EFFECT OF SHEAR STRESS, CYCLIC UNIAXIAL STRETCH AND GRAVITATIONAL UNLOADING ON THE CYTOSKELETON AND GENE EXPRESSION OF BOVINE CAPILLARY ENDOTHELIAL CELLS

Stergios C. Dermenoudis[1], Vasilis Kotsikoris[2], Stavros Topouzis[2], Yannis F. Missirlis[1]

[1] Laboratory of Biomechanics & Biomedical Engineering, Mechanical Engineering & Aeronautics Dept., University of Patras, Rion, Greece; [2] Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras,, Rio, Greece

Introduction

Endothelial cells (EC), that form a monolayer lining the luminal surface of blood vessels, interact with biochemical ligands and sense mechanical factors in their milieu. This stimulation triggers the modification of metabolic activities such as intracellular signalling and gene expression. As a response, ECs change their morphology, remodel the extra-cellular matrix and evoke functional regulations of the cardiovascular system.

The aim of this work was to expose ECs to a combination of mechanical stimuli, such as fluid shear stress (FSS) [Kataoka, 1998], cyclic uniaxial stretching [Moretti, 2004] and gravitational unloading, using our bioreactor system [Dermenoudis, 2010]. The response of ECs was evaluated by cytoskeleton reorganization and gene expression levels.

Methods

Ethylene Vinyl Acetate tubes (4mm) were coated with 4% gelatine and loaded on the bioreactor. Bovine capillary endothelial cells (BCE-HT+) were infused (50.000 cells/cm^2) (tube rotation 9rph). 4hrs later a mechanical stimulus (1,24Pa, 10% strain 1Hz, 2000rph or a combination) was superimposed for 15hrs. The cells were fixed and stained against tubulin and f-actin. The experiments were repeated in order to isolate the mRNA for RT-PCR reactions to detect the expression of ICAM-1, MMP-1, eNOS and endothelin-1.

Results

FSS caused the alignment of ECs parallel to the flow, cyclic uniaxial stretching resulted in the orientation of cells almost perpendicular to the strain direction, while, microgravity led to elongated cells towards every direction. When combined, uniaxial stretching had the strongest effect on the cells. Cytoskeletal reorganization patterns caused by a single stimulus were also present in experiments with a combination of mechanical stimuli (figure 1)

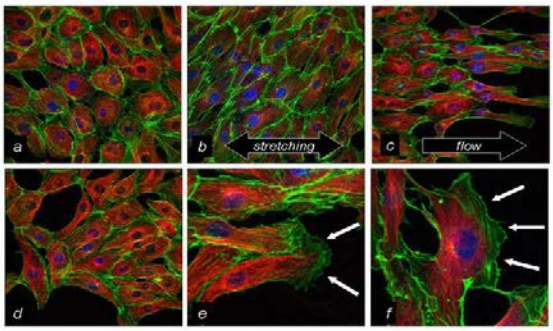

Figure 1: green:f-actin, red:tubulin a)control, b)cyclic stretching, c) flow, d)rotation, e)flow: f-actin protrusions downstream the flow(arrows), f)flow+stretching: cells aligned perpendicular to the stretching direction + f-actin protrusions downstream the flow(arrows)

Significant changes were recorded in the mRNA levels of several genes such as ICAM-1 (chart 1).

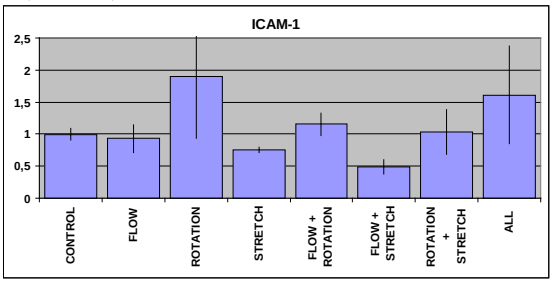

Chart 1: mRNA levels of ICAM-1.

Discussion

The use of our bioreactor system has enabled us to observe, measure, and enhance our understanding of the structural and functional responses of ECs to a combination of relevant mechanical stimuli.

References

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Moretti *et al*, J. Mater. Sci. - Mater. Med., 15:1159-1164, 2004.