A MULTIPLE-ELECTRIC-FIELD MICROFLUIDIC CHIP WITH UNIFORM FLOW FIELD FOR STUDY OF LUNG ADENOCARCINOMA CELL ELECTROTAXIS

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ABSTRACT

A new microfluidic chip (multiple electric field chip-II, MFCII) was developed to create multiple electric fields (EFs) to study the electrotaxis of lung adenocarcinoma cells. MFCII delivers high experimental throughput in comparison to conventional methods. The cell electrotaxis under three different electric field strengths (EFSs) and the control condition (no EF) can be studied in a single experiment. Also, with the homogeneous flow field in the main channel where cell migration is studied, the possible biological effect of shear force can be neglected.

KEYWORDS

Microfluidic biochip, Electrotaxis, Multiple-electric-field, Cancer metastasis

INTRODUCTION

Physiological electric fields presumably arise from transepithelial potential difference with range from tens to hundreds of mV/mm have been investigated extensively in embryo development, neurogenesis, and wound healing. [1] Under the physiological electric fields, cells may show directional migration named electrotaxis or galvanotaxis. In recent years, many cancer cells have been found to be electrotactic, but the role of physiological electric field in cancer biology is widely uninvestigated.[2 – 5]

Conventional methods to investigate electrotaxis are based on dish devices or transwell devices.[6 – 8] However, these methods have drawbacks including low experimental throughput, lack of miniaturization, complex assembly, risk of contamination, and risk of medium evaporation.

In our previous work, a microfluidic chip, MFC, was developed to create multiple EFs by manipulation of cross-sectional area. [5] MFC overcame the disadvantages of conventional methods by miniaturizing the experimental setup and increasing the experimental throughput. Cell electrotaxis under three EFSs has been studied in MFC in a single experiment. The overall experiment time using MFC is shortened than those using conventional dish-based devices.

However, the varied cross-sectional area in the consecutive segments in MFC results in a flow velocity ratio that is proportional to the EFS. In consequence, the segment with the highest EFS has the highest flow velocity and vice versa. The biological effect of shear force cannot be distinguished from the EF stimulation because electric field and flow field are coupled.

We report a new design, MFCII, in which electric field and the flow field were decoupled by interconnecting segments, as shown in Figure 1(a). The equivalent circuit of MFCII is shown in Figure 1(b). A 4 mm-wide channel were segmented so four electric fields with electric field strength (EFS) ratio of 7.9 : 2.8 : 1 : 0 were obtained with flow velocity variation of only 7.8% (coefficient of variation).

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EXPERIMENT

The electric field and flow field in MFCII was numerically simulated using a commercial finite volume method software suite, CFD-ACE+, as shown in Figure 2(a) and (c). The EFSs in the four segments were measured by inserting Ag/AgCl electrodes as shown in Figure 2(b).

MFCII was fabricated by CO_2 laser ablation on poly-methyl methacrylates (PMMA) substrates. A standard tissue culture poly-styrene dish was used as the substrate for cell migration study. Cells were injected in the microfluidic channel in MFCII and cultured on a transparent indium tin oxide heater on a phase contrast microscope.[9] The schematic diagram of MFCII setup can be seen in Figure 3.

The performance of MFCII was validated by studying the electrotaxis of CL1 lung adenocarcinoma cells. CL1 cells were subjected to *in vitro* invasion assay selection to yield CL1-0 (less-invasive) and CL1-5 (highly-invasive) cells. The electrotaxis directedness results of CL1 cells were shown in Figure 4. CL1-5 cells showed anodal electrotaxis with directedness proportional to EFS while CL1-0 cells were non-electrotactic. The directedness results of both cells in null region (EF=0) and control (no EF application) was indistinguishable suggesting that null region can be used as the control condition.

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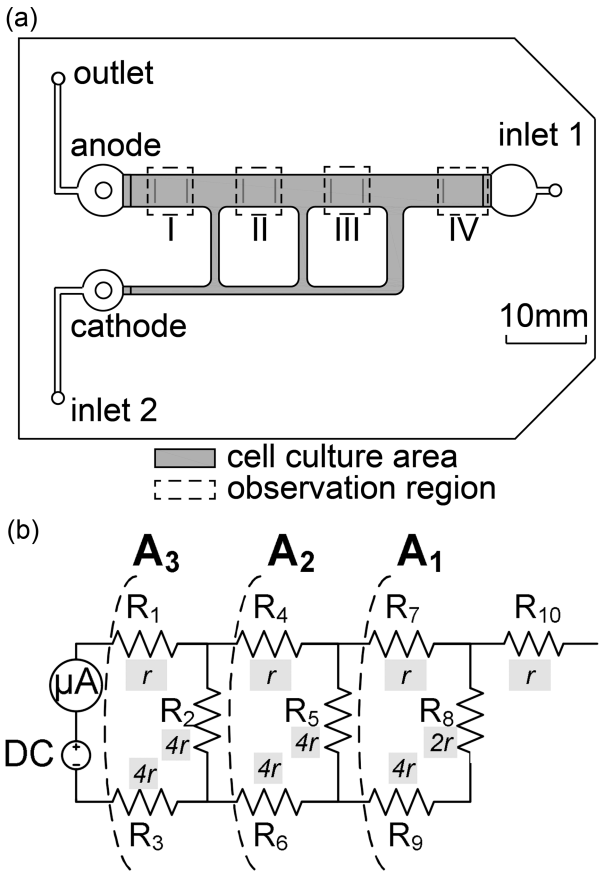

Figure 1. (a) The top view of MFCII. A 4 mm-wide channel is divided by 1 mm, 1 mm, 2 mm-wide vertical channels into segment I, II, III, IV.

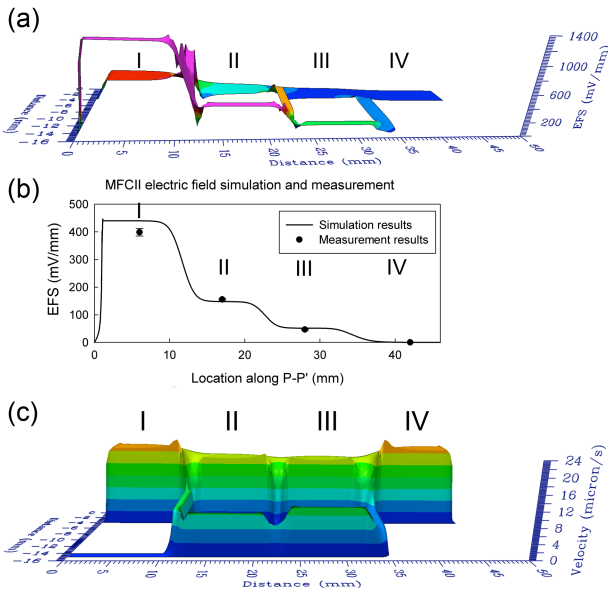

Figure 2. (a) The EF distribution of MFCII. (b) The measured EFS and simulated EFS in the middle of the 4 mm-wide channel (b) The flow field distribution in MFCII.

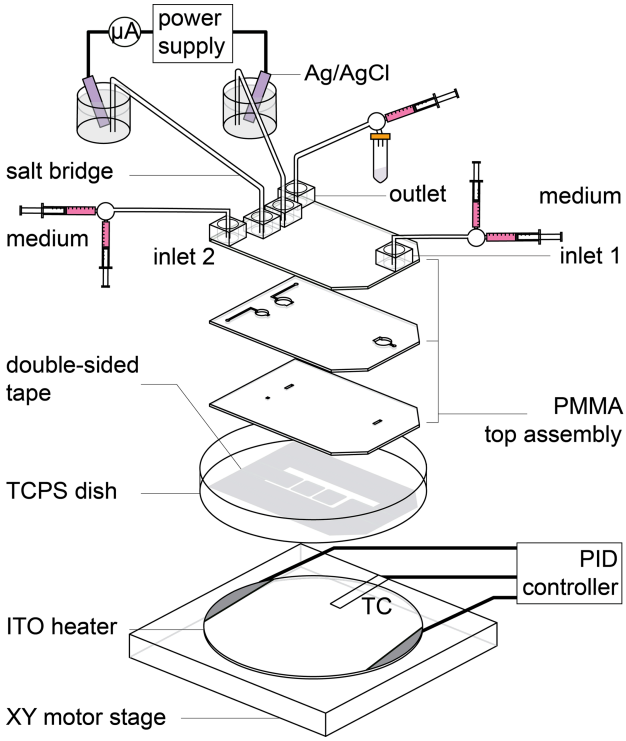

Figure 3. MFCII is composed of the PMMA top assembly, patterned double-sided tape, and the poly-styrene dish. Miniaturization of cell culture chamber in combination with an ITO heater allows easy, contamination-free cell culture and cell migration visualization. A direct current is applied through Ag/AgCl plate electrodes to introduce EF into the chip.

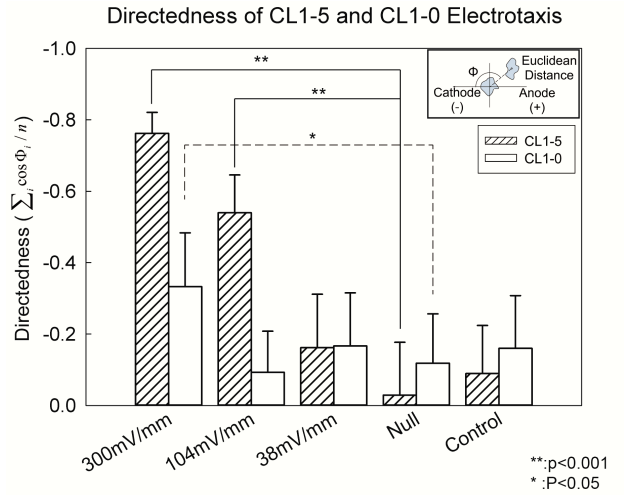

Figure 4. The directedness of CL1 cells in MFCII under three EFSs, the null region in segment IV, and without electric field application as control. The inset shows the definition of directedness as average cosine of the angle between electric field vector and the Euclidean vector