Development of Cell Culture Microdevice Actuated by Piezoelectric Thin Films for Delivering Mechanical Vibratory Stimuli to Cells

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Abstract. In order to realize a cell culture microdevice actuated by piezoelectric thin films for on-chip regulation of cell functions, this paper reported on a feasibility study by using the microdevice with KOH-etched cavities surrounded by four (111) sidewalls as microchambers in order to introduce cells to be cultured. As a result, the vibration characteristic of the PZT actuator was improved by using an electric field -150 kV/cm at 70 °C for 30 min in poling process. A feasibility study on cell culture for delivering mechanical vibratory stimuli to cells revealed the microdevice could be applicable to the culture with actual biological cells. In addition, it was found that O₂-plasma treated parylene-C process could be applicable for obtaining homogeneous surface of cell culture microdevice.

1. Introduction
Recent advances in microelectromechanical systems (MEMS) technology have opened up new possibilities in cell biology, neurobiology, pharmacology, and tissue engineering [1]. Among them, actual evidence suggests that cell functions such as cell adhesion, cell multiplication, and gene transfer can be enhanced by delivering mechanical vibratory stimuli to cells [2, 3]. This approach, which is unnecessary to provide any accelerating agents, might be free of the potential risk of this infection considering biological applications such as gene therapy and tissue engineering. However, the influencing factors of mechanical vibratory stimuli (amplitude, frequency, etc.) to cells are not obvious. Therefore, this paper focused on mechanical vibratory stimulation with nanometer-order displacement and frequency of less than 10 kHz. In order to realize screening and optimization of the factors on a chip-based system, we have been developing a cell culture microdevice actuated by piezoelectric thin films for on-chip regulation of cell functions.

This paper reports on characterization of a cell culture microdevice with KOH-etched cavities surrounded by four (111) sidewalls as microchambers in order to introduce cells to be cultured. In addition, a feasibility study on cell culture for delivering mechanical vibratory stimuli to cells was executed by using the fabricated microdevice. Moreover, deposition of parylene-C film on silicon (Si)
substrates was investigated in order to obtain a homogeneous substrate surface for fabrication of a prototype of a cell culture microdevice proposed here.

2. Cell culture microdevice actuated by piezoelectric thin film

Figure 1 shows the cell culture microdevice proposed here has a number of microactuators with a lead zirconate titanate (PZT) thin film. The PZT actuator with 1 µm in thickness is fabricated on a silicon nitride ($\text{Si}_3\text{N}_4$) diaphragm ($250 \times 250\mu\text{m}^2$ in size with 1µm in thickness) formed on a Silicon substrate (p-type, (100) single crystal) with $20 \times 20\text{mm}^2$ in size. Biological cells are seeded onto the PZT actuator covered by an insulation layer, giving mechanical vibratory stimuli to cells. The microdevice makes it possible to modify the surface condition for cell seeding and culture cells with different conditions of mechanical vibratory stimulation in each actuator because PZT actuators can be controlled independently by applying sine wave AC voltages with different amplitude and frequency. Therefore, the cell culture microdevice allows screening and optimization of the influencing factors of multiple mechanical vibratory stimuli (amplitude, frequency, etc.). Figure 2 shows a PZT actuator consisting of multiple layers ($\text{Pt}/\text{Ti}/\text{PZT}/\text{Pt}/\text{Ti}/\text{SiO}_2$) formed on a $\text{Si}_3\text{N}_4$ diaphragm, which was fabricated by an anisotropic wet etching using KOH solution, were successfully patterned and structured.

![Figure 1](image1.png)

**Figure 1.** Schematic diagram of cell culture microdevice actuated by PZT thin film; (a) Plan view of device, (b) Cross-sectional view.

![Figure 2](image2.png)

**Figure 2.** Fabricated microdevice; (a) Plan view of PZT actuator, (b) Cross-sectional view of PZT actuator.
3. Characteristics of cell culture microdevice

Figure 3 shows a typical result of time dependence of the diaphragm displacement of a PZT actuator with a size of 200×200 µm² measured by laser interferometer (Canon DS-80) which has a high frequency resolution of 500 kHz. The resulting displacement was estimated to be approximately 52 nm at an applied sine wave AC voltage of ±10 V at a drive frequency of 10 kHz. Although the diaphragm displacement was found to be disagreed with the applied AC voltage, it is considerable that the deformation of the diaphragm was restricted by the insulation layer of polyimide between top and bottom electrodes.

In order to improve drive performance of the cell culture microdevice, poling treatment was executed at electric field of -150 kV/cm at 70 ºC for 30 min, where the bottom electrode was connected to electrical ground. Figure 4 shows the diaphragm displacements of three PZT actuators, which were used for cell culture experiment of delivering mechanical vibratory stimuli to cells in this paper, with size of 200 × 200 µm² before and after poling. The average displacements of three diaphragms before the poling treatment were estimated to be approximately 25 nm (±5.6 nm) at an applied sine wave AC voltage of ±10 V at a drive frequency of 10 kHz. On the other hand, the average displacements were increased to 50 nm (±1.0 nm) after the poling treatment. As a result, the vibration characteristic of the PZT actuator was improved by using an electric field in poling process, because direction of polarization was reoriented by such a strong electrical field applied between top and bottom electrodes during the poling process. However, large displacement of diaphragm was not obtained. This was caused by the crystalline of the PZT thin film fabricated. Therefore, it is necessary to optimize the fabrication process.

**Figure 3.** Diaphragm displacement of PZT actuator with 200×200 µm² diaphragm size as a function of time.

**Figure 4.** Effect of poling treatment on diaphragm displacement of PZT actuator.
4. Delivering mechanical vibratory stimuli to cultured cells

In order to demonstrate capabilities of cell culture with the microdevice for on-chip regulation of cell functions, a feasibility study on delivering mechanical vibratory stimuli to Mouse Embryonic Fibroblast (MEF) cells was executed. In this experiment, KOH-etched cavities surrounded by four (111) sidewalls were used as microchambers in order to introduce cells to be cultured. In cell-seeding process, a frame made of polydimethylsiloxane (PDMS) was mounted on the device for storing culture solution, as shown in Figure 5. After culture solution of 300 µL Dulbecco’s Modified Eagle Medium (DMEM) was dropped into the PDMS frame, MEF cells (concentration of cell suspension: 1.0×10^5 cells/mL, volume: 100 µL) were seeded. After 5 minutes for settling the MEF cells, mechanical vibratory stimulation with displacement of 50 nm at a drive frequency of 10 kHz for 1h was applied. The MEF cells were cultured at 37 ºC in 5 % CO₂ for 48 h after the stimulation. Figure 7 shows optical micrographs of the MEF cells just after seeding into the microchamber, after 24 h cultivation and after 48 h. Immobilization treatment of MEF cells cultured for 48 h by using 2.5% glutaraldehyde was carried out. After immobilization treatment, dewatering process with dehydrated ethanol was performed in order to observe the MEF cell adhesion. As a result, 2/13 (=15.4%) of the diaphragm without delivering the mechanical vibratory stimulation was adhered by MEF cells. On the other hand, 3/3 (=100%) of that with delivering the simulation were adhered by MEF cells. It was found to be applicable to our application. Figure 8 shows a SEM microphotograph of diaphragm without delivering the simulation. A lot of MEF cells adhered to Si (111) sidewalls of the microchamber were observed. This result was considered that the different condition of the substrate surface affected the adhesion of the MEF cells because cells prefer to adhere to a substrate depending on its surface energy [4,5].

Figure 5. PDMS frame mounted on the device for storing culture solution.

Figure 6. Schematic of feasibility study on cell culture for delivering mechanical vibratory stimuli to MEF cells.
Modification of substrate surface using parylene-C

It is desirable that substrate surface is homogeneous because substrate surface effects to cell adhesion, as shown in Figure 8. Therefore, depositing of parylene-C was performed in order to obtain homogeneous substrate surface, because parylene-C is well-known as one of biocompatible materials and modification of substrate surface is easily possible [6]. Therefore, three substrates under three conditions of substrate surfaces were prepared for evaluation of cell adhesion. First, Si$_3$N$_4$ membrane was deposited on Si substrate in low-pressure CVD (LPCVD) process after SPM treatment ($\text{H}_2\text{O}_2$:$\text{H}_2\text{SO}_4$=1:3) and rinsing with deionized water. The water contact angle of the substrate was 0º. The substrate was named as “SiN(0º)”. Second, parylene-C with 1 µm in thickness was deposited on Si substrate. The water contact angle of the as-deposited parylene-C surface was 95º (named as Figure 7. Time-series photographs of MEF cells adhered on Si$_3$N$_4$ diaphragm; Upper row is without mechanical vibratory stimulation, Lower row is with stimulation of applied displacement of 50 nm at a drive frequency 10 kHz for 1 h.

**Figure 7.** Time-series photographs of MEF cells adhered on Si$_3$N$_4$ diaphragm; Upper row is without mechanical vibratory stimulation, Lower row is with stimulation of applied displacement of 50 nm at a drive frequency 10 kHz for 1 h.

5. **Modification of substrate surface using parylene-C**

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“PC(95º)”). Third, Si substrate after parylene-C deposition was treated by O₂-plasma. In the O₂-plasma treatment process, flow rate of O₂ was 50 sccm, RF power was 50W, pressure during the process was 380 Pa, and process time was 30 s. The water contact angle of the O₂-plasma treated parylene-C surface was 25º (named as “PC(25º)”).

Figure 9 shows a SEM photograph of HeLa cells adhered to the O₂-plasma treated parylene-C surface. It can be confirmed the good adhesion of HeLa cells. Figure 10 shows adhesion areas of HeLa cells on each substrate surface. The adhesion areas of the HeLa cells were calculated from binary images of obtained optical photographs by using a photo-editing software (pickmap 1.0.0.4). As a result, the substrates with Si₃N₄ surface (“SiN(0º)”) and O₂-plasma treated parylene-C surface (“PC(25º)”) were good for cell adhesion. The adhesion area of as-deposited parylene-C surface (“PC(95º)”) was extremely low. Consequently, it was found that O₂-plasma treated parylene-C process could be applicable for obtaining homogeneous surface of cell culture microdevice shown in Figure 1.

![SEM photograph of HeLa cells adhered to O₂-plasma treated parylene-C surface.](image1)

**Figure 9.** Adhesion of HeLa cells on O₂-plasma treated parylene-C surface.

![Graph showing adhesion area of HeLa cells on three types of substrate surfaces.](image2)

**Figure 10.** Adhesion area of HeLa cells on three types of substrate surfaces.

6. Conclusions

The cell culture microdevice with KOH-etched cavities surrounded by four (111) sidewalls as microchambers in order to introduce cells to be cultured was fabricated in this paper. The vibration characteristic of the PZT actuator was improved by using an electric field -150 kV/cm at 70 ºC for 30 min in poling process. A feasibility study on cell culture for delivering mechanical vibratory stimuli to cells at applied displacement of 50 nm at a drive frequency of 10 kHz for 1h revealed the microdevice could be applicable to the culture with actual biological cells for our purpose. In order to obtain a homogeneous substrate surface, deposition of parylene-C film was performed on Si substrates. As a result, it was found that O₂-plasma treated parylene-C process could be applicable for obtaining homogeneous surface of cell culture microdevice, because the adhesion area of HeLa cells on O₂-plasma treated parylene-C substrate was 90%. In future, a prototype of the cell culture microdevice proposed here will be appeared and effect of delivering mechanical vibratory stimuli to cells on cell functions will be investigated.
Acknowledgments
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