DIFFERENTIAL GENE EXPRESSION IN ENDOTHELIAL CELLS: FLUID SHEAR STRESS STIMULATED VS. ROTATING WALL VESSEL BIOREACTOR CULTURED

Amir Rezvan, Eric M. Troop, Harsh Jain, Donnie M. Simons, Donald S. Morris, Fred D. Allen and Peter I. Lelkes

School of Biomedical Engineering, Science and Health Systems
Drexel University
Philadelphia, PA

INTRODUCTION

Rotating wall vessel bio-reactors have been used as model systems to study the effects of simulated microgravity on cell culturing. In addition to the buoyancy-gravitational equilibrium of the cells achieved by RWVs there are fluid-induced low level shear stress and mass transport effects imposed on the cells. Although shear flow effects on endothelial cells has been well studied in physiological cardiovascular ranges of fluid flow, little has been examined in very low shear stress ranges such as those produced in RWVs [1,2]. This study uncouples the simulated microgravity effects of the RWV from the shear flow conditions in order to determine the impact of the different environmental signal effectors on endothelial cell gene expression. We examine the differential gene expression induced by low shear stress flow in parallel-plate steady, laminar flow culture experiments versus RWV and static cultures using cDNA gene arrays. Additionally, suspension cultures in non-adhesive bags are performed as a cell aggregation control while pulsatile flow and disturbed flow experiments are performed as flow controls.

The results from the experiments analyzed so far depict a number of genes that are differentially up- or down-regulated under the different conditions. We evaluate the possible role of the gene expression activity correlated to the applied environmental signaling conditions.

METHODS

Cells

RAME (rat adrenal medullary) cells were cultured in Dulbecco's modification of Eagle's medium with 1g/L glucose concentration and supplemented with 0.5mg/L fungizone, 0.3g/L L-glutamine, 50,000I.U./L penicillin, 50 mg/L streptomycin and 10% heat inactivated fetal bovine serum (CELLGRO) and used at passages 18-28.

HMECs (human micro-vascular endothelial cells) were cultured in MCDB 131 medium supplemented with 1mg/L hydrocortisone and 10μg/L EGF in addition to the supplements described and used at passages 18-28.

Cells were seeded and grown to confluence on microscope slides or coverslips in the case of flow experiments and on Cytodex beads in the case of the RWV and suspended bag, prior to flow exposure.

Flow setup

The cells were exposed to an average shear stress of 0.5 dynes/cm^2 produced by steady laminar, pulsatile (1.5 Hz) and disturbed flow for 24 hours. The Rotating Wall Vessel bio-reactor also produces an average shear stress equal to 0.5 dynes/cm^2, in a pattern similar to steady laminar flow [3].

Steady Laminar Flow. A parallel plate system with a rectangular chamber was used for the steady laminar flow model. Shear stress was calculated using equation 1, for a rectangular channel with very low height to width ratio.

$$\tau_{wall} = 6\mu Q/bh^2 \qquad (1)$$

The flow channel used had a width (b) equal to 24.2mm and a height (h) equal to 2.52mm. Using a fluid flow (Q) of 95ml/min, the shear stress of 0.5 dynes/cm^2 was exerted on the cell monolayer. In order to maintain a steady laminar flow, a gravity dependent system assisted by a peristaltic pump was used as shown in figure1.

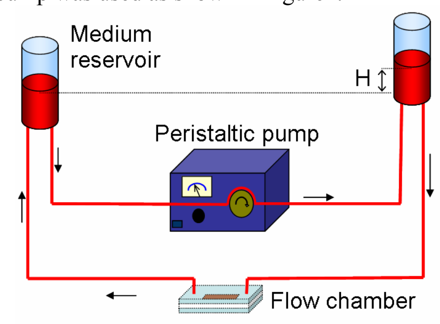

Figure 1. Steady laminar flow setup

Pulsatile Flow. The same flow chamber as in steady laminar flow was used. To achieve the desired frequency and amplitude, two independent pumps were coupled, creating an average flow of 95ml/min, ranging between 115 ml/min and 75 ml/min with 90 beats per minute.

Disturbed Flow. A flow-through cylindrical-cavity apparatus was used to produce low shear perturbed flow patterns (see figure 2). There are four distinct flow patterns in the cavity: central jet, flow impingement, flow separation and recirculating eddies. Cells grown to confluence are fixed to the 5 positions marked on figure 2 on both sides of the chamber.

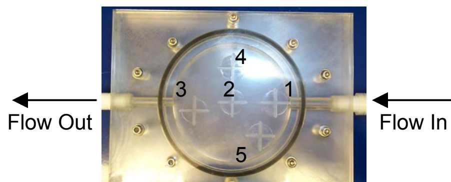

Figure 2. Perturbed Flow Chamber

RWV bioreactor culture. Cells were grown to confluence on Cytodex 3 micro-carrier beads and then cultured in 10 ml Rotating Wall Vessel Bioreactors rotating at 12 RPM (see figure 3).

Figure 3. RWV Bio-reactor

Suspension culture. Since the RWV bio-reactor brings the cell seeded beads near each other and provides for cell aggregation, a suspended culture of the same cell seeded beads in static non-adhesive culture bags was used as a control for the effects of cell aggregation on gene expression.

Static Control. All culture conditions were assessed for differential gene expression against standard static controls. These cells were grown to confluence on tissue culture flasks under standard conditions without any exposure to flow.

RNA extraction

RNA was extracted using TriReagent (Sigma) and RNeasy (Qiagen) methods according to manufacturer's instructions. RNA quantity and quality was assessed by spectrophotometry and gel electrophoresis.

Gene arrays

Clontech Atlas Rat 1.2 cDNA arrays were used to probe extracted RNA and assess differences in expression of the RAME cells between culture venues. Atlas arrays contain 1176 genes representing various physiologic functions from housekeeping to signal transduction. Clontech Atlas Image and Microsoft Excel software was used to extract expression and compare values.

HMEC gene expression is being analyzed at the Winder Research Institute, Windber, PA.

Gene expression results are verified by RT-PCR for selected genes.

RESULTS

The gene analysis performed on the RAMECs so far show genes which are similarly up- or down-regulated (compared to static culture) under both laminar steady flow and RWV cultures, including 40S ribosomal protein S17 (up-regulated) and cytovillin (down-regulated). There are also genes up-regulated in the RWV but not by laminar flow or suspended bag cultures showing an additional element in the RWVs effecting gene expression such as myelin basic protein S and GTP binding protein (Gα8). Genes which are expected to be constitutively expressed were found to vary among culture venues (see figure 4).

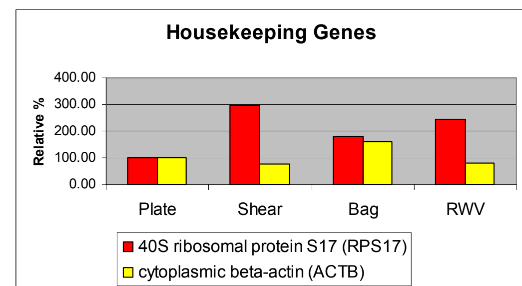

Figure 4. Examples of gene expression changes for different culturing conditions

DISCUSSION

Although changes were seen in RAMEC gene expression for the experiments analyzed so far, more analyses from experiments using a second cell type (HMEC) and the non-steady flow conditions are in progress at the time of this reporting to further evaluate the signal transduction differences between fluid flow stimulation of the cells compared to the microgravity simulation.

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