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Cross-linked polystyrene sulfonic acid and polyethylene glycol as a low-fouling material

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ABSTRACT

A negatively charged hydrophilic low fouling film was prepared by thermally cross-linking a blend consisting of polystyrene sulfonic acid (PSS) and polyethylene glycol (PEG). The film was found to be stable by dip-washing. The fouling resistance of this material toward bacterial (*Escherichia coli*) and colloidal (polystyrene particles) attachment, non-specific protein (fibronectin) adsorption and cell (3T3 NIH) adhesion was evaluated and was compared with glass slides modified with polyethylene glycol (PEG) brushes, oxidized 3-mercaptopropyltrimethoxysilane (sulfonic acid, SA), and *n*-octadecyltrichlorosilane (OTS). The extended *Derjaguin–Landau–Verwey–Overbeek* (XDLVO) theory and thermodynamic models based on surface energy were used to explain the interaction behaviors of *E. coli*/polystyrene particles–substrate and protein–substrate interactions, respectively. The cross-linked PSS-PEG film was found to be slightly better than SA and PEG toward resisting non-specific protein adsorption, and showed comparable low attachment results as those of PEG toward particle, bacterial and NIH-3T3 cells adhesion. The low-fouling performance of PSS-PEG, a cross-linked film by a simple thermal curing process, could allow this material to be used for applications in aqueous environments, where most low fouling hydrophilic polymers, such as PSS or PEG, could not be easily retained.

1. Introduction

Fouling is the process of accumulating particles, macromolecules (e.g. proteins) and microorganisms on a surface, normally resulting in a negative impact on the performance of the fouled device [1–4]. For example, medical devices that are placed in a biological environment are susceptible to surface biofouling as a result of protein adsorption and/or bacterial/cell adhesion [5]. Such fouling could result in failure of medical devices, which often require surgical removal of such implants [6]. Another example where fouling is a significant issue is membrane separation processes such as reverse osmosis (RO), ultrafiltration (UF) or nanofiltration (NF), which are often used in waste water treatment and seawater desalination. Fouling of these membranes often leads to significant decrease in permeability, which results in an increase in the energy consumption of those processes [4,7]. Most organic foulants, such as bacteria and proteins, carry a net negative charge in water and as a result, a material that is positively charged would tend to foul more easily [8,9] than a material with a net negative charge [4]. In addition, surface hydrophilicity is known to enhance the fouling resistance of many polymeric materials [4,8,10].

Many attempts have been made to increase surface hydrophilicity and incorporate a negative surface charge in order to enhance the fouling resistance of a particular surface. Surface hydrophilicity could be improved by coating or chemically modifying the surface with hydrophilic substances, or treating the surface with gamma ray, UV irradiation or plasma [4]. Some researchers incorporated zwitterions on the surface to improve both surface hydrophilicity and charge density [11,12]. Coating with poly(sodium 4-styrene sulfonate) has also been found to improve the fouling resistance of an electric dialysis membrane [8]. The authors attributed the enhancement of antifouling to the increase in negative surface charge density and hydrophilicity of the polymer. Others reported the incorporation of a negative surface charge by treating devices/membranes with polymers containing ionizable species such as carboxyl and sulfonic groups [4]. Subramanian et al. [13] have recently cross-linked electrospun fiber mats of polystyrene sulfonic acid (PSS), a negatively charged polymer, and polyethylene glycol (PEG), a highly hydrophilic polymer, in order to increase the stability of PSS for potential uses as proton exchange membrane for fuel cells. Due to the combination of negatively charged groups

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of PSS and the superior hydration of PEG [14–17], we hypothesize that this material would possess low fouling properties.

In this paper, we report a simple method by spin coating a solution mixture of PSS and PEG followed by thermal annealing to cross-link PSS with PEG, hence preparing a water-insoluble PSS-PEG film for potential biomedical and membrane applications in aqueous environments. The low fouling performance of the resulting polymer films was assessed using a bacterial species – *Escherichia coli*, a model colloidal foulant – negatively charged polystyrene (PS) particles, a protein – fibronectin, which is known to facilitate cell adhesion [18–20], and a model animal cell line – embryonic mouse fibroblast cells (NIH 3T3). A negatively charged substrate (oxidized 3-mercaptopropyltrimethoxysilane, or sulfonic acid, SA, modified glass slide) and a hydrophobic substrate (*n*-octadecyltrichlorosilane, OTS, modified glass slide) were used to serve as references for demonstrating charge and hydrophobicity effects on fouling.

The cross-linked PSS-PEG film was found to be slightly better than SA and PEG alone toward resisting non-specific protein adsorption, and they showed comparable low attachment results as those of PEG toward particle, bacterial and NIH-3T3 cells. The low-fouling performance of PSS-PEG, a cross-linked film by a simple thermal curing process, could allow this material to be used in aqueous environments, where most hydrophilic polymers, such as PSS or PEG, could not be retained due to their high solubility in water. The films could potentially serve as barriers or coatings to reduce unwanted fouling/adhesion for medical implants and membranes.

2. Experimental

2.1. Materials

75,000 g/mol polystyrene sulfonic acid (PSS) was purchased from Sigma; 20,000 g/mol polyethylene glycol (PEG) was purchased from Alfa Aesar. 2-[Methoxypoly(ethyleneoxy)propyl]trimethoxysilane ($CH_3O(CH_2CH_2O)_{6\text{-}9}(CH_2)_3Si(OCH_3)_3$, PEG-silane), 3-mercaptopropyltrimethoxysilane ($HS(CH_2)_3Si(OCH_3)_3$, MPTMS) and *n*-octadecyltrichlorosilane ($CH_3(CH_2)_{17}SiCl_3$, OTS) were purchased from Gelest. Phosphate buffered saline (PBS) tablets, each makes 200 mL of 1× PBS solution in de-ionized water, were from Sigma–Aldrich. Other chemicals used included 30% hydrogen peroxide from BDH, 98% concentrated sulfuric acid and concentrated acetic acid from VWR, and toluene, hexane, ethanol and hydrochloric acid (HCl) from EMD. The probe liquids, methylene iodide (MI) and ethylene glycol (EG) were from Sigma, and de-ionized (DI) water was purified in-house (with a conductivity of ~1 μS/cm).

Fibronectin (Green fluorescent, HiLyte 488) was purchased from Cytoskeleton Inc. 1 μm fluorescent negatively charged (carboxylate-modified) polystyrene particles were purchased from Life Technologies. 0.5 μm non-fluorescent negatively charged (carboxylate-modified) PS tracer particles were purchased from polysciences Inc. *E. coli* used was ATCC 11303. LB medium reagents used contained tryptone, sodium chloride and yeast extract. NIH3T3 cells were ATCC CRL-1658, and the cell medium used was MEME (minimum essential medium eagle) +10% FBS (fetal bovine serum) + 1% of antibiotic antimycotic solution (100x). Nylon filters (pore diameter, 0.22 μm; filter diameter, 47 mm) were purchased from GE Water & Process Technologies. Unless otherwise mentioned, all reagents were purchased from Sigma–Aldrich.

The glass slides and silicon wafers (Si-wafers) were purchased from Fisher Scientifics and Silicon Quest International, respectively. Rectangular glass tubes with a cross-sectional dimension of 12 mm × 2 mm were purchased from Friedrich & Dimmock, Inc. The tubes were cut into desired length (15–20 cm) and built in house to fabricate the parallel plate flow chambers.

2.2. PSS-PEG film preparation

The substrates (glass slides or Si-wafer) were cut into square pieces and then were immersed in a freshly prepared piranha solution for 1 h at 100 °C. The substrates were then rinsed thoroughly with deionized water and dried using an air stream. The substrates then were further oxidized in a UV/Ozone cleaner (model 42, Jelight) for 8 min. The cleaned and oxidized substrates were then coated with a 5 wt.% solution of 75:25 or 55:45 (by mass) PSS:PEG in DI water using a spin coater (p-6000 Spin Coater, Specialty Coating System Inc., Indianapolis, IN) at 2000 rpm for 30 s. Coated substrates were then placed in a vacuum oven (<100 mTorr) (VWR International, Radnor, PA) for 1 h at 40 °C then the temperature was increased to 130 °C for 12 h. After curing, the films were thoroughly rinsed with DI water to remove excess material prior to carrying the attachment experiments.

2.3. Other surface preparation

The PEG-silane modification was performed by submerging cleaned and oxidized substrates in ~0.2 wt.% PEG-silane in HPLC toluene with a small amount of HCl as a catalyst for 18 h. The modified substrates were sonicated twice in toluene and then twice in ethanol for 5 min to remove unreacted molecules. The modified substrates were then dried under air flow and stored under ambient conditions.

The MPTMS modification was performed by submerging the cleaned and oxidized substrates in 0.5 wt% MPTMS in HPLC toluene for 12 h. The substrates were sonicated in toluene and then twice in ethanol for 5 min to remove unreacted molecules and then were dried under air flow. The dried MPTMS modified substrates were submerged in 5:1 (by volume) acetic acid: hydrogen peroxide for 1 h at 50 °C in order to oxidize the thiol groups to yield sulfonic acid (SA) groups. The substrates were rinsed with DI water and dried under air flow and stored under normal room conditions.

The OTS modification was carried out by submerging cleaned and oxidized substrates in a solution of 0.2 wt.% OTS in HPLC hexane for 2 h followed by ultrasonication twice in hexane and twice in ethanol for 4 min each.

2.4. Surface characterization

The sessile drop method was utilized to measure the contact angle of water (w), methylene iodide (MI) and ethylene glycol (EG) using a contact angle goniometer (Ramé-Hart Instrument Co., Netcong, NJ) with a CCD camera attached. The images of the liquid drops were projected using the One-touch software (One-touch video capturing VC500) and captured using the Snipping Tool (Windows, Microsoft), and the angle at the three-phase contact line was measured using ImageJ software (NIH). The surface energy of each surface was estimated using the contact angles of the three probing liquids formed on that surface following the method used by van Oss et al. [21,22]. The contact angles of the PS particles were measured by depositing the particles on a nylon filter using vacuum filtration to generate a lawn of particles. The surface energy was then determined from the three liquids contact angles on the PS particle lawn (roughness effect on contact angle and interaction energies is briefly discussed in the Supplementary material section).

The zeta potential for the 1 μm fluorescent PS particles and *E. coli* was measured using Zetasizer Nano Z (Malvern Instruments) at a concentration of 1×10^8 particles or cells/mL of 0.1× PBS and 1× PBS. The measurements were performed at 25 °C. The zeta potential of the substrate was measured by cutting the substrates to obtain ~7 mm × 4 mm samples and then using the same equipment with the addition of using the flat surface zeta potential accessory