Vascular Endothelium Responds to Fluid Shear Stress Gradients

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In vitro investigations of the responses of vascular endothelium to fluid shear stress have typically been conducted under conditions where the time-mean shear stress is uniform. In contrast, the in vitro experiments reported here have re-created the large gradients in surface fluid shear stress found near arterial branches in vivo; specifically, we have produced a disturbed-flow region that includes both flow separation and reattachment. Near reattachment regions, shear stress is small but its gradient is large. Cells migrate away from this region, predominantly in the downstream direction. Those that remain divide at a rate that is high compared with that of cells subjected to uniform shear. We speculate that large shear stress gradients can induce morphological and functional changes in the endothelium in regions of disturbed flow in vivo and thus may contribute to the formation of atherosclerotic lesions. (Arteriosclerosis and Thrombosis 1992;12:1254-1257)

KEY WORDS • shear stress • vascular endothelium • disturbed flow

Vascular endothelium demonstrates a remarkable variety of responses to fluid shear stresses. The cells align with the flow direction, mobilize cytosolic free calcium, activate ion channels, organize internal cytoskeletal structures, and produce vasoactive molecules. Confluent endothelial monolayers also divide in the presence of turbulent flow. These responses have typically been studied in vitro under conditions where the time-mean fluid shear stress is uniform. In vivo, there are large gradients of surface fluid shear stress near arterial branches. The focal occurrence of atherosclerosis in such regions of disturbed flow provides indirect evidence that fluid shear stress gradients may play a role in arterial wall pathology. Our objective was to explore in vitro the specific cellular responses that may be caused by the large shear stress gradients that occur in regions of flow separation and reattachment that are characteristic of arterial bifurcations in vivo.

Methods

Bovine aortic endothelial cells were grown to confluent densities on 12-mm glass coverslips and exposed to steady fluid forces in a cone-and-plate flow chamber for times up to 48 hours. A separated flow region was created by placing a rectangular strip that was 23.8 mm long, 1.0 mm wide, and 0.4 mm high upstream from the region of interest with its largest dimension perpendicular to the flow. The strip overlapped the glass coverslip by 6 mm on each side to minimize flow edge effects. Because of the small dimensions of the model, exact numerical solutions of the complete Navier-Stokes equations were obtained using the computational program NEKTON. The cell response discussed here was obtained at an Re of 4 and 7.5 (see figure captions).

Endothelial cells committed to the cell cycle were identified by in situ monoclonal antibody detection of bromodeoxyuridine (BrdU) incorporation into cellular DNA during DNA synthesis. Immediately after exposure to flow, the coverslips were incubated in BrdU-labeled medium for 20 hours. The antigen was localized by using the avidin-biotin immunoperoxidase staining method. The originally reported method was significantly modified to improve contrast. The cell density and spatial distribution of positively labeled nuclei reported in Figure 2 were obtained from a single coverslip that was divided in small rectangular strips 0.26x0.7 mm, with the long axis of each strip parallel to the separation line. Each data block shown in the histograms of Figure 2 represents the density for one of these 0.26-mm-wide strips. Identification of individual cells and averaging over strips was performed using image-analysis software developed in our laboratory. There were typically 300–700 cells analyzed in each strip.

Results

The upper panel of Figure 1 is a photomicrograph of an endothelial monolayer in a region of flow separation and reattachment. The cells were subjected to 48 hours of laminar shear stress of magnitude \( \tau_w = 25 \) dynes/cm² (as measured away from the disturbed flow region) at Re=4. The Re is defined as \( \rho \nu h^2/ \mu^2 \), where \( h \) is the...
strip height, $\rho$ the fluid density, and $\mu$ the fluid viscosity. The spatial distributions of shear stress and shear stress gradient experienced by the cells are also shown. Cells in the vicinity of the reattachment point retained their original polygonal shape, with no preferred orientation. Elongation and alignment with the flow direction occurred where the shear stress $\tau_w$ exceeded approximately 8 dynes/cm$^2$, the threshold level for alignment found previously with uniform shear.$^5$ Cell morphology reached a steady state after 48 hours.

The spatial variations of shear studied here are analogous to those that occur in vivo in perturbed flow regions such as those existing in arterial bifurcations$^{2,20,25,26}$ and surgically created stenoses.$^6,26$ Although the shear stresses in vivo are difficult to quantify, the observed cellular shapes are quite similar to those reported in Figure 1 of Reference 26 (also Y. Yoshida, private communication).

After 48 hours, a steady-state condition was reached in which adjacent areas of significantly different cell density were observed in the monolayer (Figure 2A; shear stress $\tau_w=16$ dynes/cm$^2$ and $\text{Re}=7.5$). Cell density was lower than that of controls in regions of high shear gradients (reattachment) and was higher than that of controls downstream from the reattachment region. Far downstream (beyond eight “disturbance” heights), the shear stress gradients became negligible and the cell density and shape returned to values that were indistinguishable from those of cells subjected to purely uniform shear conditions.

Measurements from our laboratory (N. DePaola et al, unpublished observations) show that cell division begins in regions of large shear stress gradient within a few hours of the onset of flow. The region where division is enhanced increases over time, reaching a stable maximum in about 2 days. Figure 2B shows the spatial distribution of BrdU-positive-labeled nuclei in the monolayer after 48 hours. Up to 25% of the cells in the disturbed flow region showed a positive stain; this represents a 10-fold increase over the labeled cells in

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Upper panel: Photomicrograph of endothelial monolayer (stained with Gill's hematoxylin) exposed to a shear stress $\tau_w$ of 25 dynes/cm$^2$ for 48 hours. The shear stress $\tau_w$ and shear stress gradient $\partial \tau_w/\partial x$ are shown in the lower panel. Flow is from left to right, with the end of the perturbing strip at $x=0$. The Reynolds number, $\text{Re}$, defined as $\rho \tau_w h^2/\mu$, is 4. Here $h$ is the strip height, $\rho$ the fluid density, and $\mu$ the fluid viscosity. Flow separation, reattachment, and recovery to predisturbance conditions (to within 95% of the asymptotic value) are obtained within four strip heights (1.5 mm) of the separation point at this $\text{Re}$. The shear stress is zero, and the shear stress gradient is a maximum, at the marked reattachment line.
static monolayers or in regions of uniform shear (1.8±0.7%, mean±SEM). The downstream extent of the enhanced cell division appears to be limited to the area where shear stress gradients can be appreciated. These data imply that the observed increase in cell DNA synthesis is induced by local shear stress gradients.

This interpretation is consistent with the in vitro results obtained in laminar and turbulent flows. In laminar flows without shear stress gradients, enhanced cell division (above levels for static controls) was never observed at any level of shear stress smaller than that required to physically remove the cells from the surface. In turbulent flow, cell division has been induced after several hours of exposure to flow environments containing turbulent fluctuations, even though the time-average shear stress was as low as 1.4 dynes/cm², which is well below the threshold shear stress for cell shape change and alignment in laminar flow. It was postulated that the turbulence creates spatial gradients in shear and, at any instant, causes differential forces between the individual cells, thereby triggering cell division. Our data provide strong evidence to support that hypothesis. The controlled steady spatial gradients in the separated region of our experiments provide differential forces between cells that are of the same order of magnitude as those produced by fluctuations in a turbulent flow with a time-mean value for \( \tau_w \) of 1.4 dynes/cm² (see Reference 27).

The region of elevated cell density was located downstream from the region of increased DNA synthesis (compare Figures 2A and 2B). There was no evidence of cell division in the region of increased density at any time, indicating that the density increase was not due to local cell division. Furthermore, a comparison between the distributions of cell density and nuclear labeling shows that the cell density was significantly reduced in the disturbed flow region where labeled nuclei were abundant. We conclude, therefore, that cell migration downstream and out of the region of large shear gradients accounts for the density peak appearing in Figure 2A.

In addition to downstream migration, cell loss may also contribute to the local reduction in cell density observed near reattachment regions. In this region of large shear gradients, a significant fraction of cells were stimulated early enough in the experiments to have completed cell division by 48 hours. However, the average density over the entire monolayer at 48 hours was within 8% of that for controls, which had a low rate of division. It therefore appears that cell loss accompanied cell division. This presumably could occur as the cells round up during the division process, presenting a larger drag profile to the flow and fewer attachment sites to the substrate.

**Discussion**

In the early literature implicating fluid shear stress in the process of atherogenesis, speculations were made that high and/or low shear stress could be responsible for the observed pathology of the artery wall: high shear because of physical desquamation and low shear because of concentration polarization effects at the wall. Fry has presented a review of literature suggesting that disturbed flow, as found near arterial branches, is a more likely agent than either high or low shear per se. Experiments conducted under turbulent-flow conditions have led to similar conclusions. The present experiments in steady separated flow point strongly to fluid shear stress gradient as the common element in disturbed flow regions that triggers responses that may be important in the pathogenesis of atherosclerosis. A similar mechanism may explain recent in vivo observations in a model of aortic stenosis, where morphological changes of the endothelium accompanied by intimal lipid deposition were noted in regions of disturbed flow.

We have observed dramatic changes in cell shape, density, and rate of division in our in vitro model system. In addition to those morphologically apparent responses, endothelium exposed to shear stress gradi-
ents may express manifestations of dysfunction such as altered arachidonate metabolism, growth factor and cytokine production, surface adhesive properties, coagulation and fibrinolytic activities, and altered permeability to macromolecules and lipoproteins. Given the occurrence of many of the aforementioned features in the context of atherosclerotic lesion formation and progression, it is conceivable that shear stress gradients may be an important mechanistic link between hemodynamics and vessel wall pathobiology.

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