A MASS-PRODUCIBLE FILTRATION CHIP FOR ISOLATION OF CIRCULATING TUMOR CELLS FROM HUMAN BLOOD

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ABSTRACT

Enumeration of circulating tumor cells (CTCs) is a promising and harmless in vitro tool in monitoring of cancer progression and metastasis. An aim of this study is to develop a device that has the potential to solve the difficulties of CTC-isolation, such as high cost, high technical barrier, and difficulties of device mass-production. A chip with micro-filter structures and a chip-tubeless suction system was designed to separate CTCs from peripheral blood by size. This easy-to-use device made of PMMA through injection molding is a potential CTC-detection tool for wide usage.

KEYWORDS

Circulating tumor cells, CTCs, size-based cell separation, cell isolation, injection molding, mass-production.

INTRODUCTION

Isolating CTCs by the nature of larger size of most epithelial cells when compared to peripheral blood leucocytes prompts in the idea of size-based approaches, and have been demonstrated to attain high capture efficiency and high throughput [1-3]. Our group reports a mass-production filtration device had one-dimensional array apertures which could successfully capture live tumor cells, and those trapped cells could be culture on-chip after filtration. Additives like antibody, beads or special liquids are not necessary in the filtration processes. The diluted blood can introduced to the device directly for cell-separation by size. The device enables further cell analysis and other experiments of these value cells. This work provides a potential device that can be applied in routine CTC detection.

THE FILTRATION CHIP AND THE SYSTEM

Figure 1(a)-(d) show the fabrication process. A CD-like polymethylmethacrylate (PMMA) disc was first fabricated via precision micro injection molding by a CD/DVD manufacturing company (RITEK Corporation, Taiwan). One disc contains five patterns of two level microstructures, 9μm and 30μm. The patterns were cut off from disc and bonded with a PMMA reservoir using plasma-enhanced thermal bonding. Figure 1(c) shows the sketch pattern of the chip. The size of the aperture can be controlled from 0μm to 9μm, depended on different level of applied pressure and temperature as bonding conditions. Figure 2(a) shows the complete filtration chip. The size-trapping mechanism was illustrated by Figure 2(b) and (c). Small cells can flow through the aperture, and the large cells would retain on the edge or stuck into the aperture. An experimental setup for tubeless configuration was used here to ensure constant negative pressure driving sample through filter stably and high cell viability (Figure 3).

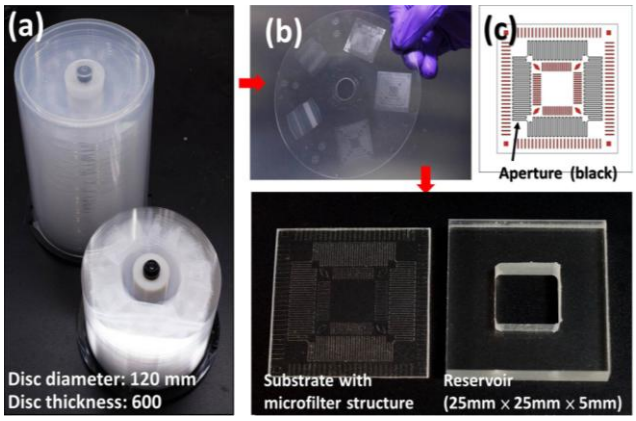

Figure 1. Process flow of the device fabrication.

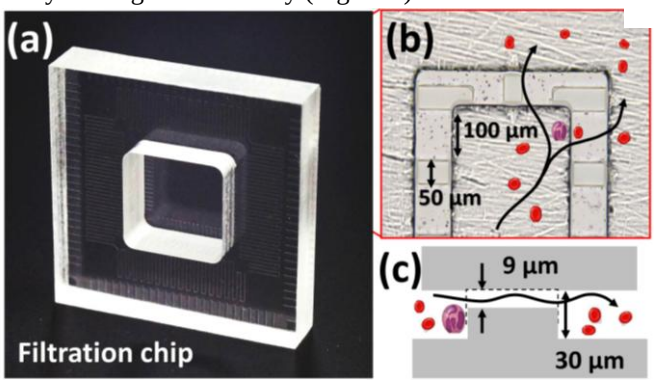

Figure 2. (a) Filtration chip (25mm X 25mm X 5mm). (b)(c) Illustration of trapping mechanism by size.

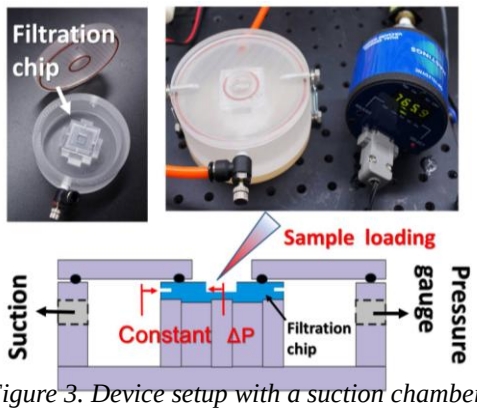

Figure 3. Device setup with a suction chamber.

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EXPERIMENTAL PROCEDURES AND SAMPLE PREPARATION

In the experimental procedure, the constant ΔP was set to a pressure value controlled by a regulator. A volume of 1ml running buffer was first introduced to the chip for rinsing. After that, the prepared cell sample was loaded into chip for filtration. The processing time was depended on the sample volume and dilution condition. After filtration, 3ml of running buffer was used to rinse the chip to remove the residual cells or other particles. If necessary, repeated the previous procedure with dye staining and buffer washing. Finally, the filtration-chip was removed to the fluorescence microscope for image detection.

To measure the diameter distribution of cell population, each of the cultured cell lines was suspended in PBS stained with Calcein-AM. With a concentration of 10^3cells/10μl, 10μl of the stained cells was sprayed on a glass slide and then wait for 20 min for cells sedimentation. For a rapid characterization, a microscope with a digital camera (Nikon D5100, 1.6 M pixels, connected with a 1.5X conversing ring) was used for image capture. The captured image resulted in a high resolution, which is around 1.5μm/pixel at 2X object lens and 0.6μm/pixel at 5X. The images were analyzed using the software of Image J.

Cultured tumor cells mixed with blood form healthy donors were filtered through the device, washed with PBS, and loaded with culture medium. Then replace the device to a petri dish cultured for 2days in an incubator at 5% CO2 and 37℃. On the 2nd day, the device was washed with PBS. Acetomethoxy derivate of calcein (calcein AM, C3100MP, Invitrogen) was added to the device for 30 min. Calcein AM can transport through the cellular membrane into cells then be hydrolyzed by an intracellular enzyme resulting in strong green fluorescence. As dead cells lack this enzyme, only live cells are marked. Fluorescent images were taken via the CCD camera (Leica 360) using a fluorescence microscope (IX-71, Olympus).

For visually distinguishing cancer cell lines or CTCs from hematic cells with fluorescence microscope, the spiked cell lines were prestained with EpCAM-PE (expressed red signal). Each staining/labeling required 20 minutes incubation at room temperature. Finally, the immune-fluorescent images were took using a 10X fluorescent microscope (Olympus IX71) fit with a CCD camera (Leica DFC360FX).

RESULTS AND DISCUSSION

Polystyrene beads (10μm) and several tumor cell lines were used for preliminary test. With very low concentration in the phosphate buffered saline (PBS) (3 beads/ml), the device had 100% capture efficiency (n=3) due to the less deformability of the beads. For live tumor cell (MCF-7 breast cancer cell line), device with 6.3μm aperture had 87.3±1.5% efficiency under a constant negative pressure of 0.1psi. We believed the small cells (<~8μm) deformed and went through the apertures. For further studies, we checked the size of cell population and found the percentage of the small cells (< 8μm) is ~15% (Figure 4). Results of other cancer cells were listed in Table 1. Those tests showed the capture efficiency would change if use different cell lines/populations or working pressure for the device.

For viability test, PC-3 prostate cancer cell line was introduced to the filtration device. After 48 hours, captured cells were stained on-chip with Calcein AM. Results showed cells are viable and even proliferating, which enable further cell culture and other analysis of rare cells, see Figure 5.

To optimize the workable pressure in our experimental setup, we have characterized the pressure of the filtration device with fixed/unfixed cell samples. Figure 6 shows the yields according to different cell conditions (fixed/unfixed) and pressure. The rigidity of cells plays an important role in size-based filtration. We thought that most unfixed cells damaged or deformed then went through the apertures exceed 0.3psi. Therefore 0.1psi pressure was chosen for following tests.

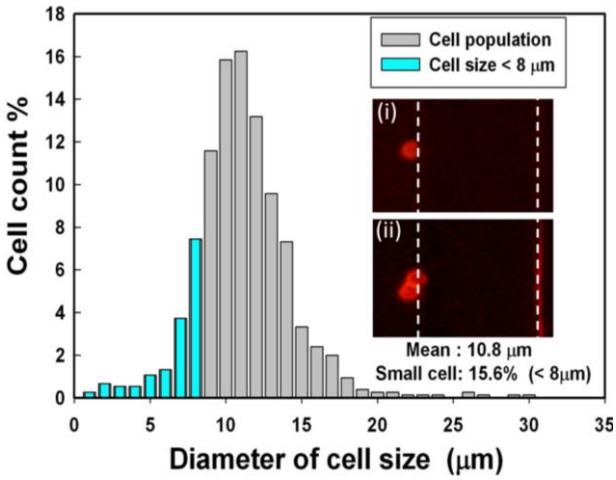

Figure 4. Size distribution of MCF-7 cell line. The inset figures show one and two cells (with PE-anti-EpCAM) captured on the aperture of the filtration chip.

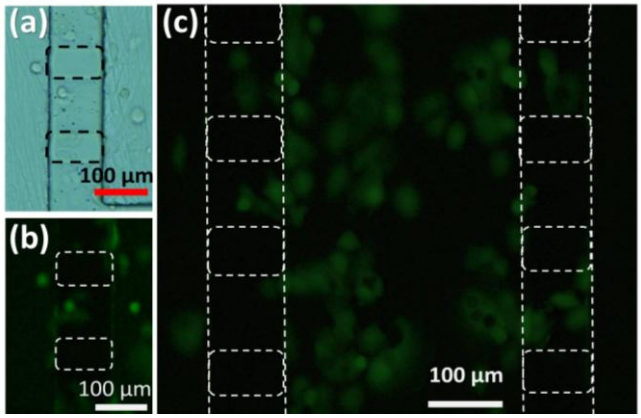

Figure 5. Viability of captured cells after 48 hours incubation. (a) Captured cells in bright filed and (b) in green fluorescence. (c) Fluorescent image (cells marked with Calcein AM) shows the captured cells are alive and proliferating around the aperture.

Tested sample	*Loaded cell/bead Number*	*Yield*	*>9um (%)*
Beads	3	100%	100%
MCF-7 (sample A)	185±8	**87.3±10.1%**	**84.4%**
MCF-7 (sample B)	92±6	**94.0±10.2%**	**92.4%**
Colo205	58±2	**74.5±14.9%**	**65.8%**
PC-3	123±6	**80.2±4.9%**	**83.5%**

Table 1. Yield PS micro-beads and three different type of cancer cell lines in PBS tested at 0.1psi.

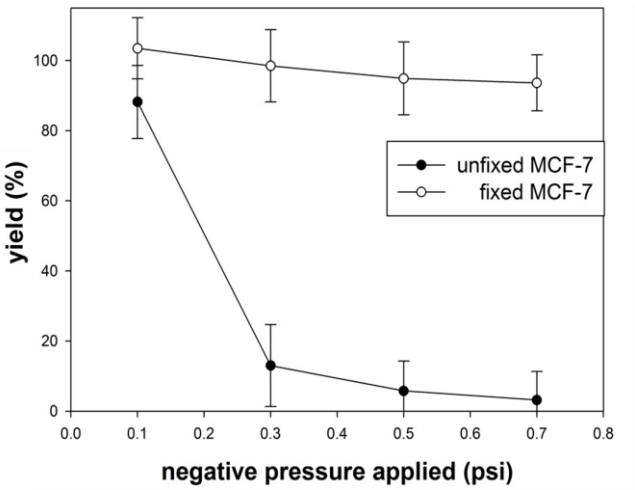

Figure 6. Effects of negative pressure and cellular deformability on yields of unfixed/fixed MCF7 in PBS tested in 0.1 psi.

We simulated different kinds of cancer patient samples by spiking cancer cell lines in 1mL of human blood from healthy donators and then diluted with 14mL PBS before processed by our device. It took about 1 to 1.5 hours to complete each filtration. The yield of each sample was 69.3±24.3% (Colo205), 78.0±14.9% (PC3) and 80.0±10% (MCF7), see Figure 7.

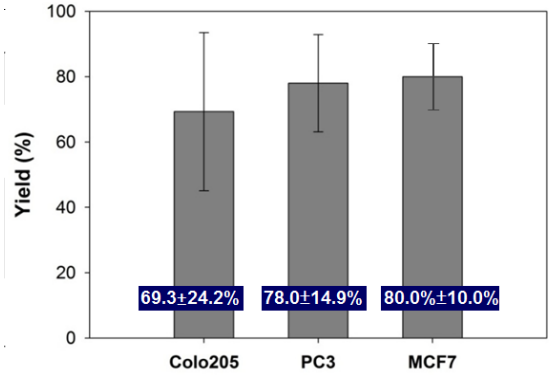

Figure 7. Yield of simulating-patient sample for CTC-detection. Three different cancer cell lines were spiked into 1mL human whole blood and diluted to 15mL.

CONCLUSION

The recovery rate of live MCF-7 cells spiked in diluted blood is 80~89%. The filtration chip could handle 15mL diluted blood (1:15) in 1~1.5 hour. For cell viability test, PC-3 prostate cancer cell line was testes to show after 48 hours filtration, and stained on-chip with Calcein AM are viable and even proliferating, which enable further cell culture and other analysis of rare cells. The system setup for chip tubeless was show the stability of performance and high cell viability. With features of low cost, tubeless, high capture efficiency and viable capture make this device potentially useful in capture of rare cell and subsequent analysis .

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