Supporting information

Protein patterning with antifouling polymer gel platforms generated using visible light irradiation

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EXPERIMENTS

Materials

Silicon (Si) wafers (100 orientation, B-doped) covered with 100-nm thermal oxide layers were purchased from Yamanaka Semiconductor Co., Ltd., Tokyo, Japan. Transmittance electron microscope (TEM) grids were purchased from Okenshoji, Tokyo, Japan. 2-Methacryloyloxyethyl phosphorylcholine (MPC) was provided by NOF Co., Ltd., Japan and used without further purification. *N*-Methacryloyl-(L)-tyrosinemethylester (MAT) was synthesised via the reaction of methacryloyl chloride and L-tyrosine methyl ester, as per a previously reported method [1,2]. Triethylamine and butyl methacrylate (BMA) were purchased from FUJIFILM Wako Pure Chemical Industries, Ltd., Osaka, Japan and distilled before use. Methacryloyl chloride was purchased from Tokyo Chemical Co., Ltd., Japan and distilled before use. 2,2'-Azobisisobutyronitrile (AIBN) was purchased from FUJIFILM Wako Pure Chemical Industries, Ltd., Osaka, Japan and purified via recrystallisation from methanol. Other commercially available extra-pure grade reagents and solvents were purchased and used without further purification. Water was purified by a Millipore Milli-Q system to obtain Milli-Q water (MQ).

Instruments

All ^{1}H NMR analyses were performed on JEOL JMTC-400/JNM-AL400 (400 MHz) using $CDCl_3$, methanol-D, or D_2O as a solvent. Molecular weight and molecular weight distribution were evaluated by gel permeation chromatography (GPC) using a Jasco GPC system equipped with a refractive index detector and size-exclusion columns, Shodex, SB-803 HQ, and SB-806 M, with a poly(ethylene glycol) (PEG, Tosoh standard sample) standard in methanol/water (70/30 vol%)

containing 50 mM LiBr. All fluorescence results were observed by fluorescence microscopy (IX71, Olympus, Tokyo, Japan). The thickness of the modified surface, the surface composition, and the roughness of the surface was determined by ellipsometer (M-200XEllipsometer, J.A. Woollam Co., Inc., Lincoln, NE, USA) operating with a 632.8-Xe-arc lamp that adjusted the incident angle to 70°–80°. Surface composition was measured by X-ray photoelectron spectroscopy (XPS) using a Shimadzu ESCA-3400 spectrometer with MgKα X-rays.

Synthesis of MPC bearing tyrosine units.

Poly[MPC-co-n-butyl methacrylate (BMA)-co-MAT] (PMBM) and poly(MPC-co-MAT) (PMM) were synthesised by conventional radical polymerisation using AIBN as an initiator in the presence of ethanol as a solvent. The monomer concentration was adjusted at 0.5 M. Then, the mixture was transferred into a round-bottom flask containing 0.5% molar ratio of AIBN. The flask was sealed with a rubber septum and purged with argon gas to remove oxygen from the system. The polymerisation reaction was conducted in an oil bath at 60°C with continuous stirring overnight. The polymers were purified by reprecipitation into diethyl ether.

Gelation test

The aqueous solution containing PMM (5 wt%), Ru(II)bpy_3^{2+} (3 mM), and APS (3 mM) was prepared, and white light (LEDWMS-EPI; Optocode Co., Ltd., Tokyo, Japan) with a nominal power of 3 W was irradiated for 15 s. The gelation was confirmed by soaking the mixtures in distilled water following visible light irradiation.

Surface modification

A silicon wafer covered with PMBM solution as a base layer, followed by a PMM solution as a contiguous layer was used as a subjected substrate. Wafers were cut into 1.0 × 1.0-cm pieces and cleaned with an oxygen plasma treatment (Model PR301; Yamato Scientific Co. Ltd., Tokyo, Japan) for 10 min. Subsequently, ethanol solution containing 0.5 wt% PMBM was coated on the wafer surface using a spin coater (ACT-220A; Active Co. Ltd., Saitama, Japan) at 3,000 rpm (30 sec). The wafer was then dried under reduced pressure for 6 hours. The wafer was soaked in water to equilibrate the surfaces, and then the aqueous solution containing PMM (5 wt%), Ru(II)bpy_3^{2+} (2.1 mM), and APS (0.9 mM) was coated on the surface using a spin coater at 1,000 rpm (20 s) and 3,000 rpm (30 s). The surface was exposed under white light for 15 s and rinsed with 3 × 10 mL distilled water and dried with N2 flow.

Regioselective multi-protein immobilisation

PMBM-coated Si wafers were prepared as described above. On the wafer surface, a solution (75 μL) of phosphate buffer saline containing PMM (5 wt%), Ru(II)bpy_3^{2+} (2.1 mM), APS (0.9 mM), and Rhodamine (Rh)-conjugated bovine serum albumin (1 mg/mL) was coated on the surface using a spin coater at 1,000 (20 s) and 3000 rpm (30 s). The specimen was exposed under the white light through a TEM grid for 15 s to regulate the photo-irradiated region and rinsed with 3 × 1 mL PBS. Subsequently, a solution (75 μL) of phosphate buffer saline containing PMM (5 wt%), Ru(II)bpy_3^{2+} (2.1 mM), and APS (0.9 mM), and fluorescein isothiocyanate (FITC)-conjugated bovine serum albumin (1 mg/mL) was coated on the surface using a spin coater at 1000 (20 s) and 3,000 rpm (30 s).

The modified surface was exposed to visible light using a normal table lamp for 15 s using the TEM grid to immobilise the second protein. Next, the modified surface was rinsed with distilled water several times to wash away the non-irradiated region from the surface, and the surface was then dried via flushing with N_2 gas. Because the non-irradiated region did not form covalent crosslinking between the first and second layers, it could be easily removed. The confirmation of the protein arrays on the modified surface was further observed using a fluorescence microscope (IX71, Olympus, Tokyo, Japan).

Immunosorbent assay

Avidin from egg whites (Sigma-Aldrich Inc., St Louis, MO, USA) was immobilised with PMM on the PMBM-immobilised Si wafer. Briefly, a solution (75 μL) of PBS containing PMM (5 wt%), Ru(II)bpy_3^{2+}, (2.1 mM), APS (0.9 mM), and avidin (1 mg/mL) was coated on the surface using a spin coater at 1,000 (20 s) and 3,000 rpm (30 s). To create avidin arrays, the white light was irradiated on the surface and rinsed with 3 × 10 mL PBS. The specimens were incubated with the primary antibody solution [1 wt% ovalbumin solution containing 0.02 mg/mL of sheep anti-BSA biotin conjugated antibody (polyclonal, Bethyl Laboratories, Inc., Montgomery, U.S.A.)] for 60 min at 37°C. After the specimens were rinsed with 3 × 1 mL PBS, PBS solutions with varied concentrations of BSA, which was the model of a target protein, were put into contact with the specimens for 60 min at 37°C. After a second rinsing with 3 × 1 mL PBS, the specimens were incubated with the secondary antibody solution [1 wt% ovalbumin solution containing 0.02 mg/mL of sheep anti-BSA FITC conjugated antibody (polyclonal, Bethyl Laboratories, Inc., Montgomery, U.S.A.)] for 60 min at 37°C. The specimens were

rinsed with 3 × 1 mL PBS and 2 × 1 mL distilled water and blow-dried with a stream of nitrogen gas flowing. Finally, the surfaces of the specimens were observed using a fluorescence microscope. The fluorescence intensity was analysed using ImageJ to clarify the correlation between the intensity and the BSA concentration.

REFERENCES

1. F. Sanda, T. Abe and T. Endo, *J. Polym. Sci., Part A: Polym. Chem.*, 1997, **35**, 2619.
2. H. Yavuz, V. Karakoc, D. Turkmen, R. Say and A. Denizli, *Int. J. Biol. Macromol.*, 2007, **41**, 8.

ADDITIONAL TABLES AND FIGURES

Table S1 Synthetic results of PMM

Polymer	MPC:MAT (mol%)		Conversion (%)	M_n ($\times 10^{-4}$) [2)]	M_w/M_n [2)]
	In feed	In copolymer [1)]			
PMM99.9	99.9:0.1	99.9:0.1	70.9	1.2	1.6
PMM99.5	99.5:0.5	99.7:0.3	75.9	2.6	1.8
PMM99	99.0:1.0	99.1:0.9	69.3	3.1	2.0
PMM95	95.0:5.0	95.7:4.3	76.0	4.1	2.3
PMM90	90.0:10.0	91.1:8.9	77.9	4.1	2.8
PMM80	80.0:20.0	81.1:18.9	73.9	3.9	3.7
PMM70	70.0:30.0	68.9:31.1	72.3	4.3	3.5

[1)] Determined by ^{1}H NMR analysis. [2)] Determined by SEC analysis.

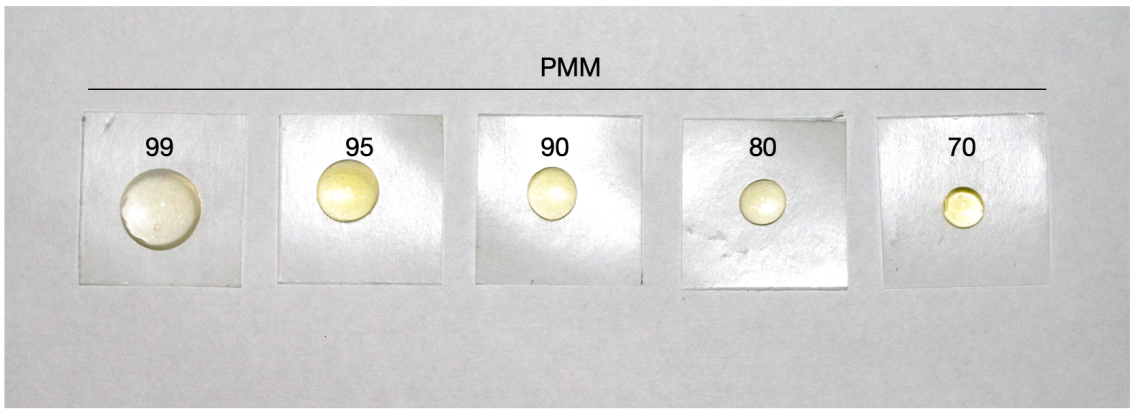

Figure S1 A photograph of equilibrium swollen hydrogels after soaking in water for 48 h.

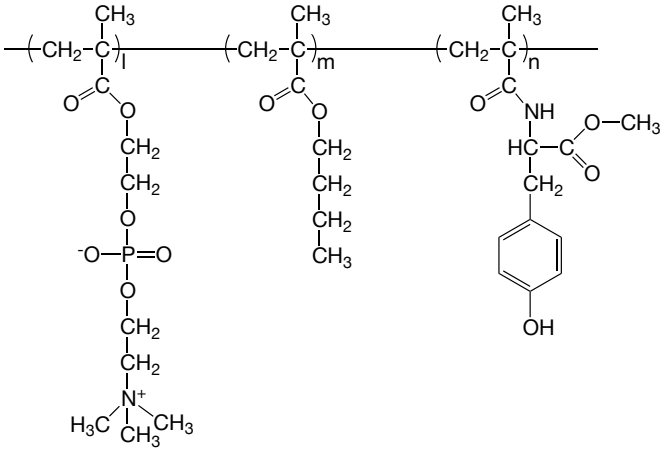

Figure S2 Structure of PMBM.

Table S2 Synthetic results of PMBM

Polymer	MPC:BMA:MAT (mol%)		Conversion (%)	M_n ($\times 10^{-4}$) [2)]	M_w/M_n [2)]
	In feed	In copolymer [1)]			
PMBM	30.0:40.0:30.0	30.7:49.4:19.9	44.7	1.5	2.8

[1)] Determined by ^{1}H NMR analysis. [2)] Determined by SEC analysis.

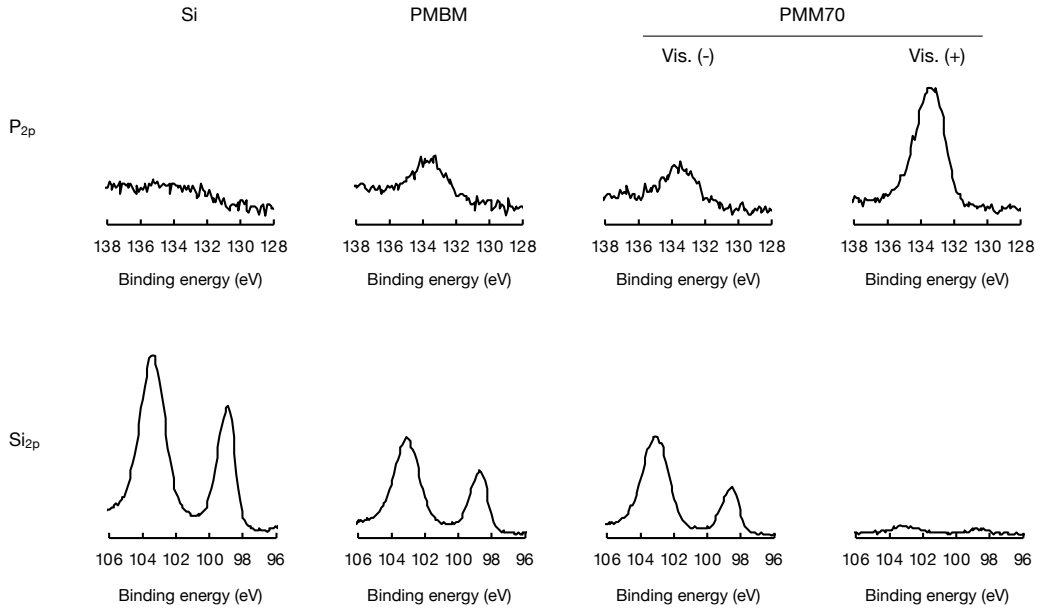

Figure S3 XPS spectra of sample surfaces.

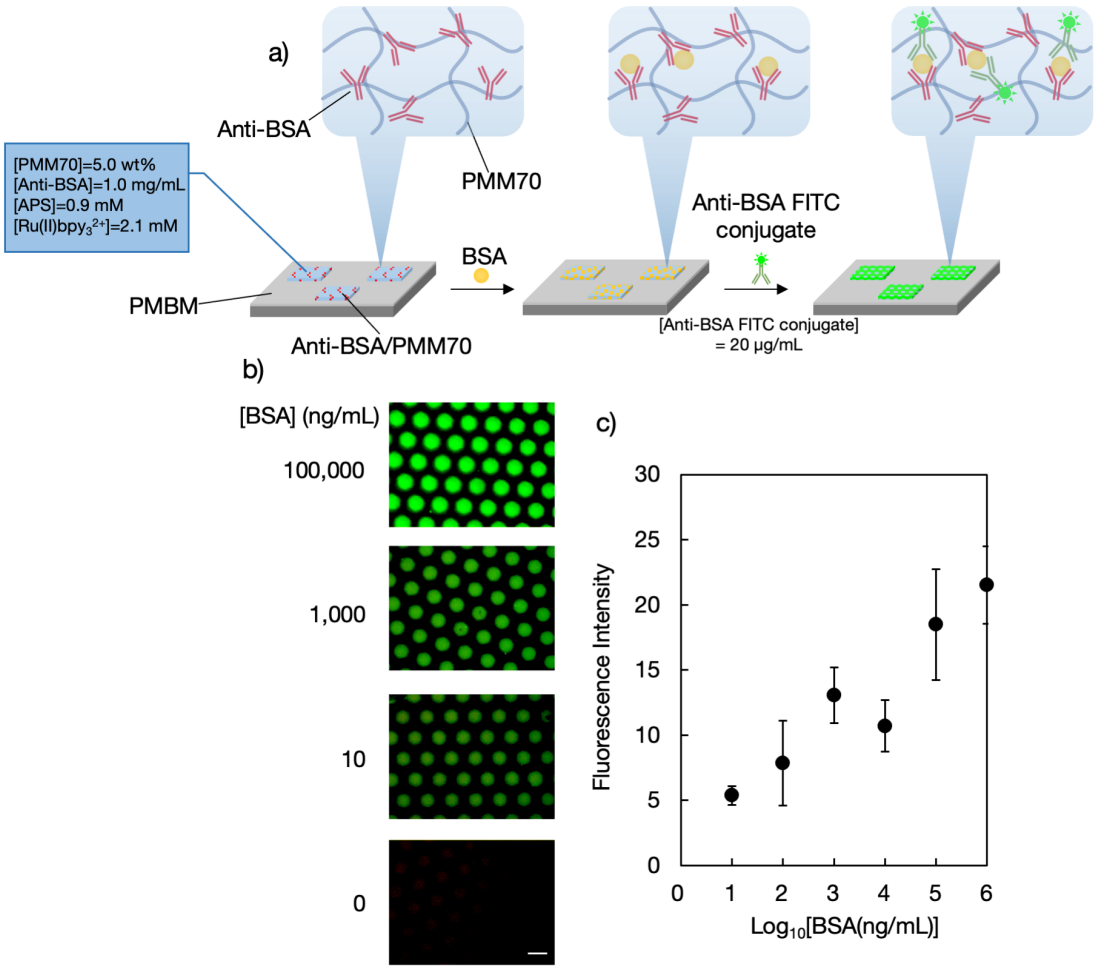

Figure S4 Simulated immunosorbent assay using antibody-immobilised phosphorylcholine polymer hydrogel platforms. a) Schematic of the simulated immunosorbent assay process. b) Fluorescent micrographs of polymer arrays after the immunosorbent assay. A scale bare represents 100 μm. c) Plotted dose–response curve for detection BSA (n=4).