

CELL POSITIONING AND SORTING USING DIELECTROPHORESIS

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INTRODUCTION: In this paper we describe a micro-fabricated cell separation and positioning system based on the AC electrokinetic technique, dielectrophoresis (DEP). The system uses non-uniform electric fields produced by arrays of microelectrodes to manipulate cells held in suspension. Depending upon the mode of operation one can either concentrate a single cell type from a heterogeneous mixture (with all other cell types passing through the device); or alternatively separate different cell types along the length of the device. In the second mode of operation the position at which a cell ends up is based on its size and the dielectric properties of its cellular membrane.

METHODS: DEP has been applied to the separation and manipulation of a vast array of bioparticles since it was first described by Pohl in 1978 [1-3]. The technique has been used to manipulate cells by a number of groups [e.g. 4-9].

The DEP force can be expressed as [2]:

$$\underline{F}_{dep} = \frac{1}{2} \alpha v \nabla |\underline{E}|^2 \quad (1)$$

where α is the effective polarisability, v is the volume of the particle and \underline{E} is the electric field.

Figure 1 shows how $\nabla |\underline{E}|^2$ varies with position above an array of interdigitated electrodes [10].

Fig. 1: Plot showing how the gradient of the magnitude of the electric field squared varies above an interdigitated electrode array. Different lines represent different heights above the array.

A schematic diagram of the DEP-separator is shown in figure 2. The system consists of two separate arrays of interdigitated bar electrodes integrated into the one device. When particles enter the device they are carried in a fluid stream and are distributed randomly throughout the chamber volume. Using negative DEP forces, the initial electrode array concentrates the wide distribution of particles entering the device into a well-defined sheet positioned midway between the upper and lower channel walls. Particles then enter the second or 'separation' electrode array, which is energised such that a positive DEP force acts upon either all the particles or a desired sub-population of

particles, pulling them out of solution onto the electrode surface. In this paper we will concentrate on the situation where all the cells are undergoing positive DEP and are being attracted to the separation electrode.

Fig. 2: Schematic diagram of the DEP particle separator. A binary mixture of two cell types are first focused into the central plane of the channel and then follow distinct trajectories banding at different positions along the channel length.

Particles held at the electrodes can be imaged on the device. All the captured cells can subsequently be eluted for further processing by turning off the electric field (or applying negative DEP) whilst continuing to flow fluid through the device.

RESULTS: Separation devices were fabricated on standard glass microscope slides. The electrode arrays were patterned using standard photolithography and wet etching techniques. A 100 μ m deep flow channel was defined in SU8 photoepoxy, with the channel lid aligned and glued in place using UV curable glue. Inlet and outlet holes were drilled prior to gluing the lid.

Experiments were carried out using mixtures of cultured human monocytes (THP-1 cell line) and human peripheral blood mononuclear cells (PBMCs). PBMCs were collected from the buffy coat after density gradient centrifugation of whole blood over a Histopaque-1077 gradient. Cells were labeled prior to mixing with CellTracker™ dyes (Molecular Probes). The THP-1 monocyte cell line was labeled green and PBMCs were labeled red.

The magnitude and direction of the DEP force depends upon the relative polarisabilities of the cells and the suspending media. Cells were therefore resuspended at known concentrations in a low ionic strength media (dH₂O containing Ficoll400 (3.5% w/v), sucrose (9% w/v), glucose (0.1% w/v) with the addition of small amounts of phosphate buffer) of pH 7.4, osmolality ~290mOs/m and conductivity ~10mS/m.

Cell suspensions were fed into the device using a syringe pump. A typical set of experimental conditions being: flow rate of 1ml/hr, 5V_{pp} @

20MHz applied to the focusing electrodes, and $8V_{p-p}$ @ 200kHz applied to the separation electrodes. Figure 3 shows a fluorescence image captured after 0.5ml of cell suspension has flowed through the device under the above conditions.

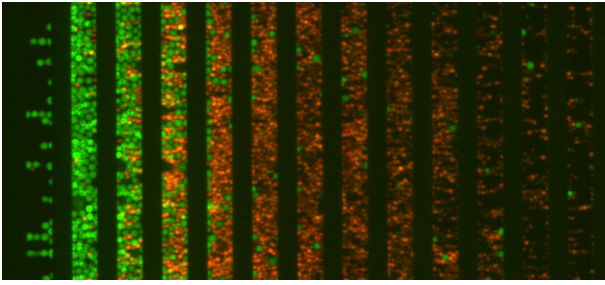


Fig. 3: Fluorescence image of THP-1 cells (green) and PBMCs (red) banding on the separation electrode array. The vertical black stripes are the $40\mu\text{m}$ wide electrodes of the separation array.

From figure 3 it is clear that the mean positions of the two cell types differ. Figure 4 shows a plot of cell numbers versus distance along the length of the separation electrode array for a similar experiment.

DISCUSSION & CONCLUSIONS: Figures 3 and 4 show a nice tight band of THP-1 cells and a more smeared out band of PBMCs. This difference in distribution profiles is due to the THP-1 cell population being relatively homogeneous; while the PBMC population is composed of a number of cell sub-types. The PBMC sub-populations are; Monocytes, T-lymphocytes and B-lymphocytes (most Granulocytes are removed in the centrifugation step). Each of these sub-populations has different size and dielectric properties [4].

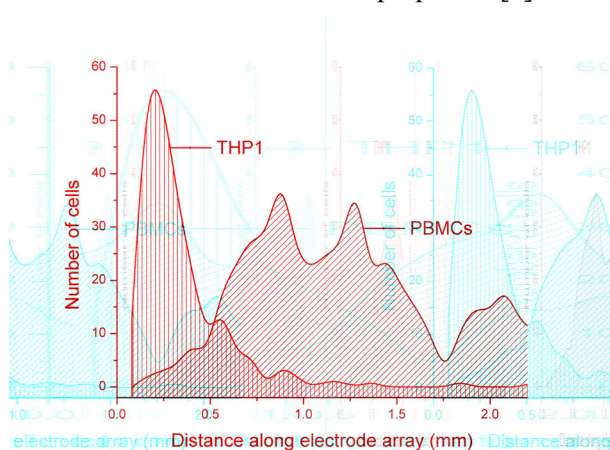


Fig. 4: Position of the different cell types along the separation electrode array for a mixture of PBMCs and cultured THP-1 cells.

If the experimental conditions are kept the same the cell types will band reproducibly in the same position on the separation array. Varying the

applied experimental conditions, such as changing the flow rate or the magnitude or frequency of the signal applied to separation electrodes results in the position of the cell band changing. Cell types other than those described will band at different positions determined by their size and dielectric properties.

In this paper we have presented a novel DEP electrode configuration, which relies upon initially focusing all the cells in the flow stream to the central plane of the flow channel. Cells then enter a second region where they undergo positive DEP and are attracted the separation electrode array where they are held.

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