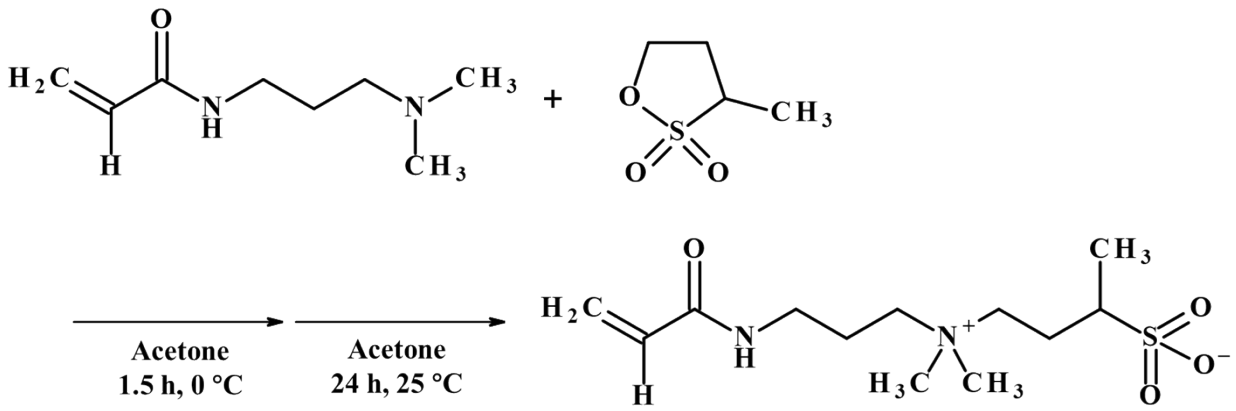
Supporting Information (SI)

Branched sulfoisobutylbetaine acrylamide polymers with hydrolytically stable amide linkages for long-term durable anti-biofouling surfaces

Ryoma Takagi, Ayaka Moroto, Toshikazu Yamamoto, Tadashi Nakaji-Hirabayashi*, Tatsuya Ishiyama, Chiaki Yoshikawa, Hiromi Kitano, Shinpei Yamamoto, and Yoshiyuki Saruwatari

*Corresponding author: Tadashi Nakaji-Hirabayashi, Ph.D. (Engineering and Nanomedicine), email: nakaji@eng.u-toyama.ac.jp



Scheme S1. Synthesis of sulfoisobutylbetaine acrylamide (SBBAm).

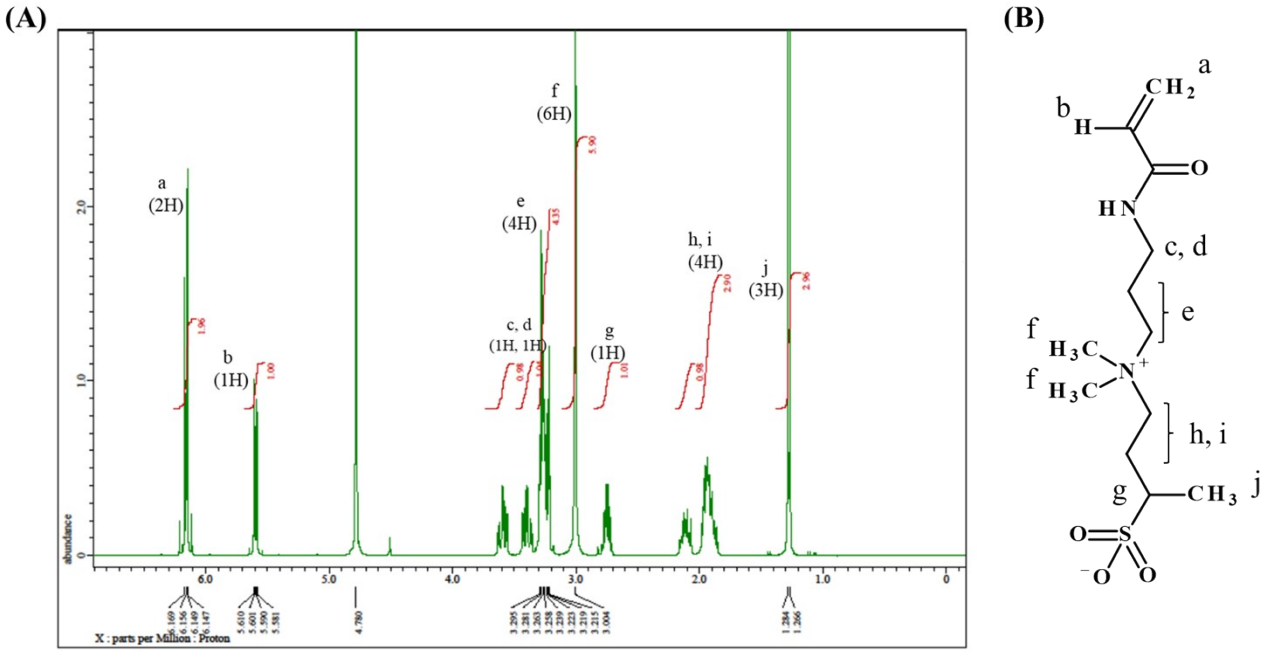
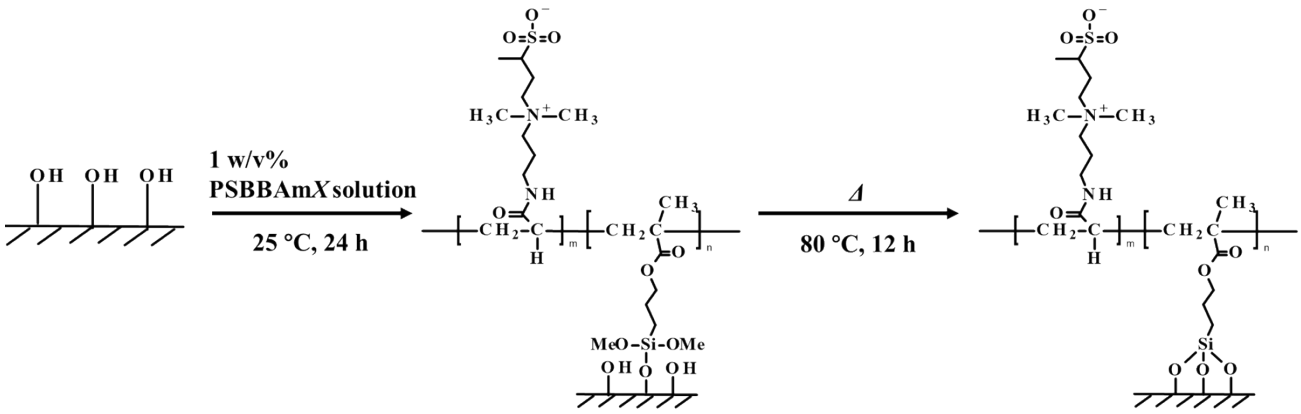


Fig. S1. (A) ^{1}H NMR spectrum of sulfoisobutylbetaine acrylamide (SBBAm). (B) Chemical structure of SBBAm and the spectral assignments of its components.



Scheme S2. Surface modification of glass and silicon wafers with various zwitterionic polymers that have silane coupling groups as side chains.

Determination of *K* and *α* values for viscosity-based molecular weight evaluation

For the viscosity-based evaluation of molecular weight, the Mark–Houwink–Sakurada (MHS) parameters (K and α) were determined using sulfopropylbetaine (SPB) homopolymers (PSPB) with known molecular weights obtained by gel permeation chromatography (GPC).

PSPB standards with different molecular weights (PSPB1–PSPB4) were synthesized via free-radical polymerization using a chain-transfer agent under the conditions summarized in Table S1. Briefly, SPB, 2-mercaptoethanol (2ME), and azobisisobutyronitrile (AIBN) were dissolved in 2,2,2-trifluoroethanol (TFE). Dissolved oxygen was removed by argon purging for 15 min, and the solution was subsequently reacted at 70 °C for 16 h. After the reaction, the polymer solution was purified by reprecipitation with 2-propanol, and the resulting PSPB was obtained as a white solid.

The molecular weights of the PSPB standards were measured by GPC with a size-exclusion column (Wakopak Wakobeads-G-50; FUJIFILM Wako Pure Chemical Co., Ltd.). Pullulan standards (Standard P-82; Showa Denko K.K., Tokyo, Japan) were used for calibration, and a 25 mM NaCl aqueous solution was used as the eluent. The number-average molecular weights (M_n) and dispersities (M_w/M_n) of the PSPB standards are summarized in **Table S1**.

Table S1. Synthesis condition and average molecular weight of SPB homopolymers.

Standard PSPB		SPB (mol)	AIBN (mol)	2ME (mol)	TFE (mL)	M_n	M_w/M_n
No.	Target M_n						
PSPB1	2500	4.5×10^{-3}	2.5×10^{-4}	0.5×10^{-4}	10	3.40×10^{3}	1.44
PSPB2	5000	4.5×10^{-3}	1.3×10^{-4}	0.3×10^{-4}	10	7.40×10^{3}	1.39
PSPB3	10000	3.6×10^{-3}	5.0×10^{-5}	1.0×10^{-5}	10	1.14×10^{4}	1.71
PSPB4	25000	4.5×10^{-3}	2.5×10^{-5}	0.5×10^{-5}	10	2.36×10^{4}	1.61

The intrinsic viscosities [η] of the PSPB standards were measured using an Ubbelohde viscometer at 37 °C (see the Experimental section for instrument details). To improve measurement reproducibility

and suppress the adsorption/reaction of polymers on the glass surface, the inner surface of the viscometer was pre-coated with 3-(dimethyl(3-(trimethoxysilyl)propyl)ammonio)propane-1-sulfonate (SPB-TMS), according to a previous report.[18] Viscosity measurements were performed independently for five runs. The MHS parameters (K and α) were determined by fitting the relationship between $[\eta]$ and M_n of the PSPB standards to the MHS equation, $[\eta] = K \cdot M^{\alpha}$. The mean values of K and α obtained from five independent runs were $(6.88 \pm 1.31) \times 10^{-3}$ and 0.627 ± 0.072, respectively (mean ± SD). A representative set of viscosity plots and the corresponding MHS fitting is shown in **Fig. S2**.

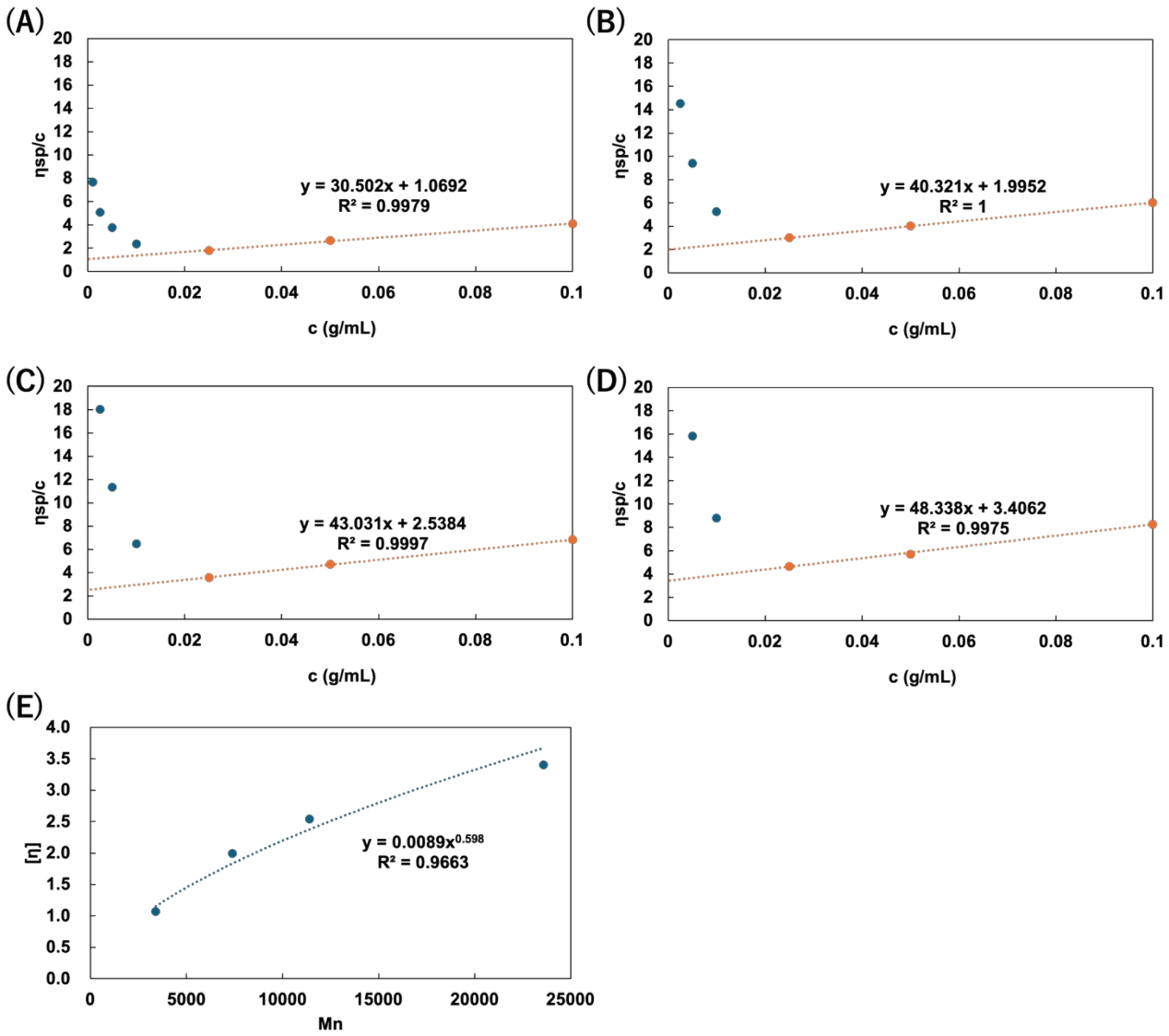


Fig. S2. Correlation of η_{red} and the concentration of (A) PSPB1, (B) PSPB2, (C) PSPB3, and (D) PSPB4 obtained by viscosity measurement. (E) Curve fitting of $[\eta]$ versus number-average molecular weight.

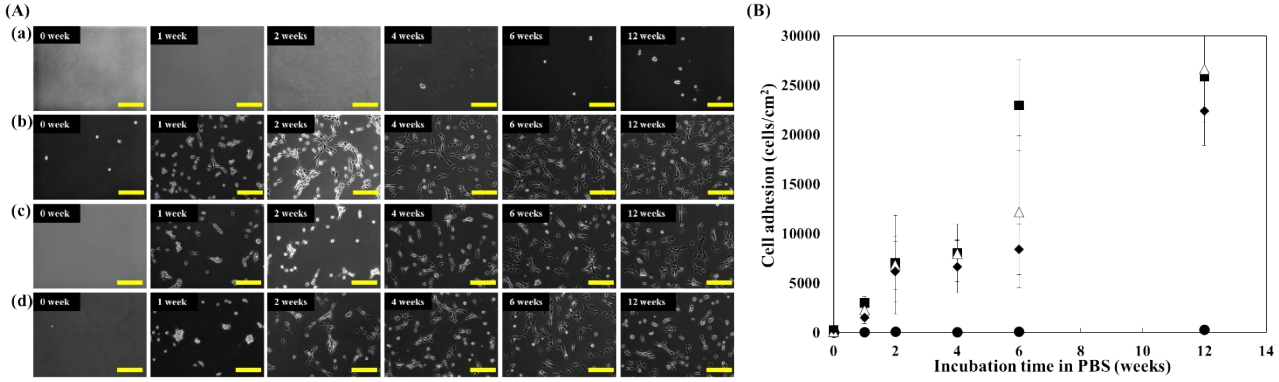


Fig. S3. Evaluation of the durability of PSBBAm70-, PSPB90-, PCMB90-, and PMPC90-modified surfaces. The modified substrates were incubated for 1, 2, 4, 6, and 12 weeks in PBS at 70 °C. The cells were then seeded on the substrates and cultured for 1 day. (A) Phase contrast images of the cells cultured for 1 day on (a) PSBBAm70-, (b) PSPB90-, (c) PCMB90-, and (d) PMPC-modified surfaces incubated in PBS at 70 °C. (B) The number of cells that adhered to the surfaces with (●) PSBBAm70-, (■) PSPB90-, (△) PCMB90-, and (◊) PMPC90-incubated in PBS at 70 °C (n = 6).

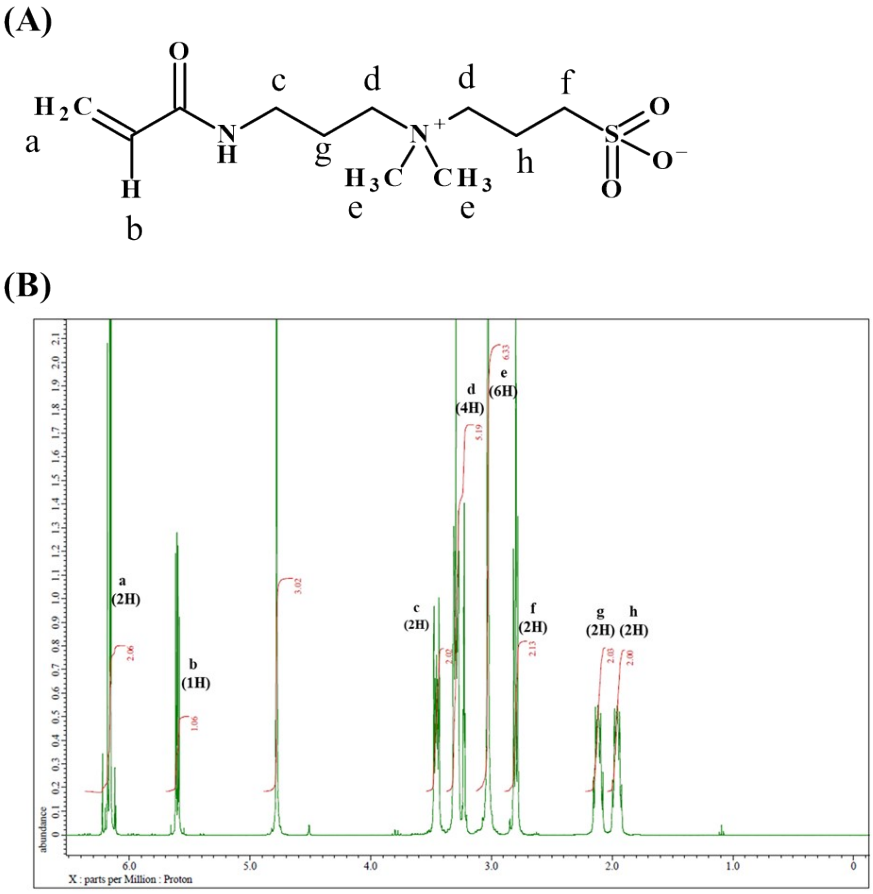


Fig. S4. Characterization of sulfopropyl betaine acrylamide (SPBAm). (A) Chemical structure and (B) ^{1}H NMR spectrum of SPBAm.

Fig. S5. Water solubility of SBBAm and SPBAm homopolymers (M_v: 5.0×10^4 g/mol for PSBBAm and 3.6×10^4 g/mol for PSPBAm). The molecular weights of the homopolymers were determined by viscosity measurements using trifluoroethanol as the mobile phase.

Ab initio calculation results

Density functional theory calculations were performed to optimize the molecular structures of SPBAm and SBBAm, and the effects of the methyl group near the sulfur trioxide group was explored for SBBAm. Calculations were performed at the B3LYP/6-31+G(d) level of theory using the Gaussian 09 program package [1]. Fig. S6 shows the optimized structure of each molecule. The distance between the S atom of the sulfur trioxide group and the N atom of the dimethylamine group is 5.32 Å for SPBAm and 4.08 Å for SBBAm. This indicates that the presence of a methyl group (denoted as C_0 in Fig. S6(b)) shortened the S–N distance. In the case of SPBAm, the S–C_1–C_2–C_3 atoms are stable in the trans conformation, whereas for SBBAm, the trans C_0–C_1–C_2–C_3 conformation is stable (that is, the gauche S–C_1–C_2–C_3 conformation is stable) because of the steric effect of the methyl group and the Coulomb interaction between the negatively charged sulfur trioxide group and the positively charged dimethylamine group. This may explain the shorter S–N distance in SBBAm than in SPBAm.

Reference

[1] M. J. Frisch, G. W. Trucks, H. B. Schlegel, et al., *Gaussian 09 (Revision A.02)*, Gaussian, Inc., Wallingford, CT, 2009.

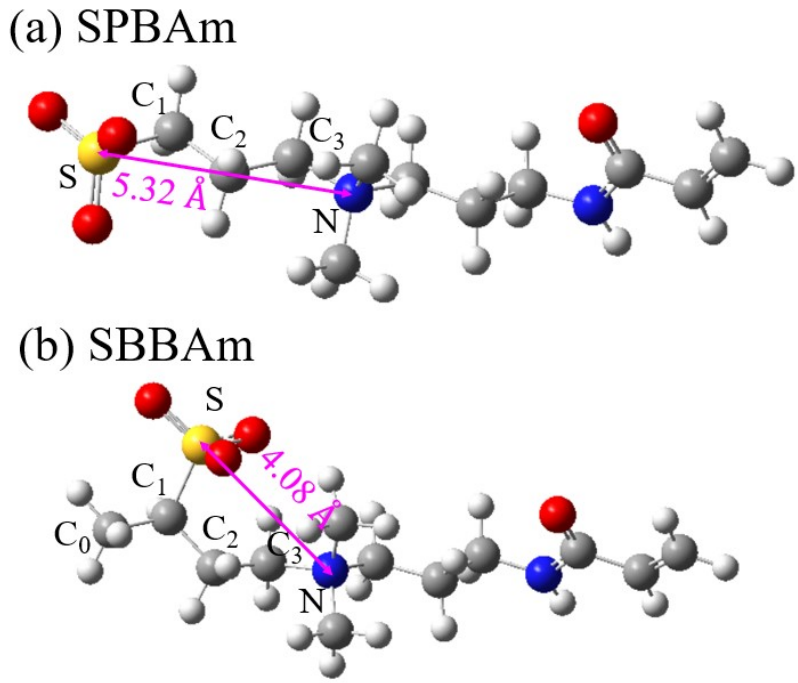


Fig. S6. Optimized molecular structures of (a) SPBAm and (b) SPBBAm.

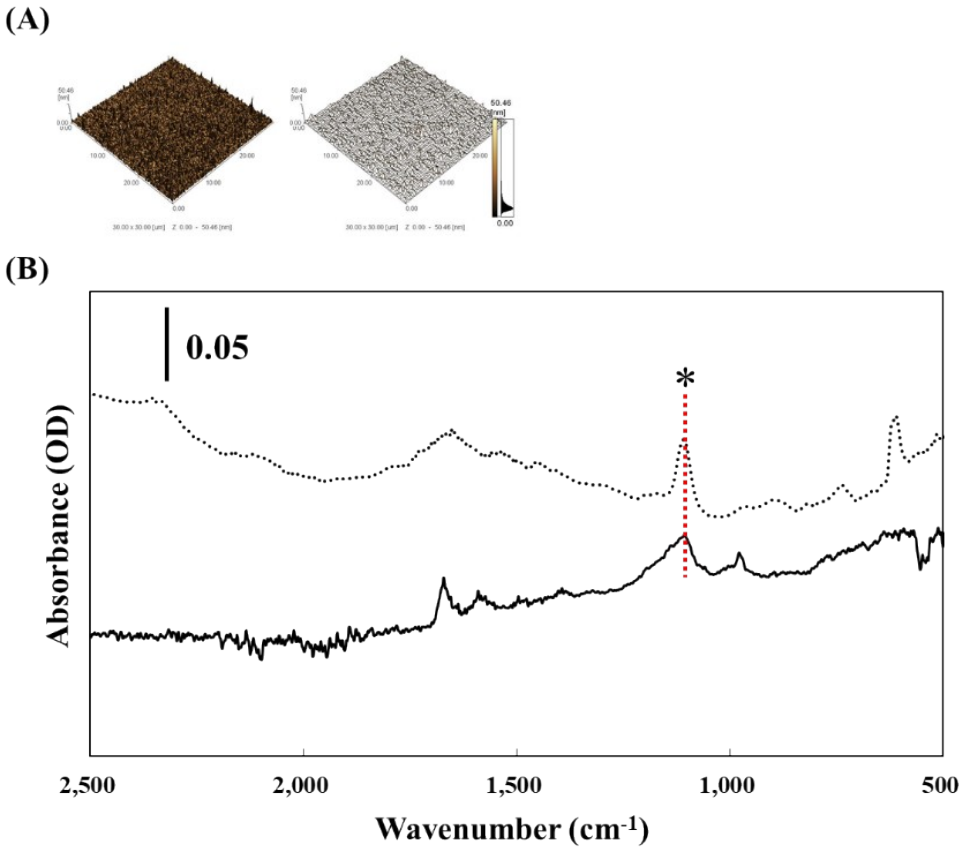


Fig. S7. (A) AFM image of a surface modified with PSPBAm70. **(B)** ATR FT-IR spectra of PSPBAm70 (solid line) and PSBBAm70 (dotted line) immobilized on a glass substrate.

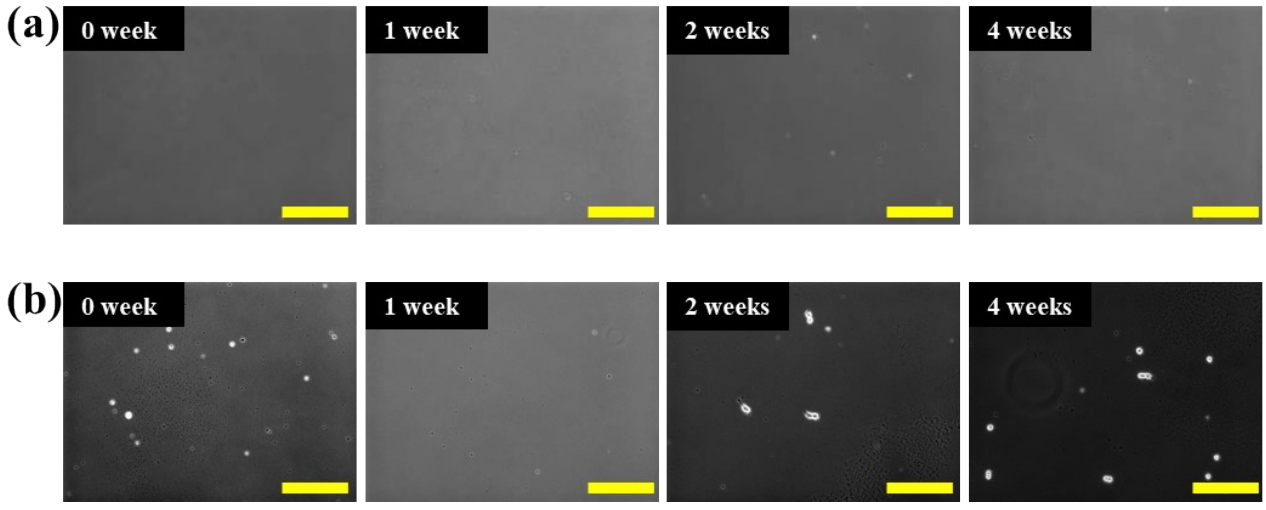


Fig. S8. Phase contrast images of the cells adhered to (a) PSBBAm70- and (b) PSPBAm70-modified surfaces. Cells were seeded onto polymer-modified surfaces and incubated in PBS at 37 °C for 0, 1, 2, and 4 weeks, then cultured for 1 day.

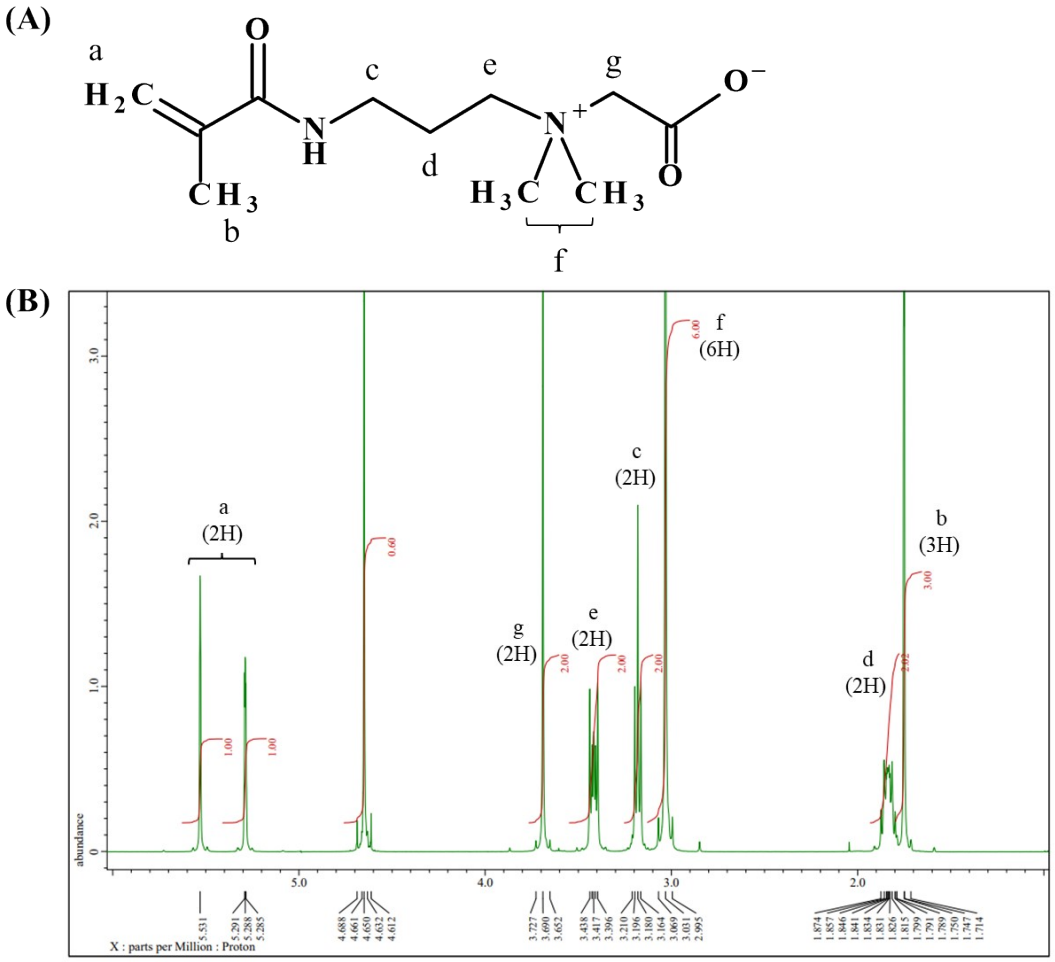


Fig. S9. Characterization of carboxymethyl betaine methacrylamide (CMBAm). (A) Chemical structure and (B) ^{1}H NMR spectrum of CMBAm.

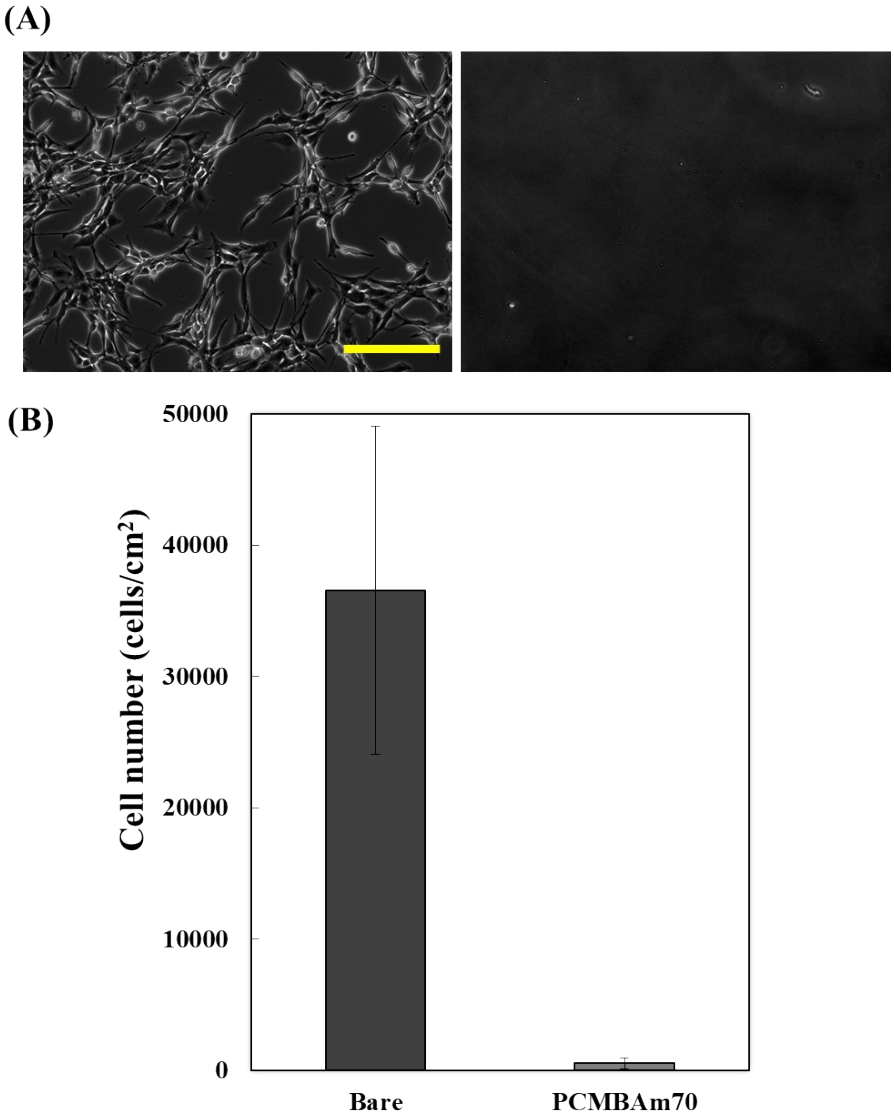


Fig. S10. (A) Phase contrast image of the cells adhered to a glass surface (left) and PCMBAm70-modified surface (right). Scale bar: 200 μm. The cells were seeded on the substrates and cultured for 1 day. (B) Number of cells adhered to the substrates (n = 3).