Microarray Detection of Rare Specific PBMC with EVB Marker via a Reversible Dielectrophoresis Technique

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Monitoring the population of the cytotoxic T cells with specific markers has become essential in decease diagnosis and vaccine development [1]. Since the concentration of specific T-cells is always very low (1~103 cells/ml), the analysis is generally conducted based on cell culture enrichment, specific staining with fluorescent dye, and then counting using a flow cytometer [2]. However, this process is time-consuming and labor-intensive, usually taking 3~7 days to yield a presumptive result. Over the past several years, various methods based on micro/nano technology have been adopted for the rapid and parallel detection of the rare cells. However, there still lack an easy and efficient way to massively monitor the population of the rare specific cells in a mixed cell suspension.

This work presents a novel method for detecting rare specific cells in a mixed cell sample based on cell-capture and reversible DEP (dielectrophoresis) scheme. First, the cells are specifically stained via a biomarker (Fig.1a) and then pipetted into the arrayed reactors after adequate washing. Second, a weak DEP field is applied to guide gently all of the cells in a suspension to interact with the bio-probes that are immobilized on a PVDF substrate in a reactor (Fig.1b). The electrical fields are simulated (Fig.2) and the DEP parameters are optimized to maximize the cell capture efficiency. Moreover, the reverse DEP scheme will redistribute the un-bonded cells into the suspension for next reaction cycle (Fig.1c). Finally, the specifically stained and captured cells are imaged and counted with the image processor (Fig.1d).

This assay is used to detect the population of rare cytotoxic Tc cells in a human blood sample; and the Tc cell has specific receptors on the membrane surface to identify the Epstein-Barr virus (EBV). The fluorescence visibility of the specific PBMC cells is also realized via quantum dots/tetramer complex staining. The experimental results (Fig. 4) indicated that the forward DEP can double the cell capture efficiency compared with that without DEP enhancement, and the reversible DEP scheme can improve the efficiency about 25% further. In addition, the number of captured specific cells in the mixed cell sample is linearly related to the number of input specific cells, even at low concentration. Since the proposed method can be parallel performed in a short period (<1 hour), it seems to be a fast and effective way to monitor the population of rare Tc cells in human blood for vaccine research.

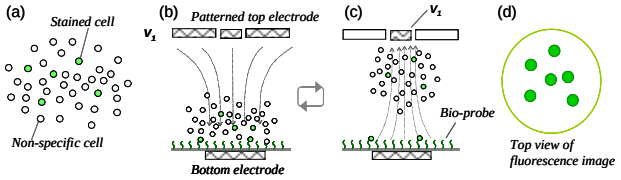

Fig. 1: Schematic diagram illustrating the proposed method for monitoring the population of specific cell in a cell suspension.

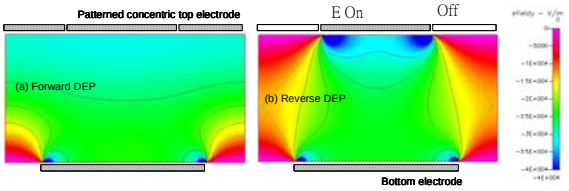

Fig.2: Simulated Grad (E^2y) in the reactor.

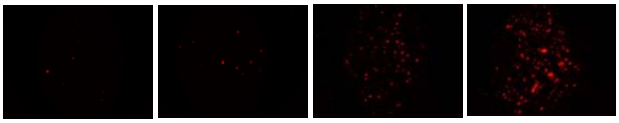

Fig.3: Fluorescent images of the stained and captured PBMC cells with various concentrations from the mixed cell suspensions.

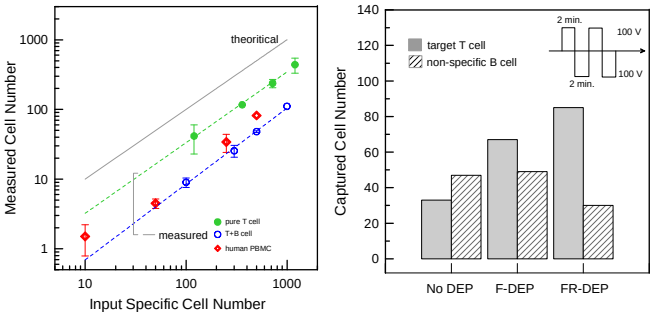

Fig.4: (a) Plot of measured cell numbers against input cell numbers with various cell samples in the forward DEP field. (b) Capture cell numbers with different DEP schemes (F- forward, FR-forward/reverse).

References

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