1P2-1

Stereo analysis of cell-cell spatial interrelationship using scanning acoustic microscopy

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1. Background

The application of artificial organs has been realized, but the problems of heterogeneous and inadequate cell-cell adhesion result in variability in patient outcomes[1)]. Then, the engineering of their quality control is required urgently. In this study, we propose scanning acoustic microscopy (SAM) as an effective tool for evaluating changes in the cell-cell spatial interrelationship. SAM is a non-invasively microscopic observation device to visualize living cells using ultrasound transmission and reflection. SAM could evaluate changes in the mechanical properties of the cells[2)] and enables intracellular observation of single cells[3, 4)]. Using SAM, we have observed three-dimensional structures of cells.

2. Material and Method

2.1 Cell culture and observation

2.1.1 Co-culture of Microglia and Granule cells

We prepared microglia and granule cells (MG-GC) derived from the cerebellum of rats on 2nd day after birth. For SAM observation, microglia and living stained granule cells were co-cultured on a polystyrene (PS) film dish 50 μm thick (Honda Electronics Co., Ltd.) to observe it using SAM. The cells were fixed, stained, and observed using the optical microscope.

2.1.2 Culture and Differentiation of Neurosphere

We prepared neural stem cells derived from the rat fetal cerebrum on the 16th day of pregnancy, The spherical form of which is called the Neurosphere (NS), and we induced its differentiation with a serum-free medium on a PS film dish to observe it using SAM. After that, the neurosphere was fixed, stained, and observed using the optical microscope.

2.2 Three-dimensional analysis

The acoustic impedance of the cell interior is basically obtained from Equations (1) and (2), using the method of Hozumi et al.[5)] (**Fig. 1**)

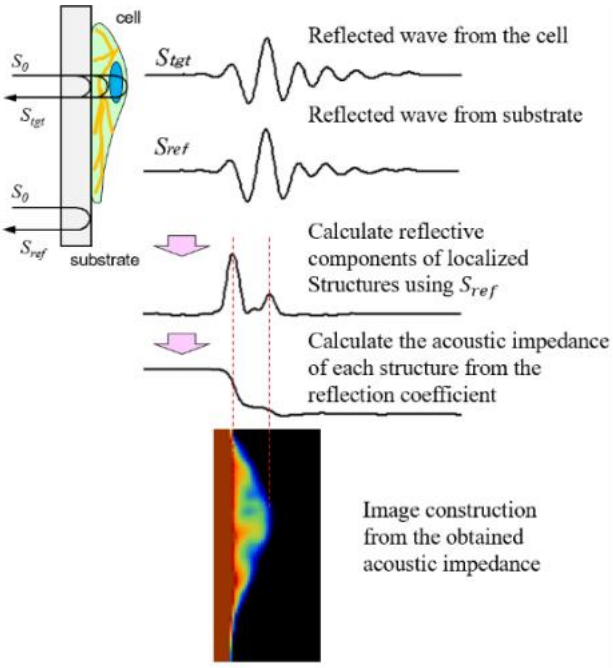

Fig. 1 Interpretation of waveforms into cross-sectional acoustic impedance profile.

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

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

3. Results

We observed that cell-cell contact in MG-GC co-culture showed a high impedance area with additional congestion over the cells. Similar congestion was observed in the high-impedance region of the differentiated NS (**Fig. 2(a, b)**). The reflection signal repeated waveforms of almost the same size (**Fig. 3(a-d)**). Neither single MG culture nor differentiating NS showed high acoustic impedance areas with signal congestion (**Fig. 2(c, d)**). Furthermore, small reflection signal waveforms followed the first significant reflection waveform, and acoustic impedance waveforms were grouped together (**Fig. 3(e-h)**).

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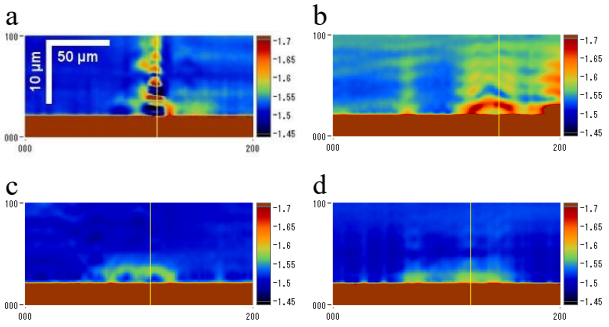
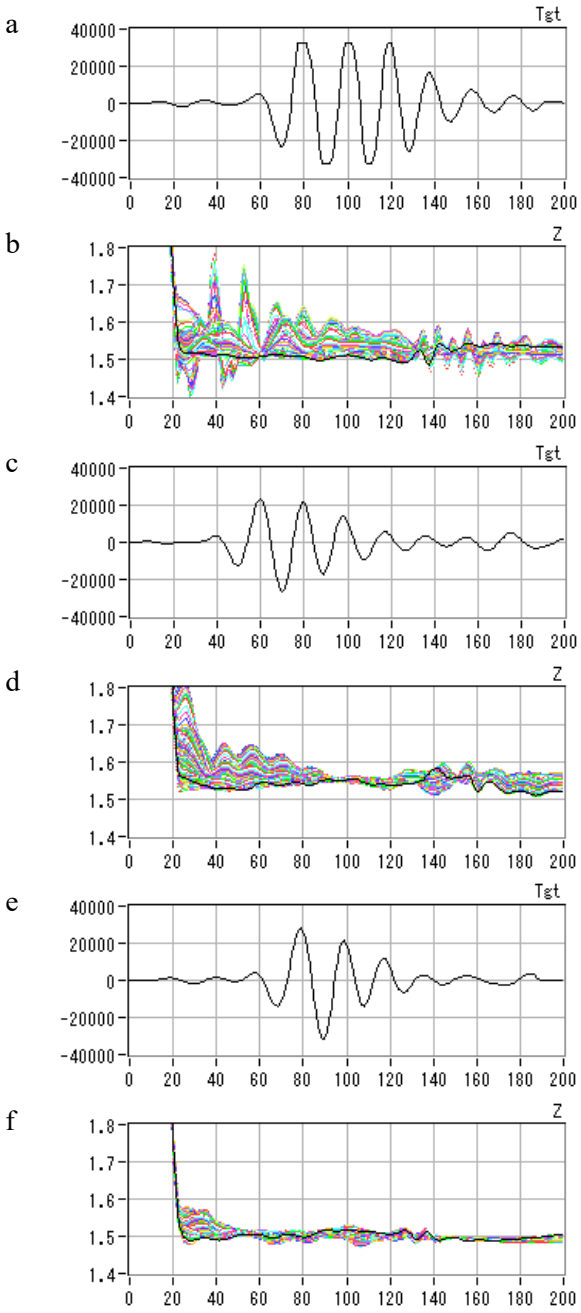


Fig. 2 Cross-sectional images. (a) MG and GC co-culture. (b) Differentiated NS. (c) MG alone. (d) Differentiating NS. Scale bar = 50 μm × 10 μm. Unit of color scale: MRayl.



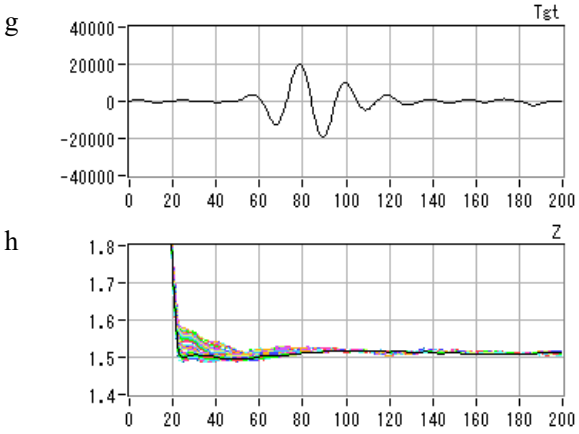


Fig. 3 Reflection waveforms (a, c, e, g. the unit of vertical axis: mV) and Acoustic impedance waveforms (b, d, f, h. the unit of vertical axis: MRayl). Note that the unit of lateral axis is point in Fig. 3. (a, b) MG and GC co-culture. (c, d) Differentiated NS. (e, f) MG alone. (g, h) Differentiating NS.

4. Discussion

SAM gives us noninvasive living cell images in C-mode and 3D-mode, and the imaging profiles differ from optical microscopes. High-density materials in the cells or cell-cell interaction between different density cells induced congestion signals over the cells. Using this imaging profile, we could evaluate matter density in and between living cells. We should proceed with an analysis of the source of congestion signals.

5. Conclusion

SAM could evaluate changes in the mechanical properties of the living cells and enable intracellular observation. In stereo visualization, high-density materials in the cells induced congestion signals over the cells, which would be useful for new applications.

Acknowledgment

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References

1) C. M. Dunn et al., Tissue Eng Part A. **29**, 594 (2023)
2) K. Kobayashi et al., Ultrasonics **54**, 1922 (2014)
3) N. Hozumi et al., Ultrasonics **99**, 105966 (2019)
4) T. K. S. Tiong et al., Jpn. J. Appl. Phys. **61**, SG1070 (2022)
5) E. B. Prastika et al., Ultrasonics **119**, 106601 (2022)