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MECHANO-BIOLOGICAL INTERACTIONS OF ENDOTHELIAL CELLS

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INTRODUCTION

Atherosclerosis is an ever-increasing cause of morbidity in the western world. Current surgical treatments include bypass grafts and coronary artery stents. However there is still a need for alternative approaches, especially for those who cannot receive conventional therapy. Tissue engineering is one such approach that may hold the key to the repair and regeneration of coronary arteries. Nevertheless, many questions need to be answered before a viable vascular tissue with the inherent properties of native tissue becomes a real contender with the surgical therapies in use today.

In vivo endothelial cells are exposed to pulsatile shear and tensile stresses. To date there have been many studies which have examined the effect of shear stress on endothelial response (Nerem, 1993, Topper, 1999, Blackman, 2002). However, the effect of pulsatile tensile stress alone has yet to be investigated comprehensively. The main aim of this research is to design and build a mechano-transduction rig to investigate the effect of tensile stress on the cell morphology and protein expression of human endothelial cells (EC).

MATERIALS AND METHODS

In this study, cultured human umbilical vein endothelial cells (HUVEC's) were cultured to a confluent level, on oxygen plasma treated silicone substrates. After an incubation period of 4 days, the cell seeded constructs were mounted in the purpose built rig and cyclically stretched by 10% at 1 Hz in a physiological environment for 4 hours, mimicking the beating of a healthy heart *in vivo*. In addition control specimens were produced in a static environment.

The cell morphology and orientation of the control samples and the cyclically stretched samples were examined using a TRITC-phalloidin stain in conjunction with confocal laser scanning microscopy (LSM-510). With respect to protein expression, samples were stained with FITC-conjugate of mouse anti-human monoclonal antibodies for ICAM-1 and VCAM-1 and qualitatively examined using the confocal laser scanning microscope. The changes in fluorescence intensity due to binding of the FITC-conjugate of mouse anti-human VCAM-1 and ICAM-1 monoclonal antibodies in the endothelial cells was taken as a measure of protein expression.

RESULTS

As shown in Figure 1 the cells subjected to cyclical strain began to elongate and orient along their long axes perpendicular to the direction of the applied strain after 4 hours. With respect to protein analysis, a decrease in the protein expression of VCAM-1 and an increase in the expression of ICAM-1 was observed when the mechanically conditioned cells were compared with the controls.

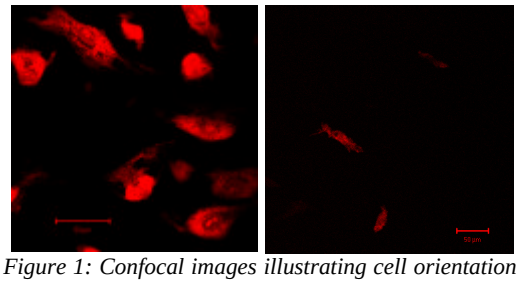

Figure 1: Confocal images illustrating cell orientation after cyclic stretch for 4 hours

DISCUSSION

The effect of tensile stress on endothelial cell morphology and protein expression was examined in this study. With respect to cell morphology, it can be seen that the cells began to elongate and orient themselves perpendicular to the direction of applied stress after 4 hours. In terms of protein analysis it can be seen that there was a downregulation of VCAM-1 and an upregulation of ICAM-1. In summary these results qualitatively demonstrate that the biological response of endothelial cells is affected by an applied tensile stress in a simulated physiological environment.

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