A Microfluidic System for the Assembly and Culture of Tumor Spheroids

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Abstract:

Multicellular tumor spheroids are 3-dimensional cell clusters that play an important role in the study of cancer. Tumor spheroids can serve as a model for *in vivo* tumor tissue because they have similar geometry and behavior to certain tumor types. Multicellular tumor spheroids have been used as an *in vitro* model for studying tumor cell response to therapy. Spheroidal cell studies have progressed to the investigation of differentiation, apo-ptosis, invasion, and other basic biological mechanisms.

The purpose of this work was to fabricate a micro-fluidic device in which multicellular tumor spheroids can be grown to a specific size and then cultured within the device for period up to one week. Initially myoblast spheroids were assembled, myoblast cells were passed through fabricated microchannels, and then trapped by perforated, semicircular wells within these channels. Arrays of wells embedded in the microchannels were fabricated with diameters ranging from 200-800 μm.

Introduction:

In vitro studies of multicellular tumor spheroids have advanced the understanding of the role of cellular microenvironments in tumor biology [1]. Studies have shown that compared to conventional monolayer cultures, tumor spheroids more closely resemble *in vivo* cells with regards to geometry and cellular environment [2]. For example, in cell-to-cell interactions and cell-to-extracellular matrix multicellular spheroids are a better model than monolayer cultures [2]. Tumor spheroids can also be used to investigate therapies for tumor cells, like drug resistance [2]. Studies have progressed to basic biological mechanisms like apoptosis, differentiation, and invasion. In particular, interest has been placed on the role of the metabolic three-dimensional microenvironment; for example, oxygen, glucose, and lactate distribution, in the development of cell quiescence and necrotic cell death [2].

Current methods for growing and culturing multi-cellular tumor spheroids are challenging. They are cultured in part by rotating them in suspension creating unequal spheroid diameters [1].

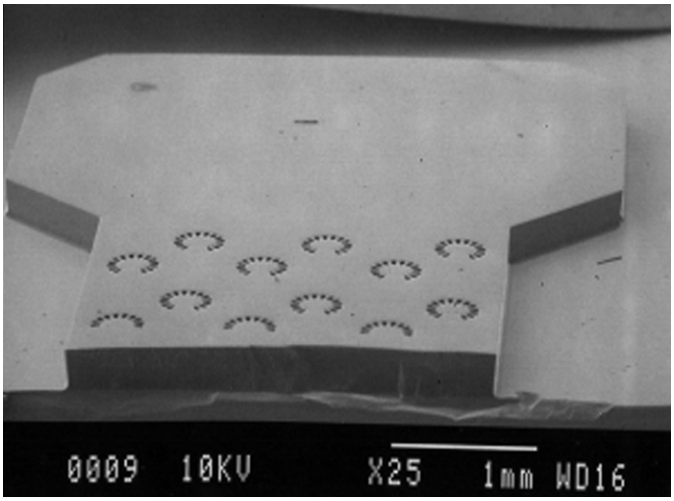

Figure 1: Scanning electron micrograph of SU-8 mold.

Experimental Procedure and Materials:

SU-8 was chosen for the mold material because it allows fabrication of structures with thicknesses greater than 100 μm, Figure 1. PDMS was used because of bio-compatibility, ease of use, and low cost of fabrication. The microfluidic device mold was made from SU-8 2050 photoresist (MicroChem, Newton, MA). The SU-8 had to be characterized for a two coat process. From Figure 3, we can see that two coats at 1600 rpm provide our specified thickness of 200 μm. Photoresist thicknesses were measured at different location on each mold using a Dektak 6M Surface Profiler.

Subsequently, the SU-8 was exposed with the non-contact ACS200 stepper and developed. The release agent 230 Fluid (Dow-Corning) was then spun on the cured mold to facilitate the removal of PDMS from the mold. The PDMS chip was fabricated using a 10:1 ratio of base to curing agent of Sylgard 184 (Dow-Corning) [3]. After PDMS was poured on the mold, it was degassed for 1 hour and baked for 24 hours at 90°C to cure. The PDMS was bonded to a glass slide by

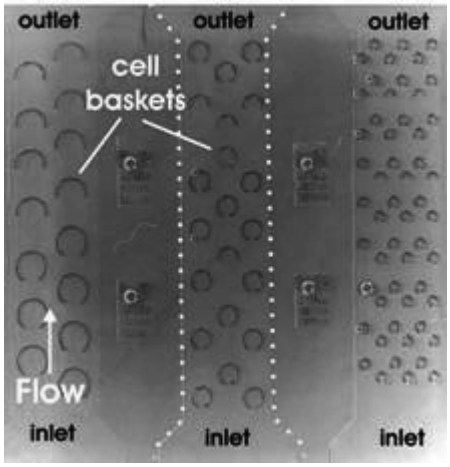

Figure 2: Optical micrograph of device.

exposing both the glass and the PDMS faces to oxygen plasma and then pressing them into each other lightly. To improve the PDMS-glass bond, the assembled device was baked for 30 minutes at 115°C.

Once the device was fully assembled, myoblast C_2C1_2 cells were flown through the channels in three different flow rate regimes of 20, 50, and 75 mL/hr. Each trial was run for 15 minutes using an automated syringe pump.

Results and Discussion:

The final device had 3 channels with well diameters of 200, 500 and 800 μm and a height of 200 μm. (See Figure 2.) Each channel was approximately 2 mm wide with 3 mm wide inlet and outlet ports. Cells were trapped in perforated, semicircular wells with pores ranging in size from 15 to 30 μm. The aspect ratio for the "pillars" comprising the wells was approximately 10 with a width of 20 μm and a height of 200 μm. 230 fluid was utilized in our process as a mold release agent between the PDMS and SU-8 to prevent rupturing of PDMS while demolding. PDMS was then baked for 24 hours at 90°C after the initial hotplate bake of 1 minute at 110°C [3]. The longer bake time ensured maximum cross-linking and Young's modulus which prevented the "pillars" from collapsing.

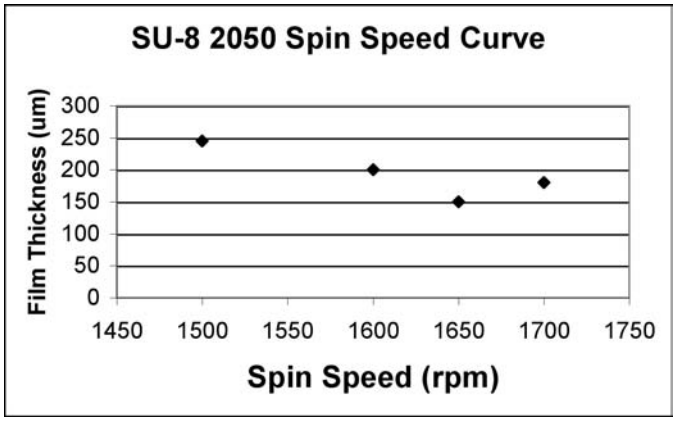

Figure 3: SU-8 spin speed curve.

Our initial cell experiments involved myoblast C_2C1_2 cells. Cells accumulated in wells for only the 20 mL/hr flow rate after approximately 10 minutes, Figure 4. At the faster flow rates, cells did not accumulate because the high rate forced the cells through the gaps in the wells. However, the slowest rate of 20 mL/hr provided enough time to allow for cells to form clusters.

Future Work:

The next iteration of the device will be fabricated with geometries that will accommodate more efficient assemble of the three dimensional cell constructs. This includes varying gap widths in between "pillars" of the wells. Also, testing on the myoblast/C_2C1_2 cells will be finalized. Finally, tumor cell experiments will be conducted.

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References:

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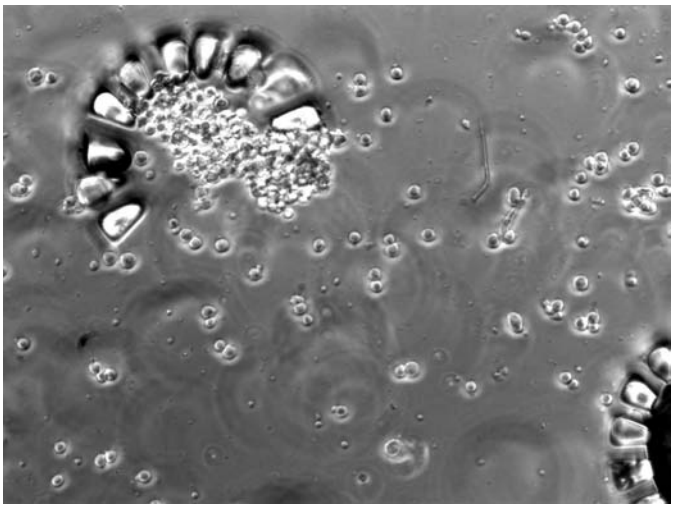

Figure 4: Myoblast cell accumulation.