AN OPTICALLY-INDUCED DIELECTROPHORETIC (ODEP) MICROFLUIDIC PLATFORM FOR ISOLATION OF CIRCULATING TUMOR CELLS (CTCS) AFTER CONVENTIONAL CTC ISOLATION PROCESS

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ABSTRACT

Negative selection-based circulating tumor cells (CTCs) isolation method can harvest the CTCs in a label-free manner that is valuable for the subsequent CTC-based assays. However, such cell isolation scheme normally suffers from low CTC purity. To address the issue, the utilization of optically-induced dielectrophoretic (ODEP) force in a microfluidic chip to further purify the CTCs was proposed in this study. The working principle was primarily based on the fact that operating conditions of ODEP for the manipulation of CTCs (prostate cancer 3 as tested cells) and leukocytes were different due to the size difference between these two cells. By fine-tuning the operating conditions of ODEP force, cancer cells can be isolated from blood cells in an effective manner. In this study, operating conditions of ODEP for manipulation the cells were first investigated. The isolation of cancer cells from cell mixtures with different cancer cell purity was carried out. Results revealed that the isolated cancer cells were viable, indicating that such CTC isolation process was relatively cell-friendly. Moreover, the proposed CTC isolation scheme was proved to be able to isolate cancer cells from the cell mixture with high recovery rate (84%-88%), and high purity (82%-89%). As a whole, this study has presented an efficient and effective method for further enhancing the purity of CTCs after the conventional CTC isolation process.

KEYWORDS

Circulating tumor cells (CTCs), ODEP, Microfluidics, Cell isolation, Cell separation

INTRODUCTION

CTCs, the rare cell species present in the peripheral blood, can be a crucial biomarker for the indication of cancer progression and the selection of proper therapeutics. With the recent advance in biomedical technologies, several positive selection-based CTC isolation schemes were proposed [1]. For example, briefly, immunomagnetic beads surface-coated with antibody recognizing specific CTC surface antigen was utilized to bind and then separate the CTCs from the leukocyte (WBC) background. Overall, however, these methods are not able to harvest all possible CTCs in a label-free manner for the subsequent CTCs-relevant assays. To address the issue, more recently, a few studies demonstrated the negative selection-based CTC isolation schemes, by which only blood cells were targeted for depletion leaving behind the un-captured CTCs [2, 3]. Nevertheless, these methods normally suffered from a low purity of CTC isolation (1%-20% CTCs in an isolated cell mixture), which could in turn hinder the use of CTCs for further biochemical or cell-based assays. To tackle the technical hurdle, this study proposed the utilization of optically induced dielectrophoretic (ODEP) force in a microfluidic platform to further purify the CTCs from the pre-isolated cell mixture. The working principle is primarily based on the ODEP conditions for the manipulation of CTCs and WBCs are different. By fine-tuning the operating conditions of ODEP force, CTCs can be isolated from the blood cell background in an effective manner.

DESIGN AND FABRICATION

Figures 1 (a) and 1 (b) show a schematic illustration and a photograph of the ODEP-based microfluidic platform for CTC isolation. Briefly, two inlets were designed for loading the cell-free sucrose solution, and the PC3 cells/WBC mixture using a syringe pump (with a flow rate of 0.1 μl/min). At the CTC isolation zone, ODEP force was applied to separate the CTCs from the WBC background. The two separated cell populations can then be further isolated through the control of laminar flow regime in the microfluidic system, and finally collected at the two downstream reservoirs. The assembly and cross section of the microfluidic chip is shown in Fig. 2. Structurally, the

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proposed platform consists of a top PDMS layer (layer A), an indium-tin-oxide (ITO) glass substrate (layer B), an adhesive tape with microchannels fabricated by CO_2 laser (layer C), and a bottom ITO glass substrate with a layer of photoconductive material coated (layer D). Briefly, the layer A was bonded with the layer B with the aid of O_2 plasma treatment, and followed by assembling with the layer D through the CO_2 laser fabricated adhesive tape (layer C). To generate the ODEP force, a function generator was used to apply AC voltage between the top and the bottom ITO layers to produce an electric field. A commercial digital projector coupled with a computer was used to display controllable optical images onto the photoconductive material to generate ODEP force for manipulating cells. The overall experimental setup is schematically illustrated in Fig. 3.

RESULTS AND DISCUSSION

The ODEP conditions for the manipulation of CTCs and WBCs are different due to the size difference between the two cells. This can be explained through the equation described below.

$$F_{DEP} = 2\pi r^3 \varepsilon_m \mathrm{Re}(f_{CM}) \nabla E^2$$

where r, ε_m, $\mathrm{Re}(f_{CM})$, and E denote the radius of the cells, the permittivity of the sucrose medium surrounding the cells, the real part of the Clausius–Mossotti factor, and the root-mean-square value of the electric field, respectively. By controlling operating conditions of ODEP force, CTCs can be isolated from the blood cell background. In this study, the operating conditions for generating ODEP force for the manipulation of PC3 and WBCs were first investigated. A moving-light pattern was used to manipulate these two cells such that they can be isolated. Figure 4 revealed the relationship between the maximum velocity of the moving-light pattern that can manipulate the cells, and the applied electric voltages. Based on this result, the control of the velocity of the moving-light pattern can be used to separate the two cells at a given operating condition. To prove this concept, experimental investigations were carried out. Briefly, the PC3 cells with different cell numbers were spiked in the WBCs to make up the cell mixture samples (total cell number: 5,000 cells; cell density: 5000 cells/μl) with different purity of PC3 cells (1%, 10%, and 20%; the purity range of negative selection-based CTC isolation methods). The cell mixture suspension was then loaded and delivered in the microfluidic platform, and followed by the ODEP force-based isolation process as described in Fig. 5. Briefly, the moving-line patterns to generate ODEP force were exerted at the boundary of two laminar flows and moved downwards continuously (I-IV) at Area-1, and upwards (V-VII) to separate the two cells (VIII) at Area-2. In this operation, the input flow rates of both sucrose solution and cell mixture suspension were 15μl/hr. Scanning velocities of the light pattern at Area-1 and Area-2 were 57μm/s and 193μm/s respectively. By using this process, the PC3 cells and WBCs were separated into two laminar flows and finally collected downstream. The collected cells were further identified through cell staining (Fig. 6 (a)). Figure 6 (b) shows that the obtained PC3 cells are viable, indicating that such CTC isolation process is relatively cell-friendly. Based on the cell staining result (Fig. 6 (a)), furthermore, the separation performances including the recovery rate, and the purity of PC3 cells were evaluated through cell counting. Results (Fig. 7) demonstrated that the proposed CTC isolation scheme was able to isolate PC3 cells from the cell mixture with high recovery rate (84%-88%), and high purity (82%-89%), within the experimental conditions explored. As a whole, this study has presented an efficient and effective method for further enhancing the purity of CTCs after the conventional CTC isolation process.

CONCLUSIONS

This study demonstrated an ODEP-based microfluidic system to effectively isolate CTCs from a cell mixture. Overall, the proposed method was proved to able to harvest viable CTCs with high recovery rates (84%-88%) and high CTC purity (82%-89%). It may provide a promising tool for isolation of CTC for diagnosis and prognosis applications.

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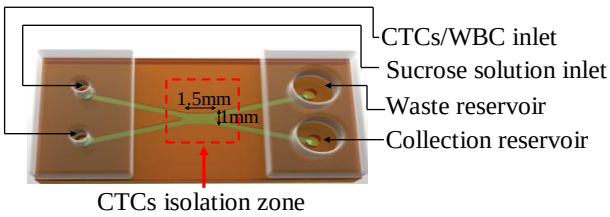
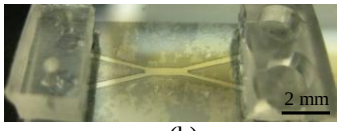


(a)



(b)

Figure 1: (a) Schematic illustration and (b) a photograph of the microfluidic platform (Top view).

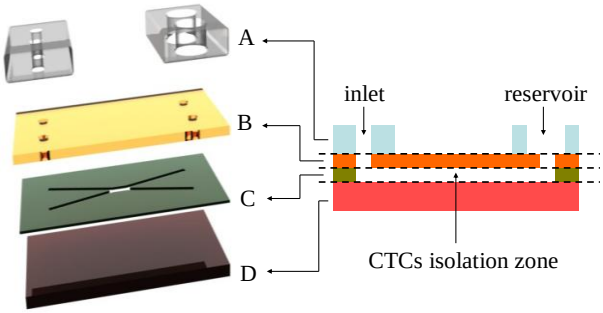


Figure 2: The structure and cross-sectional view of the microfluidic platform.

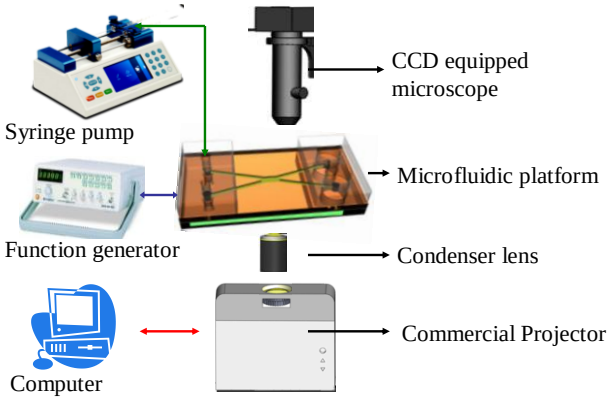


Figure 3: The experimental setup for the ODEP platform.

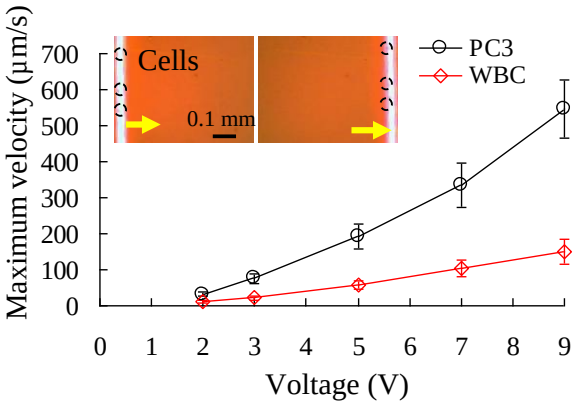


Figure 4: Evaluation of the maximum ve-locity of moving light patterns that can manipulate the cells under different operating conditions (applied electric voltage ranging from 2 V to 9 Vat a fixed frequency of 100 kHz).

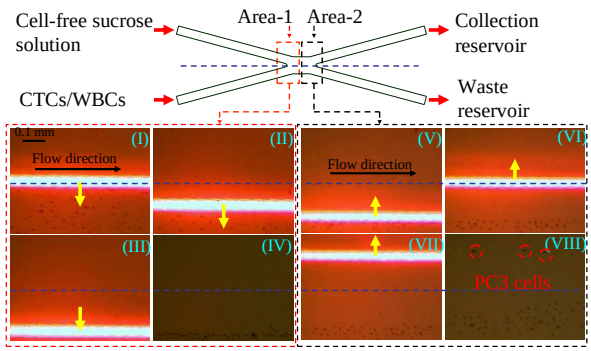
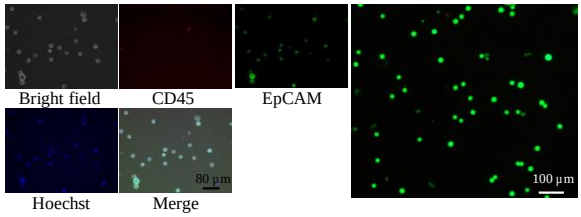


Figure 5: The microscopic images (I-VIII) of the ODEP-based cell (PC3 cells/WBCs mixture) separation process.



(a) (b)

Figure 6: (a) The images of cells under a bright-field microscope, stained with immuno-fluorescence dyes (Anti-CD45 positive (red): WBC; EpCAM positive (green): PC3 cells) and stained with Hoeches dye (blue): the nucleated cells), and (b) the collected PC3 cells stained with live and dead fluorescent dye (green and red dots represent live and dead cells, respectively).

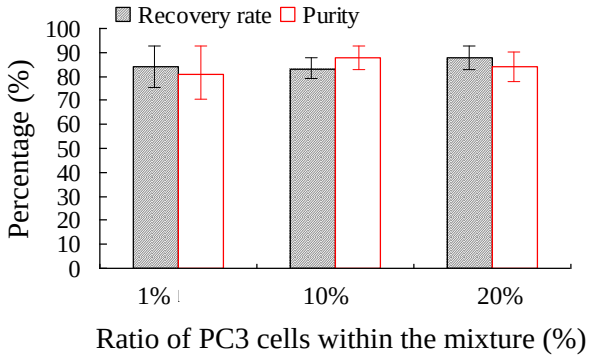


Figure 7: Evaluation of the recovery rate and purity of PC3 cells (n=20). (Recovery rate = collected PC3 cell number/spiked PC3 cell number x 100%, purity=collected PC3 cell number/collected PC3 cell + WBC number x 100%.)