EFFECTS OF SIMULATED MICROGRAVITY CULTURE TECHNOLOGY ON CELL-CELL AND CELL-SUBSTRATE ADHESION

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INTRODUCTION

The discovery of new methods for improving cell growth and function in culture is vital to the development of biomedical applications like prosthetic vascular grafts. Critical problems with prosthetic vascular grafts post-implantation include reduced endothelial cell retention, intimal hyperplasia and a reduction in graft patency. Vascular graft research is now addressing ways to improve endothelial cell retention on the lining of prosthetic vascular grafts (PVG). Extracellular matrix proteins, such as fibronectin, and shear stress have been shown to enhance cell-substrate adhesion with respect to cell seeding and retention [1, 2].

Since seeded endothelial cells have demonstrated a decrease in retention post-implantation, it is proposed that culturing human umbilical vein endothelial cells (HUVEC) seeded onto coated planar discs using simulated microgravity culture technology will result in increased adhesive strength of cell-cell and cell-substrate attachment.

The objectives of this project are to better understand the effects of simulated microgravity and to develop a protocol to enhance the adhesion of isolated endothelial cells to a PVG. This knowledge will then help in the improvement and increase the longevity of prosthetic vascular grafts post-implantation.

BACKGROUND

A novel cell culture technique has been developed which allows cells to grow in a low shear environment (simulated microgravity) [3]. Cells cultured using simulated microgravity culture technology are able to grow and differentiate to form 3-D tissue aggregates that structurally and functionally resemble parent tissue. It has been shown that cultures grown in states of microgravity promote cell-cell interaction by "up-regulating various cell-cell adhesion molecules and ECM proteins" [3]. Adhesive forces as observed in endothelial cells are produced by several interactions, including cell-matrix and cell-cell interactions. Because it has been shown that both gravity and shear stress alter cellular activity, such as gene expression and metabolic activity, it is critical to determine what effects these two parameters have on cell adhesion [4].

Since large diameter prosthetic vascular grafts (PVGs) are used to replace atherosclerotic vessels or vessels weakened by diseases such as diabetes, it is vital to develop techniques to enhance the adhesion of endothelial cells onto the inner lining of PVGs if the lifetime of the implanted graft is to be optimized. Since the isolation procedure of endothelial cells may disrupt the cell's ability to develop a normal cell-substrate and/or cell-cell adhesion, then a reduction in endothelial cell retention post-implantation can be expected, greatly reducing the potential longevity of the graft. Evidence from studies utilizing microgravity culture technology indicates that culturing endothelial cells using simulated microgravity culture technology may result in an increase in cell-cell and cell-substrate adhesion as previously reported for kidney epithelial cells [5].

MATERIALS AND METHODS

Human umbilical vein endothelial cells (HUVEC) are seeded onto 1:5 Matrigel coated ¼" circular planar discs until the cells reach a density equivalent to 80% confluence. When the cells reach 80% confluence, the seeded cells will continue to grow for 48 hrs in one of three culture environments --- simulated microgravity (low gravity and low shear stress), a perfusion flow system (gravity and variable shear stress) or a conventional culture environment (gravity and no shear stress). Following the 48 hour culture period, the cells are fixed in 4% paraformaldehyde, stained with hematoxylin and eosin (H&E) and morphometric analysis such as cell surface area, perimeter, width and length is performed. Immunocytochemistry will also be performed on seeded cells cultured in each of the three specified culture environments in order to localize and identify the presence of fibronectin (cell-substrate adhesion protein) and E-cadherin (cell-cell adhesion protein). Enzyme-linked immunosorbent assays will be performed on seeded cells cultured in each of the three specified

culture environments in order to quantify fibronectin and E-cadherin protein levels.

RESULTS AND DISCUSSION

Morphometric analysis of seeded cells cultured in either a simulated microgravity environment or in a conventional culture environment (Figure 1) illustrates that there is a statistically significant morphological difference between cells cultured conventionally or cells cultured in simulated microgravity. Significance was determined with respect to cell width, length, perimeter and aspect ratio (cell width/cell length).

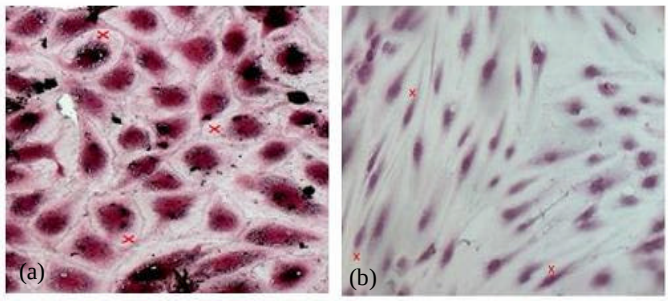

Figure 1. H&E stained HUVEC (a) conventional and (b) simulated microgravity.

The surface area of seeded cells cultured in simulated microgravity or in conventional culture is not statistically different as expected due to no change in cell volume between the cells. However, cells cultured in simulated microgravity are longer and thinner than cells cultured in conventional culture. This is most likely due to the small shear stress (~ 0.52 dynes/cm^2) the cells experience in simulated microgravity culture as opposed to none in conventional culture. In addition, one major difference between the cells cultured in simulated microgravity and those cells cultured in conventional culture is that cells cultured in simulated microgravity develop fine hair-like projections at each pole of the elongated cell body (Figure 2).

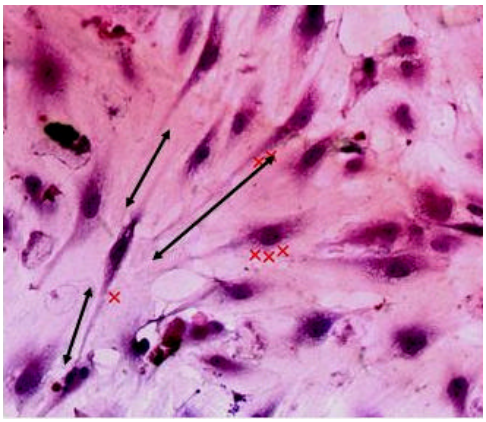

Figure 2. H&E stained HUVEC cultured in simulated microgravity. The black arrows indicate the hair-like projections.

Seeded cells cultured using simulated microgravity grow in an environment similar to that observed in space (i.e., low gravity and low shear stress). Cells cultured using conventional culture grow in an environment with gravity but devoid of shear stress. Cells cultured in a low shear perfusion flow system experience varying levels of shear stress and gravity. Therefore, by studying the morphology and the adhesion of seeded cells cultured in each of these three specified culture environments allows for the direct analysis of shear stress and gravity effects on seeded HUVEC. Morphological data is currently being collected for cells cultured in a low shear perfusion flow chamber. Future testing of cells cultured in each of the three specified culture environments includes immunocytochemistry for localization of fibronectin and E-cadherin and protein analysis, specifically fibronectin and E-cadherin adhesion proteins using ELISA's. Further study of the adhesion proteins via ELISA's and immunocytochemistry will aid us in better understanding the composition of these hair-like projections as observed in cells cultured in a simulated microgravity environment.

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