Design of Microstructure for Stimulating Mechanical Torque to Cells

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Abstract: In this study, we proposed the design for microstructure which can apply bending or torsion stimulus to cell using an external magnetic field. First, we defined "ideal bending stimulus" and "ideal torsion stimulus" for cell on a microstructure. In order to apply ideal bending or torsion stimulus to cells, the thickness of the microstructure of cell-culturing region is important. We designed and microfabricated the microstructure which consists of a thin silicon beam as cell-culturing region and a ferromagnetic material, nickel film for magneto-active structure. Then, fabricated microstructures actuated by external magnetic field and deformation of the microstructures was measured. From the results of the measurements, we calculated radius of curvature and angle of torsion respectively and we confirmed the platform almost actuated in theory. Our design of the platform can contribute to applying new kinds of mechanical stimuli to cultured cells.

1 INTRODUCTION

In this study, we designed and fabricated a microstructure to apply bending stimulus or torsion stimulus to cell on the sub-mm scale substrate. Recently, mechanobiology has been a noticed research topic. It is known that the behaviours of cells such as proliferation and orientation of the actin filament are affected by external mechanical stimulus (Kozai *et al.*, 2005; Wang *et al.*, 2001). In previous studies, several kinds of mechanical stimuli, for example, a stretching stimulus, a shear stimulus, and a hydrostatic compressive stimulus, have been applied to cultured cells (Wang *et al.*, 2014; Hagiyama *et al.*, 2017; Galbraith *et al*., 1998). However, no researches which apply a bending stimulus or a torsion stimulus by mechanical torque, have been reported. This is because the thickness of cells is very thin like 1 μm. Therefore, it is difficult to apply only ideal bending stimulus or ideal torsion stimulus while avoiding stretching stimulus to cells on a substrate.

To apply bending or torsion stimulus to cell, we propose a magneto-active microstructure. By utilizing a magnetic field and mignetic anisotropy, bending deformation or torsion deformation can apply to cells on a substrate with sub-mm scale. In addition, it is able to actuate several microstructures at the same time without any physical contact to cells. In this paper, first, we mechanically examined

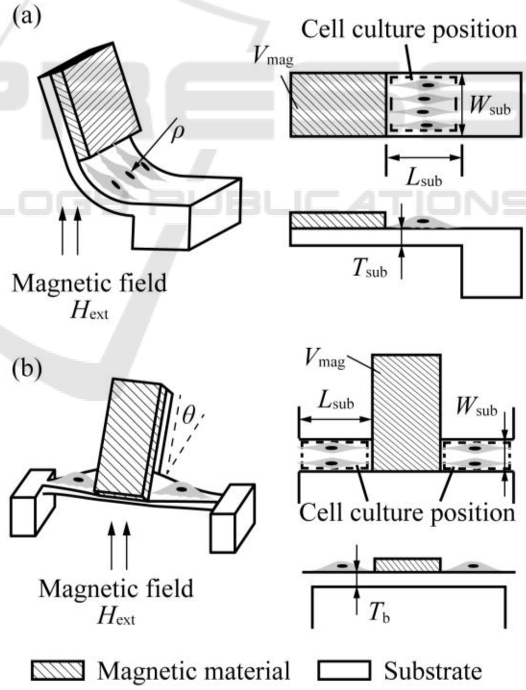

Figure 1: Schematic image of designed microstructure. (a) Microstructure to apply bending deformation. (b) Microstructure to apply torsion deformation.

bending and torsion stimulus to a cell, respectively, when mechanical torque is applied to the substrate and designed magneto-active microstructure. After that, the microstructure was actuated under external

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magnetic field. We confirmed that the microstructure can be actuated with sub-mm scale in radius of curvature or angle of torsion.

2 DESIGN

Figure 1 shows the schematic image of the microstructure which we designed and fabricated in this study. When bending or torsion stimulus is applied to a cell, it is necessary to consider the centre position of torque where have no deformation. First, we discuss about bending stimulus. As shown in Figure 2, we regard a cell on substrate as a rectangular solid. When bending stimulus is applied to cell on substrate with radius of curvature ρ, strain distribution occurs to inside of the cell (Figure 3 (a)). The strain ratio of top position of cell to bottom position of cell is expressed as,

$$\frac{\varepsilon_{\mathrm{bottom}}}{\varepsilon_{\mathrm{top}}} = \frac{y_{\mathrm{b}} - 0.5t_{\mathrm{cell}}}{y_{\mathrm{b}} + 0.5t_{\mathrm{cell}}} \tag{1}$$

where $\varepsilon_{\mathrm{top}}$ and $\varepsilon_{\mathrm{bottom}}$ are the strain of top and bottom position of the cell, y_{b} is a distance from centre of bending, i.e., neutral plane, to centre of the cell, and t_{cell} is the thickness of the cell. In the case of $y_{\mathrm{b}} = 0$, namely, the position of neutral plane is centre of the cell, we obtain $\varepsilon_{\mathrm{bottom}} / \varepsilon_{\mathrm{top}} = -1$ from Equation (1). In this case, ideal bending stimulus is applied to the cell. On the other hand, in the case of $y_{\mathrm{b}} >> t_{\mathrm{cell}}$, i.e., the position of neutral plane is too far from the cell, we obtain $\varepsilon_{\mathrm{bottom}} / \varepsilon_{\mathrm{top}} \approx 1$ from Equation (1). In this case, we can estimate the stimulus of the cell as compressive or tensile stimulus not bending stimulus. By designing y_{b} to near zero, we can apply more ideal bending stimulus to cells. By calculating an equation about the total stress on cross section of a cell and a substrate, the value of y_{b} is obtained. The equation is expressed as,

$$\begin{aligned} &\frac{E_{\mathrm{cell}}}{\rho}\{(y_{\mathrm{b}} + 0.5t_{\mathrm{cell}})^2 - (y_{\mathrm{b}} - 0.5t_{\mathrm{cell}})^2\} \\ &+ \frac{E_{\mathrm{sub}}}{\rho}(y_{\mathrm{b}} - 0.5t_{\mathrm{cell}})^2 \\ &= \frac{E_{\mathrm{sub}}}{\rho}(t_{\mathrm{sub}} - y_{\mathrm{b}} + 0.5t_{\mathrm{cell}})^2 \end{aligned} \tag{2}$$

where, E_{cell} is the young's elastic modulus. E_{sub} and t_{sub} are the young's elastic modulus and thickness of cell culture substrate, respectively.

Next, we discuss about torsion stimulus. The discussion is almost the same as the discussion about bending stimulus. In case of rectangular cross-section, strain distribution is non-linear. However, we assumed linear strain distribution as a circular cross-section to consider simply. When torsion stimulus is applied to a cell on substrate with angle of torsion θ, shear stress distribution occurs on inside of the cell (Figure 3 (b)). The shear stress ratio of top position of cell to bottom position of cell is expressed as,

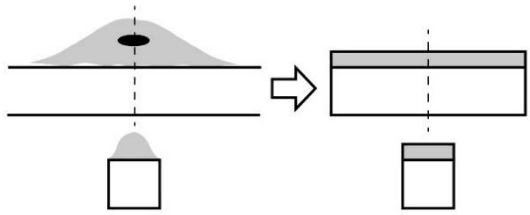

Figure 2: modelling of cultured cell on a substrate.

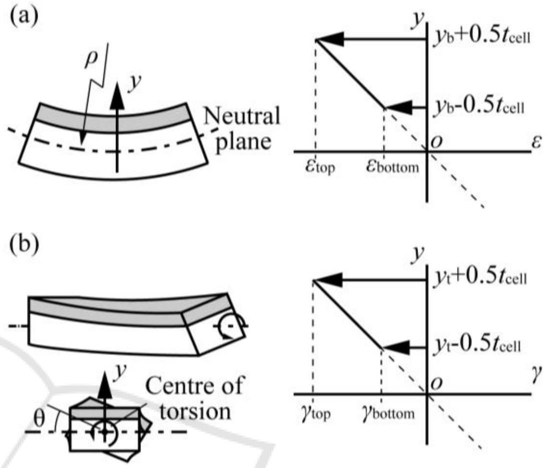

Figure 3: Schematic image of strain distribution in a cell. (a) Strain distribution during bending stimulus. (b) Shear strain distribution during torsion stimulus.

$$\frac{\gamma_{\mathrm{bottom}}}{\gamma_{\mathrm{top}}} = \frac{y_{\mathrm{t}} - 0.5t_{\mathrm{cell}}}{y_{\mathrm{t}} + 0.5t_{\mathrm{cell}}} \tag{3}$$

where, γ_{top} and γ_{bottom} are the shear strain of top and bottom position of the cell and y_{t} is a distance from centre of torsion to centre of cell. In the case of $y_{\mathrm{t}} = 0$, namely, centre of torsion is centre of cell, we obtain $\gamma_{\mathrm{bottom}} / \gamma_{\mathrm{top}} = -1$ from Equation (3). In this case, ideal torsion stimulus is applied to the cell. On the other hand, in the case of $y_{\mathrm{t}} >> t_{\mathrm{cell}}$, we obtain $\gamma_{\mathrm{bottom}} / \tau_{\mathrm{top}} \approx 1$ from Equation (3) and this equation indicates that the torsion stimulus of cell is totally same as shear stimulus. By designing y_{t} to near zero, we can apply more ideal torsion stimulus to the cell. By calculating an equation about total shear stress on cross section of a cell and a substrate, the value of y_{t} is obtained. The equation is expressed as,

$$G_{\mathrm{cell}}\theta\{(y_{\mathrm{t}}+0.5t_{\mathrm{cell}})^2-(y_{\mathrm{t}}-0.5t_{\mathrm{cell}})^2\} + G_{\mathrm{sub}}\theta(y_{\mathrm{t}}-0.5t_{\mathrm{cell}})^2 = G_{\mathrm{sub}}\theta(t_{\mathrm{sub}}-y_{\mathrm{t}}+0.5t_{\mathrm{cell}})^2 \tag{4}$$

where, G_{cell} and G_{sub} are shear modulus of the cell and the cell culture substrate.

To apply bending or torsion stimulus to cells, which cultured on sub-mm area, we utilized magnetic anisotropy. By patterning a soft magnetic material on beam, bending or torsion deformation can be obtained as shown in Figure 1. The inclination of the magnetic material is tuned by an external magnetic field applied perpendicularly to the microstructure. By calculating an equation about the magnetic torque and the torque generated by elastic deformation of the beam, magnitude of bending or torsion stimulus against an external magnetic field is obtained. In the case of rectangular beam, the equation is expressed as (Iwase et al., 2005; Roark et al., 1989),

$$\frac{(L_{\mathrm{sub}}/\rho)}{\cos(L_{\mathrm{sub}}/\rho)} = \frac{12M_{\mathrm{S}}}{E_{\mathrm{sub}}}\cdot\frac{V_{\mathrm{mag}}\cdot L_{\mathrm{sub}}}{W_{\mathrm{sub}}\cdot T_{\mathrm{sub}}^3}\cdot H_{\mathrm{ext}} \tag{5}$$

for bending stimulus and,

$$\frac{\eta\cdot L_{\mathrm{sub}}}{\cos(\eta\cdot L_{\mathrm{sub}})} = \frac{2M_{\mathrm{S}}}{32G_{\mathrm{sub}}}\cdot\frac{V_{\mathrm{mag}}\cdot L_{\mathrm{sub}}}{W_{\mathrm{sub}}\cdot T_{\mathrm{sub}}^3}\cdot H_{\mathrm{ext}} \tag{6}$$

for torsion stimulus. Where, η is an angle of torsion par unit length. V_{mag} and M_{s} are the volume and the saturation magnetization of magnetic material, respectively. L_{sub} and W_{sub} are length, and width of the cell culture substrate.

3 FABRICATION AND EXPERIMENTAL RESULT

We fabricated the microstructure. To decide detail dimensions, we targeted C2C12 as an example. We estimated the length of C2C12 on substrate about 100 µm. Therefore, the size of deforming substrate was set 100 µm in length and 50 µm in width. To fabricate the microstructure with size of micro order, we utilized silicon (Si) with sub-micron thickness as the substrate. Si is suited to microfabrication and fabricate free standing structure like Figure 1 easily. In the case of C2C12, assuming that the thickness is 1µm, on 270-nm Si substrate, y_{b} = 635 nm and y_{t} = 635 nm from Equations (2) and (4). Therefore, we obtain $\varepsilon_{\mathrm{bottom}}/\varepsilon_{\mathrm{top}} \approx 0.12$ and $\tau_{\mathrm{bottom}}/\tau_{\mathrm{top}} \approx 0.12$, respectively.

Fabrication process is shown in Figure 4. In the fabrication process, silicon-on-insulator (SOI) wafer was used. The thickness of the device Si layer, box layer, and handle Si layer of the SOI wafer were 270 nm, 200 nm, and 300 µm, respectively. First, nickel (Ni) layer with the thickness of 200 nm was sputtered on the SOI wafer as a magnetic material (Figure 4 (a)). Ni has a high saturation magnetization (M_{s} = 0.6 T). Then, the Ni layer was patterned using photolithography, and the device Si layer was etched with inductively-coupled-plasma reactive-ion etching (ICP-RIE) using the Ni layer as a mask (Figure 4 (b)). After that, the Ni layer was patterned again to remove the Ni layer from the surface of cell culture region and a negative photoresist (SU-8) was coated and patterned on the Ni layer (Figure 4 (c)). We used SU-8 to prevent a bending of beam, which is caused by a strain mismatch between the Si layer and the Ni layer. Then, the handle Si layer was patterned and etched with ICP-RIE (Figure 4 (d)). Finally, the structure was released by etching the box layer using HF vapour (Figure 4 (e)). Fabricated microstructure is shown in Figure 5. To compare with Equations (5) and (6), we fabricated some microstructures with different value of V_{mag} by designing the width of Ni layer.

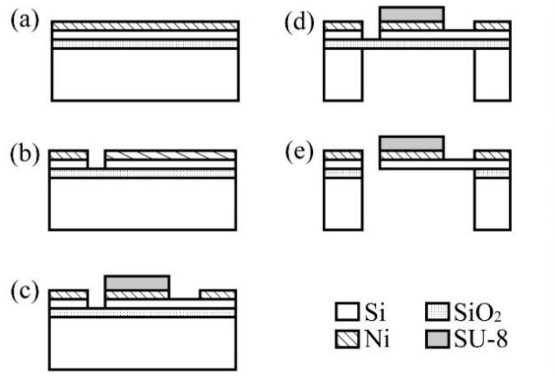

Figure 4: Cross-sectional diagrams of the fabrication process. (a) Sputtering Ni layer. (b) Etching Ni and device-Si layer. (c) Etching Ni layer and pattern SU-8 layer. (d) Etching handle-Si layer. (e) Etching SiO_2 layer.

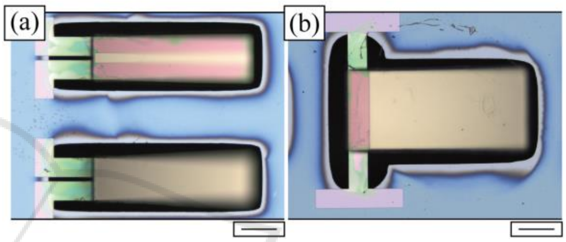

Figure 5: Images of fabricated microstructures. (a) The microstructure to apply bending stimulus. (b) The microstructure to apply torsion stimulus. Scar bars, (a) and (b): 100 µm.

We actuated the fabricated microstructures. Figure 6 (a) shows the image of the experimental

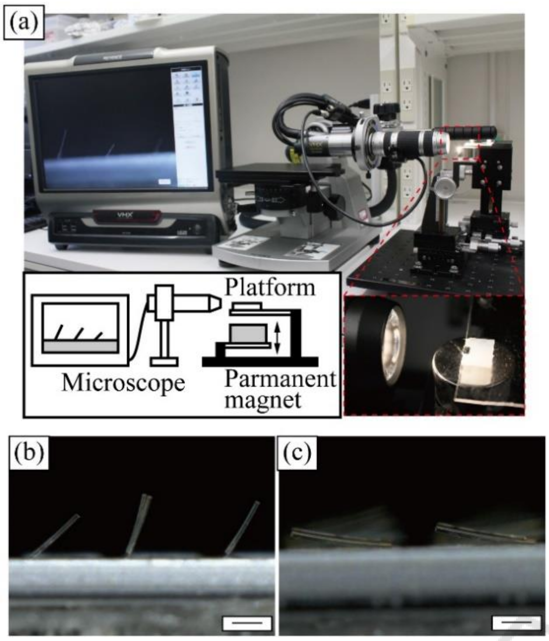

Figure 6: Images of experiment. (a) Experimental setup to measure inclination angles of microstructures. (b) Inclination of the microstructure for bending stimulus under H_{ext} = 343 kA/m. (c) Inclination of the microstructure for torsion stimulus under H_{ext} = 343 kA/m. Scar bars, (b) and (c): 100 µm.

setup. Using a permanent magnet, external magnetic field was perpendicularly applied to the microstructure and the value of H_{ext} was controlled by setting the distance from the microstructure to the permanent magnet. We observed and measured inclination angle of magnetic material from the side using microscope. Figures 6 (b) and (c) show the microscope image of the microstructure applied external magnetic field. We confirmed the actuation of the microstructure. As shown in Figures 6 (b) and (c), we can actuate some microstructures at the same time. These results indicate that we can apply some magnitudes of stimulus to cells in an external magnetic field at the sometime.

We calculated the deformation of substrate, i.e., ρ and η from the measured inclination angle. Figures 7 (a) and (b) show the relationship between H_{ext} and ρ, and the relationship between H_{ext} and η, respectively. Theoretical curves in Figures 7 (a) and (b) were obtained from Equations (5) and (6), respectively. Figure 7 (a) indicates that substrate totally deformed in theory for each microstructures, which have different value of V_{mag}. The minimum ρ was less than 100 µm, which is almost same size as C2C12. This result suggests that the microstructure might be possible to apply enough magnitude of bending stimulus to cells. On the other hand, from Figure 7 (b),

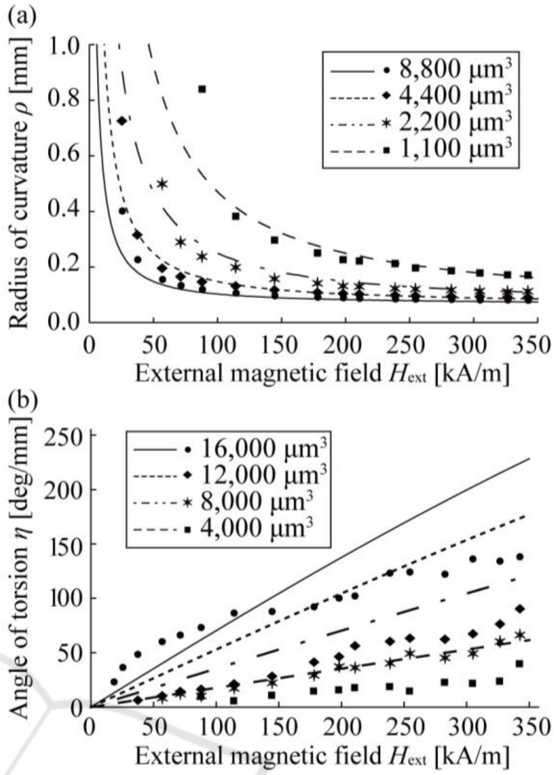

Figure 7: Experimental results. (a) Relationship between external magnetic field and radius of curvature ρ. (b) Relationship between external magnetic field and angle of torsion η.

the actuation of the microstructure for torsion stimulus was less than theoretical value for each microstructures. Causes of this result might be effect of thin rectangular-shaped cross section and/or effect of the centre position of torque in the microstructure. However, the maximum η was about 130 deg/mm. From this result, the microstructure might be able to apply torsion stimulus to the cell in some degree and increasing the V_{mag} or using other magnetic material, which has higher saturation magnetization, larger η might be able to apply to cell. By designing the dimensions of the cell culture microstructure, we can design the magnitude of bending or torsion stimulus.

4 CONCLUSIONS

We proposed the design of microstructures to apply bending or torsion stimulus to cultured cell by using magnetic anisotropy. As a result, we deformed substrate with 100 µm in radius of curvature at the least in the case of bending deformation and with 130 deg/mm angle of torsion at the most.

Firstly, we focused on the distance from centre of mechanical torque to centre of cell on substrate and we concluded that the distance is an important factor when we design bending or torsion stimulus. We fabricated the designed cell culture microstructure using 270-nm-thick silicon and actuated under an external magnetic field. We confirmed the actuation of fabricated microstructure.

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