IRES²プロジェクト研究

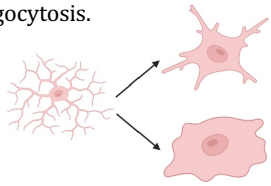
超音波顕微鏡を用いた発達期神経細胞障害の生理機能評価と医療への応用

プロジェクトメンバー：ダイバーシティ推進センター　吉田祥子、IRES² 沼野利佳、本多電子　小林和人

Materials and Methods

Microglia and microglial phagocytosis

Microglial (MG) is a type of immune cells and they maintain the homeostasis in the brain. MG's roles are promoting synaptogenesis, protecting neurons and removing targets such as inflammation substances and damaged neurons. When MG detect the targets, MG migrate and contact the targets to scavenge them by release inflammatory cytokine, a process known as phagocytosis.
In addition, MG is known that they show different phenotypes by their roles. They exhibits ramified, hyper ramified and ameboid forms during detecting, protecting, and phagocytosis, respectively.



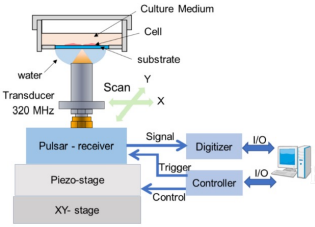
Preparation of microglial phagocytic model

We cultured MG derived from neonatal rat cerebellum for 8 days and transported them on a PS substrate to observe with SAM. Two days later, we added damaged neuronal granule cell (GC) derived from rat cerebellum to cultured MG and observed using scanning acoustic microscopy (SAM). Finally, cells were stained and observed using the optical microscope.

Scanning Acoustic Microscopy (SAM)

SAM's system

Ultrasound waves, with central frequency of 320 MHz, were sent to the target by applying a voltage to the transducer and received the reflected waves using the same device. The signals were defined as the reflected intensities.
We obtained the reflected intensities of the target by scanning the transducer two-dimensionally under the polystyrene (PS) film dish with 50 μm thickness. The scanned areas were 180 μm × 180 μm as 200 points × 200 points (X × Y).

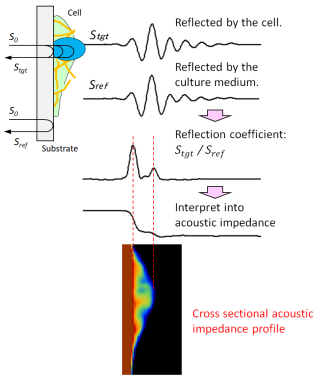


Calculation of Acoustic Impedance value

$$Z_{tgt} = \frac{1+\frac{S_{tgt}}{S_0}}{1-\frac{S_{tgt}}{S_0}} Z_{sub} = \frac{1-\frac{S_{tgt}}{S_{ref}} \cdot \frac{Z_{sub}-Z_{ref}}{Z_{sub}+Z_{ref}}}{1+\frac{S_{tgt}}{S_{ref}} \cdot \frac{Z_{sub}-Z_{ref}}{Z_{sub}+Z_{ref}}} Z_{sub}$$

Z: Acoustic impedance
S: Reflected intensity
S_0 : Transmitted signal

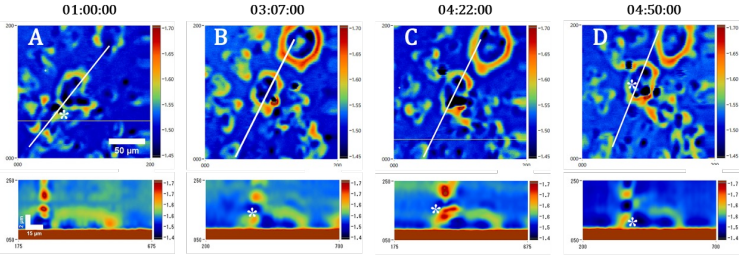
The acoustic impedance of the interface between cultured cells and substrate can be calculated from the above formulas. S_{ref} and S_{tgt} are the reflected intensities from the medium and cells, respectively. Z_{sub} and Z_{ref} are the specific acoustic impedances of the substrate and medium, respectively. The multiple signals S_{tgt} reflected from the cell include reflections due to the internal structure of the cell. Using the reflected component S_{ref} from the medium, the reflection coefficient of the locally reflected wave can be calculated as shown in the figure on the right. From the obtained reflection coefficients, the specific acoustic impedances of the structure are calculated one after another, and a tomographic image of the cells is constructed. Three-dimensional resolution is 0.2 μm in the depth direction.



Acoustic impedance change during microglial phagocytosis

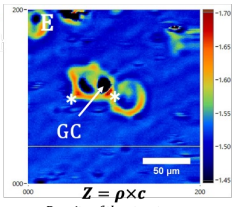
Three-dimensional consecutive observation using SAM

Two-dimensional image of **Figure A** showed MG approached GC 1hour after co-culture. Three-dimensional image of **Figure B** showed a window opened on MG three hours later. Three-dimensional image of **Figure C** showed the window was closed and there was a high acoustic impedance area four and a half hours later. Three- and two-dimensional images of **Figure D** showed decreasing of acoustic impedance values on MG and there was GC under MG five hours later. This consecutive observation suggests GC is engulfed through the window on MG.



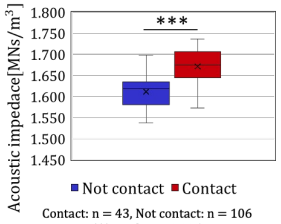
Quantitative analysis of acoustic impedance

The acoustic impedance values around GC increased significantly in MG when they contact with GC (**Figure E, F**). The difference of the values reflect the difference of densities of intercellular substances. The distribution of MG's F-actin was fibrous (**Figure G**).

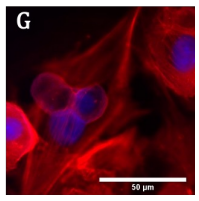


$Z = \rho \times c$

ρ: Density of the structure
c: The speed of sound in the medium



Contact: n = 43, Not contact: n = 106
*** P value < 0.005

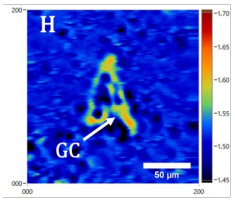


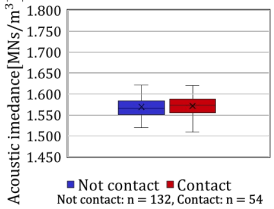
MG's F-actin/GC's nuclei

Actin polymerization and microglial phagocytosis

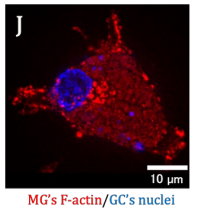
Inhibition of actin polymerization

There was no change in acoustic impedance values due to contact (**Figure H, I**), and the distribution of MG's F-actin was granular (**Figure J**). The results suggest that actin polymerization is involved in microglial phagocytosis.





Not contact: n = 132, Contact: n = 54



MG's F-actin/GC's nuclei

Conclusion

・We observed the interrelationship between microglial phagocytosis and actin polymerization using SAM three-dimensionally and consecutively. Actin polymerization associated with cell-cell interactions regulates microglial phagocytosis. Regulation of actin polymerization is a potential therapeutic target for neurodegenerative diseases.

yoshida.sachiko.gm
@tut.jp