A PHOTODEGRADABLE HYDROGEL SHEET FOR MICROSCALE OPTICAL CONTROL OF CELL ADHESION AND DETACHMENT

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ABSTRACT

Control of cell adhesion and detachment on the micrometer scale is an important issue for understanding cell-substrate or cell-cell interaction. We synthesized a photocleavable crosslinker, which composed of hydrophilic poly(ethylene glycol) (PEG), photocleavable nitrobenzyl groups and amine-reactive *N*-hydroxysuccinimide ester groups. The photocleavable crosslinker forms a photodegradable hydrogel sheet in culture dishes through reaction with amino-terminated four-arm PEG. The formed hydrogel sheet was degraded upon UV light irradiation. Optical two-dimensional cell manipulation methods, including cell micropatterning and local cell detachment, were successfully demonstrated by means of computer-controlled micropatterned light irradiation on this photodegradable hydrogel sheet.

KEYWORDS

Photodegradable hydrogel, Cell adhesion, Cell micropatterning, Optical cell manipulation, Local cell detachment

INTRODUCTION

Light irradiation can be applied to an object locally and instantaneously in a non-contact manner, and therefore light irradiation can be used as a more versatile micrometer-scale cell manipulation technique than many other techniques, including microcontact printing,[1, 2] inkjet printing,[3, 4] microfluidic patterning,[5-7] electrochemical patterning[8, 9] and optical tweezers.[10] Recently, we and other research groups have developed cell micropatterning methods using photoresponsive surfaces.[11-16] In these methods, the thin layers (typically nanometer scale) of photoresponsive compounds, containing nitrobenzyl groups, azobenzenes, or spirobenzopyrenes, are formed on the substrates. The chemical properties of the photoresponsive compounds change in response to light irradiation, and cells attaches to the irradiated area. Combining with micropatterned light irradiation system, stepwise micropatterning of multiple cells is also achieved.[17, 18]

However, there have been no reports to date that describe techniques to induce cell detachment from the photoresponsive surfaces by light irradiation; the extracellular matrix (ECM) produced by the attached cells strongly support the cell adhesion and the approach to use the nanometer-scale layer of the photoresponsive compounds does not work to induce cell detachment. Currently, local cell detachment in response to light irradiation is desired to realize versatile spatiotemporal cell manipulation techniques, such as cell separation, and dynamic rearrangement of micropattern.

In this study, we formed a micrometer-scale layer of a photodegradable hydrogel sheet on the surface of culture dishes using a newly synthesized

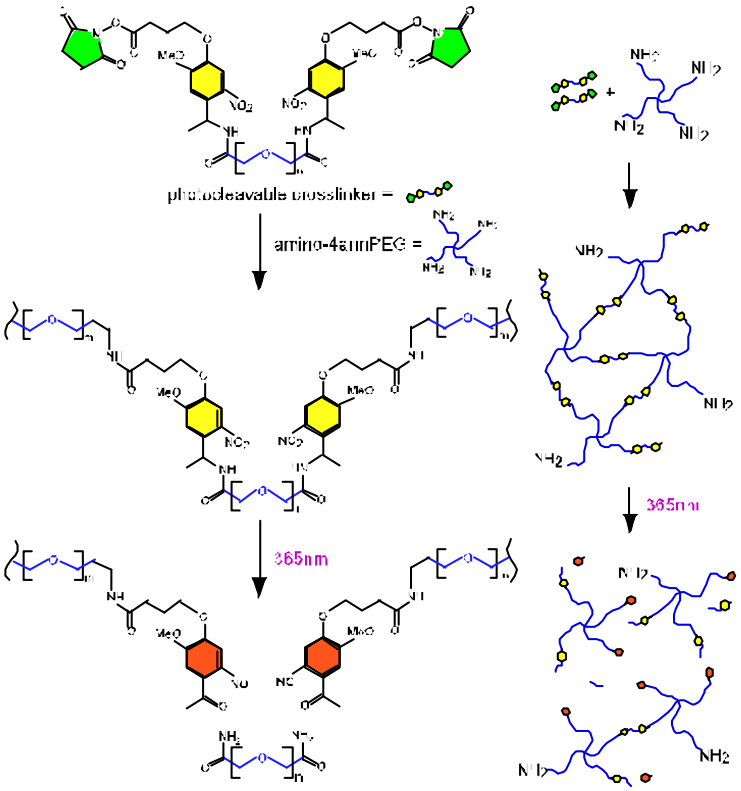

Figure 1. Synthesis and degradation of the photodegradable hydrogel.

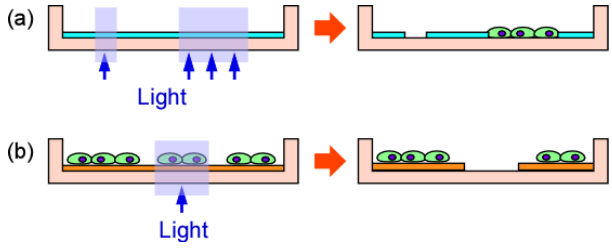

Figure 2. Schematic of optical cell manipulation on the photodegradable hydrogel sheet. (a) Cell micropatterning by means of micropatterned light irradiation on a cell-nonadhesive photodegradable hydrogel sheet. (b) Collection of target cells by means of local light irradiation on a cell-adhesive photodegradable hydrogel sheet.

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photocleavable crosslinker. The photocleavable crosslinker also enabled modification of the biologically inactive photodegradable hydrogel with a functional ECM protein. By this approach, we applied the photodegradable hydrogel both to cell micropatterning and to local cell detachment.

EXPERIMENT

We synthesized a photocleavable crosslinker that composed of the following functional groups (Figure 1): (i) poly(ethylenegrycole) (MW: 560) as a water-soluble main polymer chain; (ii) nitrobenzyl groups, which are cleavable in response to UV light irradiation; and (iii) *N*-hydroxysuccinimide (NHS) ester groups, which react with primary amine groups and form peptide bonds under physiological conditions. The synthesized photocleavable crosslinker forms photodegradable hydrogels through its reaction with a variety of polymers possessing amino or hydroxyl groups. In a typical preparation, photodegradable hydrogels were formed by reacting the photocleavable crosslinker with amino-terminated four-arm-PEG (amino-4armPEG; MW: 10,000 Da) at a molar ratio of 2:1 (Figure 1).

Figure 2 show the mechanism of cell micropatterning and local cell detachment by means of micropatterned light irradiation of the photodegradable hydrogel sheet. For cell micropatterning (Fig. 2a), a cell non-adhesive photodegradable hydrogel sheet was formed in a tissue culture polystyrene (TCPS) dish. After micro-patterned light irradiation, the cells adhered to the exposed TCPS surface in the irradiated area. For local cell detachment (Fig. 2b), the surface of the photodegradable hydrogel was modified with fibronectin. After the cells attachment on the fibronectin-modified photodegradable hydrogel sheet and local light irradiation, the cells in the irradiated area detached from the hydrogel surface (Figure 2b).

RESULTS AND DISCUSSION

Absorbance spectra of the photocleavable crosslinkers were measured under UV light irradiation (Fig. 3). Cleavage of the photocleavable crosslinker occurred after 40 s of irradiation (600 mJ/cm^2) was evidenced by an increase in the absorbance at 390 nm. Further degradation of the photocleavable crosslinker was observed after 600 s of irradiation (9000 mJ/cm^2), as evidenced by a decrease in absorbance at 365 and 390 nm.

The formed photodegradable hydrogels were swelled in aqueous solution but not solubilized in water (Figure 4, left), and were degraded into a yellow solution upon UV light irradiation (Figure 4, right). The UV light irradiation induced the cleavage of the nitrobenzyl group; water soluble compounds, four-arm-PEG derivatives and PEG derivatives, were formed.

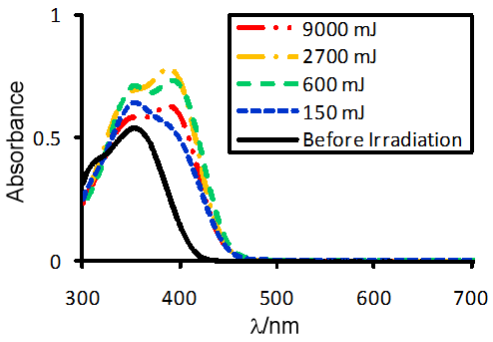

Figure 3. Absorption spectra of a 0.01 wt% solution of the photocleavable crosslinker under UV light irradiation (365 nm, 15 mW/cm2).

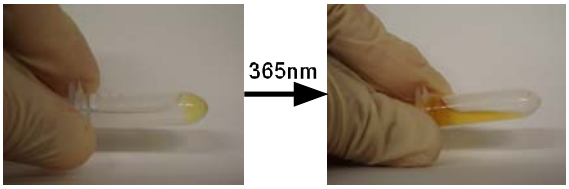

Figure 4. Degradation of the photodegradable hydrogel under UV light irradiation (365 nm, 100 mW/cm2).

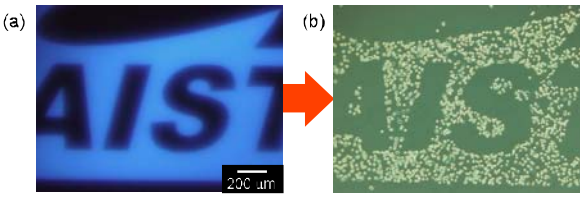

Figure 5. Cell micropatterning by micropatterned light irradiation on a cell-nonadhesive photodegradable hydrogel sheet. (a) Irradiated pattern. (b) Cells attached on the irradiated area.

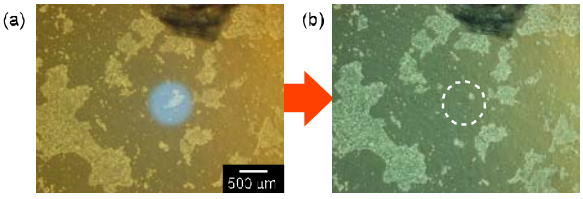

Figure 6. Local cell detachment by local light irradiation on a cell-adhesive photodegradable hydrogel sheet. (a) Irradiated pattern. (b) Cells in irradiated area detached from the surface.

For cell micropatterning, micropatterned UV light (365 nm, 60 mW/cm^2) was irradiated for 5 s on the cell nonadhesive photodegradable hydrogel sheet (Fig. 5, left panel).After washing with PBS, Chinese hamster ovary (CHO) cells were seeded on the irradiated dishes. After 2 days of cultivation in the irradiated dishes, the CHO cells grew on the exposed tissue culture polystyrene surface in the irradiated areas, and a pattern of cells identical with the irradiated micropattern was formed (Figure 5, right).

In order to demonstrate local cell detachment, the surface of the photodegradable hydrogel sheet was modified with ECM protein, fibronectin. The fibronectin reacted with the NHS moieties in the photocleavable crosslinker to be immobilized on the surface of the photodegradable hydrogel sheet. CHO cells were cultivated on the fibronectin-modified photodegradable hydrogel sheet. For local cell detachment, focused UV light (365 nm, 60 mW/cm^2) was irradiated for 15 s on a target colony of CHO cells on the fibronectin-modified photodegradable hydrogel sheet (Fig. 6, left). The cells in the irradiated area detached from the substrate within 3 min after the irradiation (Fig. 6, right).

CONCLUSIONS

In summary, a photocleavable crosslinker was synthesized, and photodegradable hydrogel sheet was formed by reacting the photocleavable crosslinker with amino-4armPEG. These hydrogels were successfully applied optical control of cell adhesion and detachment. Optical cell manipulation strategies enables cell adhesion control in a spatially and temporally controlled manner, and are promising for applications in a variety of research fields.

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