A HYBRID ELECTROKINETIC PROCESSOR FOR ISOLATING EXFOLIATED CANCER CELLS AND CIRCULATING TUMOR CELLS IN PHYSIOLOGICAL SAMPLES

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ABSTRACT
This paper reports a hybrid electrokinetic processor that combines electrophoresis (EP), dielectrophoresis (DEP) and AC electrothermal flow (ACEF) for isolating exfoliated cancer cells (ECCs) and circulating tumor cells (CTCs) in physiological samples. While most electrokinetic techniques only function in low-conductivity buffers, hybrid electrokinetics enables effective operation in high-conductivity physiological fluids (~ 1 S/m) such as blood, buffy coat and urine. Using a 3-electrode configuration with optimized AC voltage, frequency and DC offset, we demonstrate that breast cancer cells can be isolated in buffy coats and biological fluids in 10 sec.

KEYWORDS: Hybrid Electrokinetics, Exfoliated Cancer Cells, Circulating Tumor Cells, Isolating

INTRODUCTION
CTCs and ECCs are cells detached from primary tumors that exist in bloodstream and other physiological samples (e.g., urine and stool). Existing CTCs and ECCs capture strategies rely on epithelial cell adhesion molecule (EpCAM) immunoaffinity or size-based separation from other cells [1]. However, immunoaffinity assay cannot capture non-EpCAM expressing cells and size-based separation cannot isolate cells with similar size. Previously, we have developed a new technology – hybrid electrokinetic sorting (EK) – an “on-chip” platform which exploits cells’ innate structural and electrical properties to concentrate and isolate them from other cells in solution. Using the EK-chip, we demonstrated manipulation of bacterial pathogens in high conductivity samples using hybrid electrokinetics [2]. We demonstrate the applicability of hybrid electrokinetics technique, which combines EP, DEP, and ACEF for CTCs and ECCs capturing in high-conductivity physiological sample.

THEORY
Electrokinetics has been one of the most powerful approaches for microfluidic manipulation. Electrokinetic techniques, including EP [3], DEP [4], and ACEF [5,6] are ideal methods for manipulating nanoscale and biological objects, such as cells and molecules, due to the length scale matching for effective momentum coupling, label-free operation, simple fabrication processes, and small voltage requirements. For instance, EP is the movement of charged objects under external direct current (DC) electric fields. The force is proportional to the amount of charges and the electric field strength. DEP is the motion of polarizable particle under inhomogeneous electric field. The strength of the force in this technique is a strong function of the medium and particle’s electrical properties, which lead to the strong frequency dependence of DEP. The force is proportional to the second power of the applied voltage. In addition, ACEF is the fluid motion generated due to the interaction of the external electric field and thermally induced gradients of electrical properties of the fluid. The fluid motion created by ACEF is proportional to the fourth power of the applied voltage and is relatively independent of the frequency at the intermediate frequency range (~ 1 MHz) for high conductivity media. These electrokinetic techniques can co-exist during electrokinetics manipulation. This represents a challenge to understand the observed electrokinetic phenomena. Systematic investigation of the AC voltage, frequency, and DC bias dependences are required to study the dominant effects. On the other hand, the co-existence of the forces creates new opportunities in combining multiple electrokinetic techniques, i.e., hybrid electrokinetics, for enhancing the overall performance of electrokinetic manipulation for various applications.

EXPERIMENTAL
The electrode configuration for cell manipulation is shown in Figure 1 a-b. The device was fabricated according to our previous work [2]. The length, width and distance of each electrode were 2.5 mm, 50 µm and 125 µm respectively. The cell line (human mammary gland adenocarcinoma MDA-MB-231) was obtained from the American Type Culture Collection (ATCC, HTB-26). Cancer cell were pre-stained using 3,3'-dihexyloxacarbocyanine iodide (DiOC6(3), Sigma). Buffo coat and whole blood (WB) were obtained from the Innovative Research Company (IPLA-WB5 and IPLA-WB1). The lysed WB was obtained to add red blood cell lysis buffer (Biolegend, USA) into WB and incubate for 15 min at 37°C. The centrifuged WB was obtained to centrifuge WB for 10 min at 1000 rpm.

Data collection and analysis were also performed according to our previous work [2]. To estimate the capture efficiency, the number of the target cells through outlet was counted by analyzing 300 consecutive images, and the efficiency was calculated based on the fraction of cell counts.
RESULTS AND DISCUSSION

The design of the hybrid electrokinetic processor is shown in Figure 1a-b. Breast cancer cells (MDA-MB-231) can be focused on the center electrode and delivered continuously to the sample collection channel (the center channel). At the same time, 80% of the white blood cells are pushed out of the center area by hybrid electrokinetics. To optimize the electrode and microchannel designs, a series of experiments were performed to test the effects of the outer electrode width, gap distance, and the inner electrode width (Fig. 1c-k). It is found that the effective capturing region depends weakly on the outer electrode while the capture efficiency is highly sensitive to the gap distance and inner electrode width. Figure 1l-n shows focusing of breast cancer cells on the center electrode. With a combined DC and AC potential applied across the side and the central electrodes, hybrid electrokinetics was generated for cancer cells manipulation. The cancer cells were driven to the central electrode by negative DEP and concentrated along it. Observations demonstrated that most of the cancer cells in the channel could be concentrated in less than 10 sec. Under static conditions, the cancer cell continued to be aggregated on the electrode due to DEP and EP. Under dynamic conditions, the cancer cell can be entrained by the flow and collected by the channel downstream. The performance of the hybrid electrokinetic device depends on various experimental conditions. We systematically investigated hybrid electrokinetics by adjusting the frequency, voltage, DC offset, and buffer conditions. Figure 2a,b shows the voltage dependence and suggests a second power dependence on the applied voltage for the velocity at the sides. A DC offset voltage can enhance the velocity of capturing (Fig. 2c,d). Moreover, the concentration velocity maximized at 100 kHz, which is likely a result of DEP in the process (Fig. 2e,f). The cancer cell and white blood cell can have different performance and was demonstrated by the comparison of frequency dependence (Fig. 2g,h).

Figure 3a shows the capturing efficiency for continuous isolation with different flow rates. There is a trade-off between the throughput and the capturing efficiency at high flow rates. Below 0.1 μl min⁻¹, more than 95% cancer cells were trapped at the center which was similar to the static condition. In addition to capturing cancer cell in buffy coat, we have also successfully demonstrated the general applicability with other physiological samples including lysed WB, centrifuged WB and urine (Fig. 3b).

CONCLUSION

In summary, we have developed a hybrid electrokinetic manipulator that is capable of directly isolating breast cancer cell in physiological samples including buffy coats, simple treated whole blood and urine. A particular design of the device are demonstrated to address the cancer cell isolated from the white blood cell. The 3-parallel electrode design is optimized such that a strong electrode field is created while maximizing the effective manipulation region. We also systematically studied the electrokinetic conditions of capturing cancer cell under static and dynamic flow and optimized the conditions to isolate the cancer cell from most of the white blood cell.

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Figure 2. (a,c,e,g) The applied AC voltage (a), DC offset (c), Frequency (e) dependence for cancer cell capturing and blood cell separation (g). (b,d,f) Position dependence for cancer cell trapping. (h) Frequency dependence for separation of cancer cell and blood cell.

Figure 3. (a) Dependence of the capturing efficiency of cancer cell on the flow rate. (b) Capturing efficiencies of cancer cell in buffy coat, lysed whole blood (WB), centrifuged WB and Urine at the same flow rate, 0.08 μm/min.

REFERENCES

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