MECHANICAL EFFECTS ON ENDOTHELIAL CELL MORPHOLOGY: IN VITRO ASSESSMENT

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SUMMARY

Endothelial cells are subjected to fluid mechanical forces which accompany blood flow. These cells become elongated and orient their long axes parallel to the direction of shear stress when the cultured cells are subjected to flow in an in vitro circulatory system. When the substrate is compliant and cyclically deformed, to simulate effects of pressure in the vasculature, the cells elongate an orient perpendicular to the axis of deformation. Cell shape changes are reflected in the alignment of microtubule networks. The systems described provide tools for assessing the individual roles of shear stress, pressure, and mechanical strain on vascular cell structure and function.

Key words: endothelial cells; compliance; shear stress; pressure; alignment; microtubules.

INTRODUCTION

Blood flow produces a tangential force (shear stress) on the luminal surface of the vessel wall. The pulsatile nature of the flow in an artery, through its pressure waveform acting on the compliance of the wall, produces a periodic variation in vessel radius, resulting in cyclic stretching of the vessel wall cells. The endothelial cells lining the vessel wall are subjected to both fluid shear stress and the pressure-induced strain components of the flow.

Mechanical forces have been proposed as causative factors in cardiovascular disease and have been implicated in modulating endothelial cell morphology and function (1,3-9,11,17,22). However, in vivo research to establish their significance is limited by the inability to accurately measure hemodynamic factors in the vicinity of the vessel wall. In addition, the ability to monitor and control all of the appropriate physical and biochemical variables is not possible at present in in vivo models.

The aim of this work is to establish in vitro models in which the effects of fluid mechanical forces on endothelial cells can be independently studied to understand the mechanism by which shear stress and strain modulate morphology and metabolism of these cells. In studies described herein, endothelial cells were grown on a compliant substrate and then subjected to well-characterized levels of either shear stress or mechanical strain. The goal was to determine the separate effects of shear stress and pulsatile strain on endothelial cell morphology.

MATERIALS AND METHODS

Cell culture. Human umbilical vein endothelial cells (HUVEC) were harvested from umbilical cords obtained from Labor and Delivery, Methodist Hospital. The cords were kept at room temperature and utilized within 3 to 4 h of delivery. Culture procedures were adapted from those of Gimbrone (10). To remove the endothelial cells, the veins were cannulated, rinsed with 50 ml of phosphate buffered saline (PBS), and then filled with 0.03%/0 collagenase in Medium 199 (GIBCO, Grand Island, NY) and incubated for 20 min. After incubation the enzyme solution was flushed through the cord with 40 ml of PBS, the effluent was collected and centrifuged at 1000 rpm for 10 min. After centrifugation, the cell pellet was resuspended in Medium 199, supplemented with 20% fetal bovine serum (FBS, HyClone, Logan UT), 100 U/ml penicillin and 100 μg/ml streptomycin. Between 7.4 X 10⁴ and 1.5 X 10⁶ cells/cm² were seeded onto the substrate for experiments.

Bovine aortic endothelial cells (BAEC) were obtained using techniques previously described (6). The cells were cloned and used in the 4th through the 7th passage. For an experiment, cells from a T-25 flask were detached, using 0.25% trypsin in 1:5000 EDTA, centrifuged at 1000 rpm for 10 min, and the pellet resuspended in complete
FIG. 1. Schematic drawing of the stretch chamber. In chamber 1, the compliant membrane experiences the same fluid motion as in experimental chamber 2, but it is not stretched. For details, see text.

FIG. 2. Bovine aortic endothelial cells cultured on Mitrathane for 3 d—then subjected to 66 h of a, movement of the medium over the cells (control) and b, 10% cyclic stretching of the Mitrathane at 1 Hz. Axis of stretch is horizontal. ×120.