TAPERED-SLIT MEMBRANE FILTER DEVICES FOR THE HIGH-THROUGHPUT Viable ISOLATION OF CIRCULATING TUMOR CELLS

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ABSTRACT

We present tapered-slit filter devices for high-throughput viable circulating tumor cell (CTC) isolation and release. The membrane filter having an array of tapered-slits reduces the cell stress concentration at the edge of slit entrance, thus achieving viable CTC isolation with minimal stress. We fabricated the tapered-slit membrane from the UV exposure of spin-coated SU8 layers. The membrane filter device captures 82.44% of the cancer cells spiked in blood with the cell viability of 72.33% after 5 day culture of the captured cells; thus, allowing further molecular analysis and stable characterization of CTC for cancer diagnosis and prognosis.

KEYWORDS: circulating tumor cells, tapered-slit, membrane filter, high-throughput viable cell isolation

INTRODUCTION

Circulating tumor cells (CTC), detached from primary tumor site, circulate in blood vessel; thus forming secondary tumors of metastasis. Current CTC research has focused to find the clinical relevance of CTC to metastasis and cancer therapy efficacy; thus, demanding the devices for high-throughput CTC isolation or capture from patients’ blood samples. The previous CTC isolation methods, based on surface specific antibody bindings, showed the problems of tumor-dependent capture efficiencies and the difficulty of viable cell isolation due to irreversible antibody binding. The alternative methods, based on cell sizes, were proposed for stable and viable CTC isolation. However, the size-based CTC isolation methods using straight-slit filters generate stress concentration to the captured CTC; thus, resulting in low CTC purity with cell damage [1]. In order to increase the isolation efficiency and viability of CTC, we proposed the lateral tapered-slit array that isolated CTC based on both cell size and deformability with the minimal cell stress [2]. The previous lateral tapered-slit filter, however, showed the limitation on throughput due to the limited number of tapered-slits in lateral array.

In this work, we present a membrane filter device using the vertical tapered-slit array, capable to achieve high-throughput viability CTC isolation. The vertical tapered-slit filter not only provides the supporting force to the captured cell, but also increases the tapered-slit density; thus facilitating high-throughput viable CTC isolation with minimal stress.

KEYWORDS: circulating tumor cells, tapered-slit, membrane filter, high-throughput viable cell isolation

Figure 1: The vertical tapered-slit membrane filter device.
THEORY

The vertical tapered-slit filter device (Figure 1) having the size of 16mm x 12mm consists of three layers: two 2,500μm-thick PDMS chamber layers for flow guidance and a 100μm-thick SU8 filter layer for cancer cell capture. The effective region for filtration on the filter layer is 10mm x 10mm, where vertical tapered-slits are formed with the wider slit inlet and the narrower slit outlet.

Compared to the straight-slit filter, the tapered-slit filter is capable not only to differentiate CTC from white blood cell (WBC) having an identical cell size with different cell deformability, but also to reduces the captured cell stress for an identical hydraulic force generated by sample flow. From the numerical simulation, we verify that the tapered-slit filter generates the minimal stress as much reduced to 18% of the stress generated in the conventional straight-slit filter.

We design the tapered-slit filter, composed of 83x415 (=34,445) vertical tapered-slits at the slit density of 34,445/cm². The outlet width of the tapered-slits is designed as 6μm, that showed the highest CTC capture efficiency in the previous study [2].

EXPERIMENTS

We fabricate the tapered-slit filter (Figure 2a~c) with the slits having the taper angle of 2° on SU8 membrane by a simple UV exposure on spin-coated SU8 layer with the adjusted expose dose and air gap between membrane and mask. The tapered-slit filter is sandwiched by the top and bottom chambers to complete the device fabrication (Figure 2).

The performance of the fabricated device have been characterized using the human lung cancer cells of H358G, transfected with green fluorescent protein (GFP). We spike the cancer cells in the PBS buffer and the blood, diluted at the three different dilution ratios (Blood: PBS=1:0, 1:2, 1:4). We load 1ml of the sample, including 60 cancer cells, to the device at the flow rate of 5ml/h using syringe pump. We repeat the cell capture process three times and observe the enumerate the captured cells using fluorescent microscope (Figure 3). After the cell capture process, we recover the captured cells from the filter by applying reverse flow to the device, then cultured the recovered cells at the temperature of 37°C with the CO₂ level of 5%. We observed the cultured cells and their viability after 5 days of culture, flowed by the survival and growth assessment of the cultured cells based on GFP fluorescence [3].

Figure 2: Fabricated tapered-slit filter device with the enlarged views of tapered-slit membrane: (a) top view; (b) bottom view; (c) cross sectional view.

Figure 3: The cancer cells captured by the tapered-slit filter for the sample, where the cancer cells are spiked in the diluted blood (Blood:PBS=1:2) at the sample flow-rate of 5ml/h: (a) bright field image; (b) fluorescent image.
RESULTS AND DISCUSSION

We have verified that the present membrane filter captured the 89.87±5.28% of cancer cells spiked in PBS. The capture efficiencies at the various dilution ratios were studied to find an effective dilution ratio. The capture efficiency significantly decreased as the blood became less diluted (Figure 4), and the dilution ratio of 1:4 were the optimal condition showing the maximum capture efficiency of 82.44±13.16%. We also observed that captured and recovered cells showed the viability of 72.33±5.31% after 5 day culture on SU8 membrane; thus, demonstrating the possibility of the culture and further analysis of captured CTC.

CONCLUSION

The tapered-slit membrane filters demonstrate high-throughput viable CTC isolation capability with the merit of cell culturing ability; thereby inaugurating further advanced CTC research for cancer diagnosis and prognosis.

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REFERENCES


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Figure 4: Capture efficiency of the tapered-slit filter for the sample, containing cancer cells in the blood of various dilution ratios and flowing at the rate of 5ml/h.