Induction of human vascular endothelial stress fibres by fluid shear stress

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Endothelial cells of the arterial vascular system and the heart contain straight actin filament bundles, of which there are few, if any, in the venous endothelium[1–4]. Since stress fibre-containing endothelial cells within the vascular system tend to be located at sites exposed to particularly high shear stress of blood flow, we have investigated, in an experimental rheological system (Fig. 1), the response of the endothelial actin filament skeleton to controlled levels of fluid shear stress. Here we report that endothelial stress fibres can be induced by a 3-h exposure of confluent monolayer cultures of human vascular endothelium to a fluid shear stress of 2 dynes cm^{-2}, approximately the stress occurring in human arteries *in vivo*. Fourfold lower levels of shear stress that normally occur only in veins, had no significant effect on the endothelial actin filament system. The formation of endothelial stress fibres in response to critical levels of fluid shear stress is probably a functionally important mechanism that protects the endothelium from hydrodynamic injury and detachment.

Endothelial cells were collected from human umbilical vein[5] and grown on glass coverslips previously coated with a layer of extracellular matrix[6]. This coating (achieved by precultivation of the coverslips with bovine corneal endothelial cells) was important in that it allowed the formation of confluent human endothelial monolayer cultures that were firmly attached to the substrate (Fig. 2). Cells grown on glass were generally less firmly attached to the surface as revealed by occasional detachment of individual cells and even large groups of cells during exposure to fluid shear stress. Fluorescent staining of the confluent cultures with rhodamine-labelled phalloidin (a specific probe for polymerized actin[7]) and with antibodies to calf thymus myosin[2] and chicken gizzard and pectoral muscle α-actinin[8] showed a rather uniform pattern of stress fibres (Fig. 3). The stress fibres displayed the typical continuous staining for actin and an interrupted pattern for myosin and α-actinin similar to the descriptions of stress fibres found in cultured aortic endothelium[1,9] and other tissue culture cells (for a recent review, see refs 10, 11). Stress fibre staining was most prominent in the peripheral cytoplasm close to the margin of the polygonally-shaped cells. We often observed intensely stained focal areas from which numerous stress fibres were seen to radiate in various directions. Outside the marginal zone intensely stained stress fibres were rather infrequent. Most of the relatively few stress fibres projecting over the central parts of the cells appeared to be rather thin and were only faintly stained.

Controlled levels of shear stress were then applied by using a transparent rotating cone placed with its tip on the centre of the coverslips (Fig. 1). Rotation of the cone caused the culture medium to flow between the cone and the surface of the coverslip. In this study a lamina flow was chosen, producing a shear stress of 2 dynes cm^{-2}, which is about the minimum level in human arteries for basal conditions (2–20 dynes cm^{-2}, locally up to 100 dynes cm^{-2})[12]. No changes in cellular shape or position occurred during exposure to shear stress, as judged by phase contrast microscopy and TV recording of the endothelium throughout the exposure period (1, 2, 3 and 4 h). Temperature and *p*H of the medium were kept constant during the experiments.

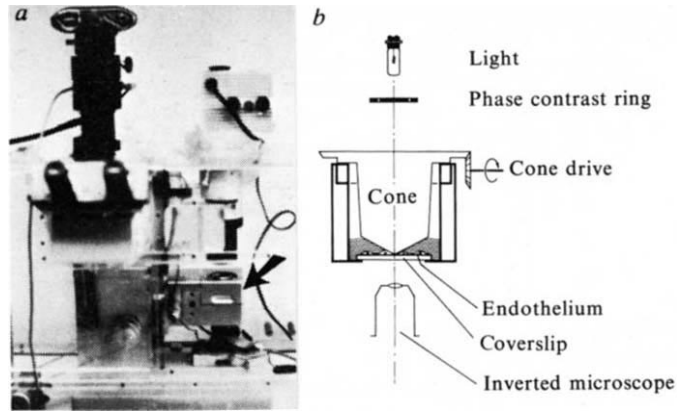

Fig. 1 *a*, Rheoscope used in this study. The construction is based on a cone and plate mechanism originally developed for microrheological studies of blood cell aggregations[20]. An arrow indicates the rheoscope chamber, which is shown in *b* in simplified cross-sectional form (not to scale). A transparent polycarbonate cone having an angle of 2.5° is fitted into a Plexiglas holder and connected to a speed-controlled motor with variable rotational velocities. The cone is placed with its tip on the surface of the coverslip under microscopic control. The direction and magnitude of shear stress were determined precisely both by direct measurements and from theory (ref. 21 and our unpublished data).

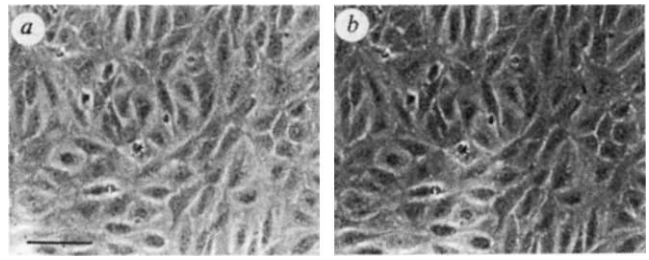

Fig. 2 *a*, *b*, Phase contrast micrographs of confluent monolayer cultures of human vascular endothelial cells collected from the umbilical vein[5], cultured in medium TC 199 containing 20% pooled human serum and 1 mg ml^{-1} endothelial growth supplement extracted from bovine brain[22]. Cells were seeded on coverslips previously coated with an extracellular matrix layer consisting of bovine corneal endothelium[6], and were grown for 8–10 days. The same area of the monolayer was monitored throughout the exposure to fluid shear stress (2 dynes cm^{-2}). *a*, Before shear stress exposure; *b*, after 3 h of exposure. No changes in cellular shape or position have occurred. Scale bar, 100 μm.

After a 3-h exposure to shear stress, the quantity and staining intensity of stress fibres had dramatically increased (Fig. 3). No preferential alignment or direction of the newly formed stress fibres was seen. The exact time course of induction of stress fibres has so far not been determined. At present we can only state that after a 1-h exposure to shear stress there was no increase in the amount of stress fibres, but a slight increase was observed after 2 h. After 4 h the quantity and density of stress fibres were about the same as after 3 h of exposure to shear stress. Thus most stress fibres seemed to have developed during the second and third hour of shear stress treatment. When the shear stress was reduced to fourfold lower levels (0.5 dynes cm^{-2}), a level normally found only in the venous system, no significant increase in the quantity of stress fibres was seen after 3 h of exposure.

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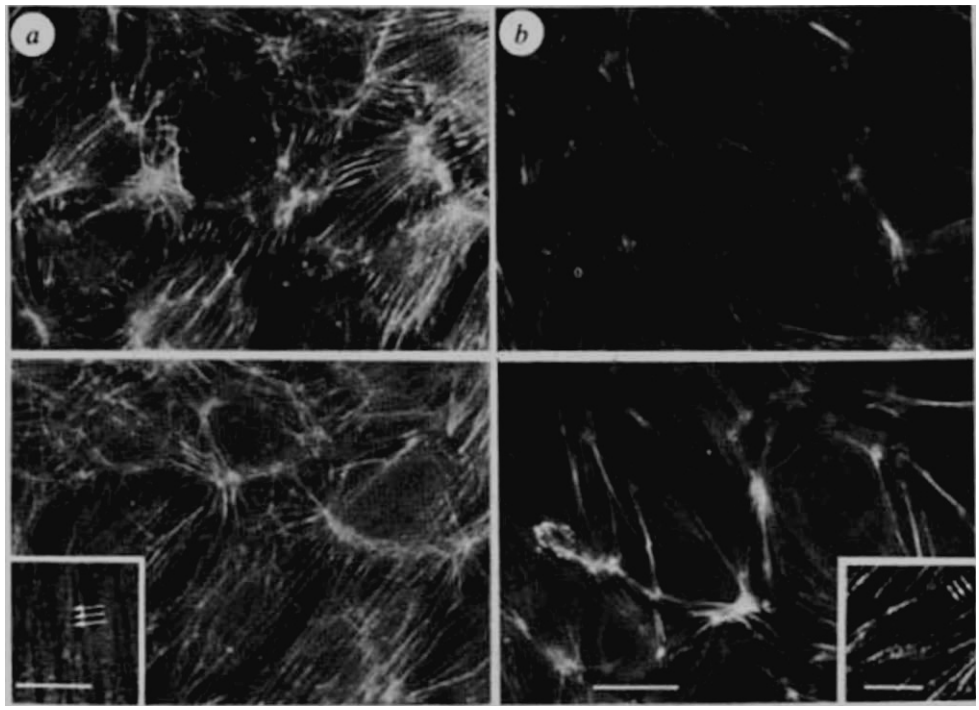

Fig. 3 *a*, Pattern of actin filament bundles (stress fibres) in human endothelial monolayer cultures (two examples are shown) after 3 h of exposure to low arterial levels of fluid shear stress (2 dynes m^{-2}). *b*, Corresponding controls. Scale bar, 20 μm. Only the central parts of the monolayers (area of ~1 cm^2) were examined, because endothelial cells located close to the margin of the coverslips were still in a subconfluent state and thus contained varying amounts of stress fibres. Each shear stress experiment was followed by a control experiment in which corresponding cultures were placed in the rheoscope chamber for the same length of time as for exposure to shear stress, but without rotation of the cone. Stress fibres were visualized by fluorescent staining with 1.4 μg ml^{-1} tetramethylrhodaminyl- which was applied to the monolayers after 5 min of fixation with 2% paraformaldehyde in phosphate-buffered saline and 3 min of permeabilization with 80% acetone (all steps were done at room temperature). The insets show stress fibres at higher magnification (scale bars, 10 μm) stained with antibodies to calf thymus myosin (inset in *a*) and chicken gizzard α-actinin (inset in *b*) using the indirect immunofluorescence technique described elsewhere[2].

Although no attempts were made to determine the threshold value of shear stress that induces stress fibre formation in this *in vitro* system, the experiments reported here clearly show that at a critical level of fluid shear force (which seems to be much lower *in vitro* than *in situ*) endothelial cells will respond by forming stress fibres. These stress fibres probably protect the cells against the shear stress of fluid flow. In view of the observation that isolated stress fibres can contract in appropriate conditions[13,14], it seems reasonable to assume that endothelial stress fibres may apply tension to resist the shear forces acting on the cells, thus allowing the cells to maintain their flattened phenotype and to remain firmly attached to the substratum. To a certain degree, stress fibre formation may prevent hydrodynamic damage to the endothelium and thus protect the vascular wall from certain pathological stimuli such as those thought to be involved in the initiation of arteriosclerosis[15].

The shear-induced stress fibres were more or less randomly distributed with respect to the direction of fluid flow. This pattern apparently differs from the alignment of stress fibres in arterial endothelial cells *in situ*, which is characterized by orientation of the fibres parallel to the direction of blood flow[1–4,16]. However, as the arterial endothelium consists of elongated cells having their long axis oriented in the direction of blood flow (this kind of orientation is probably caused by long-term shear stress in one direction)[17], and in view of the fact that in elongated, cultured cells stress fibres are mainly aligned parallel to the cellular long axis[18], it seems likely that the uniform alignment of arterial endothelial stress fibres parallel to the blood flow depends on the particular orientation of arterial endothelial cells rather than on direct actions of fluid shear stress on the orientation of stress fibres. In the experimental conditions of the present study, the confluent polygonal endothelial cells did not change their shape during shear stress; this is probably the reason why the induced stress fibres did not display a uniform alignment in the direction of the fluid flow. Support for this view comes from regenerating arterial endothelial cells of the rabbit aorta: at the edges of the outgrowing endothelium, stress fibres were frequently found oriented diagonally or even perpendicularly to the blood flow but the fibres were always aligned parallel to the cellular long axis[3]. Similar observations were obtained with subconfluent and confluent cultures of endothelium exposed to long-term (1–2 days) shear stress. Reorientation of endothelial cells in the direction of the fluid flow was accompanied by re-alignment of the stress fibres which were always (before and after the shear treatment) oriented roughly parallel to the cellular long axis (ref. 19 and our unpublished observations). However, such changes in the alignment of stress fibres seem to be only secondary effects brought about by shear stress-induced reorientation of endothelial cells.

The present study provides the first evidence that in human vascular endothelium, fluid shear stress may act directly on the stress fibre system without affecting cellular shape and orientation. The factors mediating this response of the endothelial actin filament system remain to be determined.

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