THE DISTINCT PAIRING SEQUENCE OF LIVER CELL AND FIBROBLAST ON CELL-CELL FUSION PLATFORM

Shih-Mo Yang¹, Chun-Yen Lin², Shilpa Sivashankar³, Srinivasu Valagerahally Puttaswamy³, Yen-Ta Lu⁴, Hwan-You Chang², Long Hsu¹, and Cheng-Hsien Liu³

¹ Department of Electrophysics, National Chiao Tung University, Taiwan, R.O.C.
² Institute of Molecular Medicine, National Tsing Hua University, Taiwan, R.O.C.
³ Department of Power Mechanical Engineering, National Tsing Hua University, Taiwan, R.O.C.
⁴ Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan, R.O.C.

ABSTRACT

Recently we are developing the cell-cell fusion platform which has a potential to modify cell behavior for degenerative diseases. We present a cell-cell pairing platform to trap and pair mobile HepG2 liver cells and 3T3 fibroblast cells. A 10µm×20µm metal electrode is placed precisely inside a lateral cavity. The lateral cavity design bends the electric field and enlarges the positive dielectrophoresis force. Besides, the structure provides a shelter function to diminish the damage of direct flow shear stress acting on trapped cells. Finally, we demonstrate the distinct liver and fibroblast cells pairing sequence for the future application of cell-cell fusion.

KEY WORDS: Dielectrophoresis, Cell Trapping, Cell Pairing, Liver Cell

INTRODUCTION

The development of cell-cell fusion provides a platform for hybridomas creation and production of cloned offsprings [1]. The approach to pair two different kinds of cells is a key technology[2, 3]. In this article we present an active cell pairing method which is able to trap two or more Human carcinoma cells (HepG2) and 3T3 fibroblast cells together for future cell-cell fusion applications. The lateral cavity is so designed that it bends the electric field which enhances the positive DEP(pDEP) trapping force and diminishes shear stress caused due to flow of medium on the trapped cells. With optimum applied AC voltage and flow velocity, the cells are paired as a cell chain with arbitrary sequence inside a lateral cavity having 10µm×20µm metal electrode.

METHOD

Schematic illustration of linear cell pairing chip is represented in Figure 1. The microchannel height of 80 µm is defined by using SU-8 negative photoresist via standard photolithography process. The curing agent of polydimethylsiloxane (PDMS) is mixed together (10:1), poured onto the silicon-PR mold and cured at 90°C for one hour. The electrode pattern is developed with AZ4620 positive photoresist. After exposure and development by the etching solution (NH₄OH:H₂O₂:H₂O =1:1:2), the desired electrode pattern appears on the glass substrate. After the oxygen plasma treatment on glass substrate and PDMS lateral cavity, they are aligned and bonded together carefully. The trapping location is designed in such a way that, the electrode region and lateral cavity attract and pair cells with distinct pairing sequence.

Figure 1: The illustration of linear cell pairing chip. A microfluidics channel is mounted on the electrode and the overlaped
region exposes the metal electrode inside the lateral cavity. The electric field generated from electrode is confined and distorted by the microchannel structure. The narrow space of lateral cavity enhances the magnitude of electric field in low applied AC voltage as well.

SIMULATION

The simulations of electric field and flow velocity are represented in Figure 2(a)(b) using CFD-ACE+ software. Stronger electric fields to attract mobile cells and low shear stress protect the trapped cell. Due to the feature of lateral cavities, the magnitude of electric field is enhanced and low AC voltage of 3Vpp was applied. Besides, the lateral cavity reduces the flow velocity and protects the trapped cell from being exposed to high shear stress due to flow which is important factors for efficient cell trapping method.

![Simulation results of electric field and flow velocity.](image1)

**Figure 2:** The simulation results of electric field and flow velocity. (a) When the external power provides less AC voltage (4Vpp), the electric field is enhanced by the structure to generate positive DEP force for trapping mobile cells passing in front of the lateral cavity. (b) The color of flow simulation indicates that the minimum flow velocity is inside the lateral cavity. When a cell is attracted toward this region and trapped, the structure protects the cell from experiencing the shear stress directly.

EXPERIMENTAL AND DISCUSSION

The white dotted line in Figure 3 is the microchannel. The metal electrode of 10µm × 20µm is placed precisely to expose at the inner of the lateral cavity. It provides pDEP force to trap mobile cells and two cells are able to pair together in one lateral cavity. Larger external AC voltage would generate stronger electric field to form a cell pearl chain between opposite electrodes.

![Experimental demonstration](image2)

**Figure 3:** The dotted line is the microchannel and two different cells are trapped by the opposite electrode that are revealed inside lateral cavity.

Figure 4 demonstrates the cell pairing chain of HepG2 and fibroblast cells in distinct sequence. HepG2 and 3T3 cells are pre-labeled with DiI (red) and DiO (green) respectively. Further, injected into the microchannel and paired to form a cell chain. We use binary counting to illustrate the distinct HepG2 and 3T3 pairing situation of 5, 4, and 3 cells in sequence.
Figure 4: Cell binary counting. The HepG2 and 3T3 fibroblast cells were prelabeled with fluorescent carbocyanine dyes, DiI (red) and DiO (green), which are linearly paired in various sequences between opposite lateral cavities. The binary counting is utilized to demonstrate HepG2 and 3T3 pairing situation.

CONCLUSION

We demonstrate the non-uniform electric field bending with lateral cavity structure for trapping mobile liver cells and pairing with fibroblast cells with enhanced pDEP force. These results show the convenience of observing the cell-cell interaction or cell fusion process.

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REFERENCES


CONTACT

*Shih-Mo Yang, tel: +1-886-912-967-143; g913328@gmail.com