HIGH FLOW RATE CAPTURE OF CIRCULATING TUMOR CELLS USING A SMALL FOOTPRINT POLYMER DEVICE

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ABSTRACT
High flow rate capture of circulating tumor cells (CTCs) was performed with a high recovery rate. A new concept for rare target cell capture using a micro device was investigated parametrically using numerical simulations. High flow rate device (HFRD) prototypes were fabricated based on the simulation results. The devices were coated with antibodies (anti EpCAM) after UV modification and amine functionalization. The simulated CTCs (MCF-7 cells) were collected and spiked in ~40% hematocrit solutions of human red blood cells. The polymer high flow rate device captured the CTCs with an 80% average recovery rate at a flow rate of 750 μL/min.

KEYWORDS: Circulating Tumor Cell, Micro Polymer Device, High Flow Rate, BioMEMS

INTRODUCTION
Circulating tumor cells (CTCs) will potentially offer the opportunity for a liquid biopsy for cancer diagnosis or treatment monitoring. However, current devices for capturing CTCs (10⁻⁹ concentration from human blood cells) have issues with low flow rates, low recovery rates, or low purity [1, 2]. Affinity-based CTC capture provides specific selection and high recovery rates with high purity, but the flow rates are typically low since the antigen/antibody reaction requires a low contact velocity [3-5]. Size-based approaches offer higher flow rates but low purity and clogging by white blood cells. The design of high flow rate devices for affinity-based CTC capture from human blood was investigated, with a focus on obtaining small footprint polymer devices (3 cm × 8.5 cm), which would be applicable for point-of-care use.

THEORY
Lateral location of targets is not easy to predict or manipulate, especially for the non-Newtonian flow of human blood [6]. The HFRD manipulates the laminar velocity profile by combining side ports with a main channel to maximize the encounter rate with the antigen/antibody functionalized walls. Figure 1 shows the different velocity profiles between a closed and a port combined micro channel at near the channel wall.

Computational fluid dynamics (CFD) simulations were used to maximize performance of the device; assuming that the blood flow could be modeled as a Newtonian fluid. Figure 2 shows schematic drawing of HFRD and hot embossed PMMA HFRD. The axial mean velocity was minimized (< 2 mm/s, the optimum velocity for EpCAM-antiEpCAM binding [3]) to avoid target cell loss through the 30 μm deep main outlet. Lateral velocities at collision points with the walls in the main channel were also minimized to avoid target cell exclusion through the 5 μm depth side channels. Most of the pathlines were directed toward the side channels, facilitating exclusion of the red blood cells. Figure 3 shows the lateral velocities of the target cells at the moment of collision with the side channels and the pathlines in the first section of the main channel.

EXPERIMENTAL
Antibody (anti-EpCAM) immobilization was completed on the polymethylmethacrylate (PMMA) channel surfaces after UV modification and amine functionalization process [7]. PMMA was spin coated on a thin polycarbonate sheet that was used to seal the device and avoid channel deformation (aspect ratio: < 0.01 on the side channel.)

Rare target cell capture research requires preparation of precise numbers of sample cells, since statistical sample preparation techniques would not provide accurate results. A novel rare sample (~10 cells) preparation method was introduced. Small numbers (mean = 14.7) of MCF-7 cells were collected using a silica capillary with the polyimide cladding removed and a custom chip under a microscope and transferred into the sample solution. Viable MCF-7 tumor cells were used to model CTCs. They were membrane stained and spiked in a 40% human red blood cell solution, which is comparable with normal human hematocrit, and delivered to the device using syringe pumps. The flow rate was up to 750 μL/min and followed by a

Figure 1: Velocity profile near a surface (a) in a closed channel and (b) in a port combined channel
rinse with phosphate-buffered saline (PBS) to remove any material non-specifically binding to the channel walls. Human blood cells were excluded through the 5 μm depth side channel, unlike the CTCs which bind onto the antibody immobilized polymer surfaces. Figure 4 shows the exclusion of the red blood cells during CTC spiked blood flowing and captured MCF-7 cell after PBS washing. Exact numbers of CTCs (mean = 14.7) or statistically prepared 100 CTCs were used to verify the recovery rates.

RESULTS AND DISCUSSION

The CTCs were captured with a high recovery rate at high flow rates. The flow rate did not significantly affect the recovery rate. The average recovery rate was 76% for 100 CTCs and 85% for smaller numbers (sample mean = 14.7) of CTCs at 750 μL/min and 81% at 150 μL/min (See Figure 5.) The location of each captured CTC in the device was evaluated and agreed with simulation results (See Figure 6.) Most of CTCs were captured near the side channels. Human whole blood was also used to investigate the effect of white blood cells. The white blood cells were excluded and did not block the side channels. Aged and coagulated human whole blood blocked the side channels. The sample whole blood or patients’ blood should be used within several hours for better CTC capture performance.

CONCLUSIONS

A high flow rate CTC capture device was designed and fabricated based on CFD simulation results. The PMMA HFRD captured 80% of CTCs from solutions of human red blood cells. The device could process 7.5 mL CTC blood in 10 minutes, which is the highest flow rate with affinity-based capture reported to date and applicable to the point-of-care applications.
Figure 5: Recovery rates of MCF-7 cells at different flow rates and CTC numbers.

Figure 6: Frequency of captured target cells versus location for 100 and 14.7 (mean number) MCF-7 cell cases. The flow rate was 750 µL/min and the RBC concentration was 40–42%.

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