Electronic Supplementary Information (ESI)

Design of a star-like hyperbranched polymer having hydrophilic arms for anti-biofouling coating

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1. Experimental

1.1 Materials. Ethylene oxide acrylate (EO9A, number average molecular weight (M_n) = 480, #454990, Merck KGaA, Darmstadt, Germany) was purified by passing through a column filled with basic alumina. *N*,*N*,*N*',*N*'',*N*''-Pentamethyldiethylenetriamine (PMDETA, purity > 99%) and *N*,*N*'-diisopropylcarbodiimide (DIC, purity > 99%) were purchased from Merck. 2-Hydroxyethyl 2-bromoisobutyrate (HEBiB) was synthesized following a previously reported procedure.[S1] 4,4-Dimethylaminopyridine (DMAP, purity > 99%) was purchased from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan. Copper(I) bromide (CuBr, purity > 95%) and copper(II) bromide ($CuBr_2$, purity > 98%) were purchased from Kishida Chemical Co., Ltd., Osaka, Japan. *N,N*-Dimethylformamide (DMF, purity > 99.5%) was purchased from Nacalai Tesque Inc., Kyoto, Japan. Dichloromethane (DCM, purity > 99.5%) was purchased from FUJIFILM Wako Pure Chemical Co., Osaka, Japan). A precursor hyperbranched polymer (HBP, HB) was kindly provided by Nissan Chemical Corp., Tokyo, Japan.

1.2. Polymer synthesis.

1.2.1. Synthesis of the macroinitiator. Initiator units for atom transfer radical polymerization (ATRP), as described later, were introduced at chain-ends of HB on the basis of DIC/DMAP coupling reaction. HB (5.0 g), DIC (10 mL, 12.8 mmol) and DMAP (7.8 g, 12.8 mmol) were added to dry DCM (100 mL) in a 300 mL round bottom flask. And then, while stirring the HB solution on a stir plate, a solution of HEBiB (7.6 mL, 10.7 mmol) dissolved in 20 mL of DCM was added dropwise at room temperature. After 24 h, products were precipitated in methanol three times to remove byproduct *N,N*'-diisopropylurea, DIC and DMAP. The resulting solution was poured into ethanol to precipitate the product and dried under vacuum overnight at room temperature. Finally, the macroinitiator (HB-EBiB), which was a light yellow-coloured solid, was obtained (4.4 g).

1.2.2. Polymerization. A baked Schlenk tube was charged with HB-EBiB (87 mg), EO9A (2.2 mL, 5.0 mmol), and PMDETA (0.40 mL, 0.20 mmol) dissolved in DMF (7.3 mL). The flask was repeatedly degassed by Ar bubbling. After that, CuBr (12 mg, 0.080 mmol) and $CuBr_2$ (4.5 mg, 0.040 mmol) were quickly added. The flask was sealed with a three-way cock, and then, Ar bubbling was performed for 10 min before immersing the flask into an oil bath at 353 K. The conversion of EO9A monomers was determined by ^{1}H-NMR analysis. The reaction solution was added to toluene (20 mL), and then the mixture was passed through neutral alumina to remove the copper complexes. The product was precipitated from the toluene solution into an excess amount of hexane and dried under vacuum overnight.

1.3. Polymer characterization. The chemical structures of each HBP were identified by ^{1}H- and ^{13}C-nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectroscopy using an ECZ-400S spectrometer

(JEOL Ltd., Akishima, Tokyo, Japan). As a solvent, dimethyl sulfoxide-*d6* (DMSO-*d6*) was used. The hydrodynamic diameters of HBPs dissolved in tetrahydrofuran were analyzed by dynamic light scattering (DLS) using a Photal DLS-8000 (Otsuka Electronics Co., Ltd., Osaka, Japan) equipped with an Ar laser (488 nm). The scattering angle was set at 90 º and all measurements were performed at 298 K. In order to avoid dust contamination, all dispersions were filtered through a poly(tetrafluoroethylene) membrane filter with a mean pore diameter of 0.45 μm.

1.4. Surface characterization. The surface chemical composition of HBP layers prepared on Si-wafers with a native oxide layer by a spin-coating method was examined by X-ray photoelectron spectroscopy (XPS) using a XPS-APEX (Physical Electronics Inc., Chanhassen, MN, USA) at 1.5 $\times 10^{-9}$ Pa using a monochromatic Al-Kα X-ray source of 100 W. The emission angle was fixed at 15º. The C_{1s} peak was calibrated to a binding energy of 285.0 eV for neutral carbons to correct the charging energy shifts. Static contact angle was examined using a Drop Master 500 (Kyowa Interface Science, Niiza, Saitama, Japan) at room temperature. As a liquid probe, a droplet of ultrapure water (H_2O) from a Milli-Q system (Merck KGaA, Darmstadt, Germany) was used for the measurement in air. In addition, air bubbles were used as a probe for the measurement in water. The thickness of the HBP layers coated on Si-wafers was estimated using atomic force microscopy (AFM, Cypher ES, Asylum Research, Oxford Instruments Company, Santa Barbara, CA, USA) with an intermittent contact mode at room temperature. Cantilever tips microfabricated from Si, with a spring constant of 42 $N \cdot m^{-1}$ and a resonance frequency of 285 kHz and 0.1 $N \cdot m^{-1}$and 21 kHz, were used. The former and latter were for HB and HB-PEO9A, respectively. Prior to observing the samples in water, they were soaked for at least 3 h to reach a *quasi*-equilibrium state.

1.5. Protein adsorption test. As a substrate, polyethylene terephthalate (PET) was used. The HBP layers were prepared on the PET substrates by a dip-coating method. All samples were immersed in phosphate buffered saline (PBS, pH 7.4) for 3 h. The samples were then moved into a mixture of 0.3 $mg \cdot mL^{-1}$ of fibrinogen (341576, Merck) and 0.45 $mg \cdot mL^{-1}$ of human serum albumin (A3782, Merck) in PBS for 1 h at 310 K (37 ºC). After that, the surfaces were gently rinsed with fresh PBS. Adsorbed proteins were detached in sodium dodecyl sulfate (SDS) (1 wt % in water) by sonication for 20 min. The protein concentration in the SDS solution was determined using the microplate procedure of a micro-bicinchoninic acid protein assay kit (micro-BCA, Thermo Fisher Scientific K.K., Tokyo, Japan). These measurements followed the standard protocols for microplate procedures. The absorbance of the sample solution at around 570 nm was measured using a plate reader (INFINITE 200, Tecan Japan Co., Ltd., Kawasaki, Kanagawa, Japan). A standard curve was prepared by plotting the average blank-corrected 570 nm reading in order to determine the protein concentration of each unknown sample.

1.6. Cell adhesion test. NIH3T3 cells (RCB1862, RIKEN BRC Cell Bank, Wako, Saitama, Japan), were maintained in Dulbecco's modified Eagle medium (D-MEM, 11885-084, Thermo Fisher Scientific K.K.) containing 10% fetal calf serum (FCS, Hyclone, SH30073, GE Healthcare Japan, Co., Tokyo, Japan) in a 28 cm^2 tissue-culture petri dish. Cells were washed once with PBS and then treated with an aliquot of a solution containing 0.02% ethylenediaminetetraacetic acid (EDTA) and 0.25% trypsin. After centrifugation, NIH3T3 cells were re-suspended in the D-MEM containing 10% FCS. Subsequently, cells were incubated for the PET substrate and HBP layers in 1 mL (2×10^5 cells cm^{-2}) of the D-MEM containing 10% FCS for 12 h at 310 K (37°C) under 5% CO_2. Incubated cells were observed in the culture. The samples were washed with PBS to remove non-adherent cells. After fixing adherent NIH3T3 cells using 2 wt% glutaraldehyde in PBS for 2 h at 277 K (4°C), they were stained with 0.01 wt% crystal violet in PBS overnight and washed using water. Resultant cells were observed by a phase-contrast microscope (BZ-8100, Keyence Co., Osaka, Japan).

2. Chemical structure of HBP

Fig. S1 shows ^{13}C-NMR spectrum for HB. Peaks observed at around 174 and 125 ppm were assigned to phenyl and carbonyl groups, respectively. The molar ratio of divinyl benzene units to carboxyl groups was determined to be approximately 1:1 based on the areal fraction of both peaks.

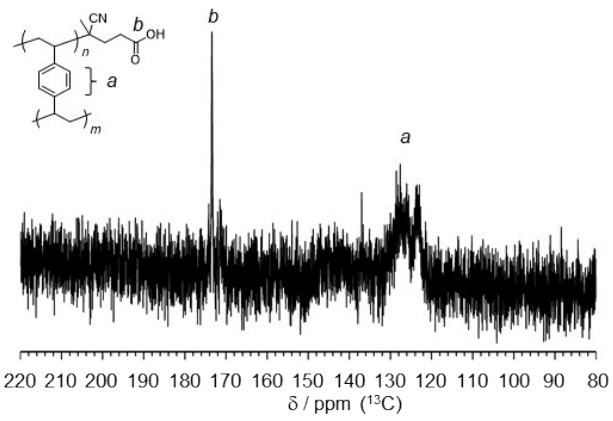

Fig. S1 ^{13}C-NMR spectrum for HB acquired in dimethyl sulfoxide-*d6*.

Fig. S2 shows ^{1}H-NMR spectra for (a) HB, (b) HB-EBiB and (c) HB-PEO9A. A peak observed at 12.3 ppm for HB (panel (a)) was attributed to dimeric carboxy groups at the chain ends. This peak disappeared after the coupling reaction, as shown in panel (b). In addition, peaks arisen from ethyl and bromoisobutyl groups in EBiB units were observed at 4.2 ppm and 1.8 ppm, respectively. These findings suggest that the coupling reaction had successfully proceeded. Panel (c) shows a characteristic spectrum for HB-PEO9A. While the broad set of signals in the range of 4.7–8.0 ppm due to poly(divinyl benzene)-core units was observed both for HB (panel (a)) and HB-EBiB (panel

(b)), this was not the case for HB-PEO9A (panel (c)). This is because the thermal mobility of core units with PEO9A chains became lower than that without PEO9A chains[S2] and the signal intensity derived from PEO9A backbones observed in the range 2.8 –5.0 ppm was remarkably strong.

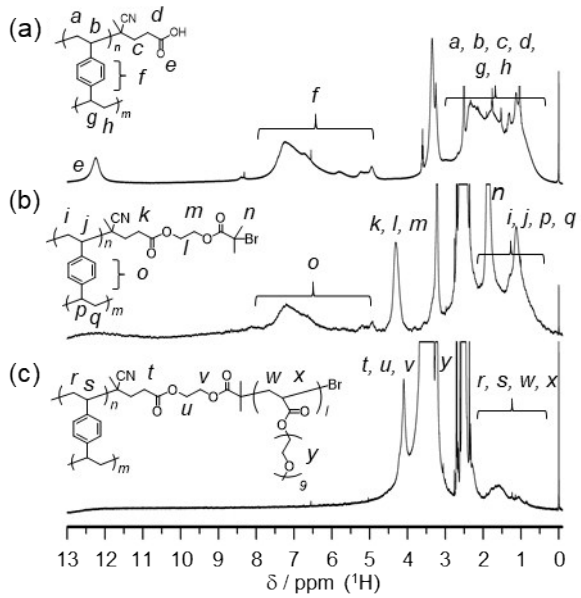

Fig. S2 ^{1}H-NMR spectra for (a) HB, (b) HB-EBiB and (c) HB-PEO9A acquired in dimethyl sulfoxide-*d6*.

3. Hydrodynamic diameter of HBP

Fig. S3 shows the number-weighted distribution of the hydrodynamic diameter for HB, HB-EBiB and HB-PEO9A determined by DLS. The value for HB-EBiB was 5.1±0.7 nm and almost the same as that for the original HB, 5.2±1.1 nm. After introducing PEO9A units onto HB, the hydrodynamic diameter increased to 15.5±2.1 nm with a narrow size distribution.

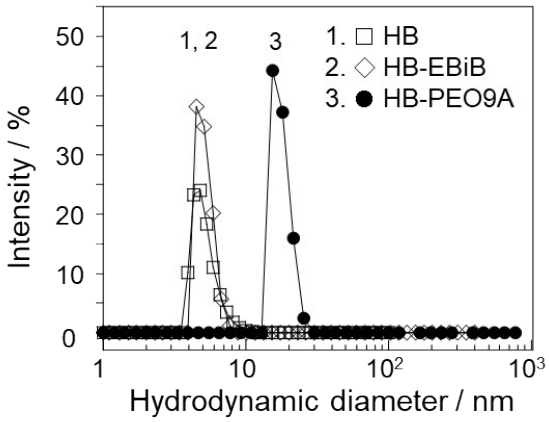

Fig. S3 Hydrodynamic diameter of HB, HB-EBiB and HB-PEO9A determined by DLS in tetrahydrofuran.

4. Cell adhesion

Fig. S4 shows phase contrast images of NIH3T3 fibroblasts on the PET substrate and the HB and HB PEO9A layers after 12-h culture before fixing by glutaraldehyde.

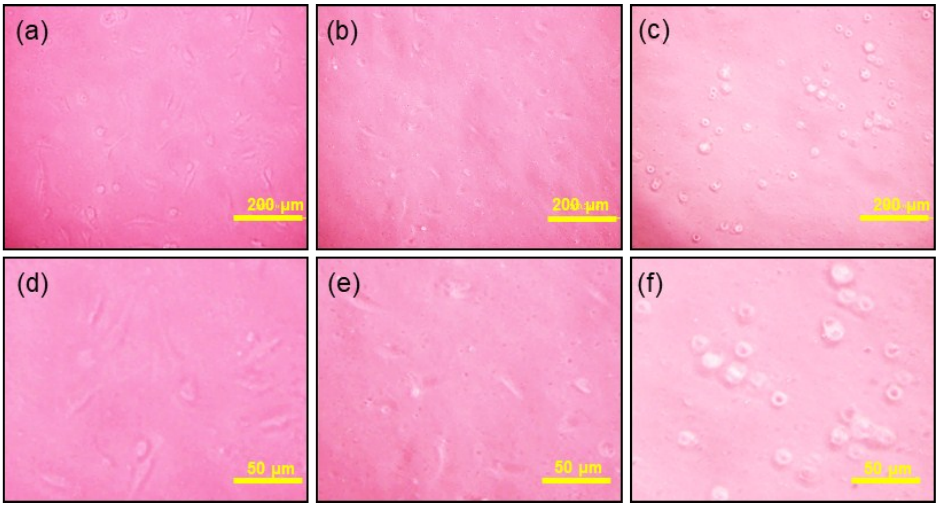

Fig S4. Phase contrast microscopic images of NIH3T3 cells cultured on (a) bare PET, (b) HB and (c) HB-PEO9A layers. Panels (d–f) are enlargements of the images shown in (a–c).

References

S1. T.-Y. Tsai, C.-F. Huang. *Data Brief*, 2015, **3**, 195–200.

S2. Y. Koda, T. Terashima, M. Sawamoto, *Polym. Chem.*, 2015, **6**, 5663–5673.