A LABEL-FREE SIZE-BASED MICRO COULTER COUNTER SYSTEM FOR CIRCULATING RARE TUMOR CELLS

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ABSTRACT

In clinical and biological fields, circulating tumor cells (CTCs) have been becoming an important topic because of their critical role in cancer metastasis. Many researchers reported that the number of CTCs is highly-related with the survival rates of cancer patients. Hence, we described a label-free size-based micro coulter counter system to count CTCs in human blood. We examined OVCAR-3 cells spiked blood samples and obtained decent results with 88% efficiency. Furthermore, we tested breast cancer patient samples and detected CTCs in 24 patient samples. Therefore, the developed system shows a facile way to count CTCs regardless of biomarkers.

KEYWORDS: Label-free, Coulter Counter, Circulating Tumor Cells, Microfluidic

INTRODUCTION

Cancer is one of the major causes of death worldwide and the cancer metastasis is a dominant factor for the death among cancer patients. In metastasis processes, CTCs play critical roles to spread the tumor from the primary site to the secondary sites. Therefore, the number of CTCs provides the valuable information about cancer activity states, cancer prognosis, and the response after therapy. To quantify CTCs, we present the micro coulter counter system on a microfluidic chip using polyelectrolytic gels (PGs) for electrical connection and the physical barriers. The proposed system is optimized to detect CTCs in undiluted blood based on the size difference among peripheral blood cells.

THEORY

Micro coulter counter system detects the resistance change in the crossed section of the sample channel and the electrical detection channel. PGs placed on the both sides across the sample channel to block the sample leakage while allowing electrical ions to pass through (Fig. 1). Applying DC voltage to two ends of the electrical detection channel generates ionic flow through PGs and the current density is concentrated on the crossed region (Fig. 2). Thus, the ionic current is influenced mostly by the resistance of the detection region. When a cell (or a particle) passes through the region, the resistance is increasing, and the amplitude of the resistance change is proportional to the volume of the cell. Thus, the developed system could provide the size information of the cell.

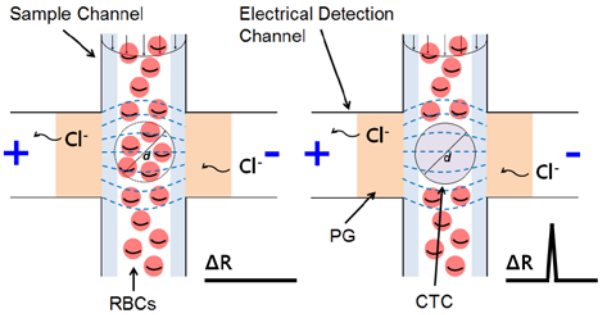


Figure 1: Schematic illustration of the micro coulter counter. The developed system detects the resistance change between PGs.

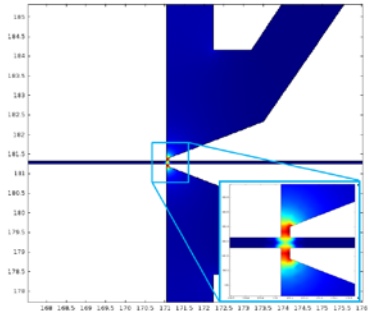


Figure 2: COMSOL simulation image of electric displacement field norm (C/m^2).

It is mostly believed that CTCs are larger than peripheral blood cells like red blood cells (RBCs), white blood cells (WBCs), and platelets. From the cancer cell line (OVCAR-3) tests, we assume that the diameter of CTC is bigger than 16 μm. Normally, a volume percentage (%) of RBCs in blood is ~45% in a specific volume, which means that the unoccupied volume is ~55%. When an OVCAR-3 cell of 16 μm in diameter passes through the detection region instead of blood, the volume difference will be about 1180 μm^3 which is the volume of ~13.1μm diameter sphere. Therefore, we set the resistance by 13.1 μm diameter sphere as a threshold for the detection of CTCs.

EXPERIMENTAL

The micro coulter counter system is comprised of a microfluidic chip, a resistance change detection system, and a data acquisition system. A microfluidic chip consists of crossed channels, one with a sample channel and the other with an electrical detection channel in which PGs are placed on the electrical detection channel. A microfluidic glass chip in this study was fabricated by conventional photolithographic procedures and PGs were fabricated on the desired sites by photopolymerization techniques with a mixture of charged monomer, photoinitiator, and cross-linker under UV. A resistance change detection system measures the resistance changes in the electrical detection channel under the consistent voltage of 0.8 V. All samples were introduced into the inlets by a syringe pump with 13.3 µl/min and 41 µl/min. Commercial micro particles (Bangs Laboratory) were utilized to calibrate the developed system and fluorescence labeled-OVCAR-3 cells were applied for spiking tests. Blood samples of 24 metastatic breast cancer patients were obtained from the National Cancer Center (NCC) of Korea (Institutional Review Board number NCCNCS-12-624). For validation of the proposed system, patient blood samples were labeled with HER2 antibody (Abcam) and secondary antibodies conjugated with Alexa 610PE (Invitrogen). For the simultaneous detection of fluorescence and the resistance, the combination of Ar laser (488 nm), fluorescence filter (Thorlabs) and a photomultiplier tube (PMT) (Hamamatsu) were utilized.

RESULTS AND DISCUSSION

We demonstrated that the developed system could generate different peak amplitudes according to the size of various particles and it is linear to the volume of a particle. To decide the counting efficiency, we spiked 10, 50, 100, and 1000 cancer cells in 500 µl blood collected from the healthy volunteers and the efficiency reached about 88% (Fig. 3). We checked OVCAR-3 cells with simultaneous detection of fluorescence and resistance.

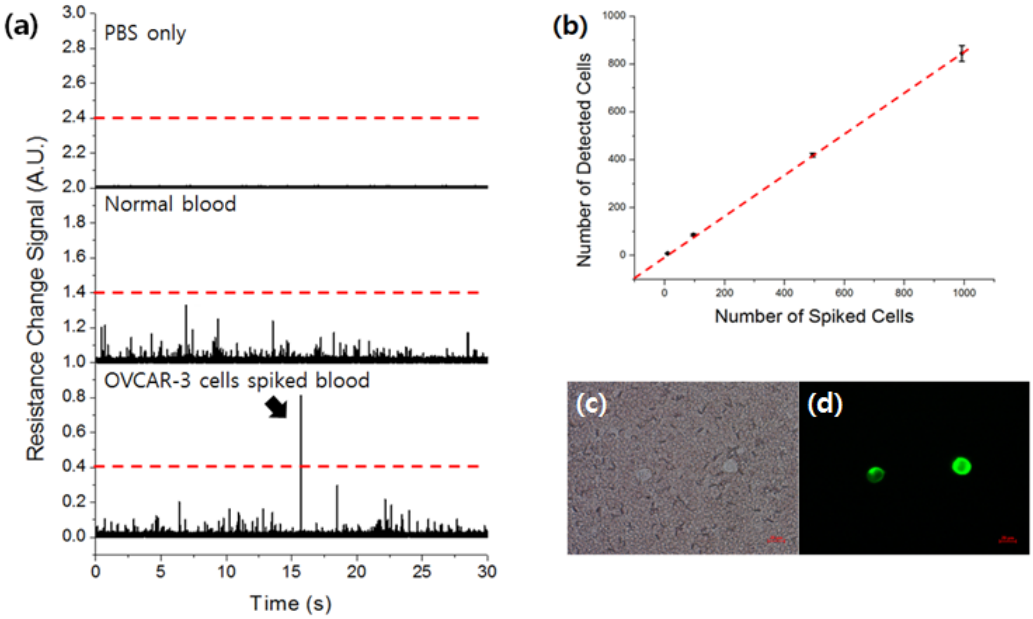
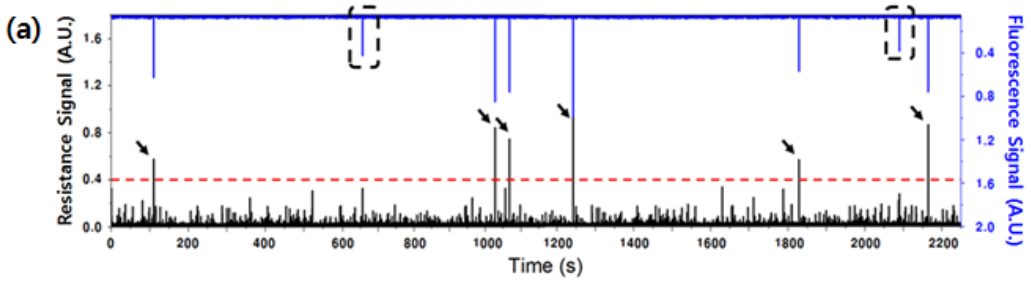


Figure 3: (a) Resistance change signals from PBS solution, normal blood samples from healthy volunteers, and OVCAR-3 cells-spiked normal blood samples. (b) The number of detected cells when a various number of cells (10, 100, 500, and 1000 cells) was spiked in healthy normal blood samples. (c), (d) Images of EpCAM labeled OVCAR-3 cell in blood samples.

In addition, we tested the blood samples from breast cancer patients and the developed system could detect CTCs in 24 out of 24 blood samples, while no CTCs were found in 5 healthy blood samples.



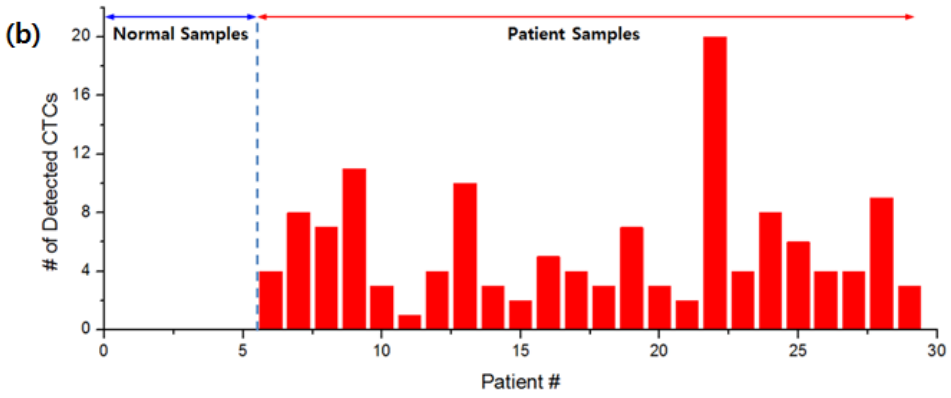


Figure 4: Detection of CTCs in breast cancer patient samples. (a) Simultaneous detection results of resistance and fluorescence. (b) The number of resistance peaks of CTCs from 5 healthy donors and 24 breast cancer patients.

As shown in Fig. 4a, all of resistance peaks (black arrow) over threshold were synchronized with fluorescence peaks, although some fluorescence peaks (dashed box) were matched with short resistance peaks, which probably indicates small-sized CTCs. Clearly, this results showed that the developed system could separate healthy people and cancer patients.

CONCLUSION

In this work, we verified that a size-based micro coulter counter system was capable of counting CTCs in blood efficiently without labeling. We successfully measured the resistance changes by passing cells in detection region. We obtained 88% efficiency for OVCAR-3 cells in blood, and acquired the positive results from all of the breast cancer patients.

Although test time for larger volume of blood is long, the simplicity of the microchannel and the system enables the system to parallelize easily. Furthermore, simple cytometry approaches allow integrating with cell sorting system for isolation to post-analyze CTCs.

ACKNOWLEDGEMENTS

We appreciated participation of cancer patients and healthy volunteers, which significantly helped our researches. This work was supported by the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (2005-2001287).

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