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Quantitative analysis of intracellular conditioning in differentiating neuronal cells by Z-scope impedance tomography

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Introduction

The application of artificial organs has been realized, and the engineering of their quality control is required urgently. Neurosphere[1)], a suspended spherical aggregate of neural stem and progenitor cells, is one of the culture models for differentiating artificial organs in vitro. In this study, we propose scanning acoustic microscopy (SAM) as an effective tool for quantitatively evaluating changes in the differentiation process of Neurosphere. SAM is a non-invasively microscopic observation device visualizing tissues and cells. The intracellular states could cause viscosity and elasticity changes during cell differentiation [2)]. SAM could evaluate changes in the mechanical properties of the cells[3)] and enables deep-depth intracellular observation[4)]. Using SAM, we evaluated the state of cell differentiation in Neurosphere and conducted to quantify internal information three-dimensionally.

Material and Method

Neural stem cells and progenitor cells derived from the fetal rat cerebrum on the 16th day of pregnancy formed spherical form called Neurosphere. Then, Neurosphere was induced differentiation. For SAM observation, Neurosphere differentiation started on a polystyrene (PS) substrate of about 50 μm thick (Honda Electronics Co., Ltd.). On days 2 and 12 after differentiation induction, we observed cultured Neurosphere using SAM. After that, fluorescence observation was performed using a confocal microscope.

The acoustic impedance of the interface between cultured cells and the substrate is obtained from the following equation

$$S_{ref} = \frac{Z_{sub}-Z_{ref}}{Z_{sub}+Z_{ref}} \times S_0 \quad (1)$$

$$S_{tgt} = \frac{Z_{sub}-Z_{tgt}}{Z_{sub}+Z_{tgt}} \times S_0 \quad (2)$$

S_{ref} is the reflected intensity from the culture medium, S_{tgt} is the reflected intensity from the cells, Z_{sub} is the intrinsic acoustic impedance of the polystyrene, and Z_{ref} is the intrinsic acoustic impedance of the culture medium.

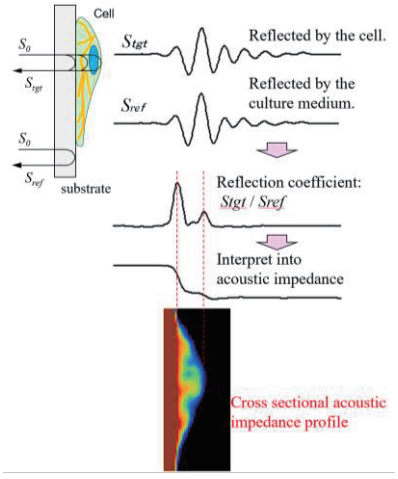

Fig. 1. Interpretation of waveforms into cross sectional acoustic impedance profile.

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The acoustic impedance of the cell interior is basically obtained from the same equations (1) and (2) as in the previous section. In the reflected wave S_{tgt} from the cell, there are reflections caused by the internal structure of the cell. Using these reflection components S_{ref}, the reflection coefficient of the localized reflection wave is obtained (**Fig. 1**). These reflection components are useful to convert the local reflection coefficients into local acoustic impedances.

Result

Before differentiation on day 2, Neurosphere showed weak impedance on the substrate surface (**Fig. 2. d**). On three-dimensional cross-sectioning views of each section, cells in

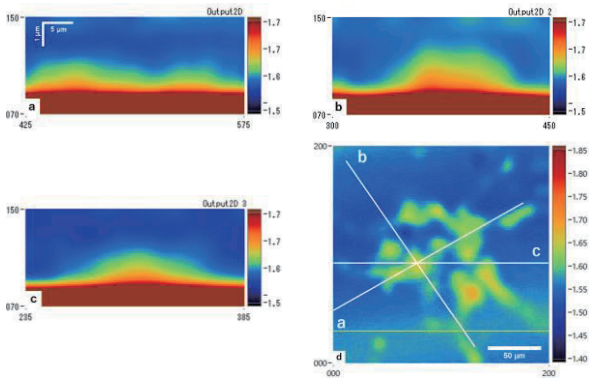

Fig. 2. Acoustic impedance measurement of Neurosphere before differentiation. a-c. Cross-sectional view of depth direction, d. XY top view

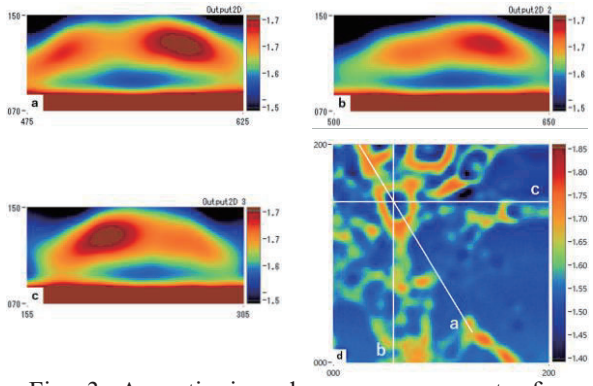

Fig. 3. Acoustic impedance measurement of Neurosphere after differentiation. a-c. Cross-sectional view of depth direction, d. XY top view. scale bar = 5μm×1μm (Fig.2, 3.a-c), 50μm (Fig.2, 3.d)

Neurosphere were thin (**Fig. 2. a-c**). High-impedance areas were near the substrate. After differentiation on day 12, conversely, the impedance of each part in the Neurosphere was increased with high adhesion to the substrate (**Fig. 3. d**). Cross-sectioning views showed that space floated from the substrate (**Fig. 3. a-c**). Furthermore, extremely high impedance areas appeared in differentiated cells.

Discussion

During differentiation, cytoskeletons develop in neurons to form neuronal circuits. In our observation, differentiated cells in the Neurosphere showed high intracellular impedance and changed three-dimensional cell structure from plane to bridge form. The structure inside the cell has become complicated. SAM three-dimensional observation could observe the differentiation and quality of artificial organs non-invasively.

Conclusion

Our results demonstrate that three-dimensional SAM analysis properly observes intracellular changes with differentiation based on acoustic impedance.

Acknowledgment

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