

MICROFLUIDIC DEVICE FOR WHOLE GENOME AMPLIFICATION OF SINGLE CANCER CELLS ISOLATED FROM WHOLE BLOOD

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ABSTRACT

Self-seeding microwell plate can sort single cells into 6400 wells based on cell size and their identity verified by immunofluorescence staining. Here, we developed a microfluidic device in which these single cells can be placed, lysed and their DNA amplified for further genetic analysis. Whole blood spiked with MCF7 tumor cells was passed through the microwell plates after leukocyte depletion and identified single cells were punched into the reaction chamber of the microfluidic device. Reagents for cell lysis and DNA amplification introduced by peristaltic pumping of micro-valves. On-chip lysis and amplification was performed in 8 parallel chambers.

KEYWORDS: Circulating tumor cells, Microwells, DNA amplification

INTRODUCTION

Genetic characterization of Circulating Tumor Cells (CTCs) offers the opportunity for a “real time liquid biopsy”. [1] Heterogeneity of CTCs command the need for individual cell characterization. Several methods have been introduced to enrich, identify, isolate and characterize these rare cells. However, following an enrichment procedure of CTC from blood, the identification, isolation and manipulation of single cells for further analysis without loss of cells remains challenging. Recently, our group developed a single cell isolation method based on self-seeding microwell plate. [2] After deposition of a cell solution on the microwell plate single cells are contained in the microwells for further single cell analysis. Here, we present a microfluidic device with open-well structures in which cells can be identified, isolated, lysed and the nucleic acids amplified following filtration of leukocyte depleted whole blood. On-chip amplification will be a powerful tool to improve genetic analysis of single cells by making use of the smaller reagent volume, automation and parallel reactions of microfluidic devices.

EXPERIMENTAL

Microfluidic devices were designed and fabricated using photolithography and PDMS multilayer soft-lithography using the approach described by Unger et al. [3] Aliquots of 1 ml of blood were spiked with 1000 MCF-7 cells and leukocytes were depleted from the blood using the RosetteSep CTC enrichment kit (Stemcell technologies, Vancouver, Canada). After depletion, the cell suspension was stained with Hoechst 33342 (Invitrogen, Breda, The Netherlands) and anti-EpCAM PE (Sigma Aldrich) for 30 min at 37 °C. Cells were washed and re-suspended in 4 ml of 1X PBS containing 1 % BSA. Repli-g single cell kit (Qiagen, Venlo, The Netherlands) was used for DNA amplification. Real-time SYBR green qPCR was used for amplifying target-specific DNA.

RESULTS AND DISCUSSION

Our microfluidic device consists of fluidic layers (blue) and micro-valve control layers (red) as shown in Figure 1. The device was bonded onto a standard glass slide (2.5 cm × 7.5 cm) in which 8 holes were drilled. The centers of the holes were positioned above the end of the channels, which connects to reaction chambers.

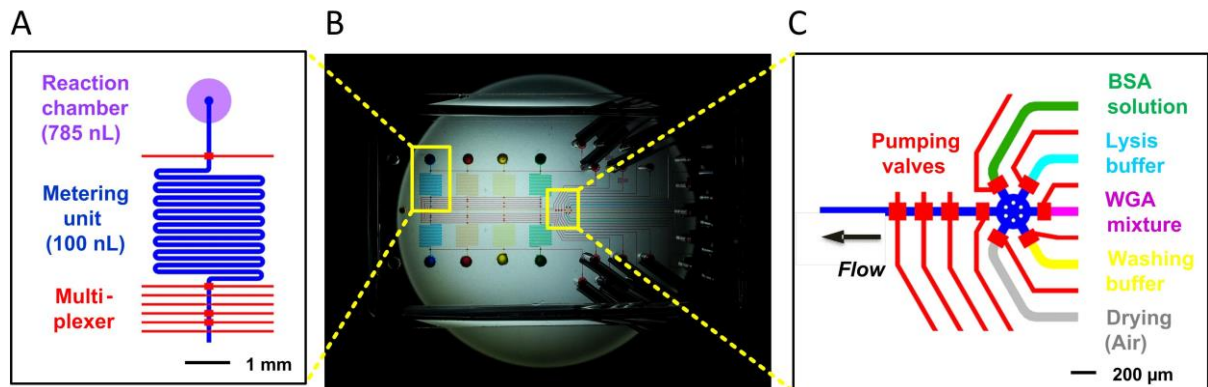


Figure 1: (A) Design of a reaction chamber (B) Photo of the actual microfluidic device. 8 individual chambers were filled with different color dyes to visualize. (C) Design of inlets and peristaltic pumps. 5 inlets were designed for different reagents and micro-valves for the peristaltic pump placed in front of the inlets.

To obtain single cells a micro-well plate containing 6400 cups with a diameter of 70 μm , a height of 380 μm and a 5 μm hole at the 1 μm thick bottom of the well were used [2].

After leukocytes depletion, the blood spiked with MCF-7 cells was filtered through the microwells. After staining with PE-labeled anti-EpCAM, and Hoechst, MCF-7 cells were identified as PE+,Hoechst+ cells in the micro-wells by inverted fluorescence microscopy. The bottoms of the micro-well containing individual MCF-7 cells were punched with a needle into the open reaction chamber of the microfluidic device. Overall experimental scheme is shown in Figure 2.

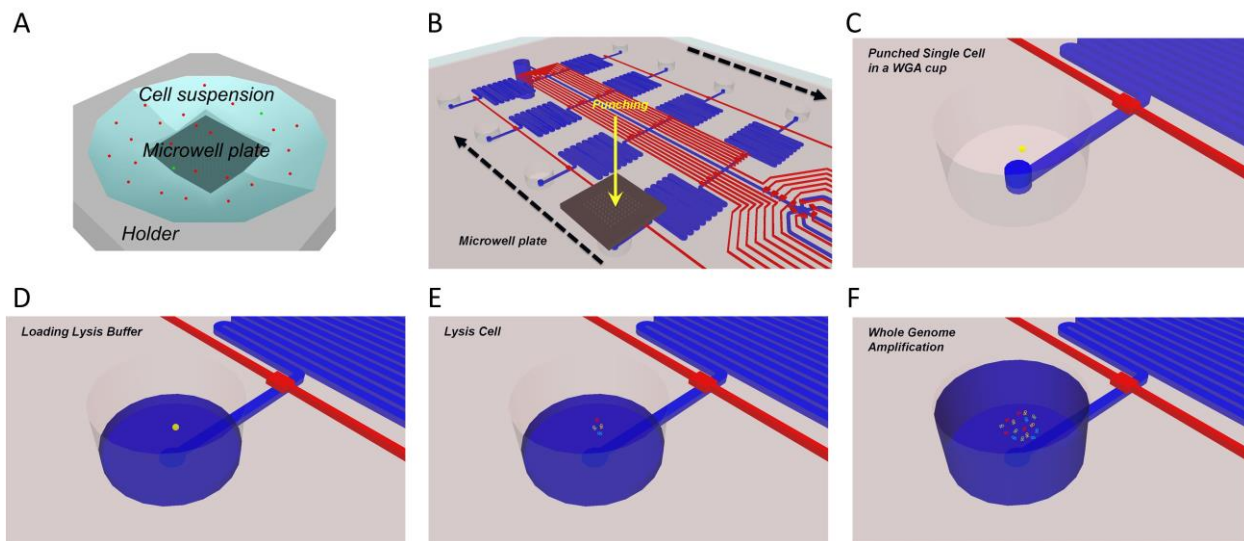


Figure 2: Experimental Scheme (A) Filtration of MCF-7 cells spiked in whole blood after leukocyte depletion. (B) Identification and punching of the bottom of the well containing MCF-7 cell (C) Isolation and observation of cells in a microfluidic chamber (D) Loading lysis buffer using peristaltic pumping (E) Cell lysis (F) Loading amplification reagents and DNA amplification.

Figure 3 shows microscope images of cells in a micro-well plate (A and B) and in a reaction chamber (D and E). Figure 3 (A) shows a section of the microwells with 2 MCF-7 cells (blue Hoechst+, green EpCAM+) indicated with orange arrows and 2 leukocytes (blue Hoechst+, green EpCAM-) indicated with yellow arrows. Figure 3 (B) shows one well containing one MCF-7 cell before punching and Figure

3 (C) shows the same well after punching in which the bottom of the well is no longer present. Figure 3 (D) is a bright-field image and (E) is a fluorescence image of an isolated MCF-7 cell in the microfluidic reaction chamber.

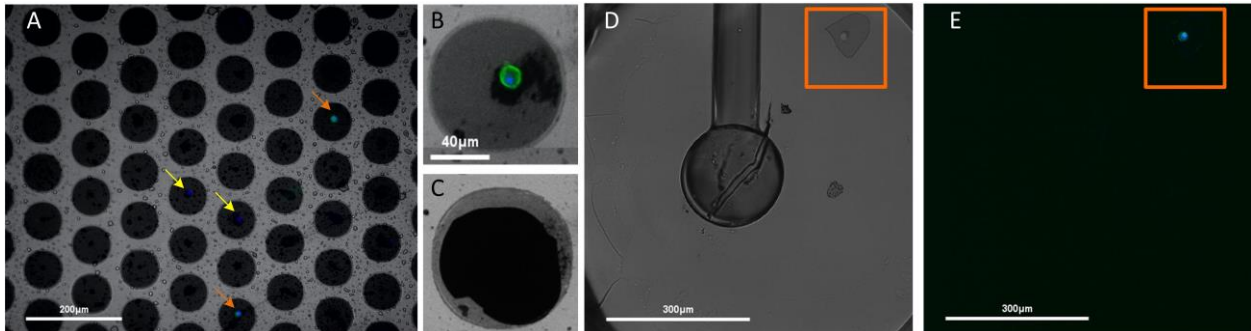


Figure 3: Microscope images of cells in a micro-well plate (A and B) and in a microfluidic chamber (D and E).

Lysis buffer solution was pumped into the reaction chambers by peristaltic pumping of the operating micro-valves. Peristaltic pumping allowed the addition of solutions from the center of chambers with precise fluid control. The maximum pumping rate was obtained at 10 Hz and is approximately 2.5 nl/sec. After 10 min lysis at 65 °C, stop solution and whole genome amplification (WGA) reagent were added sequentially using peristaltic pumping. WGA was carried out in the 8 parallel reaction chambers in a small volume (maximum chamber volume is 785 nl) for 16 hours at 30 °C. Amplified DNA were collected through open-well reaction chambers by pipetting. To verify on-chip amplification, amplified DNA samples were purified, quantified and performed qPCR targeting 8 genes present on different chromosomes. Target-specific genes were only amplified in the chambers with MCF-7 cells isolated.

CONCLUSION

We developed a microfluidic device integrated with self-seeding micro-well plate filtration. We demonstrated isolation of individual cancer cells from 1 ml of leucocyte depleted whole blood into a microfluidic chamber followed by on-chip DNA amplification.

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