Zwitterionic macro-crosslinker for durable non-fouling coating

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Supporting information

Material

2-Aminoethyl methacrylate hydro-chloride (NH_2-MA monomer, 90%), Tert-Butyl bromoacetate (98%), 2-(Dimethylamino) ethyl mathacrylate (DMA 98%), N,N-Methylenebis(acrylamide) (MBAA, 99%), Anhydrous DMF, Anhydrous Acetone (99.99%) and Anhydrous methanol were purchased from Sigma-Aldrich, St. Louis, MO. Triethylamine (TEA, 99%), trifluoroacetic acid (TFA, 99.9%) and Ethyl Ether anhydrous (99.9%) were obtained from Fisher Chemical. Co. Deuterium Oxide (D_2O 99.9%) and Dimethyl sulfoxide-D6 (D-DMSO, 99.9%) were obtained from Cambridge Isotope Laboratories, Inc. USA.

Synthesis of PCBMA-tBu monomer

The synthesis procedure was as reported previously[1]. Briefly 5 g 2-(Dimethylamino)ethyl methacrylate and 8.68 g tert-butyl bromoacetate were reacted in 20 ml acetonitrile for 24 h at 50 ^oC under N2 protection. Upon addition of 250 ml ethyl ether to the reaction mixture, the white crystals were isolated and dried. The resulting CBMA-tBu monomers were immediately stored in a desiccator at -20 ^oC (yield 96%)

Synthesis of PCBMA macro-crosslinker

The polymerization solvent was prepared by dissolving 5 mg 2-Hydroxy-4-(2-hydroxyethoxy)-2-methylpropiophenone (photo-initiator, I-2959) in anhydrous DMF. The CBMA-tBu ester monomer was first copolymerized with 2-Aminoethyl methacrylate hydro-chloride (NH_2MA monomer) at different molar ratio (10:1, 30:1 and 50:1) in the polymerization solvent (35% by weight) under UV for 10 min and the resulting polymer A was precipitated in anhydrous acetone. Unreacted monomer was washed away by re-dissolving the product in DMF and precipitated in acetone for additional two times (polymer yield: 56%). The purified copolymer A was then reacted with acryloyl chloride (3 times of the amount of NH_2 function group) in presence of triethylamine in anhydrous DMF (10% by weight) for 5 h at room temperature followed by precipitation in anhydrous ethyl ether and vacuum dry (polymer B, yield: 87%). Lastly, copolymer B was stirred in trifluoroacetic acid (20% by weight) to remove tBu ester protection and neutralized using triethylamine (TEA) to pH=7 in anhydrous methanol on ice bath. The obtained zwitterionic macro-crosslinker C was precipitated in ethyl ether and dried in vacuum (macro-crosslinker C yield: 91%; overall yield: 44%).

Characterization

The molecular weight of the macro-crosslinker and surface initiated polymer (obtained by ultrasonic treatment after coating) were determined by gel permeation chromatography (GPC) on a Waters Alliance 2695 Separations Module equipped with a Waters Ultrahydrogel Linear column and a Waters 2414 reflex detector. The mobile phase was PBS buffer solution at a flow rate of 0.7 ml/min at 35 ^{o}C. Poly(ethylene oxide) from Polymer Laboratories were used as standards. The synthesized products were examine using a Varian Mercury-400 MHz NMR. In Figure 2 of the main text, the product A and B were dissolved in D-DMSO and product C was dissolved in D_2O. The coating thickness was characterized by surface ellisometry and averaged from three measurements at different surface site (α-SETM, J.A. Woollam Co., Inc.).

Protein absorption:

To measure the fibrinogen (Fg, Sigma-Aldrich) adsorption on PCBMA macrocrosslinker coating, coated surface (either by PCBMA brush or PCBMA macrocrosslinker) and bare PU surface were incubated with 1mg/ml Fg in a well plate for 10 minutes at room temperature, followed by 5 washes with PBS buffer. Samples were then incubated with 1 mg/ml bovine serum albumin solution for 10 minutes at room temperature with 5 times wash again with PBS buffer. The tested surface were then removed from the fifth PBS wash and transferred to new wells. They were next incubated with a 1:200-dilution of horseradish peroxidase (HRP)-conjugated anti-fibrinogen in PBS for 10 minutes, followed by another 5 washes with the same buffer. After the fifth wash, the tested surfaces were transfer to new wells and SIGMAFAST OPD was added to each well at 30-second intervals. The surfaces were incubated in the OPD solution for 30 minutes away from light. The supernatant was removed from each test well, transferred to a cuvette, and its absorbance at 490 nm was measured. All samples were measured in triplicate.

Bacteria adhesion and SEM image

After bacteria adhesion on PU, the surface will be immersed in the fix solution of 2.5% gluaraldehyde, 2% paraformaldehyde in 0.1 M sodium phosphate buffer. The surface was then dehydrate in a gradient ethanol series and dried in vacuum. Before imaging, the surface sample was coated with nano-gold using a SEEVac Conductive IV sputter coater. The bacteria were imaged using a JSM - 6510LV SEM at 5 um magnification and bacteria adhesion density was calculated by counting the bacteria number per 900 um^2.

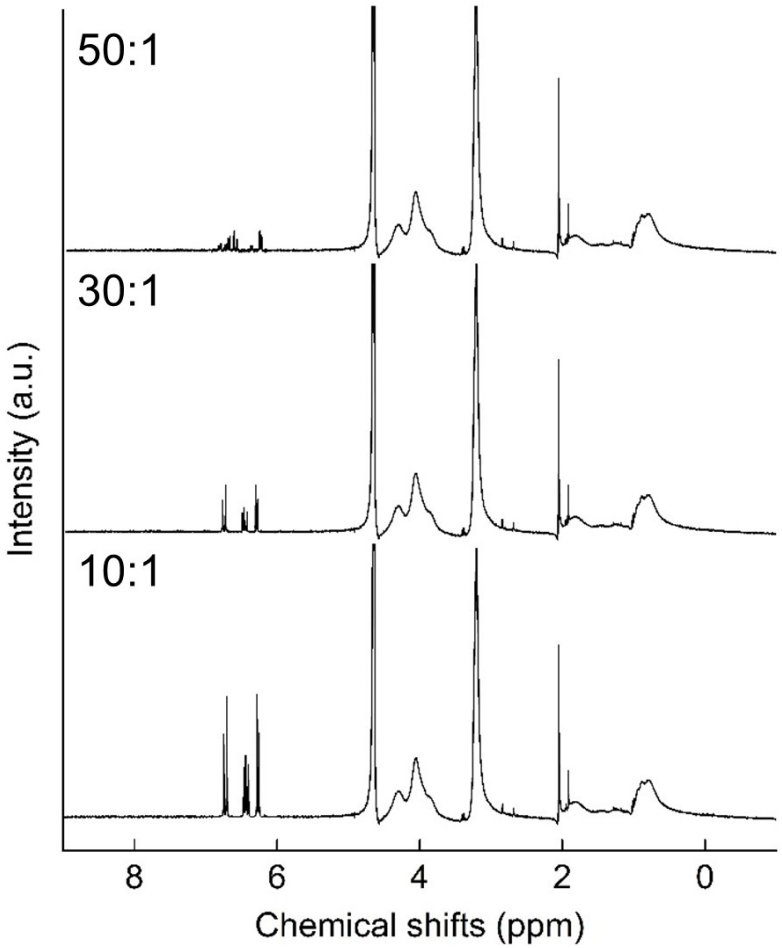

Figure S1 [1]HNMR of PCBMA macro-crssolinker with different double bond density (labeled by CBMA/double bond molar ratio).

Reference

1. Z. Cao, Q. Yu, H. Xue, G. Cheng and S. Jiang, Angewandte Chemie, 2010, 122, 3859-3864.