Identification of Endothelial Cells Using Receptor Expression Changes in Microfluidic Channels

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Statement of Purpose: This work is geared towards identifying three endothelial cell (EC) subpopulations using a microfluidic chamber. This cell-identification approach for endothelial cell subpopulations is label-free and takes advantage of the unique responses of cell receptors to microfluidic shear forces. The cell types examined in the present study are human umbilical vein endothelial cells (HUVECs), adult human microvascular endothelial cells (HMVECs), endothelial colony forming cells (ECFCs), and normal human dermal fibroblasts (NHDFs). The conventional method of EC characterization is by fluorescent labeling of surface markers and analysis by flow cytometry or microscopy.

Methods: HUVEC, HMVEC, ECFC and NHDF were obtained and ran individually in a Hele-Shaw device which gives cell adhesion as a function of shear stress. From this data an optimum shear stress was obtained. This shear stress is where the cells adhere the most. Once this optimum shear stress was obtained then this shear stress was used in a straight channel device. Each cell type studied was first incubated in varying concentrations of the tetrapeptide ligand arg-glu-asp-val (REDV). Following incubation these cells were flowed into the straight channel device, and the number of cells adhered enumerated. To evaluate the specificity of REDV to the integrin α4β1, anti α4 and anti β1 blocking antibodies were used to block these receptors prior to incubation with the varying concentrations of REDV. These cells were then flowed into the straight channel device, and the number of cells adhering enumerated. Flow cytometry was used to evaluate the number of receptors available for binding to REDV prior to and after flow.

Results Our experimental protocol called for incubating the cell suspension of interest in a solution of known REDV concentration to occupy a set number of receptors on the cells prior to flowing these cells into an REDV-coated microchannel. The expectation was that such occupation of cell receptors using solutions of increasing REDV concentration would lower cell adhesion within the REDV-coated channel, ultimately leading to zero adhesion when all receptor sites were engaged. We further hypothesized that the extent of cell adhesion suppression would be different among the different EC cell types thereby providing a means to distinguish them from one another.

The data shown in Figs. 1 and 2 indicate a counterintuitive increase in cell adhesion for all three EC types upon incubation with increasing concentrations of soluble REDV and the magnitude of this increase is different for each EC type. This difference in magnitude of increase is used to distinguish each EC type from each other. To further understand the above results, the influences of intracellular signalling pathways were investigated to better understand the combined effect of shear and ligand on these cells.

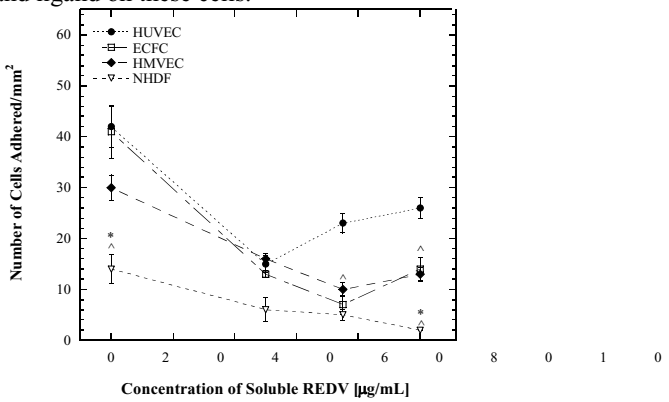

Figure 1. Cell adhesion comparison between cell types on REDV surface at 0.9 dyn/cm^2. ^ denotes significant difference with $p < 0.001$ relative to HUVEC at specified REDV concentrations and * denotes significant difference with $p < 0.001$ compared to ECFC and HMVEC at specified REDV concentrations.

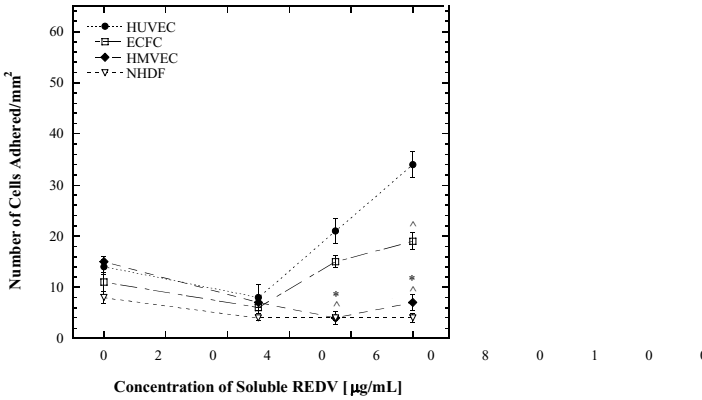

Figure 2. Cell adhesion comparison between cell types on REDV surface at 1.1 dyn/cm^2. ^ denotes significant difference with $p < 0.001$ compared to HUVEC at specified REDV concentrations and * denotes significant difference with $p < 0.001$ compared to ECFC at specified REDV concentrations.

Conclusions: The significance of the results shown in Figures. 1 and 2 is the ability to clearly differentiate between three EC sub-types that cannot be distinguished in a conventional microfluidic cell adhesion assay, using a simple label-free technique that relies only on measurements of cell adhesion using an optical microscope. This technique, when applied to EC subpopulations, can therefore serve as an effective alternative to flow cytometry-based characterization using multiple surface markers and fluorescent labels. This technique, however, would not be able to resolve the content of heterogeneous suspensions containing more than one type of EC.