Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Simultaneous Patterning of Proteins and Cells Through Bio-conjugation with Photoreactable Phospholipid Polymers

Ji-Hun Seo[†‡]*, Kazuhiko Ishihara*[†]*

†Department of Materials Engineering, School of Engineering, University of Tokyo, Tokyo 113-8656, Japan

‡Department of Materials Science and Engineering, Korea University, Seoul 02841, Korea

Tel: 81-3-5841-7124

Fax: 81-3-5841-8647

*E-mail: ishihara@mpc.t.u-tokyo.ac.jp

Experimental Methods

Materials

MPC was purchased from NOF Co., Ltd. (Tokyo, Japan), which was synthesized by a previously reported method.[1] Copper I bromide (Cu(I)Br), 4-azidobenzoic acid, and 2-aminoethyl methacrylate hydrochloride were purchased from Sigma Aldrich (St. Louis, MO, USA). Fluorescein isothiocyanate (FITC)-labeled bovine serum albumin (F-BSA) was purchased from ThermoFisher (Lafayette, CO, USA). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 2,2'-bipyridyl (bpy) were purchased from Wako Chemical (Osaka, Japan). 2-bromoisobutyric acid was purchased from TCI Co., Ltd. (Tokyo, Japan). All organic solvents (synthesis grade) were purchased from Wako Chemical (Osaka, Japan) and were used as received.

Methods

N-hydroxysuccinimide (NHS) ester-functional ATRP initiator was synthesized using a previously reported method.[2] 2-(4-azidobenzamido)ethyl methacrylate (AzMA) was synthesized as follows; in a round-bottomed flask, 2.328 g (12 mmol) of EDC was dissolved in 15 mL chloroform. To this, 0.978 g (6.00 mmol) of 4-azidobenzoic acid was added and stirred until all insoluble particles had dissolved. Following this, 2 g (12.1 mmol) of 2-aminoethyl methacrylate hydrochloride was added to the mixture, and stirred overnight at room temperature. The light red solution was then distilled using silica gel column chromatography with hexane/ethyl acetate (5/3) mixed solvent as the mobile phase. The Rf value of the TLC spot for the product was 0.7, and the final yield was 40.4%.

ATRP was synthesized as follows: in a 10 mL round-bottomed flask, 16.6 mg (0.063 mmol) of NHS-ester-functionalized ATRP initiator, 1.67 g (5.67 mmol) of MPC, and 0.182 g (0.63 mmol) of AzMa were dissolved in 5 mL degassed methanol and stirred at room temperature. After degassing by three repeated freeze-pump-thaw cycles, a mixture of 9.04 mg (0.063 mmol) of Cu(I)Br and 19.7 mg (0.126 mmol) of bpy was added to the solution and the flask was capped with a rubber septum and charged with an Ar balloon. For kinetic analysis, an aliquot (0.1 mL) of the reaction mixture was taken

at appropriate times using a long-needle syringe. After 4.5 h, the reaction mixture was passed through the 10-cm silica gel column to remove the transition metal complex, and the concentrated clear solution was then recrystallized in cold diethylether/chloroform (8/2) mixed solvent to obtain a slightly yellow powder.

For kinetic analysis, monomer conversion was calculated by comparing the ^{1}H-NMR peak due to the unreacted vinyl group and α methyl group in the polymer chain. Size exclusion chromatography (SEC) was conducted using a JASCO RI-1530 detector containing two connected TSK-GEL Super HM-M gel columns (Tosoh Co. Tokyo, Japan) and a Shodex SB-803 HQ column (Showa Denko, Tokyo, Japan). Poly(methyl methacrylate) was used as the standard in hexafluoroisopropanol as solvent.

Conjugation of PMAz with F-BSA was carried out as follows: 6.6 mg of F-BSA was dissolved in 2.5 mL 50 mM $NaHCO_3$ buffer (pH 8.2). Then 10 μmol of PMAz was dissolved in 1.0 mL of methanol, and dropped into the F-BSA solution. The solution was kept at 4^{o}C overnight, and then dialyzed using a dialysis tube (MWCO 50 k) followed by lyophilization. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 10% polyacrylamide gels. Samples were loaded in TRIS buffer containing SDS.

Patterning of F-BSA-PMAz: F-BSA-PMAz aqueous solution (0.1 wt%) was dropped onto a polypropylene (PP) disc and air dried under sterile conditions. Following this the patterned photomask was placed on the surface, and exposed to 254 nm UV (300 mW/cm^2) light for 10 sec. After thorough washing with phosphate buffered saline (PBS), the surface was observed using a fluorescence microscope (Axioskop2 plus, Carl Zeiss, Jena, Germany) at an exposure level of 1/3.5s.

The patterned surface was brought into contact with a suspension of L929 mouse fibroblasts (RCB 0081, Cell Bank, Japan) at 3.5 x 10^4 cells/mL in medium supplemented with 10 % fetal bovine serum. The sample was stored in an incubator at 37^{o}C at 5% CO_2 for one day. After gentle washing with

fresh PBS, the surface was observed using an optical microscope (Olympus Optical Co. Ltd., Tokyo, Japan)

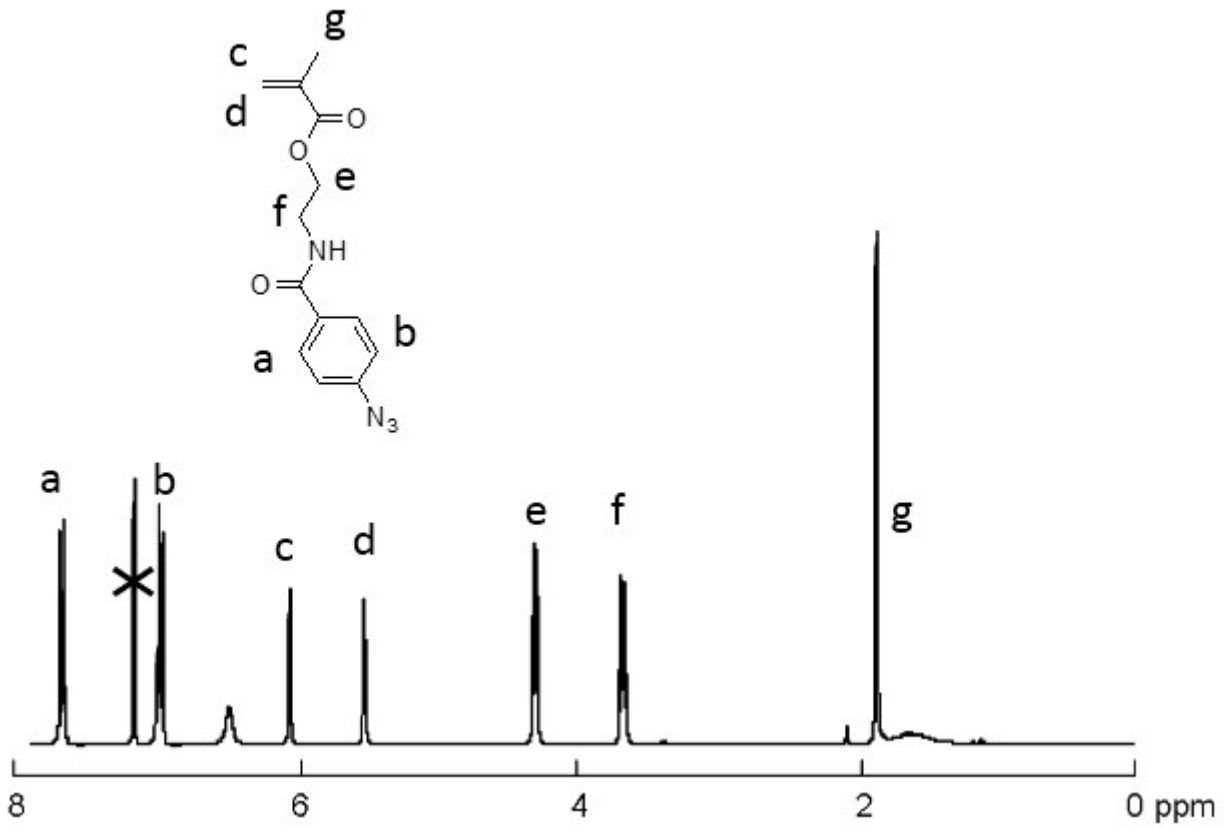

Figure S1. ^{1}H-NMR for AzMA

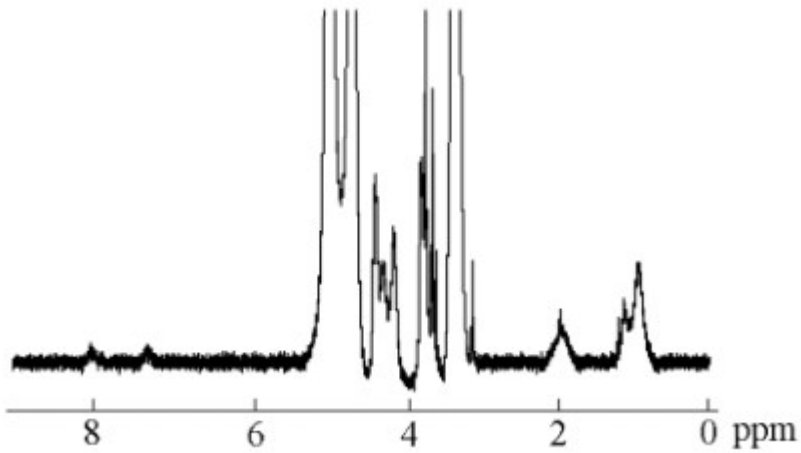

Figure S2. ^{1}H-NMR of PMAz. The broad phenyl azide group was confirmed at 7.6 and 8.1 ppm.

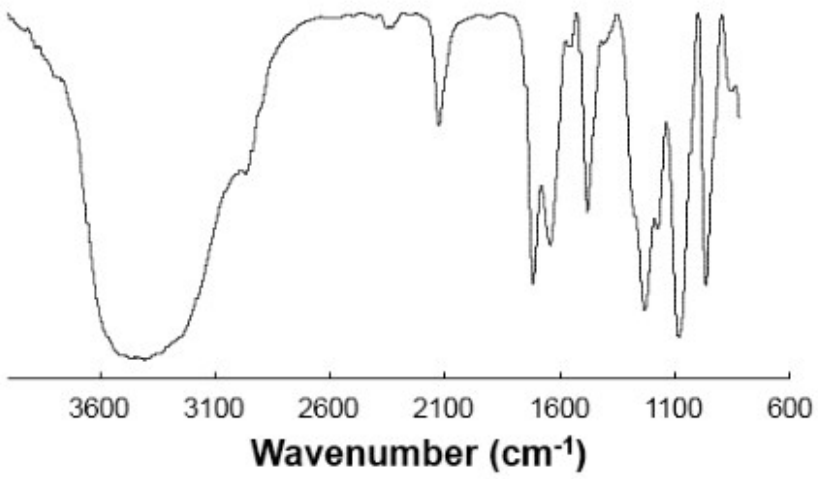

Figure S3. FT-IR chart of PMAz. A strong azide peak was observed at 2150 cm^{-1}

References

[1] Ishihara, K.; Ueda, T.; Nakabayashi, N. Polym. J. 1990, 22 (5), 355−360.

[2] Samanta, D.; McRae, S.; Cooper, B.; Hu, Y.; Emrick, T.; Pratt, J.; Charles, S. A. Biomacromolecules 2008, 9, 2891−2897.