Abstract:

We have developed a physiologically relevant, three-dimensional (3D), microfluidic in vitro model for the studies of cancer cell migration and invasion. The hydrogel-based device is composed of three parallel microfluidic channels. Flows along the two side channels were used for generating a stable chemokine gradient across the center channel. Breast cancer cells were seeded in a 3D collagen matrix, and then injected in the center channel. Cell growth, motility, as well as chemotaxis were investigated. This work is to test the hypothesis that cancer cells may use similar cellular mechanisms to migrate and invade as those employed by immune cells.

Summary of Research:

Modification of Microfabrication Procedures. Recent gene profiling work by Muller et al. [1] revealed that CXCR4 and CCR7 are highly expressed in human breast cancer cell lines. Based on this finding, we hypothesized that breast tumor cells will be chemotactic along the chemokine gradient of SDF-1α, ligand to CXCR4. To study cancer cell migration, an agarose gel device that we previously developed for bacterial chemotaxis [2] was utilized. The channel depth was increased from 100 µm to 250 µm to reduce the pressure when injecting the cell seeded collagen gel, and to minimize the slow flow in the center channel. To obtain the deeper channel, positive features on a silicon wafer were modified. More specifically, a thicker photoresist STR1045 was used together with a longer etching step. The depth was verified with both a surface and an optical profilometer.

Microfluidic versus macro scale in vitro model for cancer cell migration and invasion: current 3D in vitro models for cancer cell migration and invasion assays employ a modified Boyden chamber [3]. Although the technique is easy to implement, the results are population-based, not single-cell-based. Compared to these models, microfluidic devices with an imaging system can reveal spatial and temporal dynamics of a single cell in cancer cell migration and invasion. Microfluidic devices can also provide controllable microenvironments for the cancer metastasis processes. Another advantage of microfluidic devices is the potential for a high-throughput process.

Results and Discussions:

A malignant breast cancer cell line, MDA-MB-231, was obtained from Physical Sciences-Oncology Center at Cornell University and has been maintained in DMEM supplemented with 10% fetal bovine serum, 100 units/ml penicillin and 100 µg/ml streptomycin. Preliminary multi-well plate experiments were performed for establishing a robust 3D cancer cell culture protocol using collagen [4]. For each experimental run, four identical devices were prepared and then a cell-collagen mixture (1 × 10^6 cells/ml, 0.15% collagen) was injected into the center channel of each device. A z-stack image series (approximately 1 mm with a 5 µm interval) was taken in a bright field on an inverted fluorescent microscope (Olympus IX 81 and Hamamatsu Orca-ER). Then, the images were manipulated to reconstruct a 3D picture for visualizing cells in the collagen gel, using a commercial software (Imaris 7.0, evaluation version, Bitplane AG, Zurich, Switzerland) (Figure 1). Both the inlets and outlets were sealed with PDMS plugs to avoid any flow in the center channels. In some cases, side channels were perfused with the growth medium at a flow rate of 5 µl/min in a humidified 37°C, 5% CO₂-controlled incubator for 24 hours. Cells in the devices without the perfusion were observed to be more elongated primarily in the channel length direction (Figure 2), showing that the microenvironment considerably affected cell morphology. Time-course images revealed differential cell motility behavior when the device is with/without the perfused flows along the side channels.
References:


