THE MICROFLUIDIC TRAPPING OF ANTIBODY-SECRETING CELLS

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INTRODUCTION

In response to infection or immunization, lymphocytes of the immune system, called B-cells, develop into antibody-secreting cells (ASCs) that produce and secrete proteins called antibodies (Abs). Abs bind to foreign cells and molecules, called antigens (Ags), marking them for clearance by the immune system. The secretion of Ag-specific Abs is key to protective immunity, and Ag-specific Abs can be secreted over long periods of time in the absence of Ag, providing individuals with long-lasting protection against pathogens bearing the Ag [1,2].

Therapeutic human Abs have become one of the fastest-growing sectors in pharmacology [3-5], and there is great interest in technologies that promote the cloning of Abs that bind to a chosen Ag with high affinity. We are developing such a technology using a microfluidic system to guide and trap ASCs near nanohole arrays in a gold film. The binding of Abs to Ag immobilized on a nanohole array alters the transmission of light through the said array *via* surface plasmon resonance [6]. The Ag-binding strength (*i.e.* affinity) of Abs secreted by thousands of ASCs on a single slide could ultimately be monitored *via* the optical transmission through each array, affording real-time identification of ASCs producing the desired Abs with the strongest affinities. ASCs producing these Abs could then be isolated, and the genes encoding their Abs cloned, from these high-affinity ASCs.

Before we can perform such measurements on such a large scale, we must first develop a system capable of trapping large ASC populations within arrays of single-cell traps. Single-cell manipulation has been a major focus of microfluidics research [7]. Most relevant to our application, researchers have trapped large cellular populations within arrays of microstructures intended to trap single cells [8-10]. Lee *et al.* report cup microstructures whose concave opening faces the oncoming fluid flow, trapping cells *via* perfusion [8]. Love *et al.* and Muraguchi *et al.* both report arrays of microwells which trap cells settling under the influence of gravity [9,10].

We previously reported the integration of nanohole arrays with polymeric microfluidic channels [11]. We have since been developing polymeric microfluidic systems for the single-cell trapping of large ASC populations. Each design was fabricated using SU-8 photoresist [12] and poly(dimethyl siloxane) (PDMS) [13], and tested using polystyrene microspheres (PSS) [14] and hybridoma cell line 17/9, which secretes a peptide-binding Ab [15,16]. This paper presents our initial work developing various polymeric arrays of single-cell traps.

FLOW-THROUGH CELL TRAPS

Figure 1 presents our first flow-through trap design, inspired by the success of the u-shaped cup microstructures of Lee *et al.* [8]. The fluid flow is intended to carry a suspended cell into the concave opening of one of these cups. The 10 μm wide channel bisecting each cup is intended to be too narrow to afford cellular passage, trapping the cell against the fluid flow *via* perfusion.

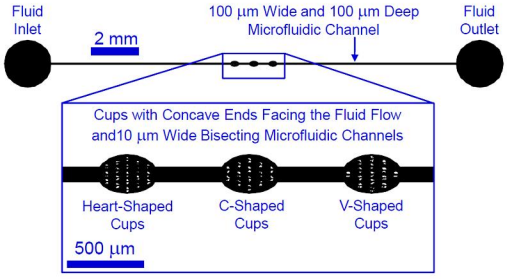

Figure 1: Cups that trap cells *via* perfusion.

Figure 2 presents our second flow-through trap design, with 50 μm by 50 μm chambers connected to a cup *via* channels too narrow to permit cellular passage. The nanoholes are to be sheltered within these chambers, to be exposed to the Abs secreted by the nearby trapped ASC and shielded from other Abs.

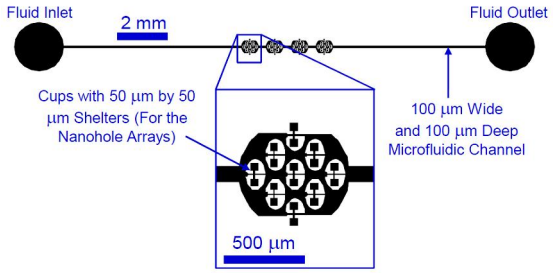

Figure 2: Cup traps that shelter the nanohole arrays.

Figures 3-5 present our other flow-through trap designs. Based upon the expectation that cells are denser than the fluid: centrifugal forces should push the cells to the channelôs perimeter, where the cup traps are located (see the insets of the channel wall).

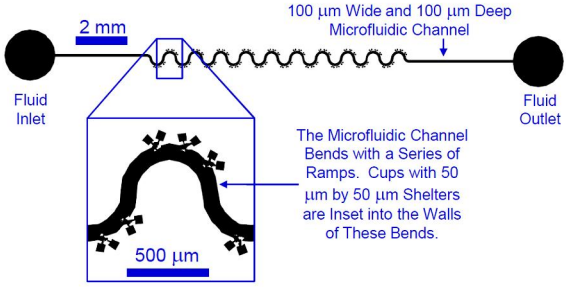

Figure 3: Cup traps and nanohole shelters inset into the walls of a serpentine microfluidic channel.

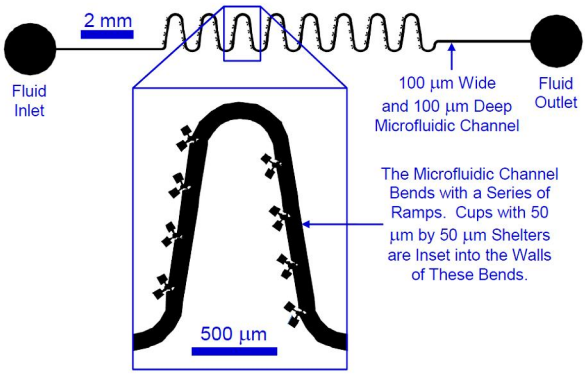

Figure 4: Cup traps and nanohole shelters inset into the walls of a ramped microfluidic channel.

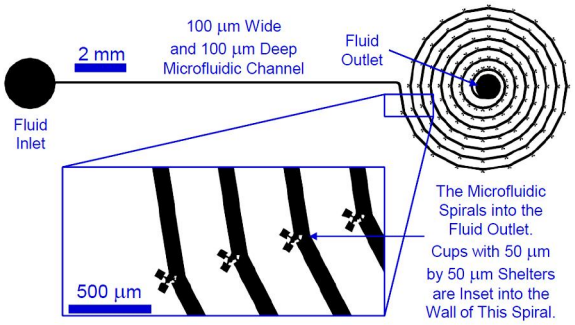

Figure 5: Cup traps and nanohole shelters inset into the walls of a spiraling microfluidic channel.

Simulation of Flow-Through Cell Traps

The fluid velocity v_f profiles within Figures 1-5 were simulated in COMSOL® Multiphysics 2D Incompressible Navier-Stokes Module [17] using: inlet and outlet pressure P boundary conditions; no-slip boundary conditions along the microfluidic channelsô perimeter; and a 100 μm deep shallow channel approximation. Mesh independence was assured in all cases. Cellular trajectories within the v_f profiles were then estimated *via* v_f streamlines and particle tracing simulations. These simulations indicated that Figures 1-5 were worthy of further consideration, whereas other designs (not presented) were not.

As an example, Figure 6 presents the simulated v_f profile within Figure 5. This simulation used a 200 Pa inlet-outlet pressure differential ΔP. Analytical flow rate calculations, based upon fluidic resistance, suggest that ΔP = 200 Pa yields a v_f profile with a mean $|v_f|$ å 8 mm/s at the channelôs center. This prediction is verified by Figure 6, in which the mean $|v_f|$ is approximately 9 mm/s at the channelôs center.

The insets in Figure 6 show that the v_f streamlines near the channelôs outer perimeter pass through the bisecting channels further down the spiral. As such, the v_f streamlines of Figure 6 suggest that cells should be trapped within the cups further down the spiral. Particle tracing simulations support the cellular behavior predicted by the v_f streamlines.

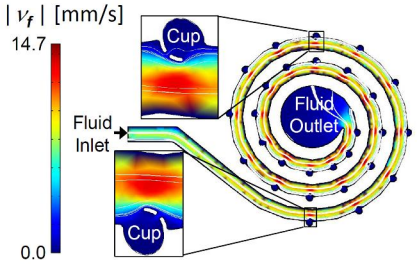

Figure 6: The v_f field (colour) and streamlines (white) within the design of Figure 5 (at t = 30 s), as simulated using COMSOL® Multiphysics with ΔP = 200 Pa.

Fabrication of Flow-Through Cell Traps

The flow-through traps were fabricated using: glass slides with and without a 5 nm titanium (Ti) or chrome (Cr) adhesion layer, Pyrex wafers, and silicon (Si) wafers. 30-122 μm thick films of MicroChemôs SU-8 2035 photoresist [12] were spun onto these substrates. These films were immediately soft-baked on a 65°C hot-plate. Following the soft-bake, each filmôs edge-bead was removed using acetone. Each film was then photolithographically patterned using a Mylar contact mask containing Figures 1-5. These films were then post-exposure baked on a 95°C hot-

plate. MicroChem's SU-8 photoresist developer was then used to develop the films. The utilized spin speeds, baking times, and exposure times were determined by the desired film thickness as based on parameters listed in MicroChem's SU-8 2025-2075 datasheet [12] that were optimized for our equipment.

SU-8 films were also patterned with the negative of Figures 1-5, forming molds for soft lithography (similar to [18,19]) used to fabricate the flow-through traps using Dow Corning's Sylgard® 184 PDMS [13].

One notable problem encountered during SU-8 trap fabrication was the poor adhesion of 10-15 μm structures (as seen in Figure 7). This lack of adhesion was partially reduced when the SU-8 film was Ò50 μm thick, when the substrate had an adhesion layer, when the substrate area was reduced, and when Si or Pyrex was used instead of glass. The PDMS trap fabrication afforded an improved small feature definition and as such did not suffer from this lack of small structure adhesion. However, PDMS features were occasionally smaller than expected, likely due to PDMS shrinkage and/or poor SU-8 mold definition. Consequently, cells were occasionally able to pass through channels intended to be too narrow to afford their passage.

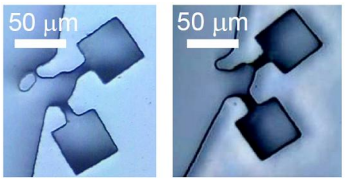

Figure 7: Cups and shelters fabricated in SU-8. The cup on the right suffered from partial delamination, due to the poor adhesion of small features within SU-8.

Testing of Flow-Through Cell Traps

The flow-through traps were tested using 20 μm diameter PSS [14] suspended in deionized water (DI H_2O) and 10-20 μm diameter 17/9 hybridoma cells [15,16] suspended in Dulbecco's modified Eagle's medium (DMEM). Concentrations on the order of 10^5 cells-or-PSS/mL and flows rates on the order of tens of μL/min were used. Most cells flowed around the SU-8 cups, with few becoming trapped. This behavior may be due to the reduction in fluid flow through the bisecting channels as a result of poor SU-8 cup adhesion. The serpent and spiral flow-through traps yielded the best results. Moreover, the PDMS traps were superior to the SU-8 traps. Figure 8 presents a PSS trapped within a cup inset into a spiraled PDMS channel's wall. Figure 8 also presents cells densely populated along a serpentine PDMS channel's wall, along with cells that passed through the cup-to-shelter channels. The removal of such extraneous cells has proven to be difficult using our current flow-through traps. We plan to try various surface treatments which may facilitate the removal of these extraneous cells.

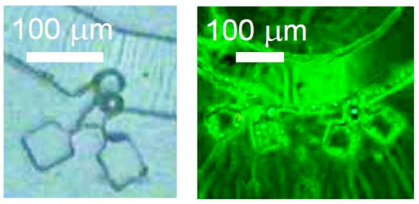

Figure 8: (LEFT) A PSS trapped in a cup inset into a spiraled PDMS channel's wall. (RIGHT) Cells densely populated around a serpentine PDMS channel's wall.

Cellular viability within the traps was evaluated after a 4 hour incubation. Cells were poisoned by Cr; but appeared to remain healthy on all other slides. Thus, Cr must not be used as an adhesion layer, to maintain cellular viability. Fortunately, Ti has been shown to more strongly adhere to SU-8 than Cr [20].

MICROWELL ARRAY CELL TRAPS

Whilst flow-through traps are desirable from the standpoint of established hydrodynamic flow cell manipulation, we are also investigating other methods for trapping large cellular populations. Inspired by Love *et al.* and Muraguchi *et al.* [9,10], we have developed cell traps composed of microwell arrays inset into the microchip's surface. Our microwell arrays feature 30, 50, 100, and 200 μm topographic diameters, vertical sidewalls, and a 3.77 mm periodicity.

Fabrication of Microwell Array Cell Traps

Our microwell arrays were fabricated in SU-8 and PDMS, *via* procedures similar to those used to fabricate our flow-through traps. Our fabricated structures consisted of 60-80 μm deep microwells inset into the surface of 400-700 μm thick PDMS films.

Testing of Microwell Array Cell Traps

As with our flow-through traps, our microwell array traps were tested using 10-20 μm diameter 17/9 hybridoma cells [15,16] suspended in DMEM. During this testing, we attempted to adhere to the procedure of Love *et al.* as much as possible [10].

Using a concentration of $4x10^5$ cells/mL, we found that 200 μm diameter microwells in PDMS would typically fill with 5-20 cells following a 10 minute settling time (as seen in Figure 9). Figure 9 also shows that the cells appeared to be viable following a 4 hour incubation. In contrast, we had difficulty trapping cells within SU-8 microwells, possibly due to SU-8's surface charge or hydrophobicity.

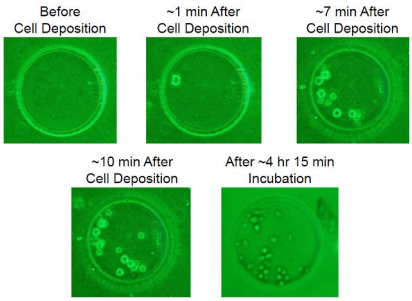

Figure 9: Cells falling into a PDMS microwell, with a 200 μm topographical diameter and a 60-80 μm depth. The cells appeared to be viable after a 4 hr incubation.

Using a peristaltic pump, we have successfully removed cells outside of the microwells without displacing the trapped cells.

CONCLUSIONS

We have designed, simulated, fabricated, and performed initial testing on a variety of first-generation trapping arrays planned for large numbers of ASCs. The basic principle behind our flow-through traps has been verified *via* COMSOL® Multiphysics simulations and testing with PSS and 17/9 hybridoma cells. Testing with the hybridoma cells has also verified the functionality of our microwell array traps.

Although we are encouraged by our initial results, our flow-through traps must be refined to yield arrays of single-cell traps capable of reversibly trapping many ASCs on a single slide. However, the cellular concentration could conceivably be tailored to achieve this performance using our microwell array traps, as the results of their initial tests are more promising.

It is necessary to remove extraneous cells near a trapped single cell, so as to isolate a given nanohole array from the Abs secreted by ASCs other than the nearby trapped ASC of interest. Such removal will require refinements to the flow-through traps, including further surface chemistry and hydrodynamic flow manipulation. However, we have successfully removed cells outside the microwells without displacing the trapped cells within the microwells.

We consider our progress thus far to be a good start towards our goal of a microfluidic system capable of trapping thousands of ASCs within arrays of single-cell traps on a single slide, each of which is near a designated nanohole array for secreted Ab detection.

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