MAGNETICALLY DRIVEN MICRO-MOVABLE ELECTRODE
FOR CELL COUPLING
Y. Yamanishi1*, T. Kawahara2, T. Iyanagi3, M. Hagiwara2, Fumihito Arai2
1JST, PRESTO, JAPAN
2Nagoya University, JAPAN
3Tohoku University, JAPAN

ABSTRACT
We developed a novel method of cell coupling on a chip by using magnetically driven microtool (MMT). It is made of nickel and is used as an electrode as well as a cell pusher. The novelty of this device is the electrode itself whose tip part is supported by a spring and is positioned by external magnetic field. We propose a new cell coupling chip which provides larger coupling force of cells by using a physical motion of MMT. The operation of the microelectrode showed that the oocyte is deformed and the donor cell is successfully coupled physically with the oocyte. The contact area between the cells was increased, and which is good for consequent fusion process. The FEM analysis on the electric field density also showed that a high concentration between the membrane of oocyte and donor cell. The proposed method is suited for cell fusion on a chip, and will contribute to high throughput cell manipulation.

KEYWORDS: Magnetically Driven Microtool, Cell Manipulation, Cell Coupling, Microelectrode

INTRODUCTION
Eliminating the manual manipulations problems of contamination, poor success, and low replicability, together with the skill-dependent human element, requires that cell manipulation be automated through the use of disposable microchips in cell manipulations such as embryo cloning and microfertilization. Complex cell manipulation such as microfertilization is currently done by skilled operators using micromanipulators. The automation of on-chip micromanipulation has been reported to ensure efficient, accurate, high cell-manipulation throughput using magnetic actuators [1][2], microfluid force [3], and dielectrophoresis[4].

We have been developed a chip for automation of embryo cloning (cloning chip) based on a new protocol of nuclear transfer. The conventional design of the coupling of oocyte and donor cell was performed only by a dielectrophoretic force which was generated by opposite electrode mounted on a chip [5]. However, due to the limited force applied for coupling cells, and the size of the oocyte is 10 times as large as the donor cells, the success rate of the continuous fusion process was limited. From the empirical investigation, it is recognized in manual operation using glass capillary that the coupling of cells with physical force increased the success rate of cell fusion. Here we propose a novel device to apply physical force between the cells using movable electrode on a chip.

EXPERIMENTAL SETTING
Figure 1 shows the concept view of the cell coupling chip. Movable electrode has a spring structure to position its tip part toward the opposite fixed electrode. Figure 2 shows the process flow to fabricate the chip. Nickel electroplating was applied after patterning of Cr/Au, then the MMT with spring structure can be obtained. This MMT was made of Nickel and it is also has a function as electrode. The novelty of the chip is the electrode itself can be moved by external magnetic field using a spring structure to use as a cell pusher.

Figure 1: Design of cell coupling chip using movable electrode.
To apply current to the electrode, a wire was connected to the electrode through a hole mounted in a chip and sealed by silver-based adhesive paste (Figure 3). The wire is completely fixed and the tip part of movable electrode can be moved by the magnetic force and precise positioning is possible without any disturbance from the wire.

The microchannel was fabricated by the conventional photolithography technique as shown in Figure 2. Before bounding the PDMS (polydimethylsiloxane) chip with cover glass by using O₂ plasma, the fabricated Nickel electrodes were assembled on a chip. The fixed electrode was assembled following a guide of PDMS columns attached with the PDMS microchannel. The movable electrode was also assembled in microchannel where the tip of the electrode aligned to a level of microchannel as shown in Figure 3.

The FEM analysis of the displacement of the movable electrode showed that the displacement of the tip was more than 240 µm and hence the tip can reach to the fixed electrode (Figure 4) at a given applied magnetic force (2 mN). Figures 5 shows the operation of the movable electrode actuated by a permanent magnet below the chip. It was confirmed that the movable electrode was moved to the PDMS wall which has a suction hole to trap an oocyte. Therefore this MMT can be used for coupling oocyte and donor cell. The transportation channel of oocyte and donor cell used the conventional design of the chip for coupling by a dielectrophoretic force [5]. This method did not provide enough force for cell coupling, because the size of the oocyte is 10 times as large as the donor cells. By using the movable electrode, we could obtain the additional physical force to increase the success rate of cell fusion.
Figure 6 shows the result of the cell coupling experiment using swine oocyte and donor cell. The oocyte is deformed and the donor cell is successfully coupled physically with the oocyte. It is important to note that the contact area between the cells was increased, and which is good for consequent fusion process. The FEM analysis on the electric field density also showed that a region of a high concentration was distributed equally between the membrane of oocyte and donor cell (Figure 7). For further work, the design of the electrode and magnitude of the applied voltage can be optimized for the consequent sufficient cell fusion process. It is also important to compare the success rate with the manual operation of cell coupling and fusion with glass capillaries.

CONCLUSION

We succeeded in a novel method of cell coupling on a chip by using MMT. This MMT was made of Nickel and it is also has a function as electrode as well as a cell pusher. The novelty of the chip is the electrode itself can be moved by an external magnetic field using a spring structure. This magnetically micro-movable electrode was fabricated by an electroplating techniques and operated successfully. FEM analysis confirmed its operation on a chip by an external magnetic force, and also it confirmed that a high concentration of electric field between the membrane of oocyte and donor cell was distributed equally due to increase of the contact area between the deformed oocyte and donor cell. This chip contributes to the high throughput cell manipulation as well as electrical measurement.

ACKNOWLEDGEMENTS

This work was supported in part by the Research and Development Program for New Bio-industry Initiatives and JST-SENTAN.

REFERENCES


CONTACT

*Y. Yamanishi, tel: +81-52-789-5656; yoko@biorobotics.mech.nagoya-u.ac.jp

Figure 6. Tucking donor cell into oocyte (a) before tucking donor cell, (b) coupling, (c) magnified view of tucking oocyte, (d) coupling of oocyte and donor cell, (e) after coupling of donor cell

Figure 7. FEM analysis of concentration of electric field (a) entire view (b) point contact of oocyte and donor cell (b) larger area contact due to deforma-