Shear stress enhances human endothelial cell wound closure in vitro

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Albuquerque, Maria Luiza C., Christopher M. Waters, Ushma Savla, H. William Schnaper, and Annette S. Flozak. Shear stress enhances human endothelial cell wound closure in vitro. Am J Physiol Heart Circ Physiol 279: H293–H302, 2000.—Repair of the endothelium occurs in the presence of continued blood flow, yet the mechanisms by which shear stress affects endothelial wound closure remain elusive. Therefore, we tested the hypothesis that shear stress enhances endothelial cell wound closure. Human umbilical vein endothelial cells (HUVEC) or human coronary artery endothelial cells (HCAEC) were cultured on type I collagen-coated coverslips. Cell monolayers were sheared for 18 h in a parallel-plate flow chamber at 12 dyn/cm² to attain cellular alignment and then wounded by scraping with a metal spatula. Subsequently, the monolayers were exposed to a laminar shear stress of 3, 12, or 20 dyn/cm² under shear-wound-shear (S-W-sH) or shear-wound-static (S-W-sT) conditions for 6 h. Wound closure was measured as a percentage of original wound width. Cell area, centroid-to-centroid distance, and cell velocity were also measured. HUVEC wounds in the S-W-sH group exposed to 3, 12, or 20 dyn/cm² closed to 21, 39, or 50%, respectively, compared with only 59% in the S-W-sT cells. Similarly, HCAEC wounds closed to 29, 49, or 33% (S-W-sH) compared with 58% in the S-W-sT cells. Cell spreading and migration, but not proliferation, were the major mechanisms accounting for the increases in wound closure rate. These results suggest that physiological levels of shear stress enhance endothelial repair.

vasculature; biomechanics; intimal healing; cell migration; cell spreading

BY VIRTUE OF THEIR LOCATION at the interface of the bloodstream and the vessel wall, endothelial cells integrate and transmit not only the neurohormonal and metabolic signals present in the circulation, but also the mechanical forces resulting from blood flow. It has become apparent during the last decade that cells can sense their mechanical milieu and respond to forces such as transmural pressure, cyclic stretch, and shear stress. Much of the work in this field has dealt with the effects of the shear forces experienced by endothelial cells in the circulation (5–8). Although it is well understood that mechanical forces can significantly alter the structure and modulate the function of the whole vessel, very little is known about how these forces affect endothelial cell structure and function during wound healing.

A link between shear of endothelial cells and vascular disease processes with poor wound healing may be seen in atherosclerosis, where wounds occur in regions of the vessel that are associated with low shear stress and/or turbulent flow (1, 5–8, 12, 14, 17, 29, 31, 33, 34). In contrast, atherosclerotic plaques rarely occur in regions of the vessel that are associated with higher shear stress and laminar flow (4, 19, 34). In addition to atherosclerosis, wound injuries to the endothelium are a critical pathogenic event in a variety of cardiovascular disorders, including vein bypass graft failure, ischemic injury, coarctation, and mechanical trauma from catheters and guidewires during medical procedures (3, 20, 26). After disruption of the intima by such injuries, the endothelial cell plays an active role in the healing process. Inward migration of endothelial cells from the margin of the lesion re-seals the internal vessel wall, decreasing shear stress on the intima and diminishing the reactive myointimal proliferation and scarring that cause vascular narrowing (25, 27). Most studies of endothelial cell wound repair have been conducted in vitro under static conditions, and cell migration has been implicated as the primary mechanism for wound closure under these conditions (9, 21).

Because endothelial cells in vivo are exposed to continual flow and must bring about wound closure under conditions of shear stress, it is important to examine the effect of shear on endothelial cell spreading, migration, and/or proliferation, which are integral to this important vascular process. Conventionally, wound closure has been studied under static conditions, without the effects of superimposed shear stress. In the present series of experiments, we employed the physiological approach of preshearing human endothelial cell monolayers in vitro to attain alignment as seen in the vasculature in vivo. After alignment, we wounded...
the monolayers and applied shear again at varying levels. In this way we have been able to compare the influence of shear vs. stasis on wound closure. To date there are no studies that have examined the effects of this physiological shear stress model on cultured human endothelial cells, nor are there studies that have examined the process of wound healing in human coronary artery endothelial cells.

Our data suggest that shear stress enhances human umbilical vein endothelial cell (HUVEC) and human coronary artery endothelial cell (HCAEC) wound closure. Enhancement of wound closure for HUVEC and HCAEC is attributable to mechanisms involving not only cell migration but also cell spreading. These results describe a role for circulatory flow in the physiology of wound closure.

**METHODS**

**Cell culture.** HUVEC were isolated from anonymous pathological specimens by collagenase digestion with the use of established techniques (30). Cultured cells were confirmed as endothelial by staining positively for von Willebrand factor and negatively for α-smooth muscle actin. All cultures tested negative for mycoplasma. HCAEC were purchased from Clonetics (San Diego, CA), and endothelial markers were verified by Clonetics. These cells were cultured in RPMI 1640 (Life Technologies, Gaithersburg, MD) supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 2.5 μg/ml amphotericin, 5 μg/ml heparin, 20 μg/ml endothelial cell growth supplement (ECGS; Collaborative Research, Bedford, MA), and 20% bovine calf serum (HyClone, Logan, UT) on 150-mm tissue culture dishes (Nunc, Intermed, Denmark). HUVEC and HCAEC were passaged with the use of trypsin-EDTA and were studied between passages 4 and 7.

**Preparation of matrix-coated coverslips.** Glass slides (37.5 cm²) were coated with type I collagen under sterile conditions. The collagen, acidified with 20 mM HCl, was used at a final concentration of 40 μg/ml (4.0 μg/cm²). The slides were coated for at least 1 h at 37°C before rinsing with phosphate-buffered saline (PBS) and plating of the endothelial cells.

**Application of shear stress.** For all of our experiments, we used the CytoShear parallel-plate flow chamber (San Diego, CA) designed by Frangos et al. (10). Briefly, the flow chamber is made of Plexiglas and is designed to subject cells grown on standard slides to well-defined laminar fluid shear stress. Fluid flow was provided by a sterile continuous-flow loop. The dimensions of our chamber were such that it could accommodate a 37.5-cm² slide with the desired monolayer of endothelial cells. We found this chamber to be ideal for use in our experiments because it provides both a constant shear stress and a large area for visualization (10). The width of the parallel-plate flow chamber is much larger than the height; therefore, the side walls have minimal impact on the shear stress distribution along the bottom surface. In addition to creating a uniform laminar shear stress, the parallel-plate chamber and recirculating flow loop are designed to limit the amount of medium circulating over a large cellular surface area. HUVEC or HCAEC at 80–100% confluence grown on matrix-coated glass slides were exposed to shear stress, determined by the following formula: \( \tau = 6 \frac{Q}{bh^2} \) (dyn/cm²), where \( Q \) is flow rate (ml/s), \( \mu \) is fluid viscosity (dyn · s/cm²), \( h \) is channel height (254 μm for standard gasket), and \( b \) is channel width (equal to 3.3 cm for our chamber). Bubbles were removed by use of a bubble trap. The perfusate consisted of normal medium.

**Imaging the wound.** Images of the wounds were collected at specified times with a Nikon Diaphot 300 inverted microscope equipped with a Hamamatsu integrating CCD camera, an Argus-20 real-time digital image processor, and a Pentium computer with a frame grabber (Fryer, Huntley, IL). Images were analyzed by use of the MetaMorph image analysis software (Universal Imaging, West Chester, PA). After the image was acquired, it was converted from pixels to micrometers with the use of a calibration image. With the use of MetaMorph, the wound edge can be traced and the perimeter and area can be tabulated. The mean width of the wound, \( w \), is given by

\[
  w = 0.25(P - \sqrt{A^2 - 16A})
\]

where \( P \) and \( A \) represent the perimeter and area of the wound in the image, respectively (28). Data are expressed as a percentage of the original wound width to normalize variability in wound healing from experiment to experiment, although similar initial wound sizes were usually observed.

**Experimental protocol.** To investigate the effect of shear stress on endothelial cell wound closure, HUVEC and HCAEC were grown to 80–100% confluence by the day of experimentation, and at least 5 days of contact between the cells and the matrix-coated slide were allowed. Wound width measurements were only made where the monolayer was 100% confluent. Cell culture media and pH were controlled for all monolayers and did not fluctuate during the experiments.

Endothelial cell responses to shear stress were evaluated under a physiological design of shear, wound, and shear (S-W-sH) or shear, wound, and static (S-W-sT) conditions. In some cases, this protocol was compared with cell monolayers that had not been sheared but were rather wounded and sheared (W-sH) or wounded and kept static (W-sT). In the S-W-sH design, HUVEC or HCAEC were sheared at 12 dyn/cm² for 18 h to attain cellular alignment, as is found in vivo in vessel walls. The monolayer was then wounded, and endothelial responses were evaluated (at 3, 12, or 20 dyn/cm²) and compared with static control cells (S-W-sT) that had been similarly presheared and wounded. The S-W-sT cells were placed in an equivalent volume of medium without application of shear forces. The responses of cells under S-W-sH and S-W-sT conditions were also compared with those of cells that were not presheared and were instead initially wounded and then subjected to the shear forces of 3, 12, or 20 dyn/cm² or static conditions.

The monolayers were kept at 37°C and 5% CO₂ during the experimental protocols. In each experiment, duplicate paired monolayers were either subjected to shear stress with the use of the parallel-plate flow chamber or maintained static as a control. Before the initiation of shear stress or maintenance in static conditions, the monolayers were rinsed once with PBS to remove cellular debris. Monolayers in either shear or static groups were exposed to the same volume of complete medium during experimentation. Each monolayer had four 150-μm-width wounds, spaced 0.5 cm apart, made in the direction of flow. Images of the cells were obtained from sheared and static cell populations on an hourly basis. Wound closure rates in monolayers exposed to shear stress were compared with wound closure rates in HUVEC or HCAEC in static culture. All images of the wound area were captured with MetaMorph software as described above. Each protocol was repeated at least four to five times for each cell type (HUVEC or HCAEC) and for each level of shear stress.
Proliferation studies. To assess the contribution of proliferation to wound closure, 5-bromo-2′-deoxyuridine (BrDU) was added after wounding or for 60 min before analysis of the cell monolayer. The BrDU was added at a final concentration of 10 μM in the recirculating flow loop for the sheared cells and added at an equal concentration to the static monolayer. Sheared and static monolayers were compared at hourly time points. The cells were washed with PBS, fixed in 70% ethanol (in glycine buffer, pH 2.0) for 20 min at −20°C, and washed again. The cells were subsequently incubated with anti-BrDU antibodies (1:10 dilution; Boehringer Mannheim, Indianapolis, IN) for 30 min at 37°C. After the rinsing, the primary antibody was detected by incubation with antimouse IgG antibody (Boehringer Manheim BrDU labeling detection kit II) conjugated to alkaline phosphatase for mouse IgG antibody (Boehringer Mannheim BrDU labeling primary antibody was detected by incubation with anti-mouse IgG antibody (Boehringer Manheim BrDU labeling detection kit II) conjugated to alkaline phosphatase). Next, the cells were subsequently incubated with the chromogenic substrate l-nitro blue tetrazolium solution and 10 μl X-phosphatase solution (50 mg/ml 5-bromo-4-chloro-3-indolyl phosphate toluidinium salt in dimethylformamide) in 3 ml of 100 mM Tris buffer (pH 9.5) for 30 min at room temperature. After the monolayers were rinsed, coverslips were applied. For each monolayer, a total of 20 fields at the wound edge and back from the edge were viewed at ×40 magnification, and the BrDU-stained cells were totaled.

Cell spreading and migration studies. As a measure of cell spreading, separation, and migration, fixed views of four groups of cells per each side of a wound were evaluated for cell area, centroid-to-centroid distance, and cell velocity, respectively. The measurements were collected hourly during the experiments. The average cell area was determined by tracing cells in the wounded monolayers at the wound edge with the use of MetaMorph software (West Chester, PA). The accuracy of the cell size measurements was compared with measurements of the same cells at higher magnifications. The distance between the geometric center of one cell (centroid) and the centroid of its two neighboring cells was also measured. For each experimental condition, four sets of cells at the leading edge of the wound were traced in each experiment (n = 12 pairs of cells in each group). The centroid-to-centroid distance between adjacent cells was evaluated as a measure of both cell spreading and migration, according to the methods of Kheradmand et al. (16). If the cell area or the centroid-to-centroid distance increases during wound closure, then the primary mechanisms of wound closure (in the absence of proliferation) are cell spreading and migration. As a measure of cell migration alone, cell velocity was measured by monitoring the same set of cells over the time course and measuring the movement of the individual centroids into the wound.

Fluorescence microscopy of fixed cells. Cell monolayers were fixed with 3.7% formaldehyde for 10 min at room temperature. After being rinsed twice with PBS, cells were permeabilized with 0.5% Triton X-100 in PBS for 5 min at room temperature. Cells were stored under PBS at 4°C. Monolayers were rinsed three times with PBS for 3 min and incubated with rhodamine-phalloidin (0.3 μM) for 30 min at room temperature. After finally being rinsed three times in PBS for 3 min each, the monolayers were mounted under a coverslip with Aquamount (H2O, glycerol, and polyvinylalcohol; Polysciences, Washington, PA).

Statistical analysis. Data are presented as means ± SE for the indicated number of experiments (n). Comparisons between mean values were made with the use of repeated-measures analysis of variance and Tukey’s modified t-test (the Bonferroni correction).

RESULTS

Shear stress and wound closure. To investigate the effect of shear stress on HUVEC and HCAEC wound closure, cultured monolayers were sheared for 18 h at 12 dyn/cm², wounded with a metal spatula, and then exposed to continued shear flow or left static. Figure 1 shows that HUVEC cell alignment was induced after 18 h of shear stress, and that shear stress influences the rate of wound closure in these cells. Figure 1A shows a representative confluent HUVEC monolayer before exposure to shear, and Fig. 1B shows the alignment of cells in the direction of flow after 18 h of 12 dyn/cm² of shear stress. After the preshear period, wounds were induced (Fig. 1C), and the cells were sheared at 12 dyn/cm² for an additional 6 h (Fig. 1D). In Fig. 1D, the HUVEC wound had closed to 45% of the original wound width by 6 h compared with the static wounds (Fig. 1E, initial wound), which closed to only 60% of the original wound width (Fig. 1F). All cell monolayers were similarly presheared. These data indicate that shear stress increases wound closure in presheared monolayers.

To determine the extent of wound closure as a function of the magnitude of shear stress, monolayers were presheared for 18 h at 12 dyn/cm² and then subjected to shear stresses of 3, 12, or 20 dyn/cm² under S-W-sH or S-W-sT conditions. These data are summarized from multiple experiments and presented in graphical form as a percentage of original wound width (Fig. 2). Differences in wound closure rate were apparent as early as 1 h after wounding. After 6 h under S-W-sH conditions (3, 12, or 20 dyn/cm²), HUVEC wounds closed to 21, 39, and 50%, respectively, compared with S-W-sT, which had only closed to 59%. Interestingly, wound closure was enhanced at lower levels of shear stress compared with higher shear stress. In HUVEC, a shear stress of 3 dyn/cm² was associated with significantly greater wound closure compared with 12 or 20 dyn/cm² or static monolayers (P < 0.05). Wound closure at 12 dyn/cm² was significantly greater than at 20 dyn/cm² between 3 and 6 h (P < 0.05). After 1, 2, and 6 h, even cells subjected to 20 dyn/cm² shear stress showed significantly greater wound closure than did cells under static conditions (P < 0.05).

After 6 h, HCAEC wounds under S-W-sH conditions closed to 29, 49, or 33% in response to 3, 12, or 20 dyn/cm² shear, respectively, and under S-W-sT conditions they closed to 58% of original wound width (Fig. 2B). It is noteworthy that a laminar shear stress of 3 or 20 dyn/cm² significantly enhanced wound closure compared with static (P < 0.05), whereas an intermediate shear stress of 12 dyn/cm² did not.

Endothelial cells that were not presheared at 12 dyn/cm², and thus not aligned before wounding, demonstrated quite different behavior in terms of wound closure. As shown in Fig. 3A, HUVEC W-sH cells show the greatest effect on wound closure with higher levels of shear (20 and 12 dyn/cm²) compared with 3 dyn/cm² or W-sT (P < 0.05). This contrasts markedly with cells that had been presheared (S-W-sH; Fig. 2A). By 6 h,
W-sH HUVEC wounds under a shear stress of 3, 12, or 20 dyn/cm² or W-sT closed to 53, 25, 19, or 52%, respectively (Fig. 3A). As shown in Fig. 3B, HCAEC demonstrate enhanced wound closure with 12 or 20 dyn/cm² shear stress compared with 3 dyn/cm² or W-sT (P ≤ 0.05). The enhancement of wound closure in HCAEC was not as pronounced as in HUVEC but followed a similar pattern of increased wound closure with increasing shear level. By 6 h, the HCAEC wounds under shear stress of 3, 12, or 20 dyn/cm² or W-sT closed to 56, 42, 44, or 65% of their original widths, respectively. To further compare presheared and “naive” endothelial cells from both arterial and venous origins, we plotted the percent wound closure after 6 h as a function of the shear stress during wound closure (Fig. 4). These data indicate that physiological laminar shear stress accelerates closure of wounded endothelial monolayers.

To determine whether shear stress enhances endothelial closure through stimulation of proliferation, HUVEC and HCAEC were subjected to BrDU labeling as detailed in METHODS. No significant differences in BrDU labeling between sheared and static monolayers were observed. On average, only five cells were labeled per high power field. Because of the short duration of these experiments, it is not surprising that there was little proliferation. We also did not detect a difference in BrDU labeling after 10 h in either protocol (data not shown).

Shear stress enhances cellular spreading and migration. To distinguish between the contributions of cell spreading and migration in wound closure, cell area,
centroid-to-centroid distance (CCD), and cell velocity at the wound edge were measured. The measurements were conducted in presheared monolayers over the course of 6 h after wounding. As shown in Fig. 5, A and B, cell area increased over time even in static monolayers, and shear stress significantly enhanced cell spreading. In both cell types, 3 dyn/cm² promoted the largest acceleration in wound closure (Fig. 2, A). In HCAEC, wound closure was significantly enhanced at 3 (○) compared with 12 (●) or 20 dyn/cm² (▲) or static monolayers (△). In HCAEC, wound closure was significantly enhanced at 3 and 20 compared with 12 dyn/cm² or static monolayers. Data are expressed as percentages of original wound width, and error bars represent SE. For HUVEC, differences in wound widths between 3 and 12 dyn/cm² compared with static conditions were significant at all time points; P ≤ 0.05, n = 4. At 20 dyn/cm², differences in wound widths were significant at 1, 2, and 6 h compared with static. For HCAEC, differences in wound widths for 3 and 20 dyn/cm² compared with static conditions are significant at all time points including and beyond 2 h; P ≤ 0.05, n = 4.

Fig. 2. Effect of laminar shear stress on wound closure in HUVEC (A) and human coronary artery endothelial cells (HCAEC; B) exposed to S-WsH conditions. In HUVEC, wound closure was significantly enhanced at 3 (○) compared with 12 (●) or 20 dyn/cm² (▲) or static monolayers (△). In HCAEC, wound closure was significantly enhanced at 3 and 20 compared with 12 dyn/cm² or static monolayers. Data are expressed as percentages of original wound width, and error bars represent SE. For HUVEC, differences in wound widths between 3 and 12 dyn/cm² compared with static conditions were significant at all time points; P ≤ 0.05, n = 4. At 20 dyn/cm², differences in wound widths were significant at 1, 2, and 6 h compared with static. For HCAEC, differences in wound widths for 3 and 20 dyn/cm² compared with static conditions are significant at all time points including and beyond 2 h; P ≤ 0.05, n = 4.

Because wound closure is also dependent on cell migration velocity, we measured cell velocity every fashion to cell area and wound closure in HCAEC under conditions of shear stress (Fig. 5D). These data show that wound closure and CCD increase in parallel.

To determine whether changes in cell area and CCD were associated with changes in actin microfilament orientation and thickening, HUVEC monolayers were presheared and wounded as described in METHODS. The cells were then fixed and stained at 6 h with rhodamin-phalloidin. Increased actin filament alignment and thickening with increasing shear stress are demonstrated in Fig. 6 (Fig. 6A, static; Fig. 6B, 3 dyn/cm²; Fig. 6C, 12 dyn/cm²; and Fig. 6D, 20 dyn/cm²). These results suggest that a lower level of laminar shear stress in HUVEC allows for increased mobility and enhanced wound closure. In HUVEC under W-sH conditions, the actin fortification was not as clear (Fig. 6E, static; Fig. 6F, 3 dyn/cm²; Fig. 6G, 12 dyn/cm²; and Fig. 6H, 20 dyn/cm²). In general, these effects on actin filament differences were similar in HCAEC (data not shown).

Fig. 3. Effects of postwounding laminar shear stress (W-sH) on HUVEC and HCAEC. A: in HUVEC, wound closure was significantly enhanced at 20 (▲) compared with 3 dyn/cm² (●) or stasis (●) (P ≤ 0.05; n = 4) and 12 (▲) compared with 3 dyn/cm² (●) or stasis (△) (P ≤ 0.05; n = 4 for 20 and 3 dyn/cm², n = 8 for 12 dyn/cm²). B: in HCAEC, wound closure was significantly enhanced at 20 compared with 3 dyn/cm² or stasis at all time points. Differences in wound widths were also significant between 12 dyn/cm² and stasis, except at 1 h. Data are expressed as percentages of original wound width, and error bars represent SE; P ≤ 0.05, n = 4.
hour by measuring the distance traveled by designated cells at the leading edge of the wound (Fig. 7). In presheared HUVEC cells, the velocity was significantly increased by postwounding shear compared with cells placed under static conditions after wounding (Fig. 7A). Migration velocity was significantly enhanced at 3 dyn/cm² compared with other levels of shear stress or stasis. These data further implicate a shear-enhanced increase in migration as a mechanism for wound closure in these cells. For HCAEC, cell velocity was significantly increased under S-W-sH conditions compared with stasis and followed a similar pattern to that of wound closure with shear stress of 3 > 20 > 12 dyn/cm² > static (Fig. 7B). Note that individual cells did not separate from the wound edge, but rather a continuous front of cells migrated.

**DISCUSSION**

In this study we investigated the role of shear stress in mechanisms of endothelial cell wound closure. We used an in vitro physiological model of human endothelial cell monolayers, which were presheared so that cells were aligned in the direction of flow, mimicking the in vivo milieu. The monolayers were then wounded and again subjected to shearing after the injury. In contrast, other models in cultured cells have generally subjected the monolayers to shear without preshearing and attainment of alignment. The major finding of this study is that laminar shear stress enhances HUVEC and HCAEC closure compared with cells in static culture. The mechanisms responsible for enhanced closure are a combination of increased cell spreading and cell migration and not cellular proliferation.

Whereas wound closure in monolayers subjected to S-W-sH was enhanced at lower levels of shear stress in HUVEC, monolayers that had not been presheared before wounding responded favorably to higher levels of shear stress (Figs. 3 and 4). HCAEC responded similarly to HUVEC, except that both lower (3 dyn/cm²) and higher (20 dyn/cm²) levels of shear stress stimulated significant enhancement of wound closure.
compared with 12 dyn/cm². Because a level of 20 dyn/cm² is more commonly seen in the arterial circulation, our findings are consistent with the hypothesis that wound closure is optimal under in vitro conditions that are similar to the conditions to which the parent cells were exposed in vivo.

Significant cell division as a mechanism of wound healing is not likely to occur in cells before 15–24 h (21) but may contribute to wound closure after this time. Extending our experimental endpoint to 10 h after wounding did not reveal a significant increase in cell division in shear stress compared with static endothelial cell monolayers, as assessed by BrDU labeling (data not shown). In contrast to proliferation, cell area and CCD significantly increased at the wound edge in sheared cells compared with static cells (Fig. 5), suggesting that cell spreading and migration occurred during wound closure. In addition, shear stress stimulated the cell migration velocity (Fig. 7), an indicator of motility. The effect on cell velocity was more marked in
HUVEC than in HCAEC. These results show that wound closure in HUVEC and HCAEC occurred primarily by cell spreading.

Endothelial cell wound repair has been studied extensively in vitro in static (non-presheared) cell culture models and in some in vivo investigations with manipulation of blood flow. In most static wound injury models, cell migration, spreading, and proliferation are each important in wound closure (9, 12). To our knowledge, there have been no previous in vitro studies examining the effect of shear on wound healing. As described by Lee et al. (21), the first stage of wound repair is a reduction of dense peripheral bands and cell substratum vinculin plaques. During this stage, the cells elongate and extrude lamellipodia. The second stage includes a reorganization of the central microfilaments parallel to the wound edge and noticeable cell spreading. Cell migration is the third stage, initiated when central microfilaments orient themselves perpendicular to the wound edge and develop vinculin plaques at the tips (21). Other studies show that when cell migration is inhibited, the wounds close purely by proliferation but at a much slower rate (9). This implies that the predominant process in wound closure is cell migration (9). These results are consistent with our model, in which we also found that cell migration was a major mechanism in wound closure for both static and sheared endothelial cell monolayers. However, in our study, we found that cell motility was significantly augmented in the sheared group compared with the static group.

Although complex patterns of flow are seen in areas of atherosclerosis, we do not yet know whether these patterns are detrimental to wound healing in other vascular disease processes. It has been hypothesized, for example, that in the arterial circulation, turbulent and low nonlaminar flows may be more responsible for endothelial cell denudation compared with laminar flows that are more physiological (>10 dyn/cm²) (5, 7, 8). However, this has not been definitively demonstrated. One in vivo study demonstrated that lowering the shear stress in rabbit carotid arteries through ligation, to produce a 70% decrease in blood flow, slowed the repair process of the endothelial cells at the wound edge (33). In that study, the wounds were made with a nylon microfilament, ~100 μm in width, and in the direction of flow. The degree to which flow was completely laminar and to which the cells maintained alignment is not clear, but it is probable that areas of flow separation occurred at the vessel lumen. These results are corroborated by the present study, because in our experiment, the W-sH monolayers showed a decrease in the rate of wound closure with low shear stress. In our S-W-sH monolayers, the lower shear stress of 3 dyn/cm² was associated with more pronounced wound closure (Fig. 2). We speculate that in non-presheared cells (W-sH), the lack of organized cytoskeletal rearrangement and alignment contributes to a slower process of closure in the presence of low shear stress. Whereas laminar shear stress in the straight part of the vessel may increase the closure of the wounded endothelium, disturbed flow with low shear stress may have opposite effects. Vessel wall pulsatility may also be a factor in closure of the wounded endothelium. Lower pulsatility in association with lower shear stress may further retard wound closure. Future studies comparing closure rates of injured endothelium under various flow patterns will enhance the pathophysiological relevance of these in vitro studies.

For our studies, we chose levels of shear stress relevant to those seen in the human arterial and venous circulations. In vivo, shear stress has previously been estimated to be in the 1- to 4-dyn/cm² range in large veins, the 20- to 40-dyn/cm² range in venules, and the 60- to 80-dyn/cm² range in arterioles (5, 11, 15, 22–24). In larger arteries, where most atherosclerosis occurs, the mean wall shear stress is typically in the range of
10–40 dyn/cm² in regions of uniform geometry and away from branch vessels (5). In regions of curvature in the arterial wall, such as the aortic arch, and at bifurcations and near branches, the steady laminar flow is disrupted to create complex forms of low laminar, disturbed laminar, and occasionally turbulent flow (5–7). Such sites are predisposed to wound injuries and can experience wall shear stresses ranging from <1 dyn/cm² to >600 dyn/cm². Endothelial cells lose their elongated orientation in the direction of flow in the presence of low or disturbed flow patterns, and the intimal wall can more easily become denuded. In our study, the finding that 3 dyn/cm² of shear stress was associated with more rapid wound closure and no cell denudation may be due to the fact that the shear was laminar and not turbulent and that this model utilized preshearing before application of the low laminar shear stress.

This is the first report to demonstrate that physiological shear stress enhances wound closure in cultured human umbilical vein and coronary artery endothelial cells and to elucidate cell spreading and migration as the mechanisms for this closure. In addition, this study demonstrates that a low level of shear stress (3 dyn/cm²), as seen at branching points and diseased areas of the circulation, is not associated with a decrease in wound closure in vitro in presheared monolayers. We speculate that the type of shear applied, laminar vs. nonlaminar or turbulent, may be more critical to the wound closure process than just the level of shear alone. Exploration of these concepts will be important to the understanding of a variety of wounds in cardiovascular disorders and to the development of functional endothelial-lined vascular grafts (2, 13, 18, 32). The conditions employed in this study, which more readily approximate those of a wound in the circulatory system, confirm a role for shear stress in facilitating wound closure.

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