Control of Single Cell Migration Using Caged Cell-Culturing Substrates

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1. Introduction

It is well known that cell migrations play a key role in the living systems. In order to understand the mechanism of cell migration qualitatively and quantitatively, control of single cell migration using caged cell-culturing substrates is one of the important and useful techniques. In this study, for the purpose of high-resolution control and observation of the cell migrations, an attempt was made to establish the fabrication process of micro-patterns on caged cell-culturing substrates using inverted microscope with high magnification objective lens. Moreover, the possibility of applications of cell migration control on the substrate was examined.

2. Materials and Methods

Figure 1 shows the schematic of the fabrication process of micro-patterns of fibronectin on caged cell-culturing substrates, where cells could easily adhere to. In this method, caged cell-culturing substrate coated with pluronic F108 was exposed to ultra violet (UV) lay under inverted microscope with 40x objective lens using Hg lump and DAPI excitation filter then incubated with fibronectin. In order to establish the formation of micro-patterns of fibronectin on the substrate, the adequate UV exposure time was obtained with the combination of fluorescence antibody technique. In addition, fibroblast-like Swiss 3T3 cells were cultured on the substrate to test whether their shape could be restricted to the micro-patterns on it.

3. Results and Discussion

First, the caged cell-culturing substrate was exposed to UV for various exposure duration and formation of micro-patterns was tested by fluorescence antibody technique. As a result, shown in Fig. 2, patterns in the immunofluorescence images were not evenly colored at shorter exposure times. This means

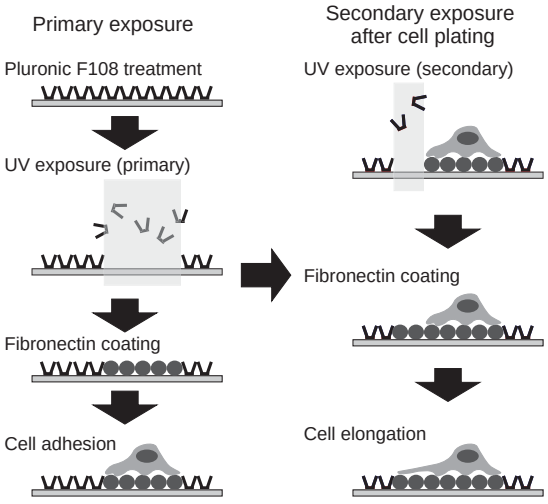

Fig. 1. Fabrication process of caged cell-culturing substrates and cell migration control.

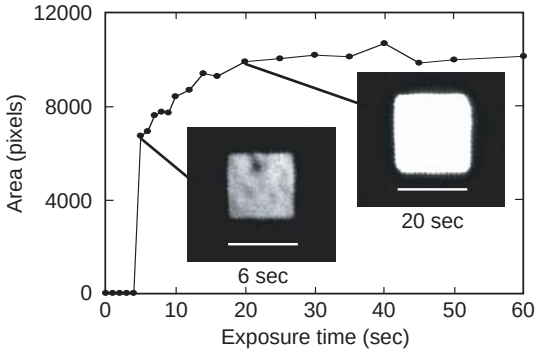

Fig. 2. Relationship between UV exposure time and absorbed are of fibronectin. Immunofluorescence images at exposure time of 6 sec. and 20 sec. are also shown in the figure. Scale bar: 20 μm.

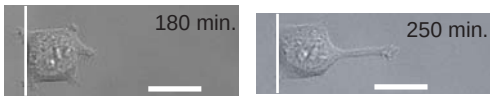

Fig. 3. Cell migration control experiment. The vertical line represents the initial position of the cell. Scale bar: 20 μm.

that fibronectin was not evenly absorbed in the exposed area on the substrate. The absorbed area of fibronectin increased with an increase in the exposure time, and it became almost constant after the exposure time of 20 seconds or longer when ND8 filter was inserted into the light path of the optical system.

Next, Swiss 3T3 cells were plated on the substrate on which micro-patterns of fibronectin were formed by the primary UV exposure. In case that the shape of the cell was successfully restricted to the area of the micro-pattern, the secondary UV exposure was tested. Cells 180 minutes and 250 minutes after the secondary UV exposure are shown in Fig. 2. The size of the micro-pattern by the primary UV exposure was 20 μm square, and that by the secondary UV exposure was 5×100 μm rectangle, respectively. We could confirm that the cell gradually elongates by forming lamellipodia along the micro-pattern by the secondary UV exposure.

4. Conclusions

In this study, the optical system for forming the micro-patterns of fibronectin on the caged cell-culturing substrates was constructed with high magnification objective lens, and the cell migration control on the substrate by the UV exposure was demonstrated. For the next step, using GFP-actin transfected cells and in combination with the laser confocal microscope, it could be possible to observe and analyze the time course of the cell migration associated with actin dynamics inside the cell.

Keywords: cell migration control, caged cell-culturing substrates, fluorescence antibody technique