Fabrication of Scaffolds for Three-Dimensional Culture of Human Endothelial Cells

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Abstract:

Guided by the natural design of the microvascular network of the human brain, we have designed and fabricated cylindrical polydimethylsiloxane (PDMS) channel networks that serve as scaffolds for the in vitro growth of artificial microvascular networks using human umbilical vein endothelial cells (HUVEC). The reconstructed networks allow us to observe how the endothelium changes when treated with tissue factors and to determine whether these changes promote the metastasis necessary attachment and passage of breast cancer cells. Varying endothelial responses towards tissue factors from different organs may hold the key to understanding organ-specific metastasis occurring in the majority of patients suffering from breast cancer.

Summary of Results:

In 40-50% of patients yearly diagnosed with malignant breast cancer, the tumor metastasizes and preferentially spreads to the lung, brain or bone [1-4]. During metastasis, breast cancer cells travel within the circulation and eventually adhere to the endothelium where they pass into the new host organ. The site of adherence is not, as one might expect, determined by the pattern of blood flow within the human body or by mechanical arrest in microvessels [3,4]. Rather, it is likely that the molecular makeup of the microvasculature in the brain, lung and bone promote breast cancer cells adhesion. These organs may also secrete tissue factors that weaken the endothelial barrier, allowing breast cancer cells to invade. To investigate these hypotheses, we are developing a device that realistically mimics the endothelial lining of the vasculature of the host organ and that allows us to observe interactions between breast cancer cells and the microvasculature in vitro. We aim to identify adhesion promoting molecules and tissue factors that weaken the endothelial barrier and thereby further metastasis.

The dimensions, shape, and fluid flow rates of the artificial microvasculature are replicated according to data observed in vivo. PDMS channel networks provide the scaffolds in which human umbilical vein endothelial cells (HUVECs) grow. The networks are fabricated by casting PDMS on negative plastic replica of silicon originals that consist of 50-150 µm wide channels with semicircular cross sectional profiles. Semicircular profiles form during dry etching of silicon with xenon difluoride [5]. We constructed round PDMS scaffolds from two mirrored PDMS casts that we aligned and bonded to each other. Square channels with feature sizes ranging from 50 µm to 200 µm were fabricated using PDMS casting of SU-8 imaged networks. Some devices contain 1-10 µm connecting channels between two main channels. These provide a diffusion path from one channel to the other and are fabricated in cured PDMS using two-photon laser lithography [6]. Using these connecting channels we will be able to manipulate the endothelium from the “tissue-side” with chemo-attractants that we deliver at precise locations via diffusion from a neighboring micro-channel.

In response to effective chemo-attractants we expect to observe an interaction between cancer cells and artificial endothelium either due to the changed molecular composition on the surface of the endothelial cells or due to the weakening of their tight junctions.
Confluent endothelial linings have been grown within square cross sectional PDMS scaffolds with dimensions 50 µm in width and height. For this purpose, the channels were coated with fibronectin and seeded with HUVECs. After 4 days in culture, the cells were assessed for confluence by immunostaining of VE-cadherin. When endothelial cells are confluent, adherens junctions form on the lateral boundaries of adjacent adherent cells. VE-cadherin is a marker of endothelial adherens junctions and the cell borders will stain positive for this protein when they are confluent [7]. The cells were also stained for actin filaments using Alexa 488-phalloidin, which binds F-actin. Images were taken with a Leica confocal microscope with intensity compensation capability (Figures 1-3).

References: