MICROFABRICATED SILICON MICROCHANNELS FOR CELL DEFORMATION STUDY

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INTRODUCTION

Investigating the cellular response to mechanical stress is a fundamental research area in cellular mechanics. Characterizing the cellular response to applied forces or pressure provides such information as cellular mobility through the vascular system and cell membrane to substrate adhesion. Subsequently the cellular response to mechanical forces can provide experimental data for computational modeling of cell behavior.

The focus of this project is to develop a tool to study cellular deformation through tight spaces, and the effect of surface roughness or topography. A set of silicon-etched microchannels have been developed to test the hypothesis that altered cell rheology affects cell motility through capillaries, which furthermore can provide information on cell metastasis. Other studies using the micropipette and similar silicon microchannels have provided evidence for this hypothesis by showing that diseased cells deform differently than healthy cells [1-3].

CHANNEL DESIGN

The microchannel system developed here improves upon the conventional micropipette in two ways. First, the smooth interior of a glass micropipette gives no information on how a cell responds to surface roughness, while sources in the literature have confirmed that surface roughness plays a role in cell motility [4-6]. Second, in the micropipette the cell travels only a short distance, perhaps 15μm, before it is ejected back into solution or continues through the pipette into waste solution, while under physiological conditions a cell would encounter additional resistance due to the length of the vessel as it travels. This microdevice meets these two limitations, and therefore provides a tool to observe cell rheology as affected by both surface patterning and channel resistance.

A yield of 44 1cm x 1cm square chips are diced from one silicon wafer. Each chip includes two independent channel systems, each with its own input and output. Utilization of the rather large $1cm^2$ area per chip is maximized in this manner. The channel system is designed for cell solution to enter from the bottom of the silicon chip, fill a large 20μm deep reservoir, and then enter the tight channel constriction, where cells are observed under a microscope. The solution then exits the channel into another large 20μm deep reservoir and leaves the bottom of the chip through the output port.

Four channel configurations are designed for the microchannel device as shown in Fig. 1. In each configuration the channel length is a constant 150μm and the depth is 6μm. Configuration 1 is a series of smooth channels that range in width from 2μm to 15μm in 1μm increments. Configuration 2 is similar to the straight configuration 1, but with 30μm x 5μm bumps patterned on the channel walls. The bumps are intended to mimic the endothelial cells that line vessel walls. Configuration 3 consists of bifurcated channels, while Configuration 4 consists of stenosed channels that originate as 100μm, 50μm, 25μm, and 10μm channels and constrict to half-widths of 50μm, 25μm, 12.5μm, and 5μm, respectively.

To achieve a line width of 2μm the channel geometries silicon etching is the preferred fabrication method. The microchannel geometries are etched into a (100) n-type silicon wafer. There are three photomasks for this process. Mask 1 includes the reservoirs and channels, Mask 2 includes the reservoirs only, and Mask 3 includes the round ports that will be etched through the wafer for fluid input and output.

A schematic of the cross-sections of the wafer during processing is shown in Fig. 2. First the device wafer (a) is RCA cleaned followed by growth of 0.5μm low quality SiO_2 (b). Mask 1 is exposed, developed, and etched into the SiO_2 by buffered oxide etch (BOE) (c). Not shown is the deposition of photoresist (PR) and developingof PR. Next Mask 2 is exposed and developed (d) followed by a 14μm deep reactive ion etch (DRIE) of the reservoirs only (e). During this etch the microchannels are protected by undeveloped photoresist. In the next step, the protective photoresist is ashed away by O_2 plasma after which a 6μm deep Lam polyetch completes the channel and reservoir etching (f).

To prepare for through-wafer etching of the fluid ports, the device wafer is flipped over (g) and Mask 3 is exposed and developed on the backside of the device wafer to provide alignment marks for the

through-wafer deep reactive ion etch (DRIE) of the fluid ports (h). To strengthen the wafer for etching of the ports, a support wafer is resist bonded to the front-side of the device wafer (i). The ports are then etched by DRIE (j). The support wafer is removed (k), remaining photoresist is ashed away(k), and the SiO_2 removed by BOE (l). Finally, to seal the channels, a Corning 7740 Pyrex wafer is anodically bonded to the top of the silicon wafer. The wafer is diced with a dicing saw to release the individual chips.

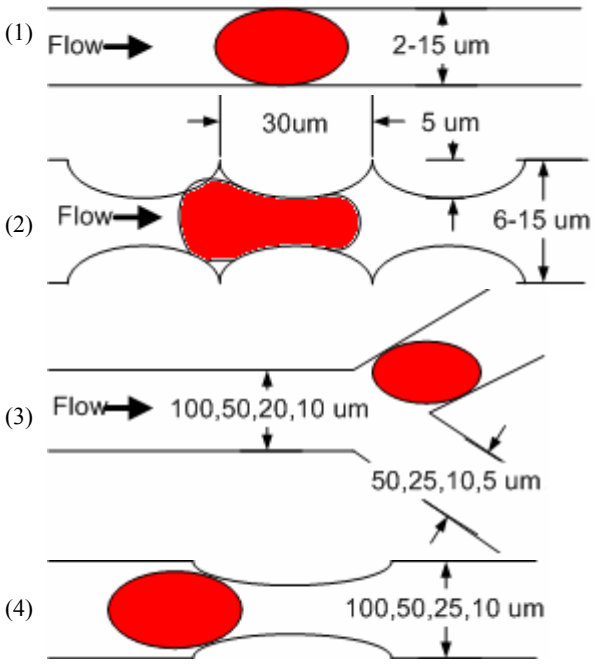

Figure 1. Channel schematics. (1) Smooth channel (2) Endothelial cell lined channel (3) Bifurcated channel (4) Stenosed channel

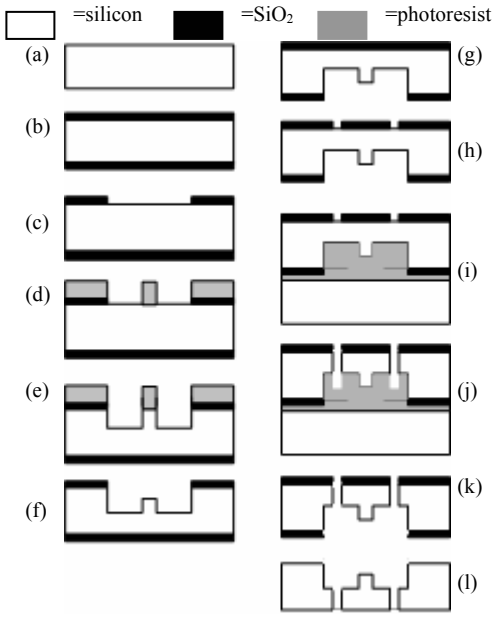

Figure 2. Cross-sections of silicon wafer during processing

RESULTS

While this method is more labor and time intensive than channels produced by soft lithography, the dimensional accuracy provided by microfabrication provides superior deformation characterization. The characterization parameters obtained are contact length, L_c, cell volume, V, transit time, t, and deformation pressure, P. Contact length is an observational measurement using tick marks etched long the length of the channels. Cell volume is the product of the contact length, channel width, and the 6μm channel depth. Transit time is measured with a stopwatch and the calibrated deformation pressure is obtained from the syringe pump.

A novel silicon-etched microchannel technique has been developed for the study of cellular deformation through tight spaces, and the investigation of surface roughness. The behavior of blood cells through different channel configurations (1-4) and experimental conditions, like shear stresses, will be analyzed and shown.

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