Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information

Efficient biotinylation of methacryloyl-functionalized nonadherent cells for formation of cell microarrays

Yasuhiko Iwasaki[1]*, Tatsuya Ohta

Department of Chemistry and Materials Engineering
Faculty of Chemistry, Materials and Bioengineering
Kansai University
3-3-35 Yamate-cho; Suita-shi, Osaka 564-8680; Japan
Fax: +81-6-6368-0090; Tel: +81-6-6368-0090
E-mail: yasu.bmt@kansai-u.ac.jp

*Corresponding author

MATERIALS AND METHOD

Materials

N-Methacryloylmannosamine (ManMA) was synthesized based on the process used for the synthesis of *N*-levulinoylmannosamine (ManLev); methacrylic acid was used instead of levulinic acid.[1] 2-Biotinamidoethyl methacrylate (BiMA) and 3-(2-bromoisobutyryl)propyl dimethylchlorosilane (BDCS) were synthesized by previously reported methods.[2] Thiol-terminated 4-arm poly(ethylene glycol) ($PEG_4$10K-SH) and 2-methacryloyloxyethyl phosphorylcholine (MPC) were purchased from NOF Corporation (Tokyo, Japan). Irgacure 2959 was kindly provided by BASF Japan (Tokyo, Japan). Silicon wafers (100 orientation, B doped) covered with a 100-nm thermal oxide layer were purchased from Chiyoda Trading Co., Tokyo, Japan. Pre-purified water was further purified using a Millipore Milli-Q system that includes reverse osmosis, ion exchange, and filtration steps (18.2 MΩ). Other reagents and solvents available in extra-pure grade were obtained commercially and used without further purification.

Cell culture experiment

Human promyelocytic leukemia cells (HL-60) were purchased from Health Science Research Resources Bank and maintained in RPMI1640 with 10% fetal bovine serum at 37°C in a humidified atmosphere of air containing 5% CO_2. The concentration

of HL-60 was adjusted to 2 x 10^5 cells/mL. The cell suspension (1 mL) was poured into a 12-well tissue culture dish and 110 μL of 50 mM ManMA/PBS was added to the wells.

After cell cultivation for 3 days, the cell suspension was centrifuged to remove free ManMA in the medium and the cells were washed with fresh medium (serum free) and centrifuged to remove any free ManMA from the medium. RPMI-1640 medium containing 2.5 wt% $PEG_4$10K-SH and 0.05 wt% Irgacure 2959 was then added to the cells and the mixture was exposed to UV light for 15 min at room temperature. The cells were rinsed again three times with the culture medium to remove any unreacted $PEG_4$10K-SH. Stock solutions of 2 mg/mL Alexa Fluor 488 C_5-maleimide (Invitrogen) and 5 mM DRAQ5™ (Enzo Life Science International) were prepared in N,N-dimethylformamide (DMF). $PEG_4$10K-SH immobilized on the HeLa cells was covalently labeled in the dark for 10 min with 0.6 μg/mL Alexa Fluor 488 C_5-maleimide dissolved in the culture medium. After rinsing with the culture medium, the nuclei were subsequently stained with 10μM DRAQ5™ in order to ascertain the position of each cell. Morphological observation of the cells was performed using a confocal laser scanning microscope (LSM 5 PASCAL, Carl Zeiss Microscopy, Jena, Germany).

Cell viability

The viability of adherent HeLa cells immobilized with polymers was investigated with a LIVE/DEAD® Viability/Cytotoxicity Kit (Molecular Probes, OR, USA). After the thiol-ene reaction of the ManMA-treated HL-60 cells with $PEG_4$10K-SH, the surface was rinsed twice with fresh medium to remove any non-reacted polymers. The cells attached to the polymer surface were then stained with 1

μM calcein dye and 2 μM ethidium homodimer-1 (EthD-1) dye. The samples were incubated for 15 min at 37°C and observed using phase-contrast and fluorescence microscopy. The number of attached cells and the percentage of live cells were determined in five randomly chosen areas using Image J (http://rsbweb.nih.gov/ij/).

Micropatterned monolayers of polymerization initiator on silicon wafers

Silicon wafers were cut into 1.2 cm × 1.2 cm pieces and cleaned before use by oxygen plasma for 30 min. Silanization was immediately performed after the wafer treatment. The cleaned silicon wafers were placed in a dry flask to which 30 ml of dry toluene and BDCS (33 μl, 0.15 mmol) were added under an argon gas atmosphere. The flask was allowed to stand for 18 h. The wafers were then removed from the solution, rinsed with toluene, 2-propanol, absolute ethanol, ethanol solution, and deionized water and dried under a nitrogen or argon stream.

A surface pattern of a BDCS monolayer on a silicon wafer was prepared with UV light (λ = 185 nm) (GL15ZH, Sankyo Denki Co., Ltd., Tokyo, Japan; 15 W) radiation through a mesh for transmission electron microscopy (hole, 45 μm; bar, 40 μm; Okenshoji Co., Ltd., Tokyo, Japan) for 3.5 h in an air atmosphere.

Preparation of prepatterned PMPC brushes via SI-ATRP

Copper(I) bromide (29 mg, 0.20 mmol) and 2,2'-dipyridyl (63 mg, 0.40 mmol) were dissolved in 9 ml of methanol with stirring under argon at room temperature. Then, ethyl 2-bromoisobutyrate (30.4 μL, 0.203 mmol) was added as a sacrificial initiator. After the solution was stirred for 30 min under an argon gas atmosphere, the BDCS-micropatterned silicon wafers were then submerged in the flask. MPC (12 g,

0.041 mol) was separately dissolved in 21 mL of methanol purged with argon for 1 h to eliminate oxygen. The MPC solution was added to the flask and polymerization occurred at room temperature with stirring under an argon gas atmosphere for 3 h. The silicon wafers were periodically removed from the polymerization mixture and rinsed with methanol and water. The number-average molecular weight of the free polymer in solution was measured with a JASCO GPC system with a refractive index detector and size-exclusion columns, Shodex, SB-803 HQ and SB-806 HQ with a poly(ethylene glycol) (PEG, Tosoh standard sample) standard in distilled water containing 10 mM LiBr. The thickness of the polymer brush was measured with an auto ellipsometer (DVA-36L3, Mizojiri Optical Co., Ltd., Tokyo, Japan) operating with a 632.8 nm He–Ne laser at a 70° incident angle. The generation of prepatterned PMPC brushes on a silicon wafer was determined by staining with rhodamine 6G or by using an x-ray photoelectron spectroscope (AXIS Nova, Shimadzu, Kyoto, Japan). The XPS data were collected at a take-off angle of 90°.

Preparation of living cell array

The silicon wafers with surface prepatterned PMPC brushes used for cell array preparation were rinsed with a copious amount of sterilized purified water. A sufficient amount of 1 mg/mL chicken egg white avidin in phosphate buffered serine (PBS) was placed on the surface and incubated for 1 h at room temperature. Excess avidin was removed from the surface by rinsing with copious amounts of PBS. The plate was stored in PBS until use.

According to a procedure similar to that of PEGylation, biotinylation of the cell surfaces was performed. When $PEG_4$10K-SH was in contact with the cells, 3 mM BiMA

was mixed with $PEG_4$10K-SH. After exposure of the cell surfaces to UV light (365 nm) for 15 min, the cells were again rinsed three times with culture medium to remove any unreacted $PEG_4$10K-SH and BiMA. The cell suspension was collected in one centrifugation tube from at least 6 wells and the cell concentration was adjusted to 2 x 10^6 cell/mL (>500 μL).

The cell suspensions were in contact with the avidin-treated wafer for 30 min at 37°C. The wafer was then gently rinsed three times with culture medium containing 10% FBS and soaked in culture medium containing 2 μM calcein AM solution for 15 min at 37°C. The wafer was again rinsed three times with culture medium and observed using a fluorescence microscope (IX-70, OLYMPUS, Tokyo, Japan).

References

1 K. J. Yarema, L. K. Mahal, R. E. Bruehl, E. C. Rodriguez, C. R. Bertozzi, *J. Biol. Chem.*, 1998, **273**, 31168.

2 R. Iwata, P. Suk-In, V. P. Hoven, A. Takahara, K. Akiyoshi, Y. Iwasaki, *Biomacromolecules*, 2004, **5**, 2308.

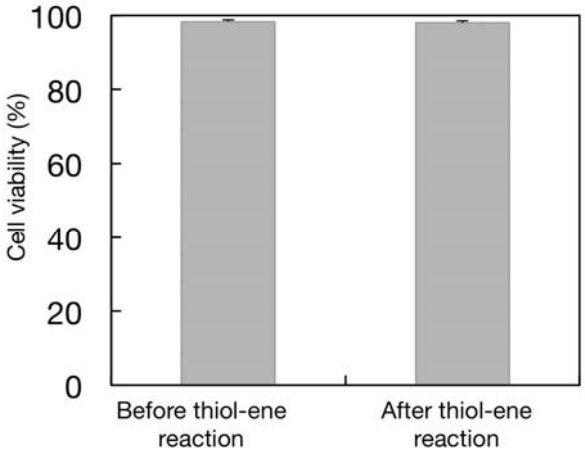

Fig. S1 Viability of HL 60 cells before and after thiol-ene reaction with thiol-terminated 4-arm poly(ethylene glycol) ($PEG_4$10K-SH). This data was determined by LIVE/DEAD® Cell Viability Assays.

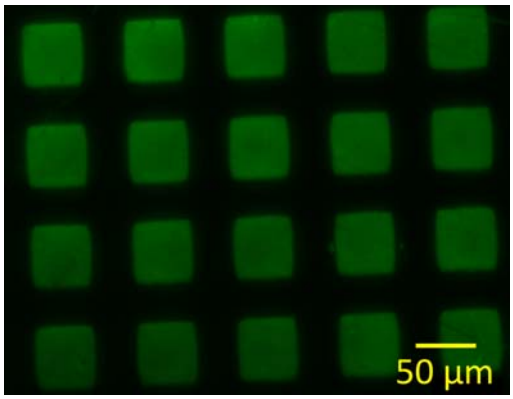

Fig. S2 Fluorescence micrograph of patterned PMPC brush surface after contact with 1 mg/mL chicken egg white avidin. To clarify distribution of adsorbed avidin, the 0.01 mg/mL of streptavidin conjugated with Alexa Fluor 488 was added to the protein solution.

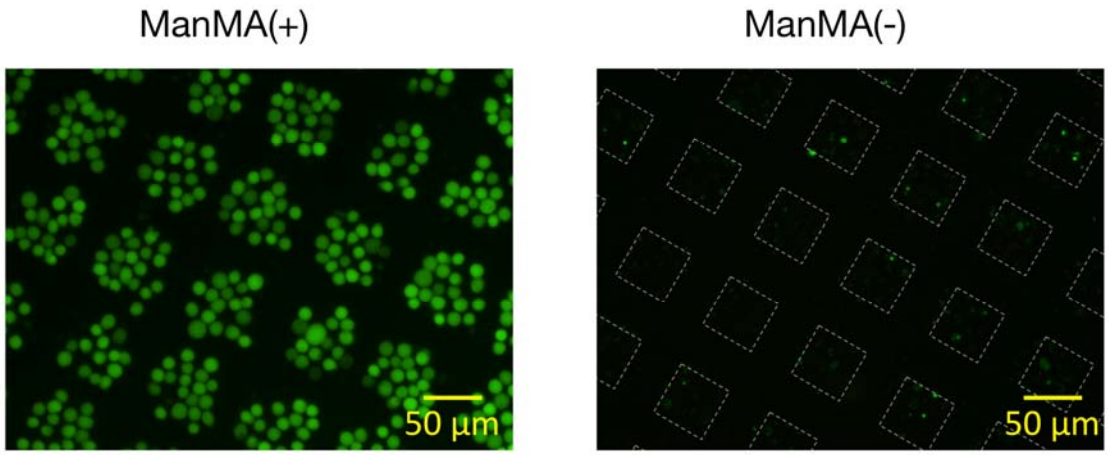

Fig. S3 Fluorescence micrograph of avidin-patterned surface after contact with ManMA treated (L) and untreated (R) HL-60 cells. The cells were treated by a process similar to biotinylation before contact with the avidin-patterned surface.