalloidin in endothelial cells remote from the wound. Blood flow is from right to left.* A: *Normal flow, 24 hours after injury. Note that peripheral band of F-actin* (arrow) *and short central microfilament bundles (stress fibers;* arrowhead). B: *Artery with low shear, 6 hours after injury, showed a similar pattern of actin distribution as* A. C: *At 12 hours, low shear, some cells were less elongated and contained fewer stress fibers.* D: *At 24 hours, low shear. Note the further decrease of stress fibers. Scale bar, 25 μm.*

at 6 hours was similar to that of normal arteries (Figure 2B). However, at 12 hours, many cells showed fewer and smaller central microfilaments, and at 24 hours, more cells showed a decrease in central microfilaments (Figure 2, C and D).

Microfilament Distribution during Wound Closure

At 6 hours after injury, the microfilament distribution in endothelial cells adjacent to the wound was similar in arteries with normal and low flow rates. The cells were slightly spread, the dense peripheral band (DPB) of actin was slightly attenuated, and the number of central microfilaments was reduced (Figure 3, A and B).

In the vessels with normal blood flow, stress fibers in the first row of cells were oriented parallel to the long axis of the cell at 12 hours (Figure 3C). The DBP in these cells was attenuated when compared with the DBP at distant sites. In the low-flow group, the cells of the first two rows had very few central microfilaments, which were very thin, and the DPB was not prominent (Figure 3D).

By 24 hours, cells in re-endothelialized areas contained abundant, long stress fibers that were oriented parallel to the long axis of the cells in arteries with normal flow. The DPB was present but not well defined (Figure 3E). In the low-flow group, endothelial cells separated by gaps usually contained very thin central microfilaments. DPB was attenuated in some places, but it delineated cell boundaries (Figure 3F).

At all time points, the distribution and organization of microfilaments in endothelial cells away from the wound, as analyzed in cells 10 rows away from the wound edge, was similar to that of endothelial cells in nonwounded arteries.

Microtubules and Centrosomes in Nonwounded Arteries with Normal and Low Blood Flow Rates

Most of the centrosomes in nonwounded controls were located on the heart side of the nucleus, a pattern previously reported.[27] After 24 hours of reduced blood flow, there was a reduction in the po-

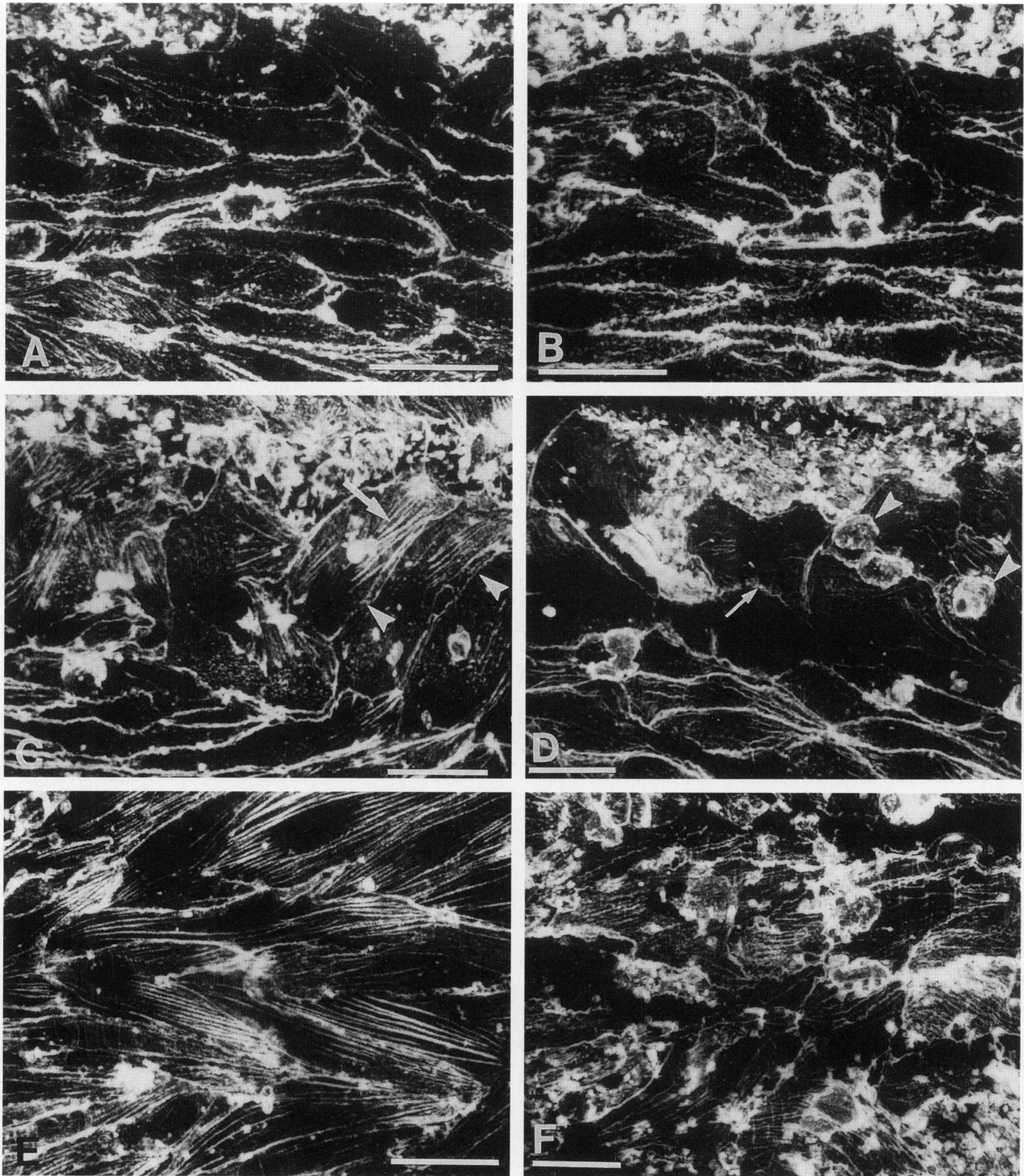

Figure 3. *Photomicrograph of endothelial cells stained for F-actin after longitudinal narrow injury subjected to normal* (A, C, *and* E) *and low* (B, D, *and* F) *flow. Blood flow is from left to right.* A: *At 6 hours after injury, the DPB in cells at the edge of the wound in arteries with normal shear are attenuated and central microfilaments are reduced.* B: *At 6 hours after injury, in arteries with low shear, the microfilament distribution was similar to arteries with normal shear.* C: *At 12 hours after injury, the cells of the first row contain thick stress fibers* (arrow) *oriented parallel to the long axis of the cell in arteries with normal shear. The DPB in these cell is attenuated* (arrowhead). D: *At 12 hours after injury, in arteries with low shear, the cells of the first row contain very few thin central microfilaments and the DPB* (arrow) *is attenuated. Note that leukocytes are attached both to the exposed subendothelium and to endothelium* (arrowhead). E: *By 24 hours, in normal shear, cells of re-endothelialized areas have abundant, long stress fibers oriented parallel to the long axis of the cells. The DPB remains attenuated.* F: *At 24 hours after injury, endothelial cells in arteries with low shear are separated by gaps. Thin, central microfilaments are generally aligned in the direction of blood flow. Scale bar, 25 μm.*

Table 1. *Centrosome Location in Endothelial Cells in Nonwounded Arteries 24 Hours after Surgery*

	% cells ± SEM		
	Toward heart	Away from heart	Lateral
Normal shear	86.5 ± 0.95	5.7 ± 0.61	7.8 ± 1.3
Low shear	71.1 ± 1.68*	5.6 ± 0.66	22.7 ± 2.33*

*Significantly different from normal shear ($P < 0.05$).

larization of the centrosomes toward the heart when compared with endothelial cells in arteries with normal flow (Table 1).

Microtubules and Centrosomes during Wound Closure

At 6 and 12 hours after injury, centrosomes in cells at the edge of the wound were redistributed toward the wound edge regardless of flow conditions (Figures 4, A–D, and 5). Centrosomes of cells distant from the wound (10th row of cells away from the wound) were located preferentially on the heart side of the nucleus at all times, and this distribution was similar to that of centrosomes in nonwounded carotid arteries (Table 2). At 24 hours, the reduction in the polarization of the centrosomes toward the heart seen in nonwounded arteries with low flow also was observed in cells distant from the wound.

Wound closure occurred by 24 hours after injury when flow rates were normal. At this time, centrosomes of cells at the midline of the wound site were downstream of the nucleus, ie, opposite to cells in nonwounded regions. However, centrosomes of cells a few rows away from the midline of the wound site faced the midline of the wound.

Our original wounding procedures did not allow interpretable comparisons of centrosome position with low *versus* normal blood flows at 24 hours because wounds had closed when blood flow rates were normal (Figure 4, E and F); therefore, we made wider wounds of approximately 150 μm in four rabbits with normal flow and four rabbits with reduced flow. The wounds remained open at 24 hours when flow rates were normal, and centrosomes in cells at the wound edge remained oriented toward the wound. When flows were low, the preferential orientation of centrosomes toward the wound was reduced at this time and significantly more centrosomes were oriented away from the wound, away from the heart, and toward the heart (Figure 6). Far from the wound, the tubulin network was compact and the centrosomes often were obscured by condensed microtubules and it was difficult to discern their position. However, some reduction in polarization toward the heart was detected (Table 2).

Discussion

The primary finding of this study is that the repair of endothelial wounds *in vivo* is impaired by simultaneous reduction in intraluminal blood flow rate and shear stress. In addition to a slowed rate of repair, abnormal features of the repair process were observed: cell-cell contact along the wound edge was reduced and the cytoskeletal reorganization that accompanies repair was dramatically altered.

The repair of small *in vivo* wounds has been studied previously. Our findings are consistent with reports that altered hemodynamics affects endothelial repair at coarctations[15]; however, complex flow conditions and wall tensions at coarctations[28] precluded conclusive statements regarding a particular hemodynamic stress. In the current study, repair of injury was related to reductions in fully developed, stable flows and their associated shear stresses.[8] Also, we restricted our examination to this early time after injury (0 to 24 hours) to selectively examine nonproliferative repair, ie, spreading and migration. This was possible because endothelial cells adjacent to denuding wounds, both *in vivo* and *in vitro*, do not enter the S phase of the cell cycle until 20 to 28 hours after wounding.[29] We observed significant effects of flow on wound repair and on associated cytoskeletal reorganization occurring between 6 and 12 hours.

With normal hemodynamics, the longitudinal injuries we created were covered within 24 hours of injury by lateral spreading and migration of adjacent endothelial cells, as reported previously.[15,30–32] Cells maintained contact with their neighbors while the wound was being closed. The cells migrated into the wound at an angle that was intermediate between the downstream and circumferential directions, so that the cells were organized into a herringbone pattern at the time of wound closure. This is unlike migration under static conditions *in vitro*, in which migration is perpendicular to the wound edge.[24] The off-axis orientation of cells seen at wound closure *in vivo* probably reflects the combined influences of normal shear stress, which promotes axial orientation,[33] and longitudinal injury, which promotes circumferential orientation.[24]

When blood flow and shear stress were reduced, the herringbone pattern did not occur, probably because there was a reduced stimulus for axial orientation. In addition, the cells along the wound edge exhibited less spreading during repair and were less

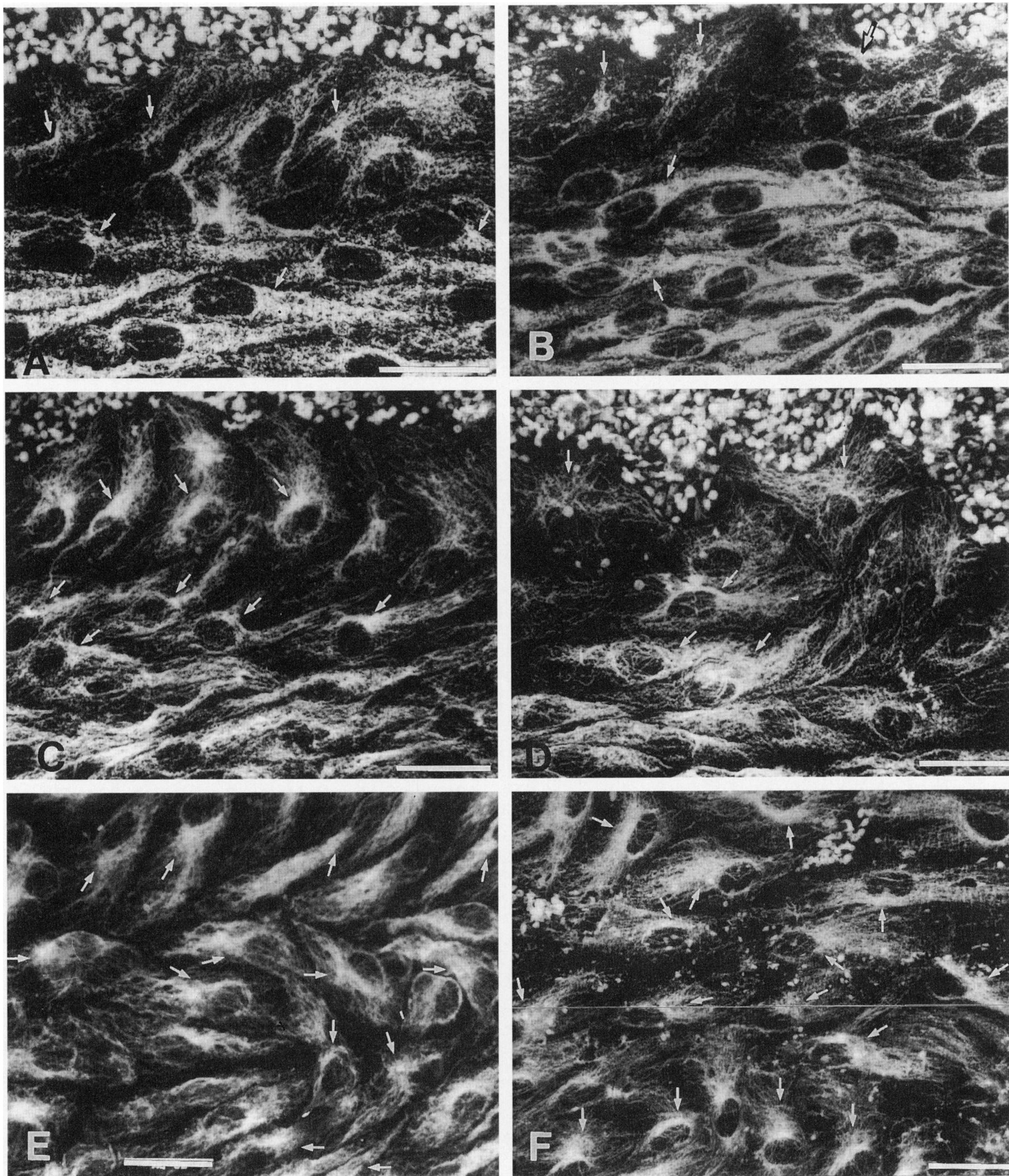

Figure 4. *Photomicrographs of endothelium stained for tubulin after longitudinal narrow injury.* **A**, **C**, *and* **E**: *Normal shear.* **B**, **D**, *and* **F**: *Low shear. Blood flow is from right to left.* **A** *and* **B**: *By 6 hours, centrosomes* (**arrow**) *in cells at the edge of the wound were redistributed toward the wound side but still on the heart side of the nucleus in arteries with normal* (**A**) *and low* (**B**) *shear. Note that the centrosomes of cells away from the wound were located preferentially on the heart side of the nucleus (on the right).* **C** *and* **D**: *By 12 hours, the centrosomes* (**arrow**) *of endothelial cells of the first row faced the wound edge* (**top**) *in arteries subjected to normal* (**C**) *and low* (**D**) *shear. Centrosome of cells of the second and subsequent rows were preferentially located on the heart side of the nucleus.* **E**: *At 24 hours after injury, centrosomes* (**arrow**) *of cells in the middle of the wound are located on the downstream side of the nucleus; centrosomes of the next row of cells faced the wound. Centrosomes in cells beyond the original width of the wound were preferentially located on the side of the nucleus toward the heart.* **F**: *In arteries with low shear, re-endothelialization was not complete by 24 hours and cells were separated by gaps. The centrosome* (**arrow**) *of the cells located over the site of injury were randomly distributed around the nucleus. Note platelets adhering to the subendothelium in the gaps. The cells in the area of the original wound edge were confluent and their centrosomes faced the central portion of the wound. Scale bar, 25 μm.*

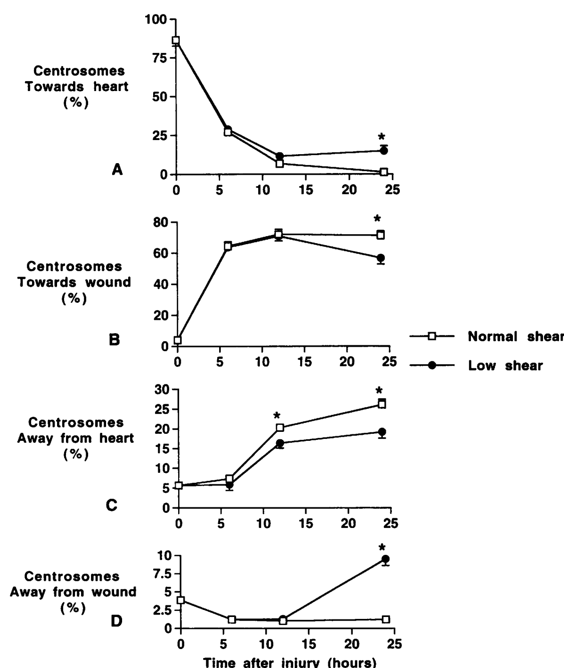

Figure 5. *The percentage of endothelial cells at the wound edge with centrosomes toward the heart* (**A**), *toward the wound* (**B**), *away from the heart* (**C**), *and away from the wound* (**D**) *at time 0 and 6, 12, and 24 hours after wounding. *Significantly different at given time point* ($P < 0.05$).

elongated. An additional striking feature of repair under low flow conditions was that intimate contact between neighboring cells was not maintained; instead, large gaps appeared between the cells.

Extensive studies of endothelial repair *in vitro* have established the critical role of cytoskeletal reorganization in this process.[26] Spreading and migration during repair are preceded and accompanied by major reorganization of the cytoskeleton; furthermore, chemical disruptions of microfilaments or microtubules impede or prevent these processes.[21,23,25]

Cytoskeletal reorganization during repair includes translocation of the centrosomes, from which microtubules emanate, to the wound side of the nucleus before cell migration.[18,20,21,23] Centrosomes in the uninjured rabbit carotid artery were oriented predominantly toward the heart.[27] This apparently is not an effect of shear as it is true in both arteries, where the heart is upstream, and in veins, where the heart is downstream[27]; nonetheless, we observed a reduction in the orientation of the centrosomes toward the heart when blood flow rate was decreased. The significance of this finding is not understood at present. As observed *in vitro*, centrosomes redistributed toward the wound edge after injury; however, significantly less reorientation occurred at 24 hours when blood flow rates were low, when compared with repair under normal flow conditions. At this time, the cells often were separated from their neighbors by gaps; therefore, they may have lost cues from other cells that are important in signaling the direction of the wound *versus* that of the intact monolayer.

Such directional cues may be transmitted between cells *via* gap junctions at the wound edge[34]; alternatively, ligand-receptor or receptor-counterreceptor interactions at the cell junction may provide information important for the directionality of migration during repair. It is not apparent from our studies how low flow or shear caused reduction or loss of cell-cell contact during repair *in vivo.* Additional studies on the regulation of cell-cell adhesion molecules and other means of communication in endothelial cells at the wound edge under low shear are warranted. *In vivo* studies probably are needed as cell-cell contact is maintained during wound repair in static cultures.[22]

Slowed repair also may be related to alterations in central microfilaments. Endothelial cells in intact rabbit carotid arteries possess well developed peripheral bands of actin microfilaments and short central microfilament bundles running parallel to the long axis of the cell and thus to blood flow.[9] The peripheral actin may be important in cell-cell adhesion whereas the prominent central filaments have been associated with both cell-substratum adhesion and with cell migration.[26] The role of microfilaments in wound repair is not fully defined, but a role for this contractile protein in cell movement has been widely implicated. Slower repair may be due to reduced

Table 2. *Centrosome Location in Endothelial Cells 10 Rows from the Wound*

Time after wounding	% cells ± SEM							
	Normal shear				Low shear			
	Toward wound	Away from wound	Toward heart	Away from heart	Toward wound	Away from wound	Toward heart	Away from heart
6 hours	4.11 ± 1.64	3.99 ± 1.49	87.05 ± 2.76	4.83 ± 0.72	3.74 ± 0.54	3.64 ± 0.64	87.55 ± 0.86	4.54 ± 0.84
12 hours	4.6 ± 0.33	4.18 ± 1.34	86.2 ± 1.95	4.98 ± 1.16	4.94 ± 0.65	5.97 ± 0.73	84.64 ± 2.42	4.45 ± 0.48
24 hours	4.74 ± 0.65	3.66 ± 0.38	86.27 ± 0.88	4.9 ± 0.23	14.64 ± 1.16*	12.49 ± 1.91*	67.23 ± 2.6*	5.89 ± 0.14

*Significantly different from 6 and 12 hours ($P < 0.05$).

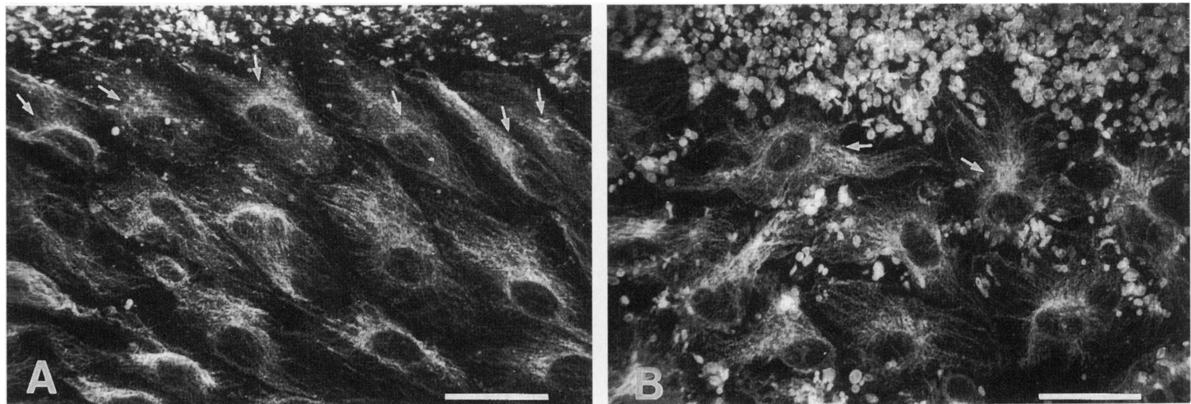

Figure 6. *Photomicrographs of endothelium at the edge of a longitudinal narrow injury 150 μm in width stained for tubulin at 24 hours after wounding. Blood flow is from right to left.* **A**: *In arteries with normal shear, centrosomes* (**arrow**) *of endothelial cells were redistributed toward the wound side.* **B**: *In arteries with low shear, cells do not maintain contact with each other and show less centrosome redistribution* (**arrow**) *toward the wound edge. Scale bar, 25 μm.*

actin-myosin force generation or reduced cell-substratum contact resulting in less effective force transmission to the substratum during such movement. We have shown previously that wounding enhances central microfilament formation in cells at the wound edge.[24] Under low shear conditions, central microfilaments are fewer and smaller,[7,9] and we now have found that their formation in response to injury is suppressed. These features of microfilament redistribution may contribute to slower repair.

It is not clear why central microfilaments fail to form at wound edges when blood flow rates are low. It is possible that redistribution of actin from other pools, such as the DPB, contributes substantially to their formation. The reduced DPB with low flow rates would then limit actin available for stress fiber formation after injury.

We have inferred that shear stress influences the orientation of migrating cells near wounds, as many *in vivo* and *in vitro* studies have demonstrated the capacity of shear stress to align cells. Other flow-sensitive aspects of wound repair, including separation of migrating cells from their neighbors and changes in cytoskeletal reorganization, may also represent direct responses to shear stress. Alternatively, they may be secondary to other effects of low flow and shear stress on vessel wall physiology. These effects include vasomotion, vessel wall remodeling, the deletion of some endothelial cells, and low-flow-induced interactions of endothelium with leukocytes and the cytokines that may be produced as a result of these interactions.[35]

In future experiments it should be possible to assess the influence of leukocyte-endothelial interactions by disrupting these interactions with antibodies[10]; however, the effects of vasomotion and remodeling are less easily assessed, as these responses are inevitable outcomes of reducing shear stress. Furthermore, it is not meaningful to allow vasomotion and remodeling to go to completion before injuring the arteries because the end result of these processes is that shear stresses are returned to approximately normal levels.[8] Consequently, novel approaches will be needed that may require more detailed information on flow-induced remodeling than is currently available.

The subadjacent vessel wall, both cells and matrix, and platelets adherent to the wound also may influence endothelial repair in a flow-dependent manner. Platelets release transforming growth factor-β, which reduces endothelial cell migration *in vitro* over short time periods.[36] An imbalance of relaxing and contracting factors, some released from platelets and endothelial cells, may affect local hemodynamics by inducing vasoconstriction.[37] Macrophages, endothelial cells, and smooth muscle cells release platelet-derived growth factor as well,[38–40] and platelet-derived growth factor delays endothelial wound repair *in vitro.*[41] Also, basic fibroblast growth factor and at least one of its receptors, fibroblast growth factor receptor-1, are expressed by repairing endothelium *in vivo.*[42] Basic fibroblast growth factor promotes endothelial repair *in vitro*[43] and is specifically associated with centrosome redistribution during *in vitro* endothelial wound repair.[44] It is possible that low shear interferes with the release of basic fibroblast growth factor from mechanically injured cells and thus disrupts repair. Also, the convection of all of these factors from the wound site, and hence their local concentrations, likely will be sensitive to local flow conditions. Recent evidence implicates the early growth response gene, *egr*-1, in mediation of

growth factor production at sites of endothelial injury,[45] so shear sensitivity of *egr*-1 expression could provide a potent means of growth regulation during repair. However, shear sensitivity of *egr*-1 has not been tested.

The role of low shear as a risk factor for atherosclerotic plaque development has been difficult to evaluate because low shears often coincide with complex flow patterns.[46,47] Our model has allowed examination of effects of low shear independent of complex flows. Our current data suggest that one mechanism by which low shear may enhance plaque growth is by delaying endothelial wound repair when frank denudation occurs on the surface of plaques,[48] thus exposing the subendothelium for longer periods to leukocytes, platelets, and atherogenic agents in the serum. This hypothesis is testable but remains to be proven.

In summary, repair of narrow endothelial wounds is impeded when blood flow rates and shear stress are reduced. Repair with low flow was associated with reduced cell spreading and separation of cells from their neighbors. The latter may cause the cells to lose important cues that define the direction of repair. In this regard, centrosomal reorientation toward the wound edge was reduced as the cells lost contact with their neighbors. In addition, low blood flow rates were associated with reduced microfilament bundles in cells throughout the monolayer, including those participating in repair. Loss of these microfilament bundles, which contribute to cell spreading and migration, could further impede repair.

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