Fabrication of a Novel Microfilter for Circulating Tumor Cell Enrichment and Culture

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Abstract:

Detection of circulating tumor cells (CTCs) provides valuable information in diagnosis and prognosis of metastatic cancers, but the rarity of CTCs in blood makes it challenging to develop a method with sufficiently high sensitivity. Filtration of CTCs based on size has shown potential as a quick and inexpensive option. This project presents a novel three-dimensional slot-patterned microfilter efficient in viable capture of CTCs. This design better supports the captured cells, eliminates stress concentration on the cell surface during filtration, and prevents cell rupture. The filter design is also optimized to simplify the fabrication process. Device characterization confirms the structure, and preliminary filtration testing with cultured tumor cells exhibits effective viable capture. Thus the process achieved successful fabrication of a microfilter that can be further evaluated for performance in enrichment of CTCs.

Introduction:

Cancer metastasis, the spread of cancer throughout the body, is the leading cause of death for cancer patients. Detection and monitoring of circulating tumor cells (CTCs) for those with metastatic cancer can provide valuable information into treatment response, patient survival, and risk of relapse [1]. However, the rarity of CTCs in blood, on the order of 1 in 10^{10} blood cells, and the necessarily large sample volume, usually 7.5 ml blood, make it challenging to develop a method with sufficiently high sensitivity and selectivity.

The two most common methods of CTC isolation, immunomagnetic selection and density centrifugation, are expensive, labor-intensive, and do not have consistently high recovery rates [2]. Isolation based on size is potentially a cheaper and quicker procedure. Tumor cells are usually larger than blood cells, so a filter can be designed to capture CTCs while allowing blood cells to pass. With commercially available polycarbonate track-etched filters, lack of control over pore locations and clogging during filtration lead to poor performance [2]. In contrast, with microfabrication, both size and location of the pores can be precisely defined by lithography and etching techniques, enabling higher capture efficiency. Viable capture is also a goal of the current filter design. By controlling the filter's microenvironment to capture CTCs without harm, post-filtration live cell studies can be made possible.

We present a novel "3D" microfilter efficient in viable capture of CTCs and relatively simple to fabricate. The device is a 10 μm-thick parylene membrane sectioned into rectangular basket-like filtration units with 15 μm high walls and two parallel 4.5 μm wide slots on the bottom adjacent to each sidewall. Compared to single-layer designs, this filter has the advantage that both the bottom and the walls of the filter will support the captured cells to prevent rupture (see Figure 1). It also reduces the number of lithography steps and overall fabrication complexity as compared to previous three dimensional (3D) filter designs.

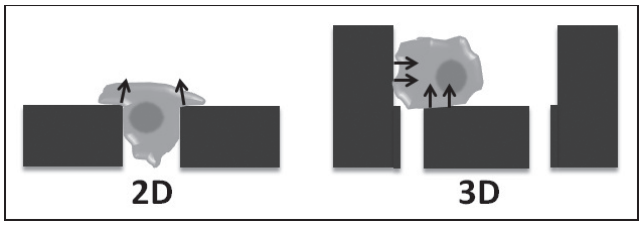

Figure 1: Forces on a trapped cell.

Experimental Procedure:

Fabrication started with 4-inch <100>-oriented *p*-type silicon wafers. As shown in Figure 2, a 15 μm-thick layer of sacrificial photoresist SPR 220-7 was spin-coated and patterned with photolithography to define the height of the basket walls. The trenches were sealed with 10 μm of Parylene-C to yield an even surface, a result of the conformal coating feature of parylene deposition. Parylene-C is very suitable for this application due to its biocompatibility, ease of processing, optical transparency, and low cost.

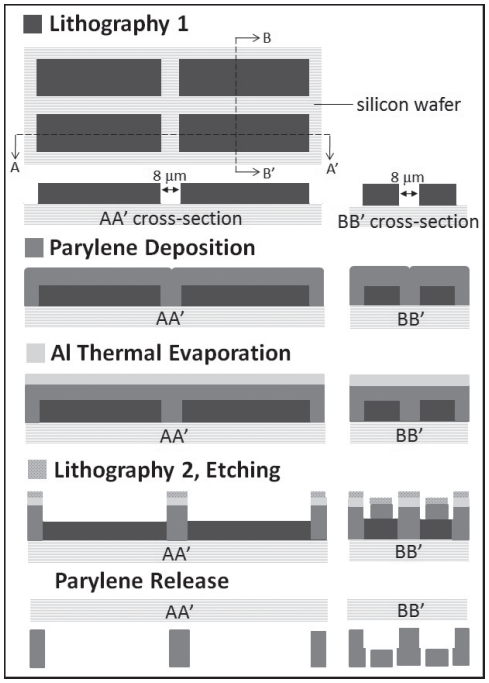

Figure 2: Micro-fabrication process flow.

A thin layer of 100 nm aluminum (Al) was then thermally evaporated and patterned by a second lithography with SPR 955 photoresist. Oxygen plasma etching with this layer of Al as the mask defined the slots in the parylene. Finally, the sacrificial photoresist was dissolved, and the parylene membrane was released from the silicon substrate and cut into individual filter devices. Dimensions of the filter design were based on previous optimization tests.

Results and Conclusions:

After careful adjustment of procedure specifics, the desired device structure was obtained, with clear-cut edges and precise patterning (Figure 3). The filters were tested by passing through solutions that contained GFP-labeled breast carcinoma UACC cells. The tests were run on several filters, all of which showed high capture efficiency (approximately > 90%). It was evident that captured cells were caught near the slots by the wall (Figure 4), supporting the design concept that both the bottom layer and wall would provide support to reduce stress on the cell. More rigorous testing of cell viability will be conducted in the future.

The filter needs to be further evaluated, but this project demonstrates that research is headed in the right direction. Ultimately, this device could aid significantly in the study of cancer metastasis in both clinical and research settings.

Future Work:

Cell culture on the device will be observed to evaluate cell viability. A functional assay can confirm ongoing metabolic activity in captured cells. Scanning electron microscopy observations of the filter can also provide more information on cell membrane integrity post-capture. Further filtration testing will be conducted with blood samples containing or spiked with tumor cells to verify that only the tumor cells are captured while blood cells filter through. Optimization of the device will increase sample volume and/or filtration speed.

Acknowledgements:

I would like to thank my PI, Dr. Siyang Zheng, for allowing me the opportunity to work in his lab and mentor, Mingda Zhou, for guiding me along the way. I would also like to thank Dr. Cheng Dong and his student, Ph. D. candidate Pu Zhang, for their help in testing. Much appreciation goes to Kathy Gehoski for coordinating the program. Funding for this project was provided by the National Nanotechnology Infrastructure Network Research Experience for Undergraduates (NNIN REU) Program and the National Science Foundation.

References:

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[2] O. Lara, et al. "Enrichment of rare cancer cells through depletion of normal cells using density and flow-through, immunomagnetic cell separation." Exp. Hematol. 32(10), 891–904 (2004).

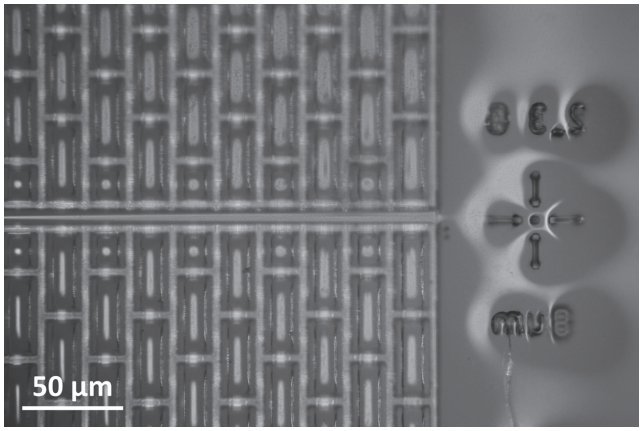

Figure 3: Fabricated 3D microfilter.

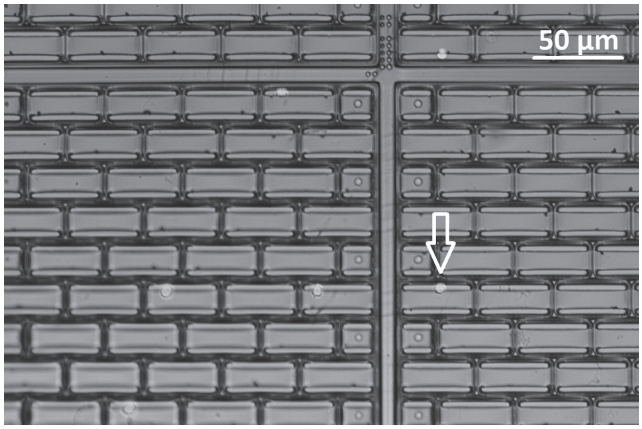

Figure 4: Device post-filtration (arrow points to a captured tumor cell).