Grafting Poly(ethylene glycol) to a Glass Surface via a Photocleavable Linker for Light-induced Cell Micropatterning and Cell Proliferation Control

Yukiko Kikuchi,[1,2] Jun Nakanishi,*[1,3] Hidekazu Nakayama,[1,3] Takahiro Shimizu,[4] Yutaro Yoshino,[4] Kazuo Yamaguchi,[4] Yasuhiko Yoshida,[2] and Yasuhiro Horiike[1]

[1]*World Premier International (WPI) Research Center Initiative, International Center for Materials Nanoarchitectonics (MANA) and National Institute for Materials Science (NIMS), 1-1 Namiki, Tsukuba 305-0044*

[2]*Graduate School of Engineering, Toyo University, 2100 Kujirai, Kawagoe 350-8585*

[3]*PRESTO, Japan Science and Technology Agency (JST), 4-1-8 Honcho, Kawaguchi 332-0012*

[4]*Department of Chemistry, Faculty of Science, Kanagawa University, 2946 Tsuchiya, Hiratsuka 259-1293*

(Received July 7, 2008; CL-080667; E-mail: NAKANISHI.Jun@nims.go.jp)

This paper describes a glass substrate having a photocleavable poly(ethylene glycol) (PEG) designed for light-induced cell micropatterning. The substrate changed from non-cell-adhesive to cell-adhesive by the photocleavage of PEG. Cellular patterns maintained for more than 17 days, and they were able to be changed to control cell migration and proliferation at multi- and single-cell levels by irradiating their adjacent regions during cell cultivation.

Functional substrates whose cell adhesiveness can be changed by an external stimulus during cell cultivation are useful scaffolds for cellular patterning in coculturing heterotypic cells and dynamic control of cell–substrate interactions.[1] Of these, those based on *glass* substrates are of practical importance in bioanalytical applications because they are compatible with fluorescence-imaging technology in a high-resolution inverted objective setup.[2] Several groups reported methods for controlling cell adhesiveness on glass surfaces.[3] However, these methods did not enable the analysis of biological processes that need long-term observation (>1 week), such as proliferation and differentiation,[4] mainly because the cell-repelling activity of the background was too weak to keep the cells attached within given regions for such a period of time. To overcome this limitation, in the present study, we developed a glass substrate where PEG was covalently bound to the surface via a photocleavable group (Figure 1). Since PEG blocks protein adsorption and cell adhesion,[5] we expected that its covalent linkage to a photocleavable group would create a surface that would resist cell adhesion for long term but would become cell-adhesive via the photocleavage of PEG. Although a similar substrate has been reported for protein patterning,[6] its PEG density is too sparse, and its PEG chain length is too short, to prevent cell adhesion for long periods of time, considering the study of Mougin and co-workers.[7]

The surface of a glass coverslip was silanized with 1-[3-methoxy-6-nitro-4-(3-trimethoxysilylpropyloxy)phenyl]ethyl *N*-succinimidyl carbonate (Figure 1a)[8] by refluxing in toluene solution for 48 h. This agent introduces a succinimidyl carbonate via a photocleavable 2-nitrobenzyl group, which undergoes reaction with primary amines and their release by irradiation (λ = 365 nm, Figure 1b). By reaction of the substrate with 0.5 mM PEG-amine (MW 10000–12000) in aqueous solution (0.1 M $NaHPO_4$, 0.4 M K_2SO_4, pH 7.1) at 37 °C for 18 h, the contact angle of the substrate changed from 67 to 47° [Figure 2a(II), exposure time 0]. This contact angle decrease reflects the introduction of hydrophilic PEG to the surface. Upon irradiation, the substrate became hydrophobic with a contact angle of 60° in 10 s [Figure 2a(II)]. On the other hand, substrate without PEG modification showed a contact angle decrease by irradiation, and the contact angle became 64° in 10 s [Figure 2a(I)]. About 90% of the immobilized PEG was photocleaved in this irradiation time (See Supporting Information).[11] In spite of this incomplete photocleavage reaction, the 10-s irradiation was sufficient to change the surface from non-cell-adhesive to cell-adhesive (vide infra). In accordance with these results, attenuated total reflection infrared (ATR-IR) spectra of the substrate exhibited an increase and decrease in peaks corresponding to the CH_2 stretching (2945 and 2894 cm^{-1}) of PEG and a broad OH stretching peak of adsorbed water during PEG immobilization and irradiation, respectively (Figure 2b). These results support the idea that PEG was immobilized on the glass surface and cleaved in response to irradiation as shown in Figure 1.

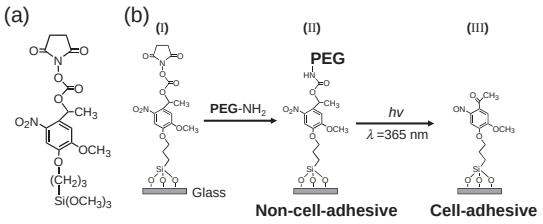

Figure 1. Photoactivatable cell-culturing substrate: (a) Silane-coupling used in this study. (b) Immobilization (I → II) and photorelease (II → III) of PEG on the glass substrate modified with the reagent shown in (a). The substrate changed from non-cell-adhesive to cell-adhesive by irradiation.

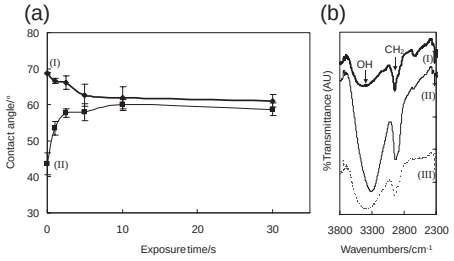

Figure 2. Surface characterization: (a) Contact angle changes of (I) silanized and (II) PEGylated surfaces against UV irradiation (UV power 1 W/cm^2). (b) ATR-IR spectrum of each of the surfaces shown in Figure 1b.

Next, we examined photoinduced cell patterning onto the

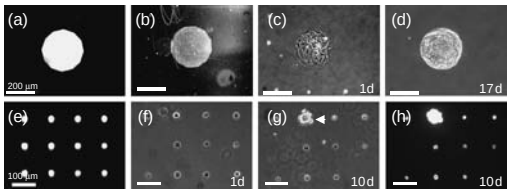

Figure 3. Cell patterning on the photoactivatable substrate: (a and e) Irradiation patterns. (b) An immunofluorescence image of vitronectin. (c, d, f, and g) Phase contrast images of NIH3T3 cells after indicated days. An arrowhead in (g) shows the cells that continued proliferation. (h) A fluorescence image of living cells stained with the LIVE/DEAD assay kit (Invitrogen).

PEG-grafted substrate and monitored cell proliferation thereon. Upon irradiation of the substrate at a circular region of 80000 μm^2 and immersion of the substrate in a serum-containing medium for 2 h, the irradiated region permitted adsorption of serum proteins, including vitronectin (Figures 3a and 3b). Since vitronectin is one of the extracellular matrix proteins that promote cell adhesion,[9] NIH3T3 cells seeded on the substrate formed a similar circular pattern (Figure 3c). The cells proliferated on the spot, remaining confined within the region for more than 17 days (Figure 3d). On the other hand, when the irradiating region was reduced to 400 μm^2, which is slightly smaller than the size of a single NIH3T3 cell, the array spots were occupied by single cells (Figures 3e and 3f). After a 10-day culture, about 85% of cells remained in singles, whereas the rest (15%) proliferated to form cell spheroids on the array spots (Figure 3g, arrowhead). Most of the cells were alive, which was confirmed by a LIVE/DEAD assay (Figure 3h). These results were consistent with a previous report showing reduced proliferation in cells with less spreading.[10] Such investigation was difficult to conduct in substrates where pattern failure occurred in less than one week.

The most important feature of the present substrate is that the cellular pattern can be changed during cell cultivation by in situ irradiation of the substrate. This feature was verified by inducing cell migration and proliferation. NIH3T3 cells were firstly placed in a circular spot, and then another circular region alongside the initial spot was irradiated (Figure 4a). The cells migrated onto the newly formed cell-adhesive region and proliferated thereon. After culture for 4 days, the cells became confluent and formed a dumbbell-like pattern (Figure 4b). As demonstrated in Figure 3d, the cells remained within the initial circular spot for more than 17 days, when such secondary irradiation was not applied to the substrate. Therefore, the secondary irradiation is essential for the induction of cell migration and proliferation. Furthermore, UV irradiation itself had little cytotoxity on adjacent cells because most of them were alive with migration activity in our previous study.[3b] The same experiments were repeated at the single-cell level. A single-cell array was formed as in Figure 3f, and a circular region of 8000 μm^2 adjacent to an arbitrarily selected cell was irradiated (Figure 4c). The irradiation switched on cell spreading and proliferation, resulting in a small colony originating from the single cell on the secondary irradiated regions (Figures 4d–4f). Nonirradiated cells remained on the original array spot, as shown in Figure 3g. Therefore, the induction of cell proliferation at the single-cell level will be useful for the selective proliferation of a specific clone from a heterotypic cell mixture.

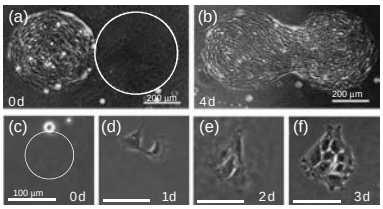

Figure 4. Induction of cell migration and proliferation at (a and b) multi- and (c–f) single-cell levels. Phase contrast images of NIH3T3 cells after culturing for indicated days are shown. White circles represent irradiated regions.

In summary, we have developed a photoactivatable glass substrate having a photocleavable PEG for cell patterning. This substrate allowed long-term cellular patterning (>17 days) as well as in situ alteration of the cellular patterns during cell cultivation. As an example of its application, we demonstrated induction of cell migration and proliferation in multi- and single-cell levels. This substrate is useful not only for the control of cell proliferation but also for engineering microenvironments (e.g., cell–cell contact and cell spreading) for stem cells since their activities and lineage commitment are highly dependent on such cellular microenvironments.

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References and Notes

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11 Supporting Information including magnified view of Figures 2b, 3, and wide-view images of Figures 4c–4f are available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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