Evaluation of Cell Viability Using a Surface Acoustic Wave Device with Silicone Chamber

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1. Introduction

In recent years, the demand for mature cellular tissue has increased in cultured meat generation and in regenerative medicine. Especially in regenerative medicine, mature tissues are required to generate robust organs that function stably in human body[1]. Therefore, it is important to continuously evaluate cell viability, which contributes to the search for a culture environment making production of mature cellular tissues efficient. However, conventional cell viability assessment methods including western blotting[2], and gene transfer[3] are invasive to cells due to the necessity of cell homogenization and genetic risks, respectively.

In this study, we propose a non-invasive method to evaluate cell viability using a Rayleigh-type surface acoustic wave (R-SAW). R-SAW is irradiated to the cell by the transmitting Interdigital transducer (IDT) , and then received by the IDT on the opposite side of the cell (**Fig. 1**). Cell viability is evaluated from the continuously acquired R-SAW waveform changes. Four IDTs were formed, two on the medium side for control and two on the cell-seeded side.

2. Experimental methods

2.1 SAW device fabrication

R-SAW was generated by the SAW device fabricated by lift-off method. To fabricate the SAW device, LN chip that is the size of 15 mm × 30 mm was cut out. After cutting, the LN chip was ultrasonically cleaned with acetone and ethanol. In the photolithography process, a negative photoresist film was coated to form the IDT. Then chromium and aluminum were evaporated onto the LN chip. After evaporation, negative photoresist on the LN chip was removed by ultrasonic cleaning with acetone.

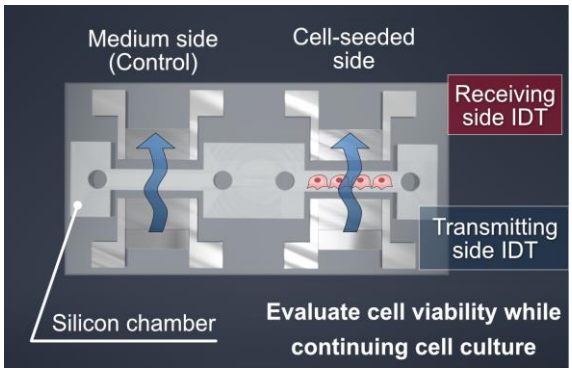

Fig. 1 Evaluation of cell viability by SAW.

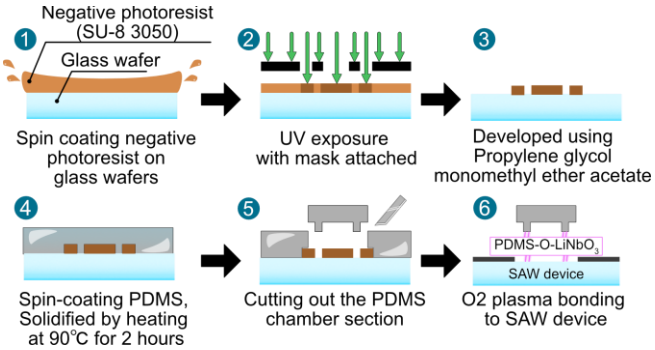

Fig. 2 Fabrication process of silicone chamber

2.2 Silicone chamber fabrication

Silicone chamber was made of polydimethylsiloxane (PDMS). Silicone chamber was fabricated with molds formed in photolithography process (**Fig. 2**).

For fabricating the mold of silicone chamber, glass wafer was ultrasonically cleaned with acetone for 1 minute. After ultrasonic cleaning, O_2 plasma was irradiated on the glass wafer for O_2 plasma ashing. Negative photoresist was coated on the glass wafer to form with thickness of 100 µm. The mold was formed by developing after UV exposure through a photomask.

To enable the release of PDMS with a high aspect ratio, the molds were coated with a fluorine mold release agent and evaporated. Then PDMS was applied to the mold and heated at 90°C for 2 h. After heating, the silicone chamber was cut out. In order to remove the mold release agent, ultrasonically cleaned with IPA for 1 minute. After cleaning, inlets and outlets with a diameter of 1.5 mm were processed for seeding cells in the channels. The bonding surface between the SAW device and the silicone chamber was irradiated with O_2 plasma. The irradiated surfaces were attached to each other and

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heated at 80°C for 4 h to enhance adhesive performance[4]. In addition, 10 µl pipette tips were fixed to the silicone chamber with a PDMS cube in order to allow for continuous medium replenishment.

Cells were seeded from one inlet. The other inlet was seeded with medium only for control. Two pipette tips of 10 µm were fitted to inlets in order to continuously refill the culture medium (**Fig. 3**). The pipette tips were supported by a PDMS supporter bonded by O_2 plasma bonding.

3. Results

3.1 SAW device with Silicone chamber

The fabricated SAW device and silicone chamber were bonded by O_2 plasma bonding (**Fig. 4**). The resonance frequency of both medium side and cell seeded side IDT was measured to be 38.4 MHz. Alternating current of the respective resonance frequency was applied to the transmitting side IDTs during SAW waveform measurement.

3.2 Cell seeding and SAW waveform measurement

C2C12 cells were used for the evaluation target. Culture medium and cell suspensions were injected from a pipette tip supported by a PDMS supporter (**Fig. 5**). On the medium side, very few C2C12 cells were found. While on the cell-seeded side, C2C12 cells were found at a density of 1.4×10^3 cells/mm^2. This suggests that there is no liquid leakage or mixing between the silicone chambers on the medium side and cell-seeded side. Note that measurement of cell density should take into account the doubling of the cells when lithium niobate is observed with a phase contrast microscope.

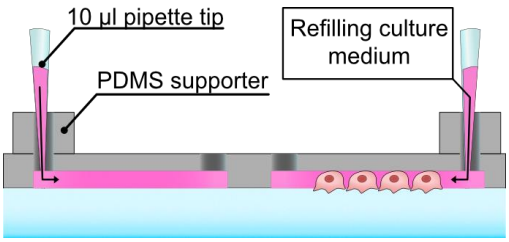

Fig. 3 Device setup for waveform measurement with 10 µl pipette tips and PDMS supporter.

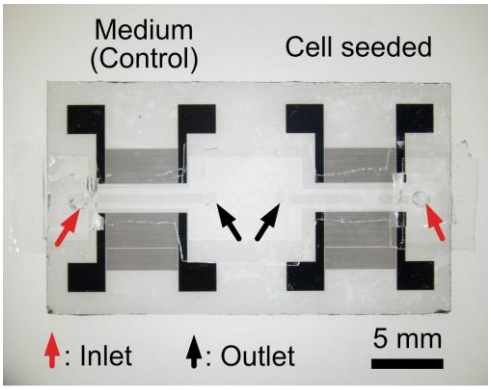

Fig. 4 Evaluation of cell viability by SAW

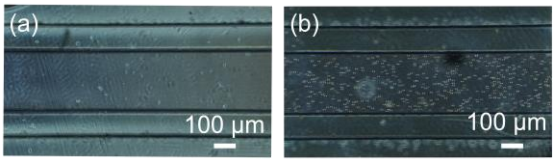

Fig. 5 Flow paths in silicone chambers after cell seeding. (a) medium side for control. (b) cell-seeded side.

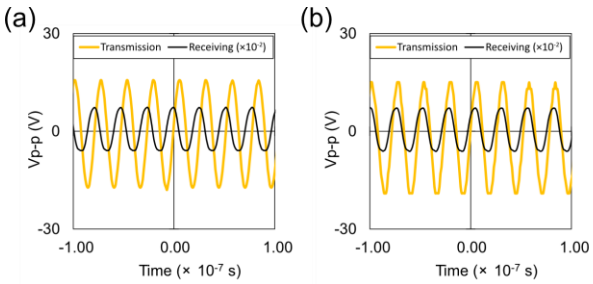

Fig. 6 SAW waveforms obtained from the cell-seeded side. (a) before cell seeding for blank data. (b) after cell seeding.

SAW waveforms were measured before and after cell seeding on the cell-seeded side. The amplitude ratio of the received wave to the transmitted wave was 4.0×10^{-3} and 3.9×10^{-3}, respectively. The phase lag of the received wave relative to the transmitted wave was 2.0×10^{-8} s and 2.5×10^{-8} s before and after cell seeding, respectively. This suggests that the increase in mass due to the injection of the cell suspension intensified the attenuation of the R-SAW.

4. Conclusion

Silicone chambers were fabricated using a photolithography process. The fabricated silicone chambers were bonded to SAW devices using O_2 plasma. After plasma bonding, the culture medium was injected from a pipette tip for inlets supported by a PDMS supporter to confirm little leakage. The attenuation of R-SAW before and after seeding cells on the cell-seeded side was confirmed. For the future work, we will measure SAW waveforms while concurrently continuing culture.

Acknowledgment

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References

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