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Using the micro-array electrode chip and AC signals to generate the electric field effect on cell migration

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ABSTRACT

We have successfully fabricated the micro-array electrode chip with different gap sizes by Micro-Electro-Mechanical-Systems (MEMS) technology and generated the moving electric field to drive cells to migrate by employing AC signal. Our strategy is to simulate the ramp waveform signal with various gap sizes (10 μm, 20 μm, and 30 μm) to examine the optimal moving electric field by the ANSYS 9.0 software. The electrode with 10 μm gap can generate the better moving electric field effect on cell migration than that of other gap sizes. In the experiments, when fixing the electric field strength at 0.6 V/mm in 100 kHz, the migration velocity of the cells was 21.25 μm/h in the micro-array electrode chip with the gap of 10 μm, and the direction of cell migration could be controlled by the moving electric field. Moreover, the cell migration was more obvious when the driving frequency is over 50 kHz. This method for cell migration could be applied to cell manipulation of bio-applications.

1. Introduction

In the cell migration, the galvanotaxis and chemotaxis were utilized to introduce the movement for cells. Chemotaxis is the phenomenon in which bodily cells, bacteria, and other single-cell or multicellular organisms direct their movements according to certain chemicals in their environment [1–3]. Galvanotaxis is a directional movement of motile cells in response to an electric field. By detecting and orientating themselves toward the electric fields, the cells are able to direct their movement toward the damages or wounds to repair the defect. Such movement may contribute to directional growth of cells and tissues during development and regeneration.

In the previous researches for galvanotaxis, some cells have characters of galvanotaxis and galvanotropism in the human body. The electric field was used to differentiate the cell migration between the normal cells and cancer cells [4,5]. In 1984, the electric cell-substrate impedance sensing (ECIS) was developed to observe the cell migration and the signal transmission between the cells [6–9]. This method utilized the gold electrode and AC field to measure electric current change of cells, and it could be applied for cancer growth, cancer transmission, and would healing. In 1985, Robinson discussed the galvanotaxis and membrane potential for various cells [10]. In 2004, when the mouse fibroblast cells were driven in the DC field (0.6 V/mm) in the ECIS sensor, the time of cell proliferation was shortened by half. Therefore, the electric field is an important role for the cell migration.

In the cell control, electric field and electrode chip were generally used for cell focusing, separation, migration, and location [11–14]. In 1998, Masuda et al. used the moving electric field to drive the cells [15]. The sine waves of phase differences (degrees of 120 and 60) which were respectively connect to the continuous array electrodes were generated the moving electric fields, and they successfully drove the red blood cells. In 1991, Fuhr et al. used the high frequency moving electric field to drive the cells move straight [16]. Recently, the moving electric field was utilized for fluid control. In 2000, Pollack et al. used the microactuation for rapid manipulation of discrete microdroplets [17]. Microactuation was accomplished by direct electrical control of the surface tension through two sets of opposing planar electrodes fabricated on glass to transport. In 2005, net flow of electrolyte induced by a traveling-wave potential was applied to an array of microelectrodes. Two fluid flow regimes were observed: at small-voltage amplitudes the fluid flow was in the direction of the traveling wave, and at higher-voltage amplitudes the fluid flow direction was reversed [18]. The moving electric field enhanced the electric field character and changed the conventional DC electric field application.

In the conventional ECIS, in order to make the cells uniformly spread in the micro-electrode chip, the chemoattractants were used to lead the cell migrate in the microchip quickly. However,

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the chemoattractants would cause the cells damage. In this study, the ramp waveform signal was used to generate the moving electric field in the micro-array electrode chip by employing AC signals. The ramp waveform signal with various gap sizes (10, 20, and 30 μm) was simulated to analyze the optimal moving electric field by ANSYS 9.0 software. The electrode gap of 10 μm can generate the best moving field effect on cell migration, and the migration velocity of the cells was 21.25 μm/h in the driving frequency of 100 kHz and the electric field strength of 0.6 V/mm. Moreover, the driving frequency had influence on cell migration, and the cell migration was more obvious when the frequency was over 50 kHz. This research was applied to control the cell migration and increase spread velocity.

2. Materials and methods

2.1. Cell culture

Mouse fibroblast cell line (NIH-3T3) is obtained from Institute of Molecular Medicine, National Cheng Kung University. These cells are cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% heat-inactivated fetal bovine serum (FBS), 1% streptomycin and penicillin, and 1% L-glutamine. The NIH-3T3 cells are sub-cultured every 2–3 days.

2.2. Simulation process

In this study, we simulated the electric field difference in various gap sizes under the ramp waveform by using the ANSYS 9.0 software. One ramp wavelength was set be 15 points (electrodes) long, as shown in Fig. 1(a). The computational domain was the 15 electrodes under the medium solution, as shown in Fig. 2(a). The simulation parameter settings were shown as follows. The material property of the gold electrode was set to 4.5×10^{-5} Ω, and the medium solution was set to 19.03 ms/cm. The element type used the Plane 67 was selected in the construction of the electric field. The boundary condition was used the phase difference of 90 degree to set the input voltage signals into every electrode, as shown in Fig. 2(b). Our purpose is to discuss the electric field distribution in the reaction region. In order to shorten the simulation time, the one period signal of the ramp waveform was simulated in the various gap sizes, respectively. Since the height of the NIH-3T3 was 8 μm when it is lying on the glass substrate, the electric field distributions of the height of 0 μm, 4 μm and 8 μm were analyzed. Comparing electric field effects of these various gap sizes in *X*-direction, the differences of the electric field between positive and negative peak and the reaction time between the positive and negative electric field were discussed.

2.3. Design and fabrication of the micro-array electrode chip

The micro-array electrode chip consisted of two components: the reaction well and thin-film micro-array electrodes. The micro-array electrode chip has 21 pairs of electrodes. The area of the micro-array electrode chip was 76 mm × 25 mm (Fig. 1(b)), and the micro-array electrode chips with various gap sizes including 30, 20, and 10 μm were designed by using the AutoCAD® 2008, as shown in Fig. 1(c). In order to form the fixed-volume well for cell migration reaction region, the glass surface with patterned micro-array electrodes was bonded to a 6.0 mm thick layer of poly-dimethylsiloxane (PDMS), which had 6.0 mm × 6.0 mm square cavity on it. The electrodes were made up of a 200 nm gold layer and a 20 nm chromium under-layer.

First, a PMMA mold was fabricated by using CO_2 laser machine. After finishing the PMMA mold, the replica mold technology was used to fabricate the PDMS reaction well. The PDMS liquid was

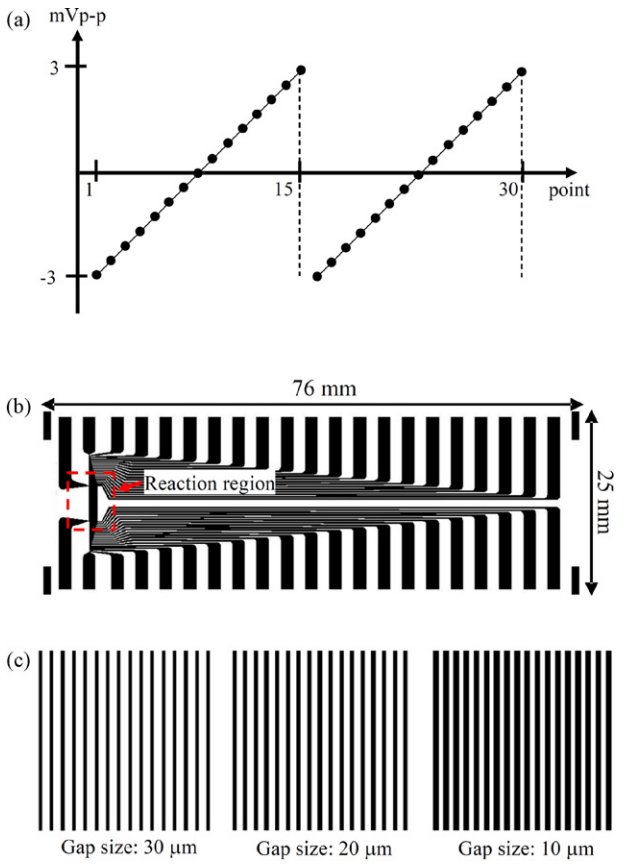

Fig. 1. (a) One ramp waveform was set be 15 points, and the voltage of the waveform was 3 mV_{p-p}. (b) Design of the micro micro-array electrode chip. (c) The reaction region of chips of various gap sizes.

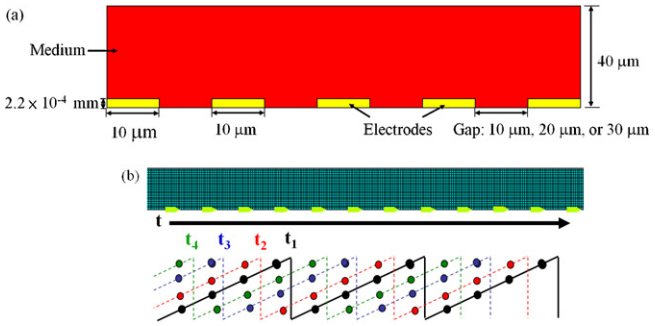

Fig. 2. (a) The dimension of the simulation domain of the micro-array electrode chip. (b) The drawing of the input voltage signals with the phase difference of 90° set to every electrode.

injected into PMMA mold, and the PDMS well was peeled off from the PMMA mold after 40 min at 70 °C in the oven. The micro-array electrodes were fabricated using micro-electro-mechanical system (MEMS) technology. The gold and chromium thin films were thermally evaporated onto glass slides. Photolithography was used to transfer the electrode pattern to the thin films. The micro-array electrodes were formed after chemical wet etching.

2.4. The experimental strategy and processes

This research strategy is to use the moving electric field to drive the cell migration on the micro-array electrode chip, as shown in Fig. 3. The electric field distributions of the various gap sizes in