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Synthesis of Catechol and Zwitterion-Bifunctionalized Poly(ethylene glycol) for the Construction of Antifouling Surfaces

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Abstract

The synthesis of catechol-containing small molecules and macromolecules always requires multiple reaction steps, coupling agents, or enzymes. In this study, a simple and scalable strategy for the preparation of catechol-containing poly(ethylene glycol) (CaPEG) by epoxide-amine polymerization of PEG diglycidyl ether with dopamine is described. The as-formed tertiary amine groups in the backbone of CaPEG can be converted into sulfobetaine structures in an alkylsulfonation step, leading to the formation of catechol and zwitterion-bifunctionalized PEG (SBCaPEG). The resulting catechol-containing CaPEG and SBCaPEG can be anchored on various substrate surfaces, including stainless steel (SS), titanium and silicon wafer, under a mild condition. Since SS is susceptible to fouling by a variety of microorganisms, the antifouling properties of the polymer-coated SS surfaces are studied in details. The CaPEG- and SBCaPEG-coated SS surfaces effectively reduced the adsorption of protein (albumin-fluorescein isothiocyanate conjugate and bovine plasma fibrinogen), as well as the adhesion of bacteria (*Pseudomonas sp.* and *Escherichia coli*) and microalgae (*Amphora coffeaeformis*), as compared to that of the pristine SS surface. In comparison to the CaPEG-coated SS surfaces, the zwitterionic SBCaPEG-coated SS surfaces exhibited even better antifouling efficiencies.

Keywords: poly(ethylene glycol), antifouling, epoxide-amine reaction, zwitterionic, catechol

Introduction

The design of low- or non-fouling surfaces to combat nonspecific adsorption of biomolecules and adhesion of microorganisms is of considerable importance for a broad range of applications in biomedical implant devices, biosensors, separation processes, and food packing and storage, as well as marine structures.[1-4] Surface coatings consisting of poly(ethylene glycol) (PEG),[5] hyperbranched polyglycerol,[6] polyvinylpyrrolidone,[7,8] neutral hydrophilic (meth)acrylate and (meth)acrylamide polymers,[9,10] zwitterionic polymers,[11-15] polysaccharides,[16] and peptides[17] have been developed to impart antifouling capability to biofluid-contacting materials. Among them, zwitterionic polymers have attracted considerable research interest due to their ultralow-fouling properties.[18] Zwitterionic polymers, such as poly(sulfobetaine),[19] poly(phosphorylcholine),[20] poly(carboxybetaine),[21] and both carboxylic acid and amine fragments-containing poly(amino acid)[22,23] have shown great promise in combating protein adsorption, cell and bacteria adhesion, and biofilm formation.

Classic strategies for surface immobilization of zwitterionic materials include: i) surface-initiated polymerization of zwitterionic monomers,[13,15,24-28] ii) surface deposition of pre-synthesized zwitterionic materials with anchoring groups,[17,29-31] and iii) surface functionalization of pre-grafted layers using strained sultones and lactones, and amino acids.[32-35] Although the existing strategies often meet the demand for the construction of antifouling surfaces, some of their specific preparation and fabrication methods may limit their use in practical applications. For example, the surface-initiated polymerization usually require the synthesis and immobilization of suitable initiating groups, prior to the polymerization step with synthetic monomers under an inert atmosphere. It is not straightforward to prepare zwitterionic materials

functionalized with anchoring groups, because they require challenging and multi-step organic synthesis and sometimes of limited yield. Thus, it remains a challenge to develop a simple, low cost and scalable method for the synthesis of zwitterionic materials for direct deposition as surface antifouling coatings.

Epoxy resins have been widely used in industry as protective coatings and for structural applications.[36] Inspired by the curing reaction between epoxy resins and primary amino moieties, an efficient and robust chain-extension reaction between bifunctional epoxy and primary amino compounds has been demonstrated to provide access to a new family of functional polymers with tertiary amine moieties as bridges for the building blocks.[37] Interestingly, the alkylsulfonation of a polymeric tertiary amine with strained sultones, usually the inexpensive 1,3-propanesultone, is a straightforward protocol for the preparation of poly(sulfobetaine). Thus, the synthesis of catechol-containing polymers via epoxide-amine chain-extension reaction, followed by alkylsulfonation with strained sultones, will allow the formation of catechol and zwitterion-bifunctionalized polymer coatings bearing both substrate anchoring capability and antifouling property. The commercially available dopamine and PEG diglycidyl ether (PEGDGE) are of relatively low cost, and the stoichiometric reaction products (CaPEG) not only contain pendant catechol groups, which are good and universal anchors for surface modification,[6,30,38-41] but also bear tertiary amine moieties in the backbone, which can be converted into sulfobetaine structures in a single post-polymerization step (SBCaPEG, **Scheme 1**). The coupling of the resulting CaPEG and SBCaPEG polymers on stainless steel (SS), titanium and silicon surfaces were explored. The protein adsorption, as well as bacteria and algae adhesion, on the polymer-coated SS surfaces were studied in details.

Experimental Section

Materials

SS foils (AISI type 304, Fe/Cr18/Ni10, 0.05 mm thick) and titanium foils (99.6%, 0.05 mm in thick) were purchased from Goodfellow Ltd., Cambridge, UK. Silicon wafer ((100)-oriented single crystal silicon) was purchased from Unisil Co. of Santa Clara, CA. Poly(ethylene glycol) diglycidyl ether (PEGDGE, M_n ~ 500), dopamine hydrochloride, bovine plasma fibrinogen (FBG), albumin-fluorescein isothiocyanate conjugate (BSA-FITC, Product No. A9771) and 1,3-propanesultone (99%) were purchased from Sigma-Aldrich Chemical Co. All other regents were purchased from Sigma-Aldrich or Merck Chem. Co., and were used without further purification.

Synthesis of CaPEG

PEGDGE (40.5 g, 81 mmol) and dopamine hydrochloride (15.4 g, 81 mmol) were dissolved in 100 mL of methanol in a 250-mL two-neck round-bottom flask, and bubbled with argon for 60 min. Pre-degassed NaOH aqueous solution (2 M, 40.5 mL) was then added dropwise into the flask. The reaction mixture was stirred for 48 h at room temperature with argon bubbling. After that, the reaction mixture was acidified to pH 4 using diluted HCl (3 M), and was dialyzed with doubly distilled water (molecular weight cut-off = 1,000 Daltons) for 48 h. During dialysis, the water was replaced every 8 h. About 35.4 g of CaPEG was obtained after lyophilization as a viscous solid (M_n = 4,800, PDI = 3.14).

Synthesis of SBCaPEG

CaPEG (20.0 g, ~31 mmol of tertiary amine) was first dissolved in 100 mL of methanol in a 250-mL round-bottom flask with argon bubbling, followed by the addition of 1,3-propanesultone (5.4

mL, 62 mmol) under stirring at 50 ^{o}C for 24 h. Upon cooling down to room temperature, the reaction mixture was dialyzed against methanol for 3 days. After rotary evaporation and vacuum drying, about 17.5 g of SBCaPEG sample was obtained as a viscous liquid (M_n = 7,800, PDI = 3.43).

Deposition of CaPEG and SBCaPEG coatings on the SS substrates

The SS foils were cut into 1 inch × 1 inch coupons, and cleaned ultrasonically with doubly distilled water, acetone and ethanol for 15 min each. The SS coupons were then rinsed thoroughly with doubly distilled water and blown dry with compressed air. The clean SS coupons were activated by immersing in the cold piranha solution (H_2SO_4 (95-97%) : H_2O_2 (30 wt%) = 3:1, v/v) for 30 min to generate hydroxyl-enriched surfaces.

The SS coupons were immersed in the CaPEG and SBCaPEG aqueous solution (20 mg/mL, pH =7.5) at room temperature for 48 h. After that, the SS coupons were thoroughly rinsed with doubly distilled water and ethanol, and dried under a stream of compressed air. The resulting CaPEG- and SBCaPEG-anchored SS substrates were denoted as SS-CaPEG and SS-SBCaPEG. The SS coupons were also immersed in an aqueous mixture of CaPEG and SBCaPEG (10 mg/mL each, pH =7.5) at room temperature for 48 h to achieve a different surface sulfobetaine content. The resulting polymer-coated SS substrates were denoted as SS-(CaPEG-SBCaPEG).

Protein adsorption on the CaPEG- and SBCaPEG-anchored SS substrates

BSA-FITC was dissolved in phosphate buffered saline (PBS, 10 mM, pH 7.4) to a protein concentration of 2 mg/mL. The pristine and polymer-anchored SS substrates were placed in a

24-well plate and immersed with the dissolved protein solution for 12 h at 37 °C. The plate was capped and wrapped with aluminum foil to provide a dark environment. The SS substrates were then washed twice with PBS and once with Millipore water to remove weakly adhered BSA-FITC. The substrates were then viewed under a Nikon Eclipse Ti microscope equipped with a 470 nm excitation filter and a 540 nm emission filter for green fluorescence. For quantitative analysis, NIH ImageJ software (http://imagej.nih.gov/ij/) was used to analyze the images obtained from fluorescence microscopy. The fluorescence intensity, which is indicative of the amount of protein adsorbed, was quantified on a per-pixel basis, summed up, and divided by the total number of pixels on the image to obtain mean values.[42]

FBG was also dissolved in PBS (10 mM, pH 7.4) to a protein concentration of 2 mg/mL. The pristine and polymer-anchored SS substrates were placed in a 24-well plate and immersed in the protein solution for 12 h at 37 °C. After rinsing successively with PBS and Millipore water, followed by drying under reduced pressure, the composition of protein-adsorbed surfaces was analyzed by X-ray photoelectron spectroscopy (XPS) measurements.

Bacteria adhesion to the CaPEG- and SBCaPEG-anchored SS substrates

Pseudomonas sp. (NCIMB 2021) and *Escherichia coli* (*E. coli*, DH5*α*) were used to evaluate the anti-adhesion characteristics of the polymer coatings to bacteria. *Pseudomonas sp.* was cultured following the methods reported in the literature.[43] Quantification of bacteria (*Pseudomonas sp.* and *E. coli*) adhesion on the pristine and polymer-coated SS substrates was carried out according to the method reported in the literature with minor modification (*E. coli* concentration = 5×10^7 cells/mL).[44] The visualization of bacterial cells on pristine and polymer-coated substrates was

assessed using a combination dye of SYTO9 and propidium iodide (PI) (LIVE/DEAD BacLight Bacterial Viability Kit, Life Technologies Corporation). The live bacteria with intact membranes would be stained green while the dead bacteria and those with damaged cell membranes would be stained red. The substrates were washed with Millipore water 2 times after the incubation period, stained with 50 μL of the combination dye for 30 min, washed with Millipore water, and then observed under a Nikon Eclipse Ti microscope, equipped with excitation/emission filters of 470/525 and 540/605 nm for green and red fluorescence, respectively.

Algae adhesion to the CaPEG- and SBCaPEG-anchored SS substrates

Amphora coffeaeformis (UTEX reference number B2080) was cultured according to procedures described in the literature.[45] The fluorescence microscopy technique and the quantification of *amphora* adhesion were described in our previous publication.[44]

Cytotoxicity of the CaPEG- and SBCaPEG-anchored SS substrates

Ten pieces of the SS-CaPEG and SS-SBCaPEG substrates (1 cm × 1 cm in size) were immersed into 20 mL of Dulbecco's Modified Eagle's Medium (DMEM) medium at 4 ^{o}C for 1, 4 and 7 days. The DMEM medium was supplemented with 10% fetal bovine serum, 2 mM L-glutamine and 100 U/mL penicillin. The cytotoxicity towards 3T3 fibroblasts was carried out via the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent. The cells were seeded at a density of 50,000 cells/wall and incubated at 37 ^{o}C for 24 h. The medium was then replaced by one pretreated with the SS-CaPEG and SS-SBCaPEG substrates. Control experiments were carried out using the complete growth culture medium, without exposure to the SS-CaPEG and SS-SBCaPEG substrates. After 24 h of incubation at 37 ^{o}C, the medium was

removed, the cells were washed thrice with PBS, and 1 mL of MTT solution (0.5 mg/mL in DMEM medium) was added to each wall. After an additional 4 h of incubation, the culture supernatants were aspirated, and the formazan crystals were solubilized with 1 mL of DMSO for 15 min. The optical absorbance was then measured at 600 nm on a microplate reader (Tecan GENios). The results were expressed as percentages relative to that obtained in the control experiment.

Characterization

The chemical structures of obtained polymers were characterized by ^{1}H NMR spectroscopy on a Bruker DRX 400 MHz spectrometer. Gel permeation chromatography (GPC) was performed on a Waters GPC system, equipped with a Waters 1515 isocratic HPLC pump, a Waters 717 plus autosampler injector, a Waters 2414 refractive index detector and a PL aquagel-OH Mixed-H 8 μm column (Agilent Technologies, S/N 0006137220-59), using Millipore water as the eluent at a flow rate of 1.0 mL/min at 35 ^{o}C. The calibration curve was generated using PEG molecular weight standards. XPS measurements were carried out on a Kratos AXIS Ultra HSA spectrometer equipped with a monochromatized AlKα X-ray source (1468.71 eV photons). The thicknesses of CaPEG and SBCaPEG coatings on silicon wafers were determined by ellipsometry. The measurements were carried out on a variable angle spectroscopic ellipsometer (model VASE, J.A. Woollam Inc., Lincoln, NE) at incident angles of 65° and 75° in the wavelength range 500 - 1000 nm. Data were recorded and processed using the WVASE32 software package. The refractive index of the polymer layers was set as 1.45 for the Normal Fit. The static water contact angle measurements were performed using an optical video contact angle system (OCA-15-plus, Dataphysics). A 3-μL droplet of doubly distilled water was

dispensed on the SS surfaces using the electronic syringe unit of the instrument. The static contact angle was measured using the sessile drop method with dedicated software (SCA20). The ζ potential of the polymers-coated SS surfaces was determined by an electrokinetic analyzer (SurPass, Anton Paar) equipped with an adjustable-gap cell using 1 mM KCl as the electrolyte solution.

Results and Discussion

Synthesis of CaPEG and SBCaPEG

The epoxide-amine chain-extension reaction is known to occur in the presence of moisture and air, and have a high tolerance for a variety of functional groups.[37,46] The robust and efficient approach can be utilized to prepare catechol-functionalized CaPEG using commercially available dopamine hydrochloride and PEGDGE as the building blocks. The primary amino group in dopamine reacts with an epoxy group to produce a secondary amine and a secondary alcohol. The secondary amine can further react with an epoxy group to form a tertiary amine. The epoxide-amine reaction follows the step-growth polymerization mechanism to form the catechol-functionalized polymers. Since catechols are easily oxidized to quinones, the reaction needs to be performed under an inert atmosphere of argon. The chemical structures of CaPEG and SBCaPEG were confirmed by ^{1}H NMR spectroscopy. The resonance peak assignments are consistent with the polymer structures (**Figure 1**). The chemical shifts in the range of 6.60 and 6.83 ppm are attributable to the aromatic protons of the catechol groups, indicating the retention of catechol moieties during the robust epoxide-amine reaction. Post-functionalization of CaPEG with 1,3-propanesultone has led to the appearance of chemical shifts (peaks *i*, *k*, and *l*),[47] attributed to the sulfobetaine segments in SBCaPEG. The extent of alkylsulfonation, estimated from the ^{1}H NMR spectrum of SBCaPEG (**Figure 1b**) through integration of the signals of aromatic protons (peaks *a*-*c*) at 6.60 and 6.83 ppm and methylene proton (peak *l*) at 1.98 ppm, is about 69%. From gel permeation chromatography (GPC) measurements, the number-average molecular weight (M_n) of CaPEG against PEG molecular weight standards is 4,800 with a polydispersity index (PDI) of 3.14, while SBCaPEG has a M_n of 7,800 and a PDI of 3.43.

Functionalization of substrate surfaces with CaPEG and SBCaPEG

The catechol-functionalized macromolecules are able to adhere to many different substrates, including metal, metal oxide, and non-metal surfaces.[48,49] In this study, the anchoring of CaPEG and SBCaPEG onto a variety of surfaces was first studied. Contact angle measurements provide supporting evidence that the surfaces have been successfully modified.[6] **Figure 2** and **Figure S1** (Electronic Supplementary Information, ESI) shows the static water contact angles and the corresponding images of polymer-coated SS, silicon wafer and titanium surfaces. The pristine SS, silicon wafer and titanium surfaces are relatively hydrophobic, with respective contact angles of 72.7°, 86.1° and 60.4°. After CaPEG and SBCaPEG coating, the contact angles of polymer-coated surfaces decrease to around 30°, due to the hydrophilic nature of CaPEG and SBCaPEG. The contact angles of SBCaPEG-coated surfaces are lower than that of the corresponding CaPEG-coated surfaces, because of the conversion of tertiary amine groups to polar zwitterionic sulfobetaine moieties. The PEG segments in CaPEG only bind water through hydrogen bonding, while the zwitterionic sulfobetaine moieties in SBCaPEG can give rise to additional ionic interactions with water molecules.[50] These results indicate that both CaPEG and SBCaPEG can be successfully anchored on SS, titanium, and silicon wafer surfaces. There are two ways to form coatings on solid substrates: i) the direct adsorption of the catechol-containing polymers, and ii) intermolecular oxidative coupling of catechol groups between the immobilized polymer chains and free polymer chains in the solution via a "grafting-to" approach.[6,38] Since silicon wafer has a relatively smooth surface, the thickness of the polymer coatings on its surface was measured by ellipsometry. The thickness of the CaPEG and SBCaPEG coatings on silicon wafer (Si(100) surfaces) are 9.3 ± 1.6 and 11.2 ± 2.3 nm, respectively.

SS is of considerable importance due to its useful applications in the food industry, biomedical devices and implants, and submerged marine structures.[35,51-53] Chemical modification of SS surfaces to confer the desired antifouling properties was studied in details. The XPS wide-scan spectra of pristine SS, SS-CaPEG and SS-SBCaPEG surfaces are shown in **Figure 3**. The enhanced C 1s signal and almost indiscernible Cr 2p, Fe 2p and Ni 2p signals in the wide-scan spectra of SS-CaPEG and SS-SBCaPEG surfaces, compared to those of the pristine SS surface, indicate the successful anchoring of CaPEG and SBCaPEG polymers on SS surfaces. The presence of N 1s signals in the wide-scan spectrum of SS-CaPEG surface and N 1s and S 2p signals in the wide-scan spectrum of SS-SBCaPEG surface also serve to confirm the successful deposition of CaPEG and SBCaPEG polymers. After immersion of the SS-CaPEG and SS-SBCaPEG surfaces in artificial seawater for four weeks, the Cr 2p, Fe 2p and Ni 2p signals (associated with the SS substrate) in the XPS wide-scan spectra of these surfaces (**Figure S2**, ESI) remain indiscernible, suggesting that the polymer coatings are intact and durable. The surface zeta potentials were also determined for the CaPEG- and SBCaPEG-coated SS substrates over a wide range of solution pH (**Figure S3**, ESI). Between pH 5 and 9, the CaPEG and SBCaPEG coatings exhibit negative surface zeta potential in the range of -3.4 to -50.4 and -24.6 to -53.8 mV, respectively.

Protein adsorption on the SS-CaPEG and SS-SBCaPEG surfaces

The adsorption of proteins to surfaces could play an important role in subsequent microorganism adhesion, as it may alter the physicochemical properties of the surfaces.[54] It is usually assumed that the antifouling surfaces to resist protein adsorption also tend to be less susceptible to bacterial adhesion. The ability of SS-CaPEG and SS-SBCaPEG surfaces to resist protein

adsorption was examined by incubating the surfaces in a solution containing fluorescent BSA-FITC, and subsequently imaged with a fluorescence microscope. **Figure 4** shows the respective fluorescence images and fluorescence intensities (bar graph) of the pristine SS, SS-CaPEG, and SS-SBCaPEG surfaces after exposure to PBS solution of BSA-FITC. Uniform and intense fluorescence is observed across the pristine SS surface (**Figure 4a**), suggesting a significant extent of BSA-FITC adsorption on the substrate surface. Weak fluorescence is present on the SS-CaPEG surface (**Figure 4b**), while almost no fluorescence is observed on the SS-SBCaPEG surface (**Figure 4c**). These results indicate that the adsorption of BSA-FITC protein on the polymer-coated SS surfaces is significantly inhibited due to the repulsive forces arising from the formation of a hydration layer on the hydrophilic surface. The enhanced antifouling effect of the SS-SBCaPEG surface may be attributed to strong surface hydration induced by ionic solvation between zwitterionic sulfobetaine moieties in SBCaPEG and water molecules.[55-57]

The adsorption of FBG on the SS-CaPEG and SS-SBCaPEG surfaces was evaluated from the XPS N 1s and C 1s core-level spectral peak area ratio ([N]/[C] ratio). Since proteins have large [N]/[C] ratios, the extents of protein adsorption can be evaluated from [N]/[C] ratio. As shown in **Figure S4** (ESI), the [N]/[C] ratios are 0.018, 0.037 and 0.035, respectively, for the pristine SS, SS-CaPEG and SS-SBCaPEG surfaces before exposure to the PBS solution of FBG. The significant increase in [N]/[C] ratio for the pristine SS surface after FBG exposure indicates that non-specific adsorption of FBG on the substrate surface is obvious. However, the adsorption of FBG on the SS-CaPEG and SS-SBCaPEG surfaces has not occurred to a significant extent, as indicated by only slight increase in the [N]/[C] ratios after exposure to the PBS solution of FBG.

Bacterial adhesion to the SS-CaPEG and SS-SBCaPEG surfaces

To further examine the antifouling properties of polymer-coated SS surfaces, the resistance to bacterial adhesion on the pristine SS, SS-CaPEG and SS-SBCaPEG surfaces was assayed. **Figures 5a-5f** shows the representative fluorescence microscope images of the pristine SS, SS-CaPEG, and SS-SBCaPEG surfaces after incubation in *Pseudomonas sp.* suspension for 4 h and straining with a combination dye of SYTO 9 and PI. There are many live *Pseudomonas sp.* cells (stained green, **Figure 5a**) on the pristine SS surface. For the SS-CaPEG surface (**Figure 5b**), the number of viable cells is less than that on the pristine SS surface. There are further reduction in the number of viable cells on the SS-SBCaPEG substrate surface (**Figure 5c**), in comparison to that on the SS-CaPEG surface. Only few dead cells with red fluorescence can be observed for bacterial stains (**Figures 5d-5f**), indicating that most of the *Pseudomonas sp.* cells are viable with the cell membrane intact on the pristine and polymer-coated SS surfaces, and CaPEG and SBCaPEG polymer coatings are not bactericidal. The bacterial-resistant properties of the SS-CaPEG and SS-SBCaPEG surfaces can be attributed to the strong hydration of the PEG segments and zwitterionic sulfobetaine moieties in an aqueous environment, induced by either hydrogen bonding or electrostatic interaction.[58]

Quantitative determination of the number of viable *Pseudomonas sp.* cells on the pristine and polymer-coated SS surfaces was carried out using the spread plate method. **Figure 5g** shows the number of adhered viable *Pseudomonas sp.* cells after exposure to the pristine and polymer-coated SS surfaces for 4 h. The number of viable cells on the respective SS-CaPEG and SS-SBCaPEG substrates decreases to about 11% and 8% of that on pristine SS surface. In comparison to the SS-CaPEG surface, the SS-SBCaPEG surface exhibit better bacterial-resistant

properties. These results are congruent with the fluorescence microscopy images. To further investigate the antifouling properties of the aged polymer-coated surfaces, the SS-CaPEG and SS-SBCaPEG substrates were immersed in artificial seawater at 25 ^{o}C for 2 weeks. The number of viable bacteria on the corresponding aged SS-CaPEG and SS-SBCaPEG surfaces increases only slightly to 15% and 11%. Thus, the catechol-containing polymer coatings on SS surfaces are stable and durable.

The number of viable *E. coli* cells on the pristine and polymer-coated SS surfaces was also assayed using the spread plate method. **Figure 5g** shows the number of bacterial cells adhered on the pristine and polymer-coated SS surfaces after 4 h of exposure to the *E. coli* culture. The number of viable cells on the respective SS-CaPEG, SS-(CaPEG-SBCaPEG) and SS-SBCaPEG substrates decreases to 35%, 27% and 19%, respectively, relative to that on the pristine SS surfaces (**Figure S5**, ESI). In comparison to the CaPEG-SBCaPEG mixed coating (XPS-derived surface [S]/[C] ratio of 0.017) and SBCaPEG only coating (XPS-derived surface [S]/[C] ratio of 0.026), the SS-SBCaPEG surface with a higher sulfobetaine content exhibits better resistance to bacterial adhesion.

Algae adhesion to the SS-CaPEG and SS-SBCaPEG surfaces

As a member of the raphid diatoms, *Amphora coffeaeformis* is widely known as a major microfouler in the benthic zone.[59] Since the CaPEG- and SBCaPEG-immobilized surfaces resist BSA-FITC and *Pseudomonas sp.* cells adhesion, it is of interest to determine the anti-adhesion properties of the polymer-coated SS surfaces to *Amphora* cells with chlorophyll autofluorescent property. **Figures 6a-6c** show the fluorescence microscopy images of pristine and polymer-

coated SS surfaces after exposure to the algae suspension. Significant *Amphora* fouling can be observed on the pristine SS surface, either in discrete or cluster form (**Figure 6a**). In contrast, *Amphora* cells only attach to a small proportion of the SS-CaPEG surface (**Figure 6b**), and even fewer *Amphora* cells settle on the SS-SBCaPEG surface (**Figure 6c**). This phenomenon is consistent with that observed for the bacterial assays, and can also be attributed to surface hydration of the PEG segments and the zwitterionic sulfobetaine moieties.[59-61]

Since the fluorescence microscopy images can only provide a visual comparison of the effect of the polymer-coated SS surfaces on *Amphora* adhesion, a more quantitative fluorescence assay was carried out via a combination of ultrasonic cell removal and chlorophyll autofluorescence detection.[44,59] **Figure 6d** shows the quantitative assay of *Amphora* cell adhesion on the pristine and polymer-coated SS surfaces. The amounts of adherent *Amphora* cells on the pristine SS, SS-CaPEG, and SS-SBCaPEG surfaces are about 12,800, 2,050, and 1,720 cells/cm^2, respectively. In comparison to the pristine SS surface, the respective adherent fractions of *Amphora* cells on the SS-CaPEG and SS-SBCaPEG surfaces are 16% and 13%. The antifouling property of the aged SS-CaPEG and SS-SBCaPEG surfaces, from exposure to artificial seawater at 25 ^{o}C for 2 weeks, to resist the attachment of *Amphora* cells were also examined. The amounts of settled *Amphora* cells on the aged SS-CaPEG and SS-SBCaPEG surfaces increase only slightly to about 2,940 and 2,400 cells/cm^2, respectively. These results also indicate the good stability and durability of the catechol-containing polymer coatings on SS surfaces.

Cytotoxicity of the SS-CaPEG and SS-SBCaPEG surfaces

The cytotoxicity of the SS-CaPEG and SS-SBCaPEG surfaces was evaluated using MTT assay

with 3T3 fibroblasts. The SS-CaPEG and SS-SBCaPEG surfaces were immersed in the DMEM medium at 4 ^{o}C for 1, 4 and 7 days to allow the toxic species to be released into the medium. Subsequently, the media were utilized for culturing the 3T3 fibroblasts for 24 h. As shown in **Figure S6** (ESI), cell viabilities, expressed in percentages relative to that obtained in the control experiment, are more than 98%, after incubation with the medium pretreated with SS-CaPEG and SS-SBCaPEG surfaces. The result of MTT cytotoxicity assay indicates that the SS-CaPEG and SS-SBCaPEG surfaces have low cytotoxicity towards 3T3 fibroblasts.

Conclusions

Catechol-containing poly(ethylene glycol) (CaPEG) was synthesized by epoxide-amine polymerization of poly(ethylene glycol) diglycidyl ether (PEGDGE) and dopamine. This finding can be extended to the design of new catechol-containing molecules and macromolecules via epoxide-amine ring-opening reaction, by replacing PEGDGE with other functional epoxides. The efficient and robust epoxide-amine ring-opening reaction will also allow for easy scale-up and production of large quantities of catechol-containing polymers. The as-formed tertiary amine moieties in the backbone of CaPEG can be converted into sulfobetaine groups in a simple post-polymerization step, leading to the formation of catechol and zwitterion-bifunctionalized PEG (SBCaPEG). The CaPEG and SBCaPEG polymers are able to tether to the SS, titanium and silicon wafer surfaces. Both CaPEG and SBCaPEG coatings significantly reduced the adsorption of BSA-FITC and FBG and the adhesion of bacteria and microalgae on SS surfaces. In addition, the zwitterionic SBCaPEG-coated SS surfaces exhibited better antifouling properties than the non-ionic CaPEG coated substrates. The CaPEG and SBCaPEG polymers can be developed into universal and versatile antifouling coatings on many substrate surfaces of interest.

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Legends for Schemes and Figures

Scheme 1. (a) Synthesis route of CaPEG and SBCaPEG; (b) Schematic illustration of a biomimetic approach to prepare the antifouling coating on SS surface.

Figure 1. ^{1}H NMR spectra of (a) CaPEG and (b) SBCaPEG in D_2O.

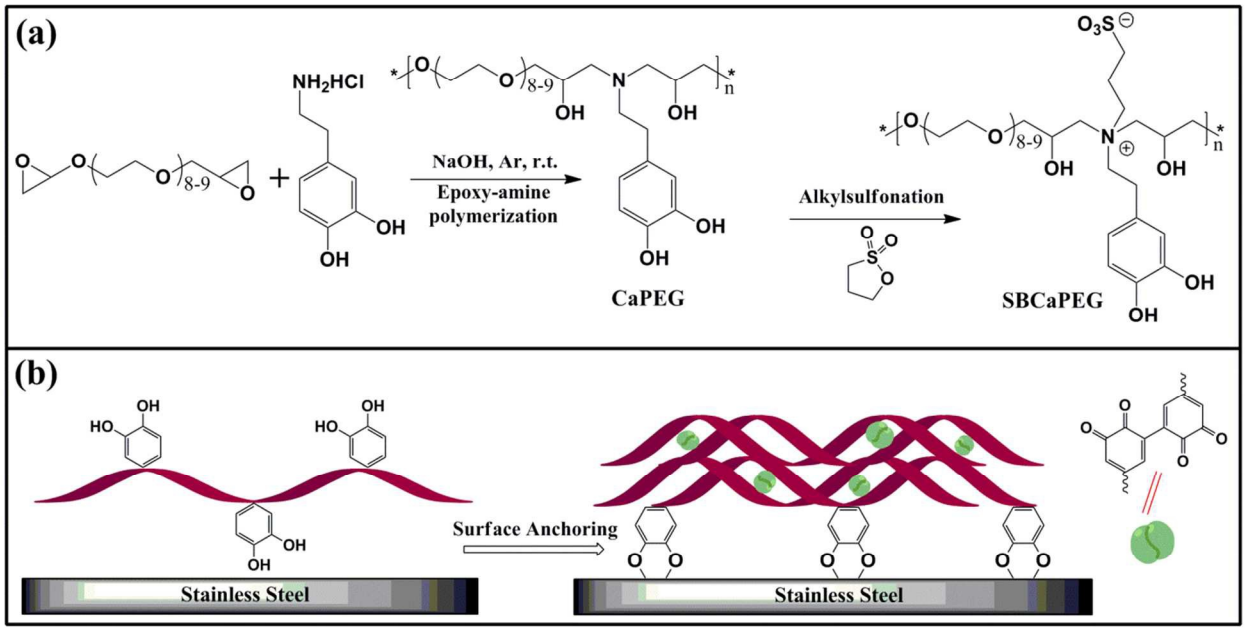
Figure 2. Static water contact angles of the pristine and polymer-coated SS, titanium, and silicon wafer surfaces.

Figure 3. XPS wide-scan spectra of the (a) pristine SS, (b) SS-CaPEG, and (c) SS-SBCaPEG surfaces.

Figure 4. Fluorescence microscopy images of the (a) pristine SS, (b) SS-CaPEG, and (c) SS-SBCaPEG surfaces after exposure to BSA-FITC solution; (d) fluorescence intensities of the corresponding SS surfaces.

Figure 5. Representative fluorescence microscopy images of the pristine SS, SS-CaPEG, and SS-SBCaPEG surfaces under the green filter (a,b,c) and the red filter (d,e,f), respectively, and (g) the number of adhered *Pseudomonas sp.* cells per cm^2 of the pristine SS, and as-prepared and aged SS-CaPEG and SS-SBCaPEG surfaces, after exposure to the bacterial suspension in artificial seawater (10^8 cells/mL) for 4 h.

Figure 6. Fluorescence microscopy images of the (a) pristine SS, (b) SS-CaPEG, and (c) SS-SBCaPEG surfaces, and (d) number of adhered *Amphora* cells on pristine SS, and as-prepared and aged SS-CaPEG, and SS-SBCaPEG surfaces after exposure to a 30‰ salinity, filtered seawater suspension of *Amphora coffeaeformis* (10^5 cells per mL) for 24 h.



Scheme 1

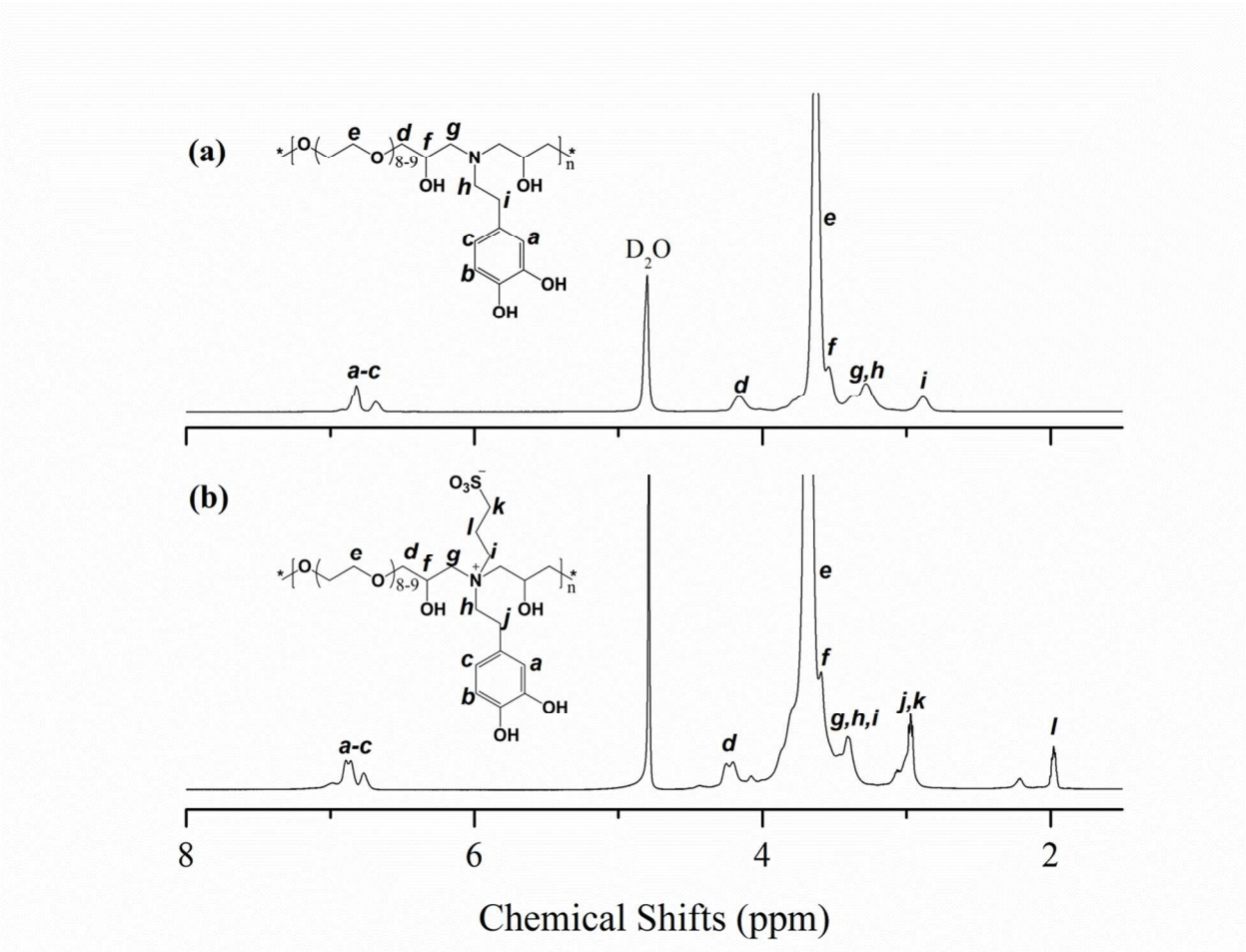


Figure 1

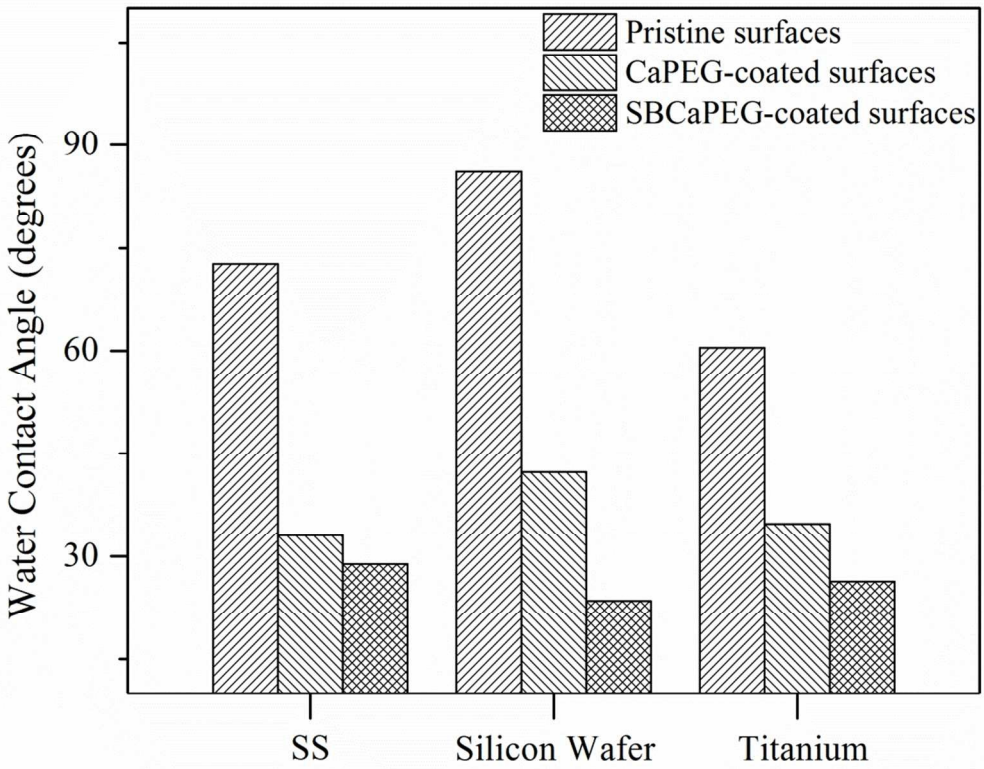


Figure 2

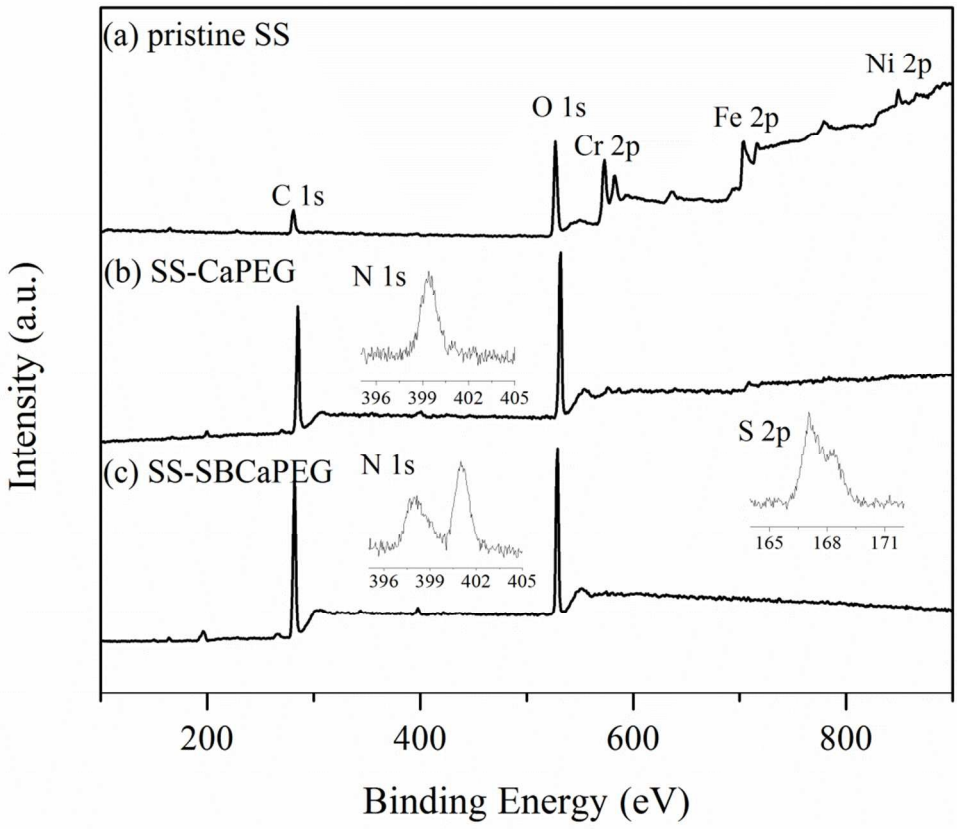


Figure 3

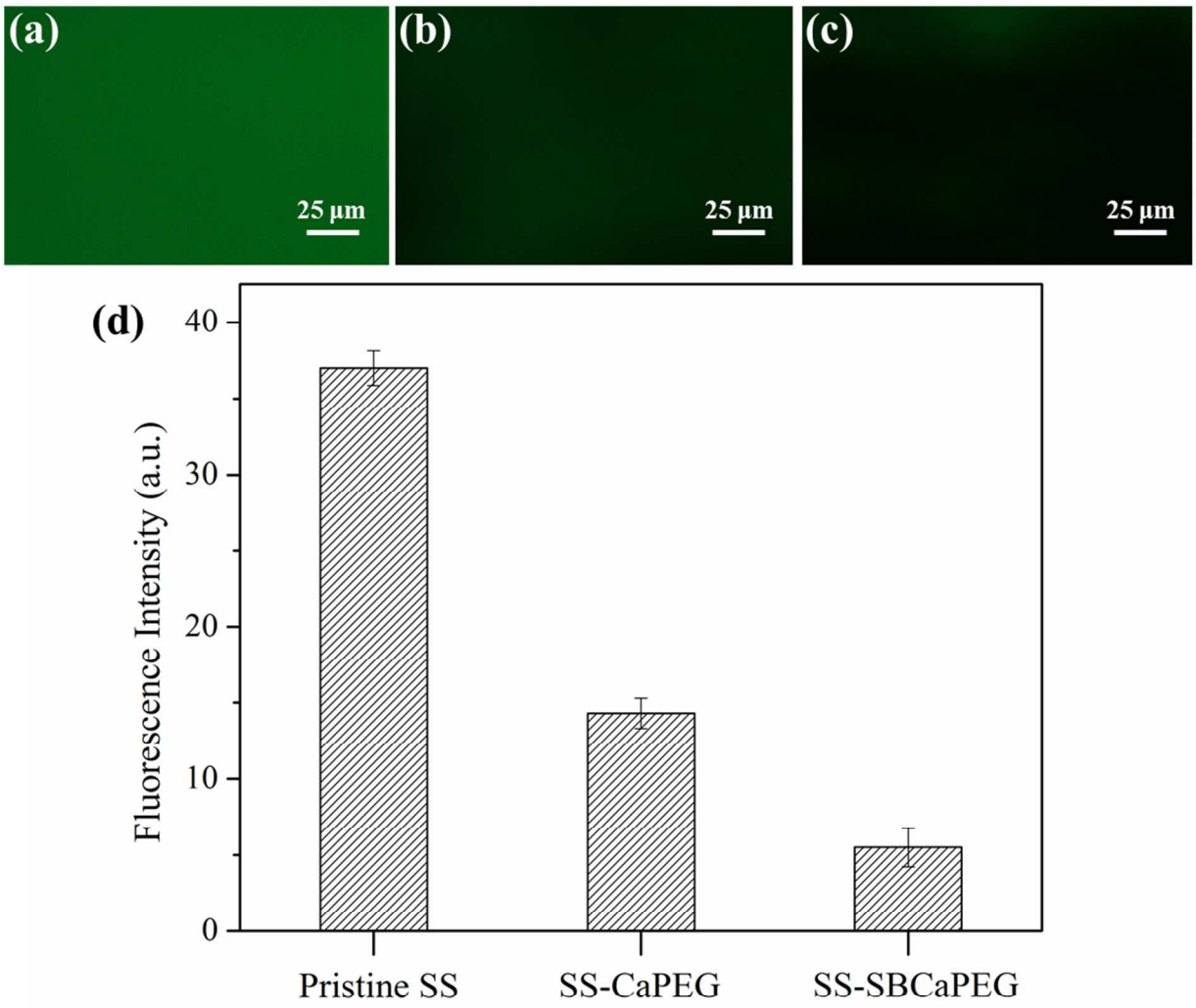
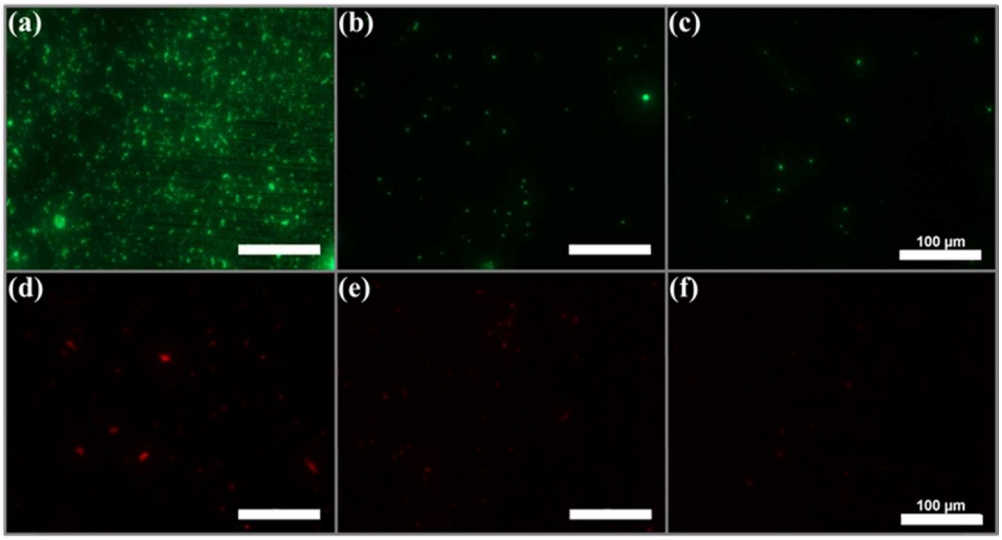


Figure 4



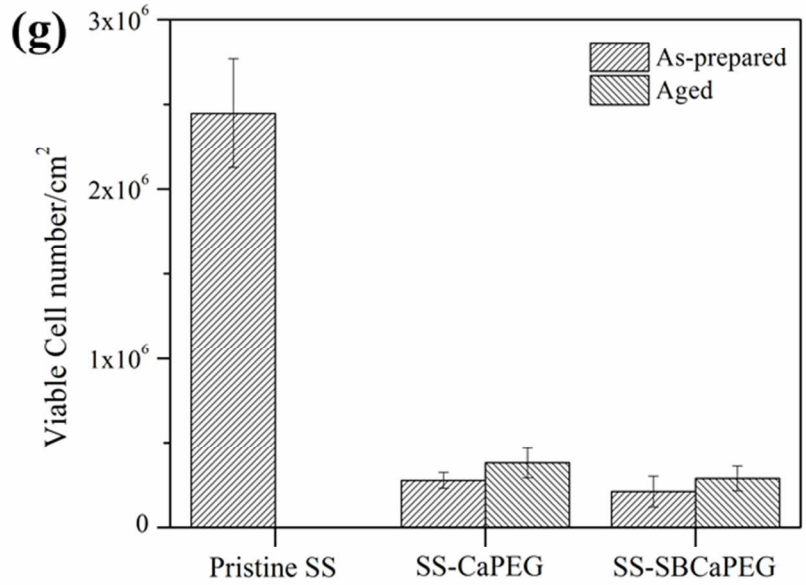


Figure 5

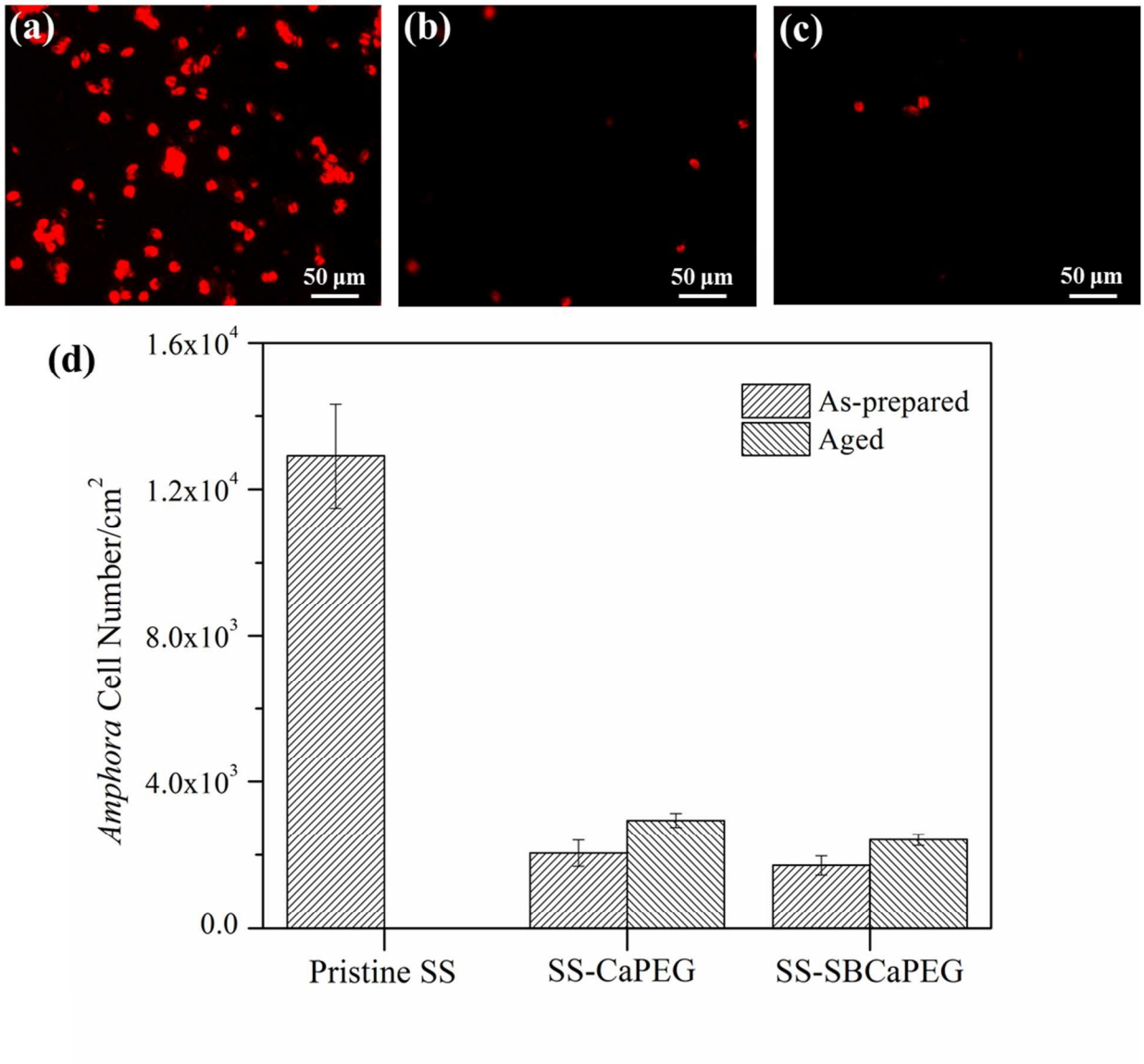


Figure 6

TOC

Title: Synthesis of catechol and zwitterion-bifunctionalized poly(ethylene glycol) for the construction of antifouling surfaces

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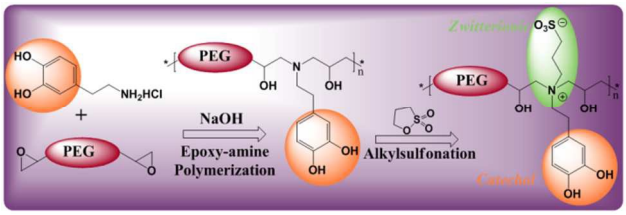

Versatile antifouling coatings from catechol and zwitterion-bifunctionalized poly(ethylene glycol)