Lectins: A Means of Stem Cell Identification via Flow Cytometry and Microfluidics

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Introduction

Human embryonic stem cells (hESCs) are pluripotent cells derived from the inner cell mass of blastocyst-stage embryos.[1] They are capable of proliferating and differentiating to form cells of the three embryonic germ layers namely endoderm, mesoderm and ectoderm. Cells of the three germ layers include vascular, muscle and neural cells respectively. Human embryonic stem cells are a model system for studying early events during human development and are a powerful resource of non-transformed human cells for use in diagnostic and therapeutic applications. The successful exploitation of hESC derivatives requires the ability to direct differentiation to specific lineages in defined, efficient, and scalable systems. There is a need for better stem cell differentiation markers. Some of the current markers are not effective at tracking initial differentiation. Hence this work utilizes lectins as stem cell differentiation markers.

Lectins are a group of proteins that bind specifically and reversibly to mono- and oligosaccharide carbohydrate structures. They have been used to identify, characterize, and isolate novel cell subpopulations on the basis of the presentation of specific carbohydrate groups on the cell surface. Reliable readouts for initial differentiation events have been obtained with the use of lectins. In this work a microfluidic platform with immobilized lectins is used to characterize glycan patterns on both human embryonic stem cells, and differentiated endodermal cells (DEs). This platform is implemented as a cheap alternative to the standard technique (flow cytometry).

Experimental

Firstly, flow cytometry was performed on hESCs and DEs to determine lectin surface receptor expression. This was followed by the use of Hele-Shaw devices with immobilized lectins, to characterize cell adhesion as a function of shear stress. Cells were captured in this device and the adhesion enumerated. Comparisons were made between the flow cytometry and the Hele-Shaw data.

Subsequent experiments were performed to determine the binding strength of cells within the device. This was achieved by loading cells into microchannels and incubating for 0, 15, 30 and 60 mins. Fluid was flowed into the device to detach weakly bound cells. The number of cells adhered after flow was then enumerated.

Results and Discussion

Initial characterization of cell surface marker expression was performed using flow cytometry. The results were compared to that obtained from cell adhesion with microfluidic devices designed to generate Hele-Shaw flow. As described in prior work by our group, this device is shaped with a flared geometry that provides a linear gradient of shear stress along the flow channel axis.[2] This geometry provides a versatile platform for the evaluation of cell adhesion to surface-immobilized ligands as a function of shear stress. Hence, comparisons of

adhesion levels on different ligands at a fixed surface density could be made. A higher level of adhesion indicates the cell type has a higher affinity for the ligand.

Comparisons were made between the data obtained from flow cytometry and the adhesion based microfluidic cell capture system. Both techniques are able to provide information about cell surface receptors affinity for a ligand. However for some comparisons both methods displayed varied results. This inconsistency was thought to be as a result of changes in binding strength within the microchannels. Flow cytometry mainly addresses the question of receptor presence. Subsequent experiments were performed to determine the binding strength associated with hESCs and DEs bound to the lectin DBA and SSEA3. This was achieved by allowing cells to adhere within the microchannel and determining the amount of cells that remained adhered after high flow. The results indicate that both cell types bind stronger to the lectin DBA than to SSEA3 (Figure 1). It also indicates that binding strength plays a major role in cell adhesion assays. A cell type may express high levels of a marker and still adhere minimally to that ligand. Hence the significance of this work lies in the ability to determine whether a cell type adheres due to high marker expression or strong binding strength.

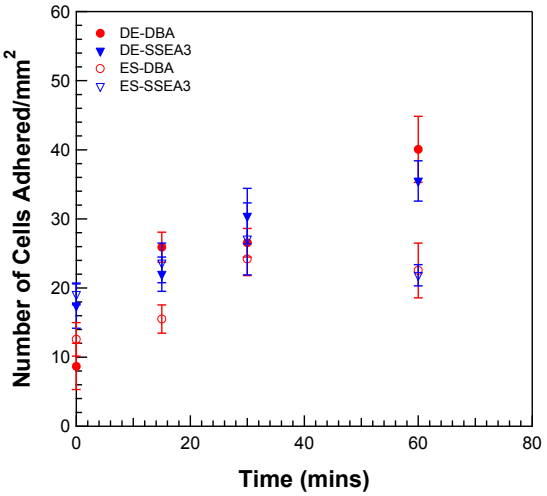

Figure 1: Human embryonic cells and differentiated cells are detached after incubation at 0, 15, 30 and 60 mins.

References:

1 Peerani, R. *et al.* Niche-mediated control of human embryonic stem cell self-renewal and differentiation. *Embo J.* **26**, 4744-4755, doi:10.1038/sj.emboj.7601896 (2007).

2 Plouffe, B. D. *et al.* Peptide-mediated selective adhesion of smooth muscle and endothelial cells in microfluidic shear flow. *Langmuir* **23**, 5050-5055, doi:10.1021/la0700220 (2007).