Quantitative evaluation of cell adhesion toward RAD16RGDS peptide coated substrate

Y. Tagawa, Y. Morita, Y. Hirano, E. Nakamachi
Faculty of Life and Medical Sciences, Doshisha University, 1-3 Tatara-Miyakodani, Kyotanabe, Kyoto, Japan
E-mail: ymorita@mail.doshisha.ac.jp

Introduction
Materials used in scaffolds, must act as substrate to enhance cell adhesion and cell proliferation. Since surface of these materials can be modified by coating cell adhesion peptide, we focused on RGDS sequence which is related to integrin mediated cell adhesion and presented in the cell-attachment domains of fibronectin, vitronectin and collagens. Although we have reported that RGDS peptide has affected cell adhesive activities, the effect of the peptide on cell adhesive force was not clear quantitatively.

The purpose of this study is to develop the measurement system for adhesive force of single cell and evaluate quantitatively the effect of cell adhesion peptide on cell adhesive force.

Results and Discussion

Materials and Methods

Materials
- 24 well dish
- 4 well chamber
- Ultra thin glass plate (t=30 μm, l=45 mm, b=1.5 mm)
- Non coat
- Pronectin F coat
- RAD16RGDS coat

Adhesive force measurement for single cell

Cell adhesion
Tissue regeneration
Scaffold structure

Number of adherent cells measurement
Cell spreading area measurement
Adhesive force measurement

Immuno fluorescent stained (Actin filament, Vinculin)

Materials
- 10% FBS
- Antibiotic

Cell (NIH3T3)
DMEM
Subconfluent
Cell suspension

Ultra thin glass plate
(t=30 μm, b=1.5 mm)
(l=45 mm)

CO2 Incubator

Number of focal adhesion
Culture time (h)

Number of focal adhesion
Culture time (h)

Cell adhesive force depend on not the spreading area but the number of focal adhesion.

Conclusion
Increase in the adhesive force due to RAD16RGDS was measured using the developed measurement system. RAD16RGDS accelerated cell adhesive force and improved cell adhesion activities in early stage.