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*Short Communication*

# Electrochemical Cell Entrapment Device for BioMEMS Applications Using Benchtop Fabrication Techniques

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A microfluidics-based device that has the capability to trap cells has been designed and constructed and its capabilities have been demonstrated using dielectrophoresis, a promising tool for the selective manipulation and separation of cells, bacteria and other bioparticles. The device consists of a dielectrophoretic chamber and an interdigitated electrode geometry that is fabricated from gold-coated glass slides. It was designed with the intention of providing a device that was compatible with standard laboratory equipment and had the capability to prevent leakage and discriminate cells. Experimental results reveal that the device is capable of trapping leukemic K562 cells at an average efficiency of 94.2%. In addition to this, the design offers a number of advantages, which include the ability to exchange microscope slides and change separation chamber height. It is also reusable, easy to assembly and cleaning and provides a simple method of connecting electrodes and a power source. The device has the potential to become very beneficial in the preliminary stages of many immunosensing applications that require separation of different types of cells.

**Keywords:** Biotechnology, BioMEMS, Microfluidics, Dielectrophoresis

## 1. INTRODUCTION

Microfluidics-based biological microelectromechanical systems (BioMEMS) is a device that integrates laboratory functions and components such as reservoirs, integrated electrodes, fluid handling, reactors, heaters, pumps, separators and sensors, on one, small scale chip [1]. These integrated microsystems have a wide range of applications in areas such as biomedical research, clinical diagnosis, food pathogen detection, rapid drug discovery and environmental analysis [1,2]. For

example, they can be used to provide cancer prognostic information by conducting a molecular analysis of cancer cells within a small volume of pathologic fluid.

In addition, the ability to perform particle counting, analysis, trapping, manipulating, separating and sorting systems, creates rapid development of the design and fabrication of the device. A technique called dielectrophoresis (DEP) has been proven to be an effective method for the separation of bioparticles such as cells, viruses and proteins. This separation technique is particularly useful in the biotechnology industry as it offers many beneficial biological applications. One of the most successful of which is the separation of cancer cells. The simplest method of separating these cells is through the utilization of a technique that allows the solution containing the cell sample to flow through an array of electrodes, subsequently using DEP to trapping the cells at specified locations within the electrode geometry.

In the early 1990s, Gascoyne *et al.* demonstrated the first use of positive and negative DEP as a separation technique for cancer cells [3]. They measured the dielectrophoretic properties of normal, leukaemic, and differentiation-induced leukaemic mouse erythrocytes by selecting a suitable frequency and cell suspension medium. As a result of their research findings there is an increasing interest in the separation of human cancer cells.

The revolution in microfabrication techniques as a consequence of developments in the electronics industry, are now poised to revolutionize the pharmaceutical, biotechnology, and biomedical device industries. Photolithographies, etching techniques and deposition methods have been successfully used to create large numbers of microscopic components such as micropumps, microfluidics, microsensors and microelectrodes on silicon or glass substrate. The components are then integrated to form BioMEMS devices. The first device to use microfabrication techniques to build a dielectrophoretic laboratory-on-chip was the "dielectrophoretic fluid integrated circuit", which was developed by Washizu, Masuda and Nanba [4]. This device was able to store and transport cells step-by-step around microfabricated channels and sort them into different outlets.

Gascoyne *et al.* designed a device for separating several different cancerous cell types from blood [5]. The device consisted of two 2-mm thick glass walls that were sealed along their long edges with UV-curing epoxy glue. The lower glass was equipped with an interdigitated electrode. An inlet and an outlet hole were drilled in the top chamber wall and Teflon tubes were glued in place to form ports for the injection and removal of cell suspensions. A separation chamber was maintained by a Teflon gasket of 100μm thickness running along the active length of the separator.

Gasperis *et al.* described a microfluidic device for separating MDA-435 human breast cancer and peripheral blood mononuclear (PBMN) cells according to their dielectric properties [4]. Their device operated by combining 2-dimensional dielectrophoretic forces with field flow fractionation (FFF) and comprised a thin chamber and multilayer microelectrode array at the bottom. The fluidic chamber was assembled from a top plastic plate and a bottom plastic substrate that squeezed in a 250μm thick latex gasket. Pressure-loaded metal wires were used in order to provide electrical connections to the electrodes. This device can be readily integrated with other microfluidic components, such as micro polymerase chain reaction (PCR) and capillary electrophoresis devices for microscale sample preparation and analysis.

Li *et al.* constructed a microfabricated device to separate and manipulate live and heat-treated non-pathogenic *Listeria innocua* cells with great efficiency [6]. A rectangular electrode chamber was created by using wax to affix a silicone rubber (0.3 mm thick) to a glass substrate that contained interdigitated microelectrodes. Such on-electrode manipulation and separation of cells by using a combination of dielectrophoretic and electrohydrodynamic forces can be very useful in diagnostic applications.

Doh *et al.* suggested a continuous cell separation chip using a hydrodynamic DEP process [7]. The design consists of three planar electrodes, a microchannel, two inlets for medium solution and one inlet for the cell mixture to be pumped in and three outlet ports for separated cells to be pushed out. The three electrodes were fabricated on the glass substrate to provide the maximum electric field region on the centreline of the middle electrodes, and the minimum region on the centreline. In addition, they created a polydimethylsiloxane (PDMS) microchannel where the continuous cell separation occurred via the use of a miromoulding technique. The device is useful for high throughput biological analysis systems.

Although these reviewed systems and devices are capable of successfully trapping the intended cell populations, the majority of them are laboratory-bound because of various fabrication constraints and complexities, with cost factors being the most dominant of these. The objective of this paper is thus to document the development of a low-cost lab-on-chip device that is capable of trapping cell populations using the DEP technique alone.

## 2. MATERIALS AND METHODS

### *2.1. Device Design*

The device is constructed of two pieces of Perspex that allows a fluid (mixture of cells) to flow in between. The top and bottom covers are separated by a gasket and pressed together by fasteners. The thickness of the gasket defines the depth of the separation channel.

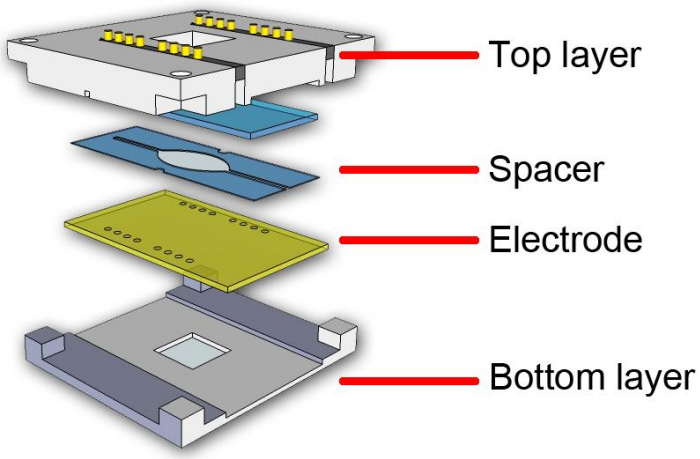


**Figure 1.** Schematic diagram of the developed device

*2.2. Electrode Fabrication*

There is a single inlet and outlet port, located laterally at the top cover of the flow cell. The fluid is pushed into an enclosed separation chamber that has an electrode at the bottom via the use of an external source, such as a syringe pump. In order to provide a secure connection between the electrodes and the power source, eight spring mounted probes are proposed. Particular attention is given to ensure the design addresses the following details: leakage prevention, the ability to exchange the microscope slide, the need for a device that is reusable, the requirement for the device to be easy to assembly and the need for it to be compatible with different gasket thicknesses. Fig. 1 shows the schematic diagram of the developed device and the overall setup of the various experimental components.The selection of interdigitated geometry was based on its ability to efficiency trap and separate bioparticles. Decay in the values of the electric field gradient in this type of geometry occurs exponentially with the distance from the electrode plane, at a rate inversely proportional to the characteristic electrode periodicity [8].

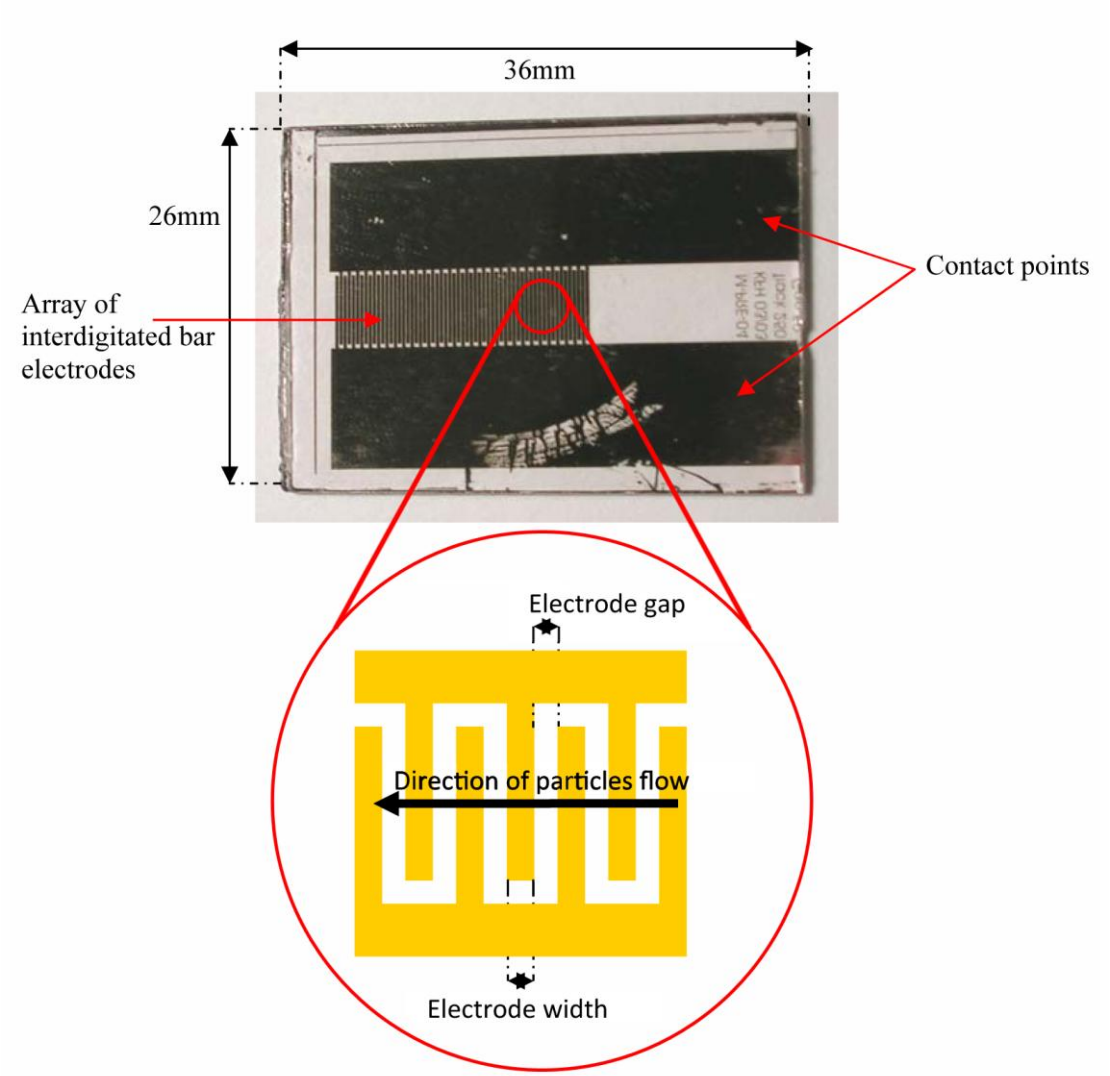


**Figure 2.** The completed electrode with the interdigitated geometry design

The geometry design was printed onto a gold-coated microscope slide (polished 100-200 nm; XanTec bioanalytics GmbH, Duesseldorf, Germany) using the photolithography method and the slide was cut according to the dimensions of the slide holder located on the bottom cover of the flow cell. The electrodes would be connected to the head of the spring-mounted probes once the device is assembled. The power source can then be applied via the probes.

The process of making the electrodes begins with chemically cleaning the slide in order to remove any surface contamination. This can be done by firstly agitating it for approximately 30 min in a mixture of 10% distilled water and 90% propan-2-ol before rinsing the slide with distilled water and agitation in a 100% propan-2-ol solution. This step has to be repeated three times. Fig. 2 shows the completed electrode following the fabrication process.

### *2.3. DEP Experiments*

Chronic myelogenous leukemic K562 cells were used as the sample population of choice to be trapped within the chamber. The conductivity medium consisted of 8.5% sucrose and 0.3% dextrose buffer (after Gascoyne *et al.* [5]) and was adjusted to 25 $\mu Scm^{-1}$ using phosphate buffered saline (PBS). Final conductivity was verified with a conductivity meter.

The cell density in each sample was set at $50 \times 10^4$ cells per ml (±20% difference) for the DEP measurements. 5 ml of cell solution were centrifuged at 1,500 rpm for approximately 6 minutes, and the supernatant was removed prior to resuspension with the conductive medium. The process of centrifuge and washing were repeated two times. Cell density was measured once more prior to the delivery of the cell solution into the chamber.

Following this, the separation chamber, inlet and outlet tubes were filled with approximately 0.7 ml (total volume of the chamber and tubes) of conductivity media. In order to perform the DEP experiments, the flow cell was energized with 20 V (peak-to-peak) at the optimum frequency of 1 MHz (after Labeed *et al.* [9]) using a waveform generator through spring-mounted probes. A syringe pump was used to push flow into the flow chamber. Next, 0.5ml of cells were pumped at a flow rate of 1.02 $mlh^{-1}$. This flow rate was chosen as a result of earlier research performed by Gascoyne *et al.* [5], who revealed that flow rates of between 5 and 100 $\mu lmin^{-1}$ were optimal.

In order to push the cells, which remained in the tubes and separation chamber, 1.4 ml (two times the total volume of the chamber and tubes) of conductivity media was pumped into the device. The medium and cells that were not trapped were collected at the outlet port and counted to determine the efficiency of the trapping process.

## 3. RESULTS AND DISCUSSIONS

Once the syringe pump was turned on, the sample was pushed through the inlet tube; it took some time before flowing and trapping could be observed on the electrodes. After 15 minutes, some trapped cells were observed, as shown in Fig. 3. As time passed, more cells were trapped at the edge of

the electrodes. The total time taken to inject the whole sample was found to be 30 minutes using 0.5ml of cells (approximately $14 \times 10^4$ cells in total) at a flow rate of 1.02 $mlh^{-1}$. In order to test that no cells were trapped inside the tubing and flow cell itself, 1.4 ml of conductivity medium was used to further flush the system, and the number of cells collected outside the system was consequently counted. The efficiency of the number of cells collected was found to have an average of 94.2% (SD ± 0.01) (Table 1), and the values are significantly correlated ($p<0.05$).

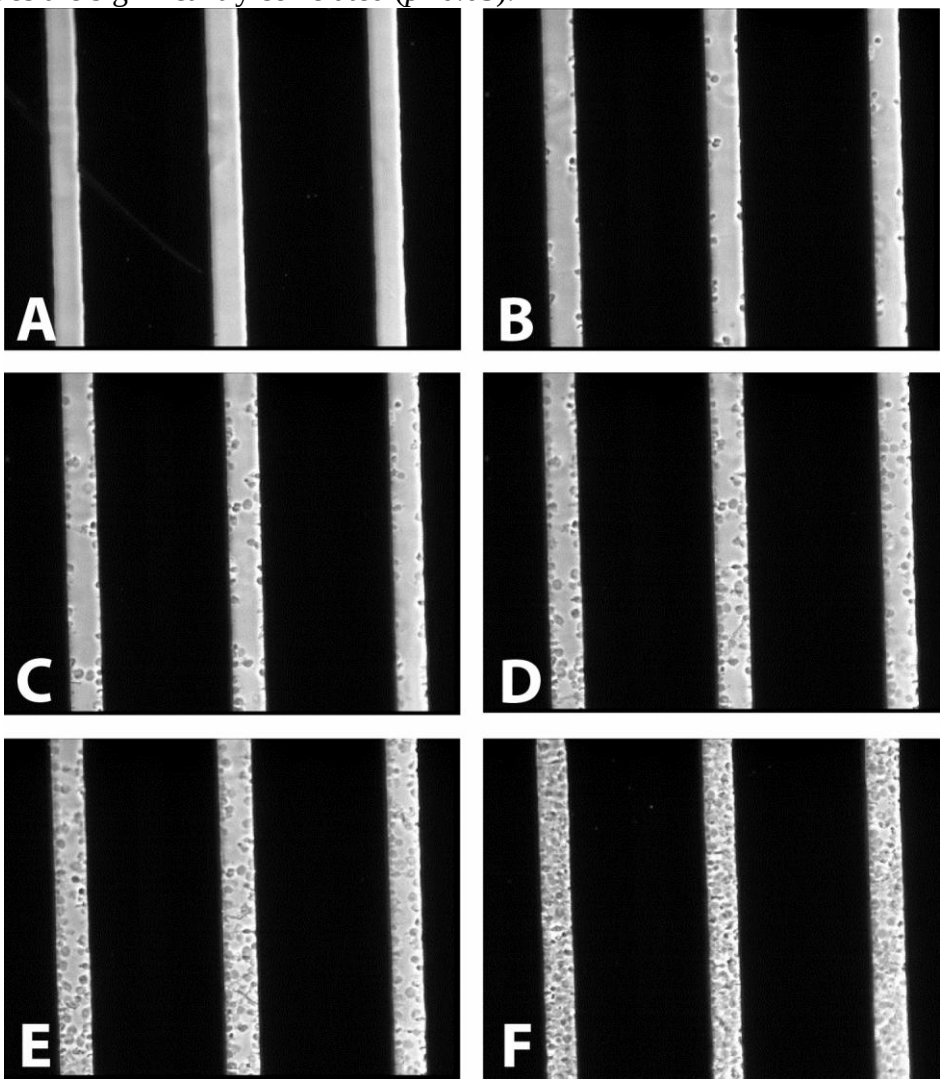


**Figure 3.** Cell collection at specified time intervals: a) 7 min, b) 15 min, c) 20 min, d) 25 min, e) 30 min, f) completion of experiment

**Table 1.** Cell collection efficiency of the device

| Number of cells injected, In (cells) | Number of cells collected, Out (cells) | Cell collection efficiency = Out/In x 100% |
|---|---|---|
| $14.5 \times 10^4$ | $13.5 \times 10^4$ | 93.2% |
| $13.3 \times 10^4$ | $12.7 \times 10^4$ | 95.4% |
| $14.2 \times 10^4$ | $13.4 \times 10^4$ | 94.6% |
| $11.2 \times 10^4$ | $10.5 \times 10^4$ | 93.9% |
| $15.0 \times 10^4$ | $13.9 \times 10^4$ | 92.9% |
| $13.4 \times 10^4$ | $12.8 \times 10^4$ | 95.2% |

An additional observation from the experimental procedure revealed that a certain amount of conductivity medium had to fill inside the flow cell. To calculate this volume, it was necessary to calculate the length and diameter of the inlet and outlet rubber tubes and bores inside the flow cell, the thickness of gasket and the area of chamber:

$$V_{push\ in} = 2\pi(r_{tube})2l_{tube} + 2\pi(r_{bore})2l_{bore} + wl_{gasket}h \quad (1)$$

where r is radius, l is length, w is width and h is the height of gasket used. If $r_{tube}$, $r_{bore}$, $l_{tube}$, $l_{bore}$, $l_{gasket}$, w and h are 1mm, 0.5mm, 90mm, 17mm, 28mm, 6mm and 0.04mm respectively, the total volume to push would be 641.4 $mm^3$. This means that almost 0.7 ml of medium was filled inside the flow cell before the cells were pushed through in order to ensure that the cells were suspended through all the way. Later, 1.4 ml of medium needed to be pushed in to collect those cells that were not trapped. The volume of the collected cells were at par with previously developed devices [2-4], thus paving the way for the fabrication of low-cost cell entrapment device that may be used at point-of-care settings and as part of a complete diagnostic tool.

## 4. CONCLUSION

In conclusion, a device that can trap cells through the use of a DEP flow separation technique has been developed and successfully tested with a sample population of K562 cells. The device was proven to resist any leakage caused by high amounts of pressure inside the separation chamber. Particular consideration such as selection of material, design construction, material and work limitations were given to ensure the design was able to offer a number of advantages. These include the ability to exchange microscope slides, the ability to offer connectivity between electrodes and power sources, reusability, the ability to adjust separation chamber heights, ease of assembly and ease of cleaning.

Further studies are warranted with regards to the consistency in the fabrication of the gasket (to ensure uniformity of chamber volume), and to determine the optimal experimental parameters, namely height for the chamber, cell density, and flow rate. Additional experimental procedures are also required to determine the capability of the said device in separating a desired cell type from a heterogeneous cell mixture.

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