論文の内容の要旨

MEMS多軸センサを用いた細胞のトラクション力計測

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1. Introduction

Cell adheres to a substrate and generates the forces against the substrate for migration or growth. These traction forces relate to cell functions such as cell shape, morphology, extracellular matrix (ECM) generation, mechanical signal transmission and cell migration. For example, the traction force of a cancer cell was larger than it of a normal cell. Hence, the traction force measurement is the key for understating cell status or behaviors. The traction force changes by mechanical characteristics of a surrounding environment. The stiffness of the substrate is one of the elements to influence the cellular traction force. Especially, human body has broad stiffness of the substrate from soft brain tissue [E: 10^3 GPa] until bone [10^{10} GPa]. In previous studies, the traction forces have been measured using flexible substrates such as polydimethylsiloxane (PDMS) pillars, micro cantilevers and embedded micro beads in the gel. However, these methods were based on observation with microscopy. Therefore, the substrate has to be flexible for large deformation. The traction force on the rigid substrate is difficult to measure. In this work, we present a piezoresistive type sensor to measure the traction forces which is formed a piezo-resistor on a Si layer, then the applied forces can be obtained as the resistance change. Figure 1 illustrates the concept of the piezoresistive force sensor for the traction force measurement. We proposed the piezoresistive type sensor which was doped on a side-wall in order to measure the horizontal and vertical directional forces. Smooth muscle cells spread on the sensor pad, and then the sensor measures the traction forces.

2. Principle

Figure 2 illustrates the simulation results of strain change when x and z axis forces are applied to the sensor pad. When x directional forces are applied on the sensor pad, a strain is concentrated on the side wall of the beam. The two resistances of the beams change in a reverse direction. On the other hand, when z directional forces are applied to the sensor, the two resistances decrease. The piezoresistors were formed on the surface and the side wall of the beams. The sensor beams are composed of two resistors and one wiring to detect two resistances.

3. Design・Fabrication

We designed two device chips (Figure 2). One is a sensor chip which has the piezoresistive cantilever. The other is a cover chip. It prevents cells from adhering on the sensor beams because cells settle down on the sensor

randomly in the dish. The piezoresistive cantilever is divided into two parts. First part is a sensor pad for culturing cells. Second part is the sensor beams for detecting forces. The size of the sensor pad was designed 125×15×5 μm (length×width×thickness). The diameter of a single cell is about 20 μm so that cells are spreading at least on the two pads. The gap between the sensor pads is 4 μm. The total length of the sensor and the width of each beam are 1130 μm and 5 μm, respectively. The spring constants of the proposed sensor in *x* direction and *z* direction were 0.2 N/m and 0.08 N/m, respectively. The sensor chip was coated with parylene-C (thickness: 0.5~1 μm). The actual gap between sensor pads was reduced down to 3 μm due to the parylene coating. Figure 4 (a) shows photographs of the sensor chip, sensor pads and beams. Force sensors and compensators were arrayed the same chip. Figure 4 (b) shows the cover chip which have three holes. One through hole was for cells to settle down on the sensor pads. Two holes were for liquids to remove air under the sensor. The sensor was bonded on the flexible polyimide substrate with Al wire.

4. Properties of a traction force sensor

The force sensitivity of the sensor was calibrated using a load cell and a reference cantilever. The spring constant of the reference cantilever was 2.0 N/m. We calibrated fractional resistance change of the reference cantilever using the load cell. After calculating the relationship between the fractional resistance change and the reference cantilever, the proposed sensor was calibrated with the reference cantilever. Figure 5 shows the graphs of the resistance changes of the proposed sensor to the applied *x* and *z* force. The force sensitivities in *x* directional forces of the sensor beams corresponding to R_1 and R_2 are -7.4×10^{-6} nN^{-1} and 4.7×10^{-6} nN^{-1}, respectively. The force sensitivities in *z* directional forces of the sensor beams are -1.6×10^{-6} nN^{-1} and -2.2×10^{-6} nN^{-1}, respectively. We can detect 10^{-5} of $\Delta R/R$ using an oscilloscope thereby the force resolution of the sensor is 10 nN. The characteristic matrix calculated from the fitting line is;

$$\begin{pmatrix} F_x \\ F_z \end{pmatrix} = 10^5 \times \begin{pmatrix} -0.9 & 0.7 \\ -2.0 & -3.1 \end{pmatrix} \begin{pmatrix} \Delta R_1 / R_1 \\ \Delta R_2 / R_2 \end{pmatrix}$$

The unit of force is nN. Hence, the cellular traction force can be calculated from the measured resistance changes using this characteristic matrix.

5. Traction force measurements

Figure 6 shows photographs of an experiment setup and the wire bonded sensor chip in the dish. The output signals from the sensor input to a lock-in amplifier for cut down the noise. Figure 7 shows procedures before the experiment. (i) The sensor was fixed to the dish with bees wax and rinsed with 70% ethanol, PBS and Earle's balanced salt solution (EBSS). (ii) Then, the surface of the sensor was coated with fibronectin for 1 hour at room temperature. (iii) Smooth muscle cells (BAOSMCs, CAB35405) were suspended in the dish. (iv) After 1 hour, the medium was replaced by Leibovitz's L-15 medium with 20 mM HEPES pH 7.4. L-15 medium was filtered (φ:450 μm) to prevent particles from touching the piezoresistors area. Figure 8 shows photographs of the sensor pads through the cover chip and adhered cells on the sensor chip. We confirmed that there were no cells under the cover chip. Figure 9 shows output signals of the sensor without cells in the L-15 medium at 37°C for 20 min. The sensor had a temperature compensator thereby the output signals were stable. The noise levels were corresponding to 10 nN in *x* direction, 20 nN in *z* direction, respectively.

Figure 10 shows photographs of a bovine smooth muscle cell for 30 min. The cell moved to the fixed frame as shown in Figure 11. Figure 12 shows the relationship between (a) cell perimeter and *x* axis forces, (b) cell area and *x* axis forces and (c) the cell area and the perimeter. When the cell area increased 282 μm^2, the measured force was increased about 1 μN. The relationship between cell perimeter and the traction force was also linear. The same tendency has been shown more than three cells.

6. Conclusion

In conclusion, we proposed the force sensor using the piezoresistive cantilever to measure the traction forces of BAOSMCs on the rigid substrate. The sensor was fabricated with an SOI wafer (5/2/300 μm). The piezoresistive layer was deformed on the surface and side-wall area thereby the sensor can detect *x* and *z* axis forces. We used parylene-C for insulation to use in the culture medium. The force resolution of the sensor was 10 nN with 1 μm of the parylene layer. We measured the traction force of a bovine aortic smooth muscle cell. From the traction force measurements, the calculated traction forces of the smooth muscle cell divided by unit area was 3.6~22 μm^2. The experimental results show the linear between the traction force and the cell size.