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Photoactivation of a Substrate for Cell Adhesion under Standard Fluorescence Microscopes

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Dynamic control of cell adhesion onto substrates during cell cultivation is important for tissue engineering,[1,2] cell-based drug screening, and fundamental cellular studies.[3−5] To realize such dynamic control, several functional substrates have been developed, where cell adhesion can be controlled in response to external stimuli, such as heat,[6] voltage,[7−10] light,[11−13] and enzymatic activities.[14] However, their dynamically controllable regions are predetermined unless additional equipment is used to apply external stimuli.

Here, we show a new method for photoactivation of any regions of a substrate for cell adhesion under standard fluorescence microscopes. The strategy is based on a silane coupling agent having a photocleavable 2-nitrobenzyl group, (1-(2-nitrophenyl)ethyl-5-trichlorosilylpentanoate, NPE-TCSP),[15] and on two commonly used proteins that either prevent or promote cell adhesion. The procedure was as follows (Figure 1a). A glass coverslip chemically modified with NPE-TCSP was coated with bovine serum albumin (BSA) in order to make the surface inert to cell adhesion. The coverslip was then irradiated, causing the 2-nitrobenzyl group to be photocleaved and BSA to be dissociated from the surface. The addition of fibronectin, a protein that promotes cell adhesion by the integrin-mediated interaction, led to its selective adsorption at the vacancy of BSA, causing the region to become cell-adhesive. The 2-nitrobenzyl group is a common photocleavable protecting group for surface modification[16−18] or instantaneous release of physiologically active compounds,[19] which is cleaved by UV light (∼365 nm) from a mercury lamp equipped on standard fluorescence microscopes (Figure 1b). This feature allowed remote control of cell adhesion by simple operation of the widely available equipment.

We first evaluated whether cells selectively attached to the irradiated region on the substrate. A glass coverslip chemically modified with NPE-TCSP was coated with BSA and irradiated over a circular region (Figure 2a) for 2.5 min under a fluorescence microscope. This irradiation changed the contact angle of the substrate from 65 to 46°, and the change in wettability was nearly completed in this irradiation time (Figure S1 in the Supporting Information). After incubation of the substrate with fibronectin for 30 min, HEK293 cells selectively attached to the circular region in 2 h (Figure 2b). The attached cells were confined to grow within the region and became confluent at 63 h (Figure 2c). Almost the same results were obtained with other cell types, such as COS7 and NIH3T3 (Figure 2d,e). On the other hand, no cell-adhesive region was formed on a glass coverslip modified with a nonphotolabile 2-(carbomethoxy)ethyltrichlorosilane (Figure S2 in the Supporting Information). Moreover, the addition of fibronectin is essential for the formation of cell-adhesive spots because the cell adhesiveness to the irradiated area is very weak when fibronectin is omitted from the experimental procedure (Figure S3 in the Supporting Information).

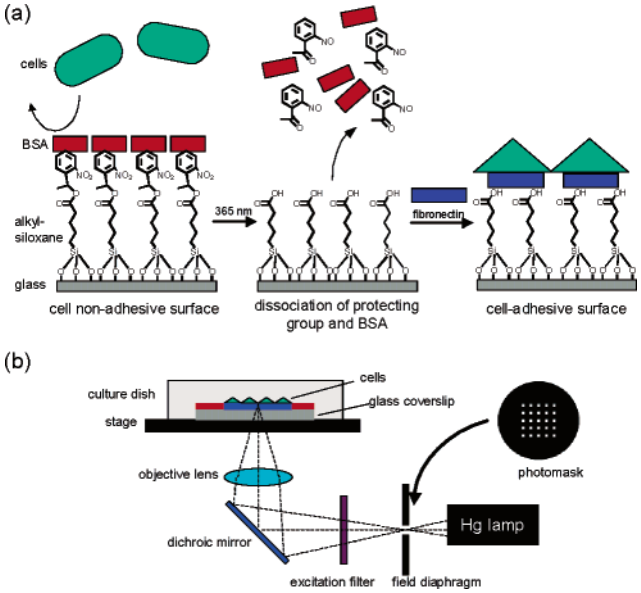

Figure 1. Schematic representations of photoactivation of cell adhesion. (a) UV-directed replacement of BSA with fibronectin changed the surface from a state that prevents cell adhesion to a state that promotes cell adhesion. (b) Experimental setup for irradiation under a standard fluorescence microscope.

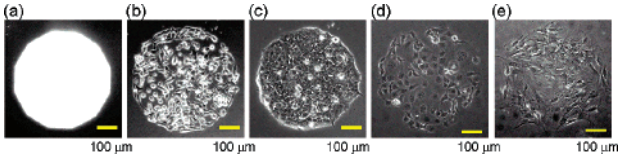

Figure 2. Formation of cell-adhesive spots in response to light. (a) UV-irradiated region. (b and c) Phase-contrast images of HEK293 cells 2 h (b) and 63 h (c) after seeding. (d and e) Phase-contrast images of COS7 cells (d) and NIH3T3 cells (e) 18 h after seeding.

Exchange of the surface protein from BSA to fibronectin was confirmed by immunofluorescence microscopy. BSA dissociated from the surface by UV irradiation, and the following addition of fibronectin resulted in its selective adsorption onto the irradiated region (Figure S4 in the Supporting Information). The possible mechanism for the dissociation of BSA is as follows. BSA is adsorbed on the surface in contact with the 2-nitrobenzyl groups. The photocleavage of the 2-nitrobenzyl groups allows BSA to diffuse into the bulk, accompanying the cleaved protecting groups. Moreover, the photocleavage reaction changes the chemical species from the hydrophobic nitrobenzyl ester to the polar carboxyl group at the very surface. This increase in the hydrophilicity may diminish

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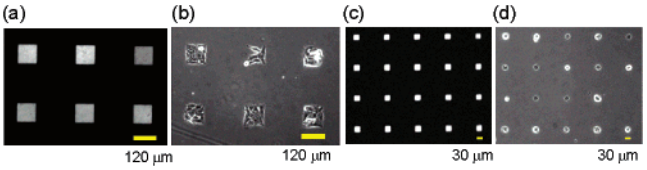

Figure 3. Cell attachment to array-patterned spots corresponding to photomasks inserted at the location of the field diaphragm of a fluorescence microscope. (a and c) Fluorescence images of glass coverslips painted with a fluorescent pen, which were irradiated using corresponding photomasks. (b and d) Phase-contrast images of HEK293 and COS7 attached on the substrates 18 and 2.5 h after seeding, respectively.

the affinity of BSA to the surface because the protein was presumably attached onto the surface via hydrophobic interaction.

To form more complex cell patterns, we inserted a photomask at the field diaphragm of the fluorescence microscope. In this configuration, light from the mercury lamp was projected to the sample in the same pattern as that of the inserted photomask (Figure 3a,c). The sizes of the irradiated regions were proportional to the sizes of the spots on the photomasks and were inversely proportional to the magnification of the objective lenses. On the basis of this relationship, a cell array of 120-μm-square spots was formed by using a photomask of an array pattern of 400-μm-square spots (Figure 3b). A photomask of 100-μm-square spots gave 30-μm-cell-adhesive spots, which is comparable to the size of single COS7 cells (Figure 3d). These results demonstrate that the present strategy offers a simple method for forming cell-adhesive spots, and their sizes can be reduced to the single cell level.

The most important point is that the present strategy allows formation of new cell-adhesive regions during cell cultivation. After culturing unstained single HEK293 cells for 1 day, other regions with a size comparable to that of single cells were irradiated in proximity to the cells attached in advance (Figure 4a). The medium was replaced with that containing fibronectin, and fluorescent HEK293 cells were seeded. The fluorescent single cells selectively attached to the irradiated regions (Figure 4a). To our knowledge, this is the first demonstration of positioning of single cells in proximity to cultivating single cells.

Moreover, two single cells of different types can be positioned in a similar way. In Figure 4b, a single fluorescent HEK293 cell was placed in proximity to a cultivating COS7 cell. Such positioning of two single cells of different types is physiologically interesting and will be useful for studying cell−cell interactions at the single cell level.

In summary, we have developed a simple method for spatiotemporal control of cell adhesion on a substrate modified with a photochemically active compound, whereon a protein preventing cell adhesion is replaced with one promoting cell adhesion in response to light. The present method makes it possible to form various cell patterns using standard fluorescence microscopes. Positioning of single cells in proximity to other single cells attached in advance is also achieved. The sizes, shapes, and locations of the cell-adhesive regions can be designed during the observation of cultivating cells, and thus the substrate will be a useful dynamic scaffold for studying cell−cell interactions.

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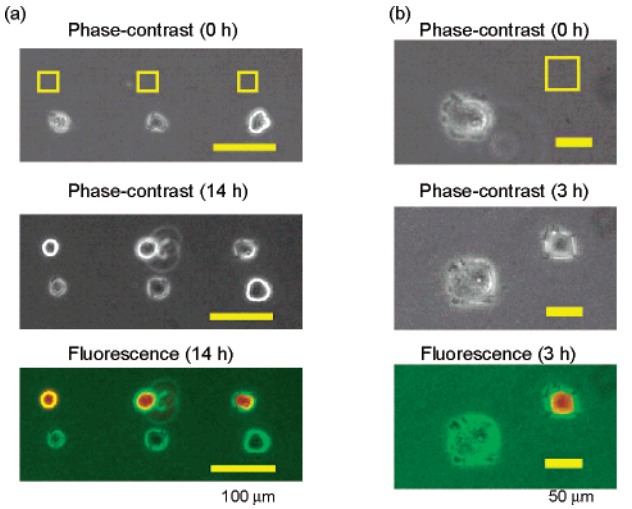

Figure 4. UV-directed positioning of single cells in proximity to cells attached in advance. (a and b) Phase-contrast and fluorescence images of substrates before and after seeding of fluorescent HEK293 cells where unstained HEK293 cells (a) or COS7 cells (b) were attached in advance. Second cells were stained with CellTracker Green CMFDA (Molecular Probes). Yellow squares represent the irradiated regions. A fluorescence image (red) is merged with the corresponding phase-contrast image (green).

Supporting Information Available: Experimental detail, contact angle measurement, immunofluorescence study (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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