

# Basic Study on Sensing Mechanism of Substrate Elasticity by Cells: Effects of Substrate Elasticity and Thickness on the Behavior of Rat Aortic Smooth Muscle Cells



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## Abstract

To study sensing mechanism of substrate stiffness by cells, we observed changes of the projected area of rat aortic smooth muscle cells (RASMs) cultured on polyacrylamide (PA) gel substrates having various elastic moduli  $E$  and thicknesses  $h$ . Cell area decreased when  $h$  increased, and reached a plateau when  $h > 7.5 \mu\text{m}$  for a hard gel ( $E=92 \text{ kPa}$ ) and  $h > 30 \mu\text{m}$  for a soft gel ( $E=6 \text{ kPa}$ ). We then performed a finite element (FE) analysis to know the relationship between displacement of focal adhesions (FAs) and gel thickness, and found that stiffness at FA level correlates well with the cell area. These results suggest that RASMs could sense the elastic properties of the substrate only in a shallow region. If the cells sensed mechanical properties of the substrate by force-deformation relation spanning whole cell length, then the sensing depth might be comparable to their dimensions. Cells may sense substrate elasticity not with deformation of the whole cell body but with local deformation at FAs.

## 1. Introduction

Elasticity of substrate has significant effects on cells on it. For example, smooth muscle cells (SMCs) increased their adhesion area and spread well on a hard gel substrate, not on a soft substrate [1]. SMCs on the gel substrate with gradient elasticity moved to the harder region [2]. A recent study revealed that the differentiation of mesenchymal stem cells (MSCs) depends on the substrate elasticity [3]. Thus, it is very important to investigate how substrate elasticity affect cell behavior, although sensing mechanism of substrate elasticity is still unclear.

Cells adhere to substrates at their FAs. They exert traction forces of  $\sim 10 \text{ nN}$  at FAs to the extracellular matrix [4]. Thus, it can be hypothesized that cells sense substrate elasticity by changes in traction forces and substrate deformations at FAs. In this study, to investigate sensing mechanism of substrate stiffness by cells, we observed the changes in the projected area of RASMs cultured on polyacrylamide gel substrates having various elastic moduli and thicknesses. We then performed finite element analysis to know the relationship between displacement of focal adhesions and gel thickness.

## 2. Methods

### 2.1. Preparation of RASMs

RASMs were isolated from rat aortic tissue using an enzymatic digestion method. The cells were cultured in

Dulbecco's modified Eagle's medium (DMEM, Wako) supplemented with 10% fetal bovine serum (Biowest) and 1% penicillin-streptomycin (Sigma) at  $37^\circ\text{C}$  in 5%  $\text{CO}_2$  and 95% air. Cells at passages 8–16 were used for all experiments.

### 2.2. Thin gel substrate on glass

PA gel layers with various elastic moduli and thicknesses were formed on glass plates. Elasticity of the gel was adjusted by varying the relative amount of acrylamide and bis-acrylamide crosslinker. In this study, we used a hard gel ( $E=92 \text{ kPa}$ ) and a soft gel ( $E=6 \text{ kPa}$ ), whose Young's moduli were measured with a laboratory-made pipette aspiration system. An acrylamide/bis-acrylamide solution mixture was placed on cover glasses (Matsunami) that had been aminosilanized to increase the binding to the polyacrylamide, and covered with a normal cover glass. Gel thickness was controlled with the volume of the mixture or the thickness of spacers. Ammonium persulfate and TEMED (Wako) were then added to the mixture to induce gel polymerization. Fluorescent beads ( $\phi 2.5 \mu\text{m}$ ) were added to the mixture as markers for the thickness measurement. The upper cover glass was removed after polymerization. The gel surface was then treated with a sulfo-SANPAH (Pierce), and coated with 0.05% type I collagen solution (Koken) to improve cell adhesion. Collagen-coated cover glasses ( $E \sim 70 \text{ GPa}$ ) were also prepared for comparison.

### 2.3. Measurement of cell area

RASMs were plated on the substrates at  $100\text{--}200 \text{ cells/mm}^2$ , and cultured for 24 h at  $37^\circ\text{C}$  in 5%  $\text{CO}_2$  and 95% air. Their images were taken with a phase contrast microscope (IX-71, Olympus) equipped with a digital CCD camera (DP70, Olympus). Cell area was measured manually with an image analysis software (ImageJ, NIH). The cells were then fixed with a 10% neutral buffered formalin. Vertical distance between the beads dropped on the top surface of the gels and the beads deposited at their bottom was measured with an inverted microscope (TE2000E, Nikon) to measure gel thickness.

### 2.4. FE analysis of FA displacement

Displacement of a FA following loading of a shear force of  $10 \text{ nN}$  was analyzed with a 3D finite element model (Fig. 2 inset). The following assumptions were made: 1) the gel is a linear elastic material with  $E=6 \text{ kPa}$  and Poisson's ratio=0.45; 2) FA is an elliptic rigid plate whose major and minor axes are 5 and  $2.5 \mu\text{m}$ , respectively [5]. The FA

model was set at the center of the surface of a 200 x 200  $\mu\text{m}$  gel, and the shear force was applied in the major axis direction of the FA. The gel thickness  $h$  was varied between 2.5–100  $\mu\text{m}$ . The bottom surface of the gel adhered to the glass was fixed completely. The lateral surface perpendicular to the shear force direction (Fig. 2 AA' cross-section) was also fixed in symmetry assumption. Other surfaces were set free. The finite element model was developed using HyperMesh 9.0 (Altair Engineering) and analyzed with Altair Optistruct (Altair Engineering).

### 3. Results and Discussion

Fig. 1 shows the relationship between projected area of RASMs and gel thickness. The cells cultured on the glass substrate spread well and their area reached  $>2500 \mu\text{m}^2$ . The cells cultured on the both gels also spread well when  $h=5 \mu\text{m}$ . Their area gradually decreased when  $h$  increased, and reached a plateau when  $h>7.5 \mu\text{m}$  for the hard gel (HG) and  $h>30 \mu\text{m}$  for the soft gel (SG). As the gel gets thicker, the cells on the gels may become less sensitive to the underlying rigid glass substrate, i.e., the apparent elastic modulus of the substrate becomes smaller, causing the decrease in their adhesion area. Such effects may be more prominent in the soft gel than in the hard. Thus in the SG, the area continued to decrease until the gel thickness reached 30  $\mu\text{m}$ . The cell area did not decrease over 30  $\mu\text{m}$  even in the SG. These results indicate that cell-sensing depth may be smaller than the size of cells (50–100  $\mu\text{m}$ ). If the cells sensed mechanical properties of the substrate by force-deformation relation spanning the whole cell length, the sensing depth should be comparable to their whole dimensions due to Saint-Venant's principle. Thus cells may sense substrate elasticity not with deformation of the whole cell body but with their local deformation.

Fig. 2 shows the relationship between displacement of FAs and gel thickness obtained in the FE analysis. FAs displacement decreased remarkably when gel thickness was decreased to  $<50 \mu\text{m}$ . Fig. 3 shows relationships of the normalized cell area  $A^*$  obtained experimentally and the stiffness at FAs  $S_{FA}$  obtained numerically to gel thickness.  $A^*$  was defined as average cell area on each SG normalized by the area at maximum gel thickness (550  $\mu\text{m}$ ).  $S_{FA}$  was defined as the reciprocal of each FA displacement normalized by the displacement at maximum gel thickness (100  $\mu\text{m}$ ). The two relationships look very similar: as the gels become thicker, both  $A^*$  and  $S_{FA}$  decreased similarly. However, the amount of the decrease was not similar between the two parameters. It was much larger in  $A^*$  than in  $S_{FA}$ , indicating  $S_{FA}$  may have drastic effect on cell spreading. And also, some of the cells remained unspread although they were cultured on thin gels with high  $S_{FA}$ . These results may indicate that the RASMs are very sensitive to the stiffness at FAs in general, but some populations of cells are not sensitive to the stiffness.

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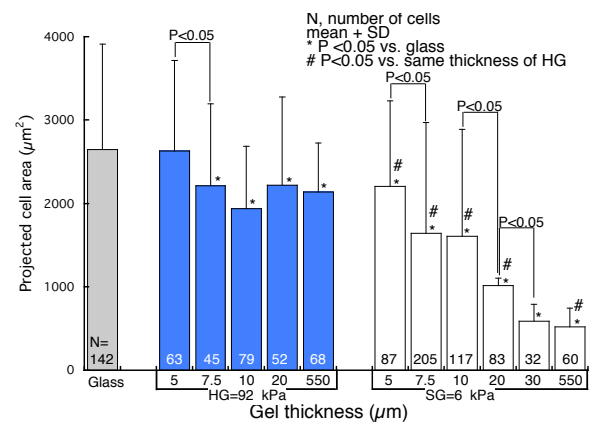


Fig. 1 Projected area of RASMs cultured on glass substrate and thin polyacrylamide gel layers with various thicknesses formed on a glass surface.

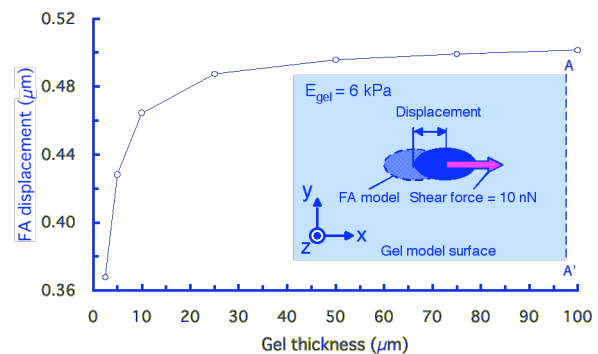


Fig. 2 Relationship between displacement of focal adhesions and gel thickness obtained in the FE analysis. Inset shows the model of a focal adhesion on the soft gel.

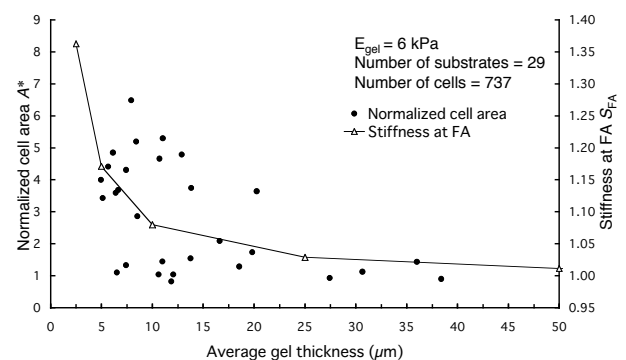


Fig. 3 Relationship between average projected area of RASMs and gel thickness. Changes in stiffness at focal adhesion  $S_{FA}$  obtained in the FE analysis was also shown for comparison.

### References

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