INVERTED OPEN MICROWELLS FOR ANALYSIS AND FUNCTIONAL SORTING OF SINGLE LIVE CELLS

Massimo Bocchi[12*], Laura Rambelli[2], Enri Duqi[2], Andrea Faenza[2], Luca Giulianelli[2], Nadia Lopez[2], Nicola Pecorari[2] and Roberto Guerrieri[2]

[1]MindSeeds Laboratories, ITALY

[2]ARCES – University of Bologna, ITALY

ABSTRACT

We present a novel method for the isolation, functional analysis and sorting of single cells or small cellular aggregates. Inverted open microwells featuring a microchannel on top, an open air-fluid interface on bottom side and embedded electrodes were implemented on flexible-PCB technology. Microwell arrays in pitch with standard microtiter plates were implemented. K562 cells were delivered to the microwells and trapped at the air-flow interface. Cell delivery and aggregate formation were controlled by dielectrophoresis. Continuous fluid replacement around the cells during the experiment was demonstrated. After analysis, live cells were easily recovered to standard plates where cell growth was demonstrated.

KEYWORDS: Dielectrophoresis, Microwell, Cell Sorting, Cell-cell Interaction

INTRODUCTION

The analysis of cell-cell interactions and sorting of single cells having a desired functionality is major unmet need in the development of new anticancer therapies and treatments, such as adoptive cell transfer therapy and discovery of new drugs aiming at activating the immune system and inducing interactions among cells within aggregates. Microsystems based on microwells or specific microfluidic structures have been proposed to isolate single cells [1-6]. Only a few of them showed the possibility to isolate multiple cells providing their contact [7]. Moreover, in most of these systems the recovery of single cells from the platform remains a manual task, limiting the number of single cells which can be retrieved [4-5].

We present here a novel microwell architecture, open at both top and bottom sides, enabling a method to isolate single cells or small cellular aggregates, analyze cells within aggregates and easily transfer single cells from multiple sites to a standard 384-well plate. The combined use of microfluidics and negative dielectrophoresis (DEP) provides a gentle method to handle cells keeping them alive throughout the analysis and sorting processes.

THEORY

An inverted open microwell system (Figure 1A) includes a microchannel placed on top of a microwell array. The miniaturized size of the wells introduces a surface tension which prevents fluid leakage from the bottom. The microchannel can be sized as to introduce a limited fluidic resistance and a relatively low pressure in the upper part of the microwell for flow rates of up to 8µL/min. Hence, the meniscus at the bottom side remains stable even when the fluid flows in the microchannel, thanks to the surface tension generated at the air-fluid interface. In such a device, cells can sediment in the microwells and lean on the air-fluid interface. Cells can remain alive at the air-fluid interface as demonstrated by the well-known hanging-drop method [9] where cells are cultured in a similar condition.

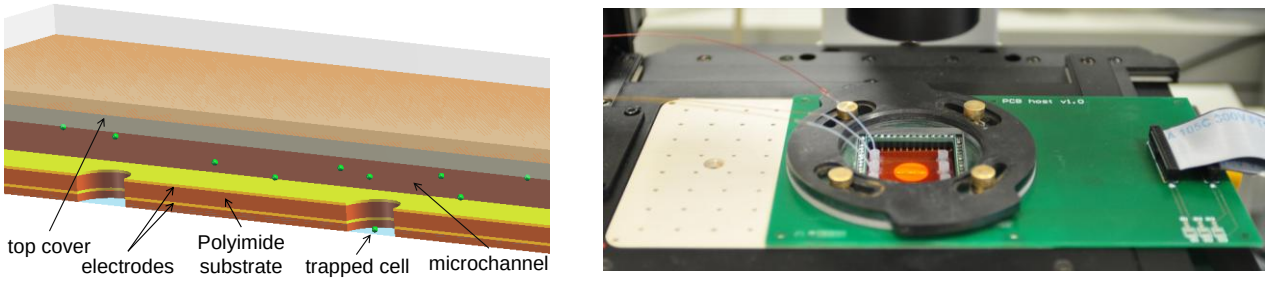

Figure 1: (A) Schematic representation of the inverted open microwell architecture. (B) Prototype of the inverted open microwell array mounted on a package which provides electrical and fluidic connections.

EXPERIMENTAL

Microwell arrays featuring three microchannels with four microwells each were fabricated on a 2-layer flexible PCB substrate using a set of biocompatible materials [8]. Microwells had a diameter of 75µm and a depth of 93µm and were drilled mechanically. Microchannels (W = 300µm, H= 150 µm, L = 2700 µm) were structured in the PCB and closed on top with a

polycarbonate sheet containing inlet/outlet holes and bonded with a biocompatible transparent adhesive. The chip was mounted on a custom package providing electrical and fluidic connections (Figure 1B).

K562 leukemia cells were suspended in physiological medium and delivered to the microwells (Figure 2). Two annular electrodes were polarized with sinusoidal voltage during the delivery procedure (Figure 2A-2D) to create a non-uniform electric field. By changing the amplitude of the signals, two DEP configurations (BLOCK and LOAD) were selectively activated to control the delivery of cells and the formation of cellular aggregates.

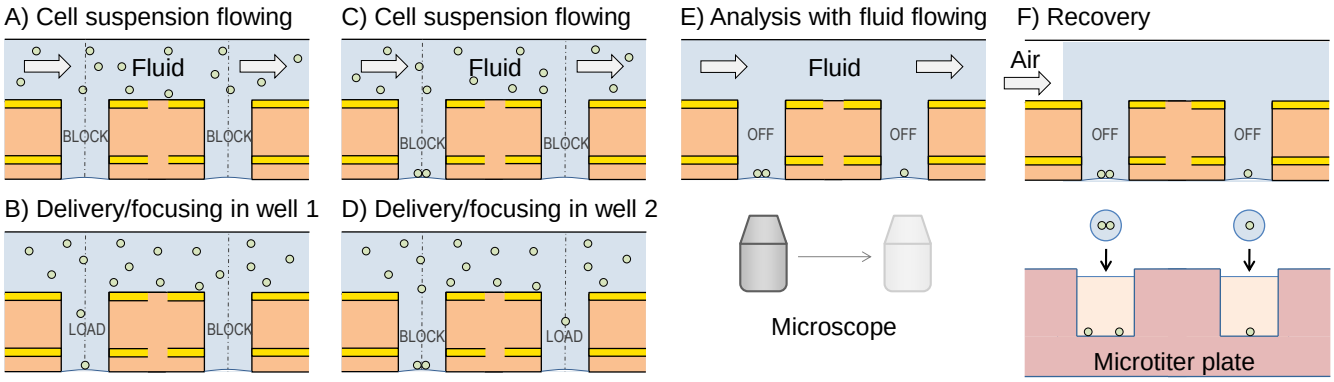

Figure 2: Workflow of the inverted open microwell system. (A) A cell suspension flows the microchannels, (B) the flow is stopped and cells sediment in the first microwell focusing along the central axis, (C-D) similarly a cell is delivered in a second microwell, (E) cells trapped at the air-flow interface remain stable even if DEP force is absent and the fluid flows in the microchannel and can be analyzed by a microscope, (F) after analysis live cells are recovered in parallel from multiple microwells to multiple wells of a standard microtiter plate.

RESULTS AND DISCUSSION

Numerical simulations were performed to compute the electric field distribution in the microwell and determine the DEP force. Particle radius was set to 5µm. A sinusoidal voltage with a frequency of 100kHz was applied between the two annular electrodes of each microwell. In BLOCK mode the amplitude is set to 1V and the vertical component of the DEP at the top of the microwell results to be high enough to contrast the gravity force. Cells are thus prevented from entering in the microwell, as shown by the arrows pointing upwards in Figure 3A. In LOAD mode the amplitude is set to 0.5V and the vertical DEP is reduced allowing cells to descend into the microwell (Figure 3B). A radial component of the DEP is still present in this configuration and is sufficient to push cells along the central vertical axis and enable the formation of cellular aggregates at the air-fluid interface.

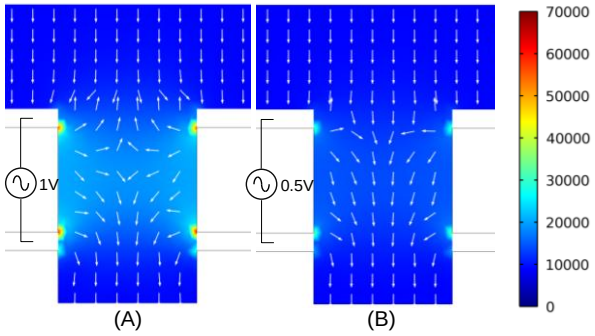

Figure 3: Slice plot of the electric field and arrow plot of the sum of DEP and gravity forces acting on a particle (A) in BLOCK mode and (B) LOAD mode. The two configurations are set by changing the amplitude of the signal applied.

Experiments with K562 cells demonstrated that the two DEP configurations acted as expected. Cells delivered to the microwells could lean on the air-fluid interface remaining stable even after deactivating the electric field and maintaining a fluid flow of 2-8µL/min in the microchannel. If LOAD mode was kept active during cell delivery, aggregates of two or multiple cells formed at the air-fluid interface and cells resulted to be in contact each other (Figure 4). Single cell delivery was achieved by statistical means, controlling the concentration of the cell suspension. Results on cell delivery statistics are reported in Table 1.

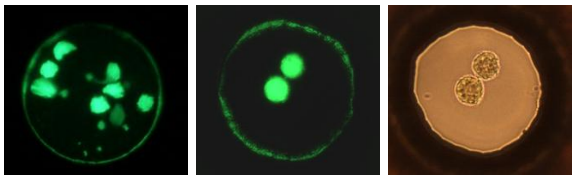

Figure 4: Cells trapped in an inverted open microwell. (A) multiple cells sedimented without DEP focusing, (B-C) two cells under fluorescent and visible lighting, respectively, after being focused by DEP.

Experimentally, the DEP activation produced a temperature increase of less than 2°C, confirmed by numerical simulation. Viability was assessed by performing a Calcein-AM viability assay on-chip and measuring the fluorescence intensity on single cells with a microscope and a custom image analysis software. An average signal decrease of 8%, compatible with the standard physiological loss [10], was measured after 1h. The content of multiple microwells was transferred to a portion of a 384-well microtiter by connecting each microchannel to an external pneumatic system and applying a 5ms-pulse of air with a 600mbar pressure into the microchannel. Cell proliferation after recovery was monitored on single cell clones and showed the formation of clusters of more than 20 cells after 4-5 days. This result shows the possibility to implement an entire cell sorting work flow with a macro-to-micro interface supporting easy transfer of single cells to standard lab equipment.

Table 1. Statistics of cell delivery in inverted open microwells (total of 41 samples) at 1.6 × 10^6 cells/mL

Number of cells loaded	Frequency
0	51%
1	42%
2 or more	7%

CONCLUSION

Cell manipulation, analysis and recovery was demonstrated in a novel microstructure, the inverted open microwell. The controlled creation of cell clusters and the continuous fluid replacement followed by analysis and recovery steps enable the functional sorting of cells showing a desired response to cell-cell or cell-molecule interactions.

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CONTACT

*M. Bocchi; massimo.bocchi@mindseedslabs.com