Quantitative evaluation of cell adhesion toward RAD16RGDS peptide coated substrate

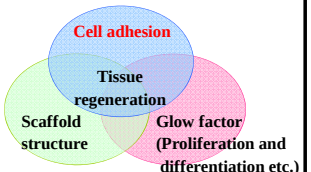
Y. Tagawa, Y. Morita, Y. Hirano, E. Nakamachi

Faculty of Life and Medical Sciences, Doshisha University, 1-3 Tatara-Miyakodani, Kyotanabe, Kyoto, Japan

E mail : ymorita@mail.doshisha.ac.jp

Introduction

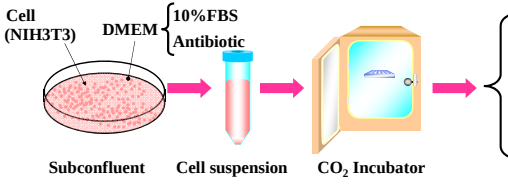
Materials used in scaffolds, must act as substrate to enhance cell adhesion and cell proliferation.
Since surface of these materials can be modified by coating cell adhesion peptide, we focused on **RGDS sequence** which is related to integrin mediated cell adhesion and presented in the cell-attachment 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.



Cell adhesion to the scaffold is important

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.

Materials and Methods

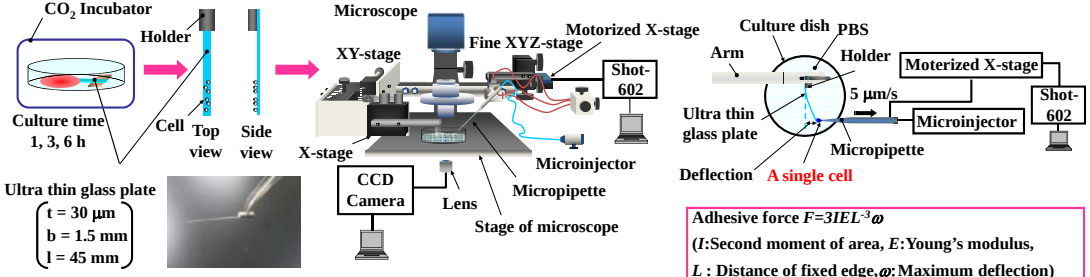


Materials

- 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

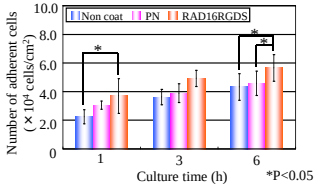


Adhesive force $F=3IEL^{-3}\varpi$

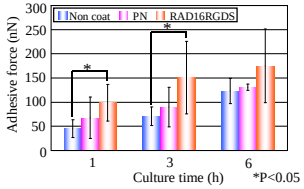
(I:Second moment of area, E:Young's modulus, L : Distance of fixed edge, ϖ:Maximum deflection)

Results and Discussion

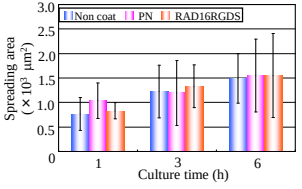
[The Number of adherent cells]



[The adhesive force]



[Cell spreading area]



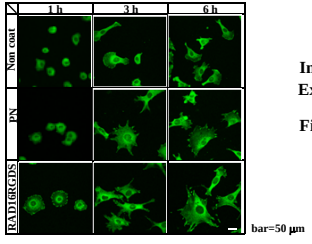
The number of adherent cells and the adhesive force was highest for RAD16RGDS coat.

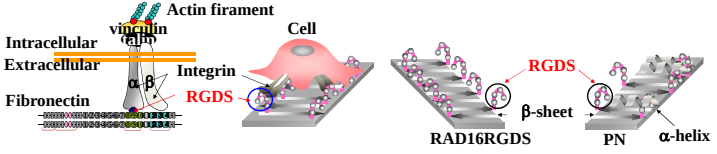
➡ **Cell adhesion is enhanced by RAD16RGDS coat.**

The cell spreading area on RAD16RGDS, PN and non coat weren't difference.

➡ **The adhesive force didn't depend on the cell spreading area.**

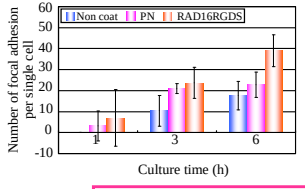
[Immunofluorescence staining of vinculin]





- RGDS is the principal integrin-binding domain present within ECM proteins such as fibronectin.
- Although both RAD16RGDS and PN peptides have β-sheet structure, a part of PN peptides formed α-helix structure. RGDS sequence expressed on surface of PN coated substrate was less than that on surface of RAD16RDGS coated substrate.

[The number of focal adhesion]



The expression of focal adhesion was accelerated due to RGDS sequence containing in RAD16RGDS having β-sheet structure.

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.