Cell Patterning

DOI: 10.1002/anie.201106106

Photocontrollable Dynamic Micropatterning of Non-adherent Mammalian Cells Using a Photocleavable Poly(ethylene glycol) Lipid**

Satoshi Yamaguchi, Shinya Yamahira, Kyoko Kikuchi, Kimio Sumaru, Toshiyuki Kanamori, and Teruyuki Nagamune**

Cell micropatterning has become an important technology for a wide variety of applications, ranging from tissue engineering[1] and cell microarrays[2] to fundamental studies in cell biology.[3] In addition to conventional patterning methods, such as photolithography, soft lithography, and inkjet printing, patterning methods with dynamic substrates, in which cell adhesive properties can be changed by an external stimulus, such as heat,[4] voltage,[5] and light,[6] at any desired position and any point in time, are currently the focus of many studies. These spatiotemporal patterning methods can easily construct patterns of multiple cell lines and be useful for analyzing dynamic cellular activity.[7] In particular, in contrast to heat and voltage, light can be readily applied to anywhere in transmissive spaces with high spatial and temporal resolution, and light-induced fine control of biomolecules or living cells has been widely reported,[6,8] even at a single-molecular level.[8b] Therefore, cell patterning with light-responsive substrates potentially offers a practical tool for biologists. However, almost all reported cell micropatterning methods have a major limitation in target cells. In conventional methods, the adhesiveness of cells is used to attach them onto bare or ligand-coated surfaces. Therefore, the existing methods cannot be applied to non- or weakly adherent cells, which include blood cells (especially immunocytes), some cancer cells, and stem cells. These cell lines are important as research targets in biological and medical fields, and for this reason expansion of an applicable range of current micropatterning methods to non-adherent cells is an important challenge.

We report herein a light-induced in situ cell micropatterning method that can be applied to non-adherent cells. Recently, we reported a cell patterning method for non-adherent cells using a cell membrane binding reagent consisting of poly(ethylene glycol) (PEG) and an oleyl group.[9] This compound can bind to any type of cell without cytotoxicity, because the oleyl moiety can be inserted into ubiquitous lipid bilayer membranes in a noncovalent manner.[9a] In the current study, a photocleavable PEG-lipid was newly designed and synthesized for light-induced cell patterning, and then cell immobilization on the substrate coated with the photocleavable PEG-lipid was confirmed to be regulated by the dose of light exposure. Moreover, the present method allows the preparation of arbitrary and fine patterns of non-adherent cells. Furthermore, the cell micropattern on the present light-responsive substrate can be altered by light irradiation at a desired point in time.

First, we designed and synthesized a photocleavable PEG-lipid. In our design, a photocleavable unit was incorporated between the PEG and oleyl moieties, and at the opposite end of the PEG segment an amino-reactive ester group was added for attachment onto the substrate through an amide coupling reaction (Figure 1a). After coating, the oleyl moieties are expected to be exposed and to anchor living cells (Figure 1b). Moreover, this molecule can be cleaved by irradiation, and then the PEG moiety is exposed at the light-irradiated area (Figure 1b). It has been reported that a PEG-coated surface inhibits cell adhesion.[10] Therefore, cell-adhesive and non-adhesive surfaces were expected to be prepared by light irradiation (Figure 1b). A photocleavable PEG-lipid was synthesized from a commercially available *o*-nitrobenzyl photocleavable linker[11] and characterized by using standard methods (see the Supporting Information). The photolytic property of the PEG-lipid in solution was confirmed by means of ^{1}H NMR spectroscopy after irradiation with UV light at 365 nm (see the Supporting Information). Furthermore, substrate coating by the photocleavable PEG-lipid and its photolytic degradation on substrates were confirmed by water-drop contact-angle measurements (see the Supporting Information).

Cell immobilization on a dish coated with photocleavable PEG-lipid was investigated by fluorescent microscopic observation before and after irradiating the dish with UV light (365 nm). On the nonirradiated dishes, a non-adherent cell line, the human hematopoietic cell line BaF3, was densely

[*] Dr. S. Yamaguchi, Prof. T. Nagamune
Department of Chemistry and Biotechnology
School of Engineering, The University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656 (Japan)
E-mail: yamaguchi@bio.t.u-tokyo.ac.jp
nagamune@bioeng.t.u-tokyo.ac.jp
S. Yamahira, Prof. T. Nagamune
Department of Bioengineering
School of Engineering, The University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656 (Japan)
K. Kikuchi, K. Sumaru, T. Kanamori
Research Center for Stem Cell Engineering
National Institute of Advanced Industrial Science and Technology
Tsukuba Central 5th, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8565 (Japan)

[**] This work was supported by the Center for NanoBio Integration in The University of Tokyo.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201106106.

© 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

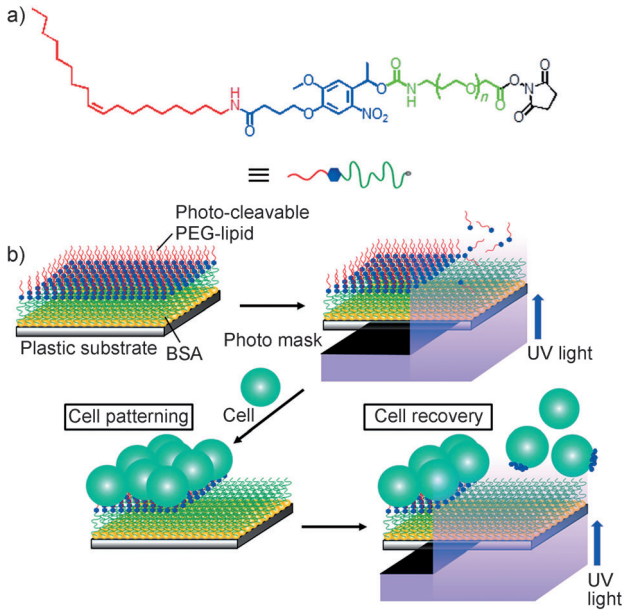

Figure 1. Schematic diagram of light-controllable cell micropatterning and recovery on a substrate coated with photocleavable PEG-lipid. a) The chemical structure of a photocleavable PEG-lipid consisting of an oleyl group (red), an *o*-nitrobenzyl photocleavable linker (blue), a PEG chain (green), and an amino-reactive *N*-hydroxysuccinimide ester group (black). b) The substrate is coated with bovine serum albumine (BSA) and the photocleavable PEG-lipid, and then the PEG moiety is exposed to light in the unmasked area. When the cell suspension is placed on this substrate and excess rinsed away, living cells are patterned on the nonirradiated area through an interaction between cell membranes and the exposed oleyl groups. The immobilized cells are selectively recovered by light irradiation.

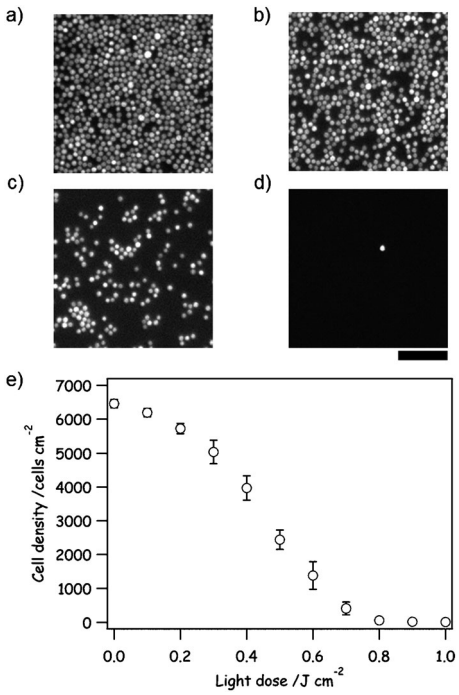

Figure 2. Light-induced inhibition of cell immobilization on a substrate coated with photocleavable PEG-lipid. Fluorescent micrographs of immobilized living cells on a substrate irradiated with UV light of a) 0, b) 0.3, c) 0.6, and d) 1.0 J cm^{-2}. Enhanced green fluorescent protein (EGFP)-expressing BaF3 cells were used in this experiment. The scale bar represents 100 μm. e) Relationship between the density of immobilized cells and the light dose.

immobilized by placing a cell suspension (6.1×10^6 cells mL^{-1}) on the surface for 15 min and rinsing with PBS (phosphate-buffered saline, Figure 2a). On the irradiated dishes the density of immobilized cells decreased corresponding to the dose increase of UV light (Figure 2b,c,d). To demonstrate the versatility of the present method, other non-adherent or adherent cells, such as the human leukemic cell line K562 and cervical carcinoma cell line HeLa, were immobilized on the same substrates, and we found that the cell densities similarly depended on the light dose. Figure 2e shows the relationship between the density of immobilized cells and the light dose in detail. A light dose above 0.8 J cm^{-2} was required to inhibit cell immobilization. This result indicates that cell immobilization can be controlled by irradiating the PEG-lipid-coated substrate with a low dose of light. Furthermore, almost all immobilized cells (98%) were alive on the present substrate after incubation for one day, and cell growth was also confirmed (see the Supporting Information). This result indicates that the PEG-lipid-coated substrate is biocompatible.

On the present substrates coated with photocleavable PEG-lipid, cell patterns were successfully obtained by irradiating with UV light (1 J cm^{-2}) in an arbitrary pattern before exposing the substrate to a cell suspension. We employed a light-irradiation system based on a computer-controllable microprojection unit in an optical system using a microscope.[12] Figure 3a shows the microscopic image of the irradiated pattern. A highly contrasted cell pattern, in which cells were immobilized only on the nonirradiated region, was obtained as expected (Figure 3b). This result strongly indicated that cells were firmly captured on the nonirradiated region, which was attributed to interaction between cell membranes and the oleyl moiety of the photocleavable PEG-lipid. On the irradiated region, where the oleyl group was removed through the cleavage of a nitrobenzyl group, cells did not interact or weakly interacted with the exposed PEG moiety and then they were washed off by rinsing. The present finding of a high-contrast cell pattern is thought to be derived from an adequate difference between the strength of the interaction of the cell surface with the nonirradiated and irradiated surfaces.

To evaluate the precision limit of the present patterning method, we attempted to prepare cell arrays with various sizes of cell spots (Figure 3c,d,e). The substrates were irradiated with inverted dot patterns of light with various dot diameters (100, 50, and 25 μm). As a result, cell arrays consisting of uniformly sized spots were obtained corresponding to the diameters of the irradiation pattern, and the precision of a single cell size was achieved by irradiating with the finest pattern of light (Figure 3e). Consequently, by high-resolution irradiation an extremely precise pattern of non-adherent cells can be prepared by the present patterning method.

© 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

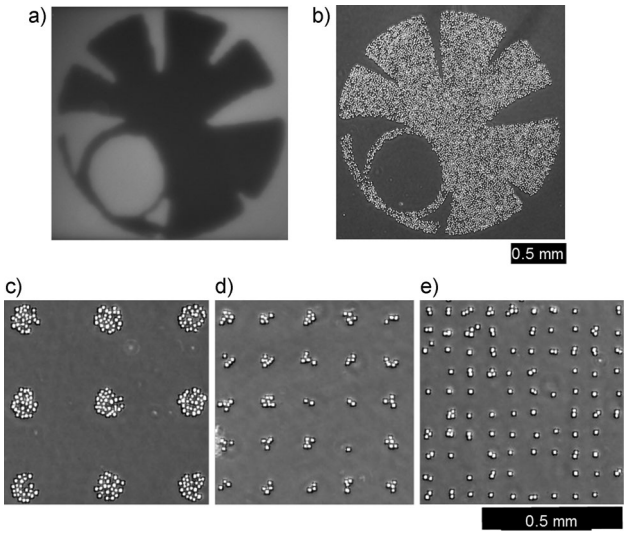

Figure 3. Micrographs of cell patterns on a substrate coated with photocleavable PEG-lipid. a) Micrograph of the substrate from irradiating a pattern with UV light (365 nm). At the irradiated area of the projected pattern, the light was reflected on the substrate and detected as a blue pattern (light area). b) A phase-contrast micrograph of the cell pattern on the substrate irradiated as shown in (a). c–e) Phase-contrast micrographs of a cell microarray on a substrate irradiated by inverted dot patterns of UV light (365 nm) with a diameter of c) 100, d) 50, and e) 25 μm.

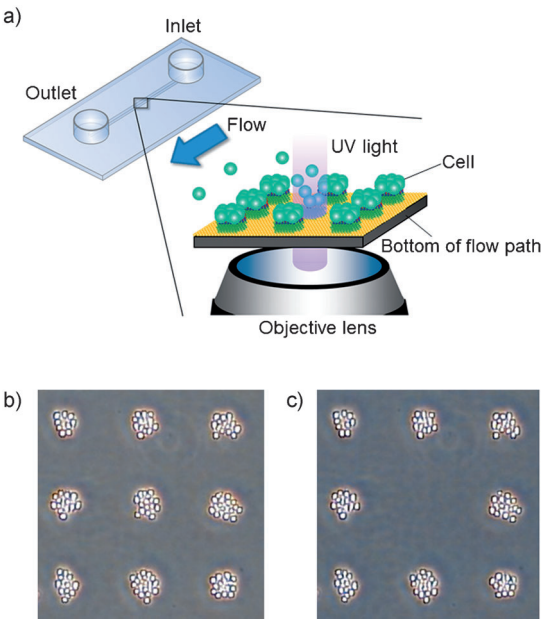

Figure 4. Light-induced cell detachment from a cell microarray on a substrate coated with photocleavable PEG-lipid. a) Schematic illustration of light-induced cell detachment in a microfluidic device. b) A phase-contrast micrograph of the microarray of BaF3 cells before and c) after light-irradiation at 5.2 J cm^{-2}. The linear flow rate was 20 cm s^{-1}.

Finally, to demonstrate that the immobilized cell pattern can be spatiotemporally altered by light irradiation, we attempted to detach immobilized cells from a targeted area by light irradiation. The fluid shear stress on irradiated cells, which was generated by rinsing treatment after light irradiation, is critical for the detachment of cells from the substrate.[13] Therefore, to precisely control the shear stress, the cell pattern was constructed on a microchannel in a microfluidic device, and the immobilized cells were irradiated under constant shear stress by microfluidic control (Figure 4a). A cell microarray with a spot size of approximately 100 μm was prepared on the PEG-lipid-coated microchannel by irradiation with an inverted dot pattern of light and subsequent introduction of cell suspension into the microchannel. At a linear flow rate of 20 cm s^{-1} we found that the cells were tightly immobilized on the nonirradiated dot spots, whereas no cells remained on the irradiated area (Figure 4b). At the same flow rate, a target cell spot was then continuously irradiated with a spot of light (365 nm, 84 mW cm^{-2}) until all cells in the target spot were detached. During light irradiation, the cells on the irradiated spot were detached one by one and swept away in the flow (see Movie S1 in the Supporting Information). After irradiation at 5.2 J cm^{-2}, all cells at the irradiated spot disappeared; however, almost all cells remained at the nonirradiated spots (Figure 4c). This light-irradiation experiment was also performed at 20 other spots on the same microchannel or other microchannels. We observed that all cells in the irradiated spots could be reproducibly detached by light irradiation below 3.2 J cm^{-2}, while 94.8% of the immobilized cells at the nonirradiated spots ($n = 69$) remained. Therefore, the immobilized cells were selectively detached only from the irradiated area. We assessed the viability of the photoirradiated-detached cells by trypan blue exclusion. The viability of photoirradiated cells ((90 ± 6.4) %) was almost the same as that of cells recovered from a nonirradiated surface ((90 ± 6.7) %; see the Supporting Information). These results show that, in principle, immobilized cells can be selectively collected from the present substrate by photoirradiation without cell damage.

Cell-sorting technology is a necessary tool for screening and purifying target cells. However, in existing methods based on flow cytometry, cells are evaluated only by the intensity of fluorescence derived from a probe or marker gene, and therefore false positive or negative cells are often sorted because of their background fluorescence. Recently, a single-cell microarray has been reported to enable high-throughput screening of target cells with a higher accuracy, because the same single cell can be analyzed before and after stimulation or staining to cancel the background noise in probe signals.[14] Moreover, on a single-cell microarray, high-content screening can be performed by observing the changes in cell morphology and cellular localization of specific proteins; such changes cannot be detected by a flow cytometer. Although further optimization of cell patterning and recovery conditions is required, the present method using a photocleavable PEG-lipid is a useful and promising tool for screening and retrieval of target cells at the single-cell level. Furthermore, in principle, the present micropatterning technology can be applied to liposomes, exosomes, bacteria, and others that are coated with a lipid bilayer membrane.

In summary, we have developed a light-controllable micropatterning method for non-adherent cells by using a

 © 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

photocleavable PEG-lipid. Cell immobilization on a substrate coated with a photocleavable PEG-lipid could be controlled by the dose of UV light and perfectly inhibited above 0.8 $J\,cm^{-2}$. On the present photocontrollable substrates, a highly contrasted cell pattern with the precision of a single cell size was easily and rapidly obtained without cell damage by irradiating an arbitrary pattern using a computer-controllable light-irradiation system. Furthermore, light-induced cell detachment from the substrate was successfully accomplished, and this result indicated that target non-adherent cells can be selectively recovered by light irradiation. Therefore, the present cell patterning method is promising for applications to fundamental studies of non-adherent cells at the single-cell level and to cell screening and sorting technologies.

Received: August 29, 2011
Published online: November 18, 2011

Keywords: cell adhesion · cell patterning · microarrays · microfluids · photolysis

[1] a) J. Lahann, M. Bacells, T. Rondon, J. Lee, I. S. Choi, K. F. Jensen, R. Langer, *Langmuir* **2002**, *18*, 3632 – 3638; b) A. Rosenthal, A. Macdonald, J. Voldman, *Biomaterials* **2007**, *28*, 3208 – 3216.

[2] a) R. Z. Wu, S. N. Bailey, D. M. Sabatini, *Trends Cell Biol.* **2002**, *12*, 485 – 488; b) D. Falconnet, G. Csucs, H. M. Grandin, M. Textor, *Biomaterials* **2006**, *27*, 3044 – 3063; c) A. P. Quist, S. Oscarsson, *Expert Opin. Drug Discovery* **2010**, *5*, 569 – 581.

[3] a) M. N. Yousaf, B. T. Houseman, M. Mrksich, *Angew. Chem.* **2001**, *113*, 1127 – 1130; *Angew. Chem. Int. Ed.* **2001**, *40*, 1093 – 1096; b) A. Brock, E. Chang, C. C. Ho, P. LeDuc, X. Jiang, G. M. Whitesides, D. E. Ingber, *Langmuir* **2003**, *19*, 1611 – 1617; c) M. Okochi, S. Takano, Y. Isaji, T. Senga, M. Hamaguchi, H. Honda, *Lab Chip* **2009**, *9*, 3378 – 3384.

[4] M. Yamato, C. Konno, M. Utsumi, A. Kikuchi, T. Okano, *Biomaterials* **2002**, *23*, 561 – 567.

[5] a) M. N. Yousaf, B. T. Houseman, M. Mrksich, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 5992 – 5996; b) W. S. Yeo, M. N. Yousaf, M. Mrksich, *J. Am. Chem. Soc.* **2003**, *125*, 14994 – 14995.

[6] a) J. Edahiro, K. Sumaru, K. Tada, T. Ohi, T. Takagi, M. Kameda, T. Shinbo, T. Kanamori, Y. Yoshimi, *Biomacromolecules* **2005**, *6*, 970 – 974; b) J. Nakanishi, Y. Kikuchi, T. Takarada, H. Nakayama, K. Yamaguchi, M. Maeda, *J. Am. Chem. Soc.* **2004**, *126*, 16314 – 16315; c) J. Nakanishi, Y. Kikuchi, S. Inoue, K. Yamaguchi, T. Takarada, M. Maeda, *J. Am. Chem. Soc.* **2007**, *129*, 6694 – 6695; d) M. Wirkner, J. M. Alonso, V. Maus, M. Salierno, T. T. Lee, A. J. García, A. del Campo, *Adv. Mater.* **2011**, *23*, 3907 – 3910; e) G. Pasparakis, T. Manouras, A. Selimis, M. Vamvakaki, P. Argitis, *Angew. Chem.* **2011**, *123*, 4228 – 4231; *Angew. Chem. Int. Ed.* **2011**, *50*, 4142 – 4145.

[7] J. Nakanishi, T. Takarada, K. Yamaguchi, M. Maeda, *Anal. Sci.* **2008**, *24*, 67 – 72.

[8] a) G. Mayer, A. Heckel, *Angew. Chem.* **2006**, *118*, 5020 – 5042; *Angew. Chem. Int. Ed.* **2006**, *45*, 4900 – 4921; b) S. Matsumoto, S. Yamaguchi, S. Ueno, H. Komatsu, M. Ikeda, K. Ishizuka, Y. Iko, K. V. Tabata, H. Aoki, S. Ito, H. Noji, I. Hamachi, *Chem. Eur. J.* **2008**, *14*, 3977 – 3986; c) S. Hashiro, S. Tsukiji, T. Nagamune, *J. Am. Chem. Soc.* **2009**, *131*, 13568 – 13569; d) S. Yamaguchi, Y. Chen, S. Nakajima, T. Furuta, T. Nagamune, *Chem. Commun.* **2010**, *46*, 2244 – 2246.

[9] a) K. Kato, K. Umezawa, D. P. Funeriu, M. Miyake, J. Miyake, T. Nagamune, *BioTechniques* **2003**, *35*, 1014 – 1021; b) K. Kato, C. Itoh, T. Yasukouchi, T. Nagamune, *Biotechnol. Prog.* **2004**, *20*, 897 – 904.

[10] H. Du, P. Chandaroy, S. W. Hui, *Biochim. Biophys. Acta Biomembr.* **1997**, *1326*, 236 – 248.

[11] C. P. Holmes, *J. Org. Chem.* **1997**, *62*, 2370 – 2380.

[12] K. Sumaru, J. Edahiro, Y. Ooshima, T. Kanamori, T. Shinbo, *Biosens. Bioelectron.* **2007**, *22*, 2356 – 2359.

[13] J. Edahiro, K. Sumaru, Y. Ooshima, T. Kanamori, *Biotechnol. Bioeng.* **2009**, *102*, 1278 – 1282.

[14] S. Yamamura, H. Kishi, Y. Tokimitsu, S. Kondo, R. Honda, S. R. Rao, M. Omori, E. Tamiya, A. Muraguchi, *Anal. Chem.* **2005**, *77*, 8050 – 8056.

 © 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim