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Optimization of Breast Tumor Cells Isolation Efficiency and Purity by Membrane Filtration

Bhuvanendran Nair Gourikutty Sajay, Liu Yuxin, Chang Chia-Pin, Poenar Daniel Puiu, Abdur Rub Abdur Rahman

***Abstract*—**Size based filtration is one of the common methods employed to isolate circulating tumor cells (CTCs) from whole blood. It is well known that this method suffers from isolation efficiency to purity tradeoff. However, this tradeoff is poorly understood. In this paper, we present the design and manufacturing of a special rectangular slit filter. The filter was designed to retain maximal amounts of nucleated cells, while minimizing the pressure on cells, thereby preserving their morphology. The key parameter, namely, input pressure, was optimized to retain the maximal number of tumor cells, whilst maximizing the depletion of normal blood cells (red and white blood cells and platelets). Our results indicate that for a slit geometry of 5 × 40 μm on a 13 mm circular membrane with a fill factor of 21%, a pressure of 6.9 mBar yields the optimum for maximizing isolation of MCF-7 and depletion of normal blood cells.

***Keywords*—**Circulating tumor cells, Parylene slit membrane, Retention, White Blood Cell depletion.

I. Introduction

CTCs are extremely rare cells found in the blood stream of cancer patients [1]. These cells are dislodged from the primary tumor and enter into circulation during a process called metastasis [2], [3]. CTCs provide key insights for understanding the metastasis process [2]-[5]. CTCs detection is emerging as a promising diagnostic and prognostic tool for clinical cancer management [1]. They also hold the potential for personalized and targeted therapy and therapeutic efficacy monitoring. CTCs are currently FDA approved biomarker for cancer prognosis. Consequently, interest in technology development to isolate CTCs as well as investigation of their clinical utility has grown exponentially. There are several physical properties that distinguish CTCs from most normal blood cells.

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Bhuvanendran Nair Gourikutty Sajay is attached to BioElectronics Programme, Institute of Microelectronics, A*STAR (Agency for Science, Technology and Research), Singapore and with Microelectronics Centre, School of Electrical and Electronics Engineering, Nanyang Technological University, Singapore 639798(phone: 65 92394614; sajay001@e.ntu.edu.sg).

Liu Yuxin is with Division of Bioengineering, Nanyang Technological University, Singapore 637457(liuy0070@e.ntu.edu.sg).

Chang Chia-Pin is with BioElectronics Programme, Institute of Microelectronics, A*STAR (Agency for Science, Technology and Research), Singapore 117685(changcp@ime.a-star.edu.sg).

Poenar Daniel Puiu is with Microelectronics Centre, School of Electrical and Electronics Engineering, Nanyang Technological University, Singapore 639798(epdpuiu@ntu.edu.sg).

Abdur Rub Abdur Rahman is with BioElectronics Programme, Institute of Microelectronics, A*STAR (Agency for Science, Technology and Research), Singapore 117685(abdurr@ime.a-star.edu.sg).

These include the larger size of most epithelial cells, differences in density, charge, and migratory properties [6]-[10]. CTCs can be efficiently captured by using size based filtering because of their unique morphological characteristics [1], [11], [12]. WBCs (diameter >5 μm) are typically larger than RBCs (thickness ~ 2 μm) and CTCs generally have size bigger than WBCs and RBCs with a rigid body. Size based filtering can be used as a negative enrichment method which is preferred over positive enrichment since it has less risk of losing CTCs. In negative enrichment components in the blood like WBCs and RBCs are depleted to enrich the CTCs unlike directly targeting CTCs as in positive enrichment methods [13].

II. Design and Fabrication

13 mm diameter circular Parylene slit membranes with slit dimension of 5μm width and 40μm length were fabricated in Institute of Microelectronics (IME) [11], [14], [15]. Figure 1 shows the different views of the Parylene filter membrane. Silicon-based technology can manufacture precise and uniform micro slit filter membranes which can be used to enrich CTCs. Silicon wafer deposited with 1μm thick silicon dioxide (SiO_2) by using plasma-enhanced chemical vapor deposition (PECVD) was used as substrate.

Monolayer of adhesion promoter, gamma-Methacryloxypropyltrimethoxysialne (A-174 silane) was functionalized on the substrate to promote subsequent poly-p-xylylene (parylene-C) deposition. As shown in Figure 2, 10 μm-thick Parylene-C layer was deposited by Parylene Deposition System (PDS 2010 Labcoter 2, Specialty Coating System, Inc).

Herein, Parylene was employed as membrane material because it possess high bio-compatibility, excellent mechanical properties with Young's modulus (4 GPa) and inert chemical property, which is resistant to moisture and most chemicals. More importantly, Parylene deposition is completely conformal of uniform thickness and pinhole free. 100nm Chromium (Cr) layer, serving as hard mask, was deposited on top of Parylene-C film via electron beam evaporation. With assistance of photolithography, thin Cr layer was defined by CEP-200 Chrome etchant with an optimum etching time of 50 s. Subsequently, micro slits were developed on Parylene-C membrane via reactive ion etching (RIE).

Finally, filter membrane patterned with rectangular micro slits was released from silicon dioxide substrate by using buffered oxide enchant (BOE) or hydrogen fluoride (HF) vapor.

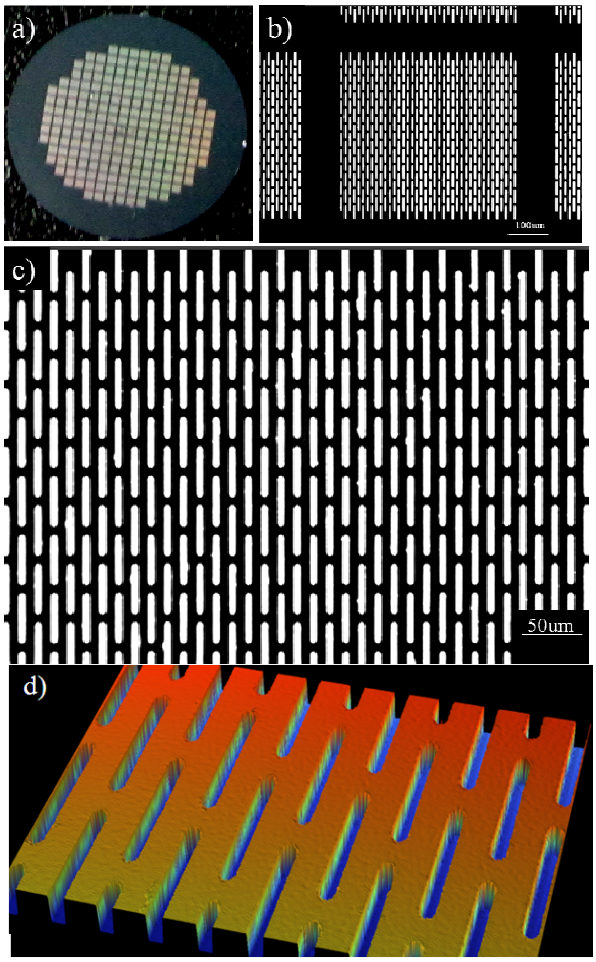

Fig. 1 Parylene Slit membrane with slit dimension 5 × 40 µm with thickness around 10 µm and its fill factor is 21 %. (a) by digital camera (b) 50x optical microscope (c) Zoomed image at 200x optical microscope (d) Image by optical profiler

RBCs have no nucleus and are highly deformable; they are able to easily squeeze through the slits even at small driving pressure. While designing slit geometry main consideration is to retain all the CTCs while depleting most of the WBCs. Furthermore, capturing viable cells is very important; hence avoidance of cell fixation is highly desirable. However, unfixed cells can also pass through the slits more easily than the fixed cells. The unique design of rectangular slit as opposed to the commonly used circular pores prevents the damage to cell morphology, by providing pressure relief points along the edges.

In selecting the optimal slit width, we found that Slit width more than 5µm can lead to CTCs escaped through slits even at low applied pressure. Another consideration for selecting 5µm slit width was that, CTCs would undergo deformation due to wedging within wider slit due to the flow pressure, which will adversely affect the viability of the captured CTCs [11]. One of the main advantages of slit design is that they will allow the cells to deform easily in the slit length direction.

This will facilitate the passage of deformable cells across the filter. This geometry of the slit helps the passage of easily deformable RBCs and WBCs when compared to that of rigid CTCs. The slits are arranged such a way as to provide good fill factor (21%) so as to reduce the flow resistance across the filter. This will help to enhance continuous flow without clogging and reduce the damage on the cells.

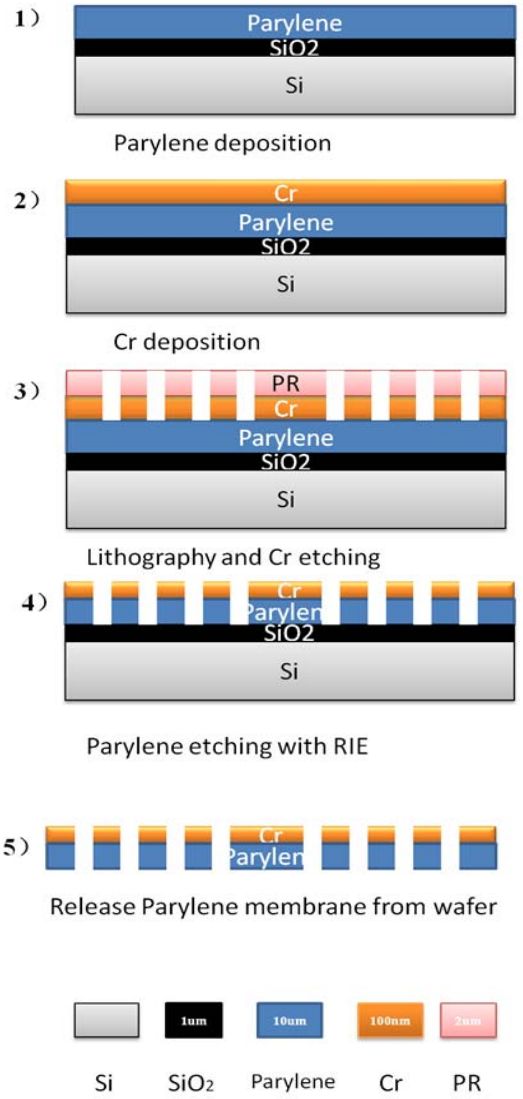

Fig. 2 Parylene micro filter membrane fabrication process flow: (1) Parylene deposition (2) Cr deposition (3) Lithography and Cr etching (4) Parylene etching with RIE (5) membrane release with BOE or HF vapor.

III. Experiment

The filter membrane was placed on a commercial filter holder and attached to the fluid delivery system via a standard luer connection. Assay is filled in a 10 ml syringe which is connected to a pressure source for positive displacement pumping of the fluid through the membrane holder.

The membrane is supported by a mesh-like structure within the filter holder to prevent buckling of the membrane under fluid pressure. To ensure that the pressure input to drive the assay is precise, it was measured by calibrated external pressure gauge. The pump used was also equipped with a real time display of applied pressure. The Jurkat cells were used as surrogate cells for human WBCs. They were cultured in our lab and are average 10 µm in diameter. Breast cancer cell lines MCF7 were employed in the experiment for CTCs retention experiments. The average size of MCF7 cell is 16 µm in diameter. The experiments were done with cells suspended in Phosphate buffered solution (PBS). Figure 3 shows the experimental setup with air pressure supply, assay containing tubes and filter holder.

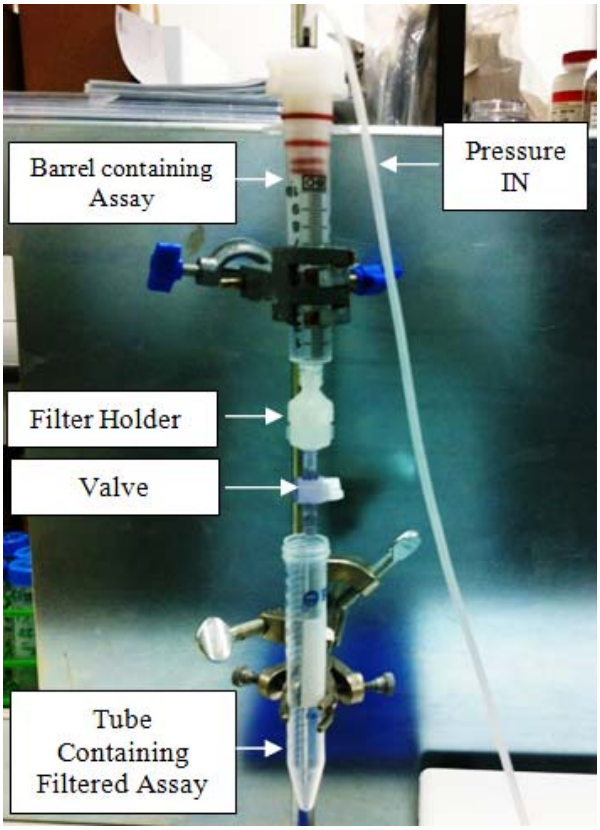

Fig. 3 Experiment setup with precise pressure input

All Parylene membranes were inspected before use to ensure the appropriate slit dimension and absence of hairline cracks and other defects. To investigate the depletion and retention efficiency of our filter membrane, input and output cell counts were monitored, before and after passage through the membrane. Cells were counted by using automated cytometer (Tali, Invitrogen Inc) and manual haemocytometer (C-chip, NanoEnTek Inc). To study the effect of pressure, the following pressures were applied to the system, 3.5 mBar, 6.9 mBar, 13.5 mBar and 20.7 mBar.

IV. Results and Discussions

5 ml of Jurkat cells at concentration of 5×10^5 cells/ml were employed with a range of pressure mentioned previously. Each experiment was repeated two times. Figure 4 shows the results of depletion efficiency of Jurkat cells at different pressure. From the experiments it was found that the depletion efficiency of Jurkat cells increased when applied pressure is increased.

At lower pressure, depleted cells are mostly cells with smaller diameter which does not need much driving pressure to escape across the slits. So at lower pressure ranges most of the big cells do not pass through. The larger cells are retained on the top surface of the filter. But with increased pressure, depletion efficiency was increased because of deformation of larger cells through the slits.

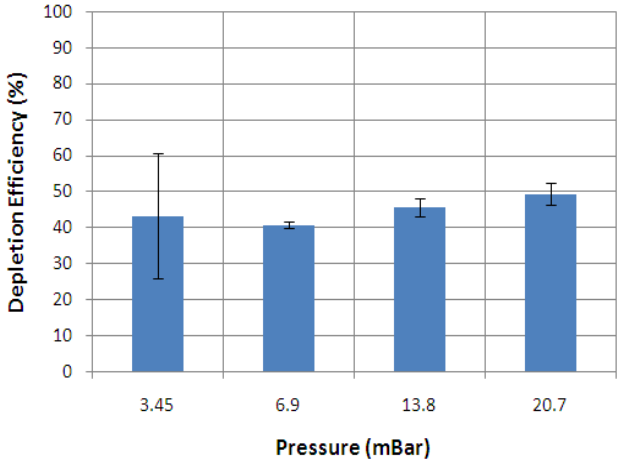

Fig. 4 Depletion efficiency of Jurkat cells at different pressure using 5ml assay with total 2.5×10^6 cells suspended in PBS

Retention efficiency of CTCs at different pressure was found out by using 5 ml assay at concentration of 10^5 MCF7 cells/ml. Series of experiments were conducted to find out the retention of MCF7 cells at different applied pressure mentioned previously. One of the challenges in CTCs retention efficiency experiment is that the cells cannot be counted at the output since there were few or no cells went through the membrane. In the end, cells were counted by manual heamocytometer chips after centrifugation of the assay to collect MCF7 cells at the bottom of the tube. Centrifugation was done for 4 minutes at 4000 rpm speed. The results were shown in the Figure 5.

From the experiments higher retention efficiency was achieved at lower applied pressure and retention efficiency decreased as applied pressure increased. Even though MCF7 cells are rigid in nature when compared to other cells, they were deformed at higher pressure by the force and escaped through the slits.

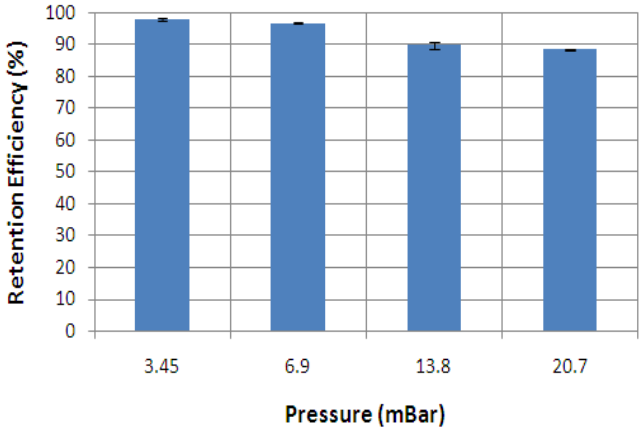

Fig. 5 Retention efficiency of MCF7 cells at different pressure using 5ml assay with total 5×10^5 cells suspended in PBS

V. Conclusion

Here, we presented the findings of our study to understand the effect of pressure in isolation and depletion of tumor cells and normal cells, using a Parylene slit filter membrane with the help of a high precision pressure delivery system. Depletion of the RBCs was nearly100% as they easily deform even at low pressures and easily pass through the membrane. It was found that the depletion efficiency of Jurkat cells (surrogate for WBCs) increases when applied pressure increases as expected, but adversely affects tumor cell retention. However, it is to be noted that at a high pressure of 20.7 mBar, ca. 10% of MCF-7 cells are lost, while nearly 50% WBCs are lost. Thus, for situations where time is critical and there may be significant CTCs load in blood (in the order of few 100), one may employ higher pressures. However, in situations where extremely low cell numbers of CTCs are encountered, low pressures should be employed and additional WBCs depletion steps may need to be employed to improve purity.

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