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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 towards 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 towards resisting non-specific protein adsorption, and showed comparable low attachment results as those of PEG towards 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.

Graphical abstract

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Keywords

polystyrene sulfonic acid; polyethylene glycol; fouling; negative charge; hydrophilicity; hydrophobic interactions; XDLVO; protein adsorption

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 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 towards resisting non-specific protein adsorption, and they showed comparable low attachment results as those of PEG towards 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.

Experimental

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]trimethoxy-silane ($(\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_{6-9}(\text{CH}_2)_3\text{Si}(\text{OCH}_3)_3$, PEG-silane), 3-mercaptopropyltrimethoxysilane ($(\text{HS}(\text{CH}_2)_3\text{Si}(\text{OCH}_3)_3$, MPTMS) and n-octadecyltrichlorosilane ($(\text{CH}_3(\text{CH}_2)_{17}\text{SiCl}_3$, 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 $\sim 1 \mu\text{S}/\text{cm}$).

Fibronectin (Green fluorescent, HiLyte 488) was purchased from Cytoskeleton Inc.. 1 μm Fluorescent negatively charged (carboxylate-modified) polystyrene particles were purchased from Life Technology. 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 (100×). 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 $\times$ 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.

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 hour at 40 °C then the temperature was increased to 130 °C for 12 hours. After curing, the films were thoroughly rinsed with DI water to remove excess material prior to carrying the attachment experiments.

Other surface preparation

The PEG-silane modification was performed by submerging cleaned and oxidized substrates in ~0.2wt.% PEG-silane in HPLC toluene with a small amount of HCl as a catalyst for 18 hours. The modified substrates were sonicated twice in toluene and then twice in ethanol for 5 minutes 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 hours. The substrates were sonicated in toluene and then twice in ethanol for 5 minutes 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 hour 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 0.2wt.% OTS in HPLC hexane for 2 hours followed by ultrasonication twice in hexane and twice in ethanol for 4 minutes each.

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 water 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 and co-workers [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 $0.1 \times$ PBS and $1 \times$ PBS. The measurements were performed at 25 °C. The zeta potential of the substrate was measured by cutting the substrates to obtain ~ 7 mm by 4 mm samples and then using the same equipment with the addition of using the flat surface zeta potential accessory (Malvern Instruments). 0.5 μm negatively charged PS particles in 16.5 mM ($0.1 \times$ PBS) and 165 mM ($1 \times$ PBS) solution were used as a tracer particle/medium. Both mediums had a pH of ~ 7.4 .

PSS-PEG film characterization

The stability of the cured PSS-PEG film was assessed by dip-washing the coated silicon wafer in DI water and then measuring the thickness of the film using an ellipsometer (Rudolph Instruments, Inc., Fairfield, NJ). Additional assessment was performed by inspecting the substrates using naked eye and an optical microscope (OM). The detailed morphology of the films was examined using OM under different magnifications, and further confirmed using an atomic force microscope (Nanoscope III multimode, Veeco Corp. Santa Barbara, CA). The chemical compositions of the cured 55:45 PSS/PEG blended films were analyzed using X-ray photoelectron spectroscopy (XPS) (PHI VersaProbe II Scanning XPS microprobe) with an Al K α excitation source. The aromatic C1s having the lowest binding energy at 284.5eV was used for calibration.

Bacterial and particle attachments

The cells were cultured using 2 loops in 10 mL of LB medium utilizing a shaker (Thermo/Barnstead Lab Line MaxQ 4000) at 37 °C and 280 RPM for 4 hours. The cells were re-suspended in $0.1 \times$ PBS or $1 \times$ PBS to yield a final concentration of 7×10^6 cells/ml. The bacterial concentration was monitored using optical density measurements. The bacterial attachment experiment was performed by placing the rinsed substrates in a parallel plate flow chamber (a cross section of 12 mm $\times$ 2 mm) with a cell concentration of 7×10^6 cells/ml in $0.1 \times$ PBS and $1 \times$ PBS at room temperature using a flowrate of 6 mL/min (i.e., a shear stress ~ 12.5 mN/m 2). The cell concentration and circulating flowrate were chosen based on the results of our previous studies on bacterial/particle attachment using flow chambers. The chosen flow would minimize the gravitational settlement of the cells [1, 2]. The cells were counted, with the aid of an optical microscope (Olympus IX 70, with a $400 \times$ magnification) video system connected to a computer monitor, by randomly selecting 10 spots on the monitor screen and by capturing the images using ImageScope 9.0 followed by manually counting the individual cells on the images. The cells were normalized based on the image view area.

The 1 μm (diameter) fluorescent particle attachment experiment was performed using 7×10^6 particles/ml in $0.1 \times$ PBS and $1 \times$ PBS at room temperature using a flowrate of 6 mL/min.

Under the fluorescent light, the particles were counted by randomly selecting and counting 10 spots using the microscope eye piece and then normalizing by the viewing area.

Protein adsorption and cell attachment

The substrates, modified with OTS, PEG, PSS-PEG and SA, were rinsed and then completely covered with fibronectin solution (2 µg/ml in 1× PBS). The adsorption was carried out for 1 hour to 7 days at 37 °C. The samples were placed in an enclosed water bath to prevent liquid evaporation during the adsorption period. After the adsorption period, the samples were rinsed thoroughly with DI water to remove non-adsorbed fibronectin molecules and residual salt from the buffer. The samples were then submerged in DI water and the fluorescence of fibronectin was imaged using a microscope fitted with appropriate filters and a camera (Olympus D-SLR digital camera model E-420). To determine the fibronectin adsorption, the mean-gray-values of adsorption images were quantified by ImageJ. The mean-gray-values were obtained from 8-10 images.

For cell attachment, 10×10^3 NIH-3T3 cells/ml were seeded on tissue culture polystyrene plate (TCP), OTS, PEG and PSS-PEG and were incubated in a 5% CO₂ incubator. Phase contrast images were taken at different locations after 12 hours of incubation.

Results and Discussion

Film and substrate characterization

Curing of the 55:45 PSS:PEG film on Si-wafer or glass slide resulted in a uniform film. After dip-washing the cured films in water, a slight color change of the films was observed (Fig. 1A) and a residual film with a thickness of ~400 nm was retained. Also, the film was fully intact with no appearance damage along the edge or corners. Uncured 55:45 PSS:PEG film completely disappeared with a single dip-wash indicating that thermal curing was needed to obtain a water-insoluble film. The curing of the 75:25 PSS:PEG film resulted in a film with a similar thickness to that of cured 55:45 film. However, after a single dip-wash, excessive peeling and breakage of the film along the corners and edges was observed, as shown in Fig. 1A. When this sample was submerged in DI-water for 1 minute, the film broke into several small pieces and detached from the Si-wafer. Therefore, the 55:45 ratio of PSS:PEG, which resulted in stable cured films, was chosen to prepare films and carry out the fouling assessment study.

The morphology of the prepared films after curing and washing appeared to have no noticeable macro or micro scaled features as shown by optical and atomic force microscopic images (Figs. S1 and S2 in the Supplementary Material section). This suggests that the phase separation of the 55:45 PSS:PEG blend at 130°C for an annealing period of 12 hours might not occur. The Gibbs free energy of mixing of the 55:45 PSS:PEG blend estimated (see details on the estimation in the Supplementary Material section) using the Flory-Huggins theory was -7.7 J/mole at a temperature of 130°C, confirming the 55:45 blend was miscible (i.e., would not phase separate).

The cross-linking of the thermally cured PSS-PEG film shown in Fig. 1A was verified using X-ray photoelectron spectroscopy (XPS). The scans were conducted on the cured and dip-

washed 55:45 PSS:PEG film. The survey scan (Fig. 1B, left) for the binding energy (BE) ranging from 100 eV to 700 eV shows the peaks for C1s, O1s, and the signature peaks (S2s and S2p) of sulfur. The atomic percentages for C, O and S are 68.6, 26.5, and 4.8%, respectively. These atomic percentages are in good agreement with the C:O:S in the 55:45 mass ratio of PSS ($M_w = 75000$ g/mol) and PEG ($M_w = 20000$ g/mol), which would be 66.7, 29.1 and 4.3%, respectively. The result suggests that most of the PSS and PEG in the un-cured film were retained in the cross-linked film. To verify the cross-linking, the high resolution O1s spectra (Fig. 1B, right) were de-convoluted. Two main peaks are observed. The first is at a BE of 531.5 eV, which is associated with O=S=O from PSS. The second is at a BE of 532.7 eV that is related to C-O-C bonds in PEG. These two peaks suggest the presence of both PSS and PEG in the cured film. The area ratio of the two peaks (i.e., O=S=O:C-O-C) is 42:58, which is very close to the expected value (44:56) based on 55:45 PSS:PEG. This result agrees with the results obtained from the survey scan. In other words, most of the PSS and PEG from the original solution (55:45) were present in the cured film. There seemed to be a small peak at a BE of ~ 533.9 eV, which is likely associated with S-O-C. The presence of this peak suggested the cross-linking of the two polymers. The ratio of the area of the O=S=O peak to the area of the S-O-C peak is roughly 13:1, which would suggest that for every 14 oxygen atoms in PSS, one of them is linked to PEG. Since each PSS monomeric unit contains three oxygen atoms linked to the sulfur, the result indicated that $\sim 21\%$ of the PSS monomeric units were linked to PEG. These results illustrated that the cross-linking of PSS and PEG occurred when the blend film was annealed at 130°C in vacuum for 12 hours or longer. The remaining unlinked sulfonic acid groups in the film would result in a net negative charge.

The surface properties of PSS-PEG cross-linked film, along with other substrates used to demonstrate that the PSS-PEG cross-linked film could potentially be a low fouling substrate, were characterized using contact angle and zeta potential measurements. All substrates, apart from OTS, were hydrophilic with SA being the most hydrophilic with a θ_w of $\sim 8^\circ$ followed by PSS-PEG with a θ_w of $\sim 20^\circ$, PEG with a θ_w of $\sim 33^\circ$ and finally OTS with a θ_w of $\sim 100^\circ$. The surface energy of the investigated surfaces was mainly contributed by the LW component, with a small contribution from the AB component (17% for *E. coli*, 7% for SA, and 0 – 1 % for other surfaces). All surfaces were negatively charged, as verified by the negative zeta potential and the large value of the negative surface tension parameter (γ^-). Other than SA, all other substrates, as well as *E. coli* and PS particles, had a dramatic change in zeta potential when the ionic strength of the solution decreased from 165 mM ($1\times$ PBS) to 16.5 mM ($0.1\times$ PBS). The decrease in zeta potential (absolute value) at a higher ionic strength could be attributed to the decrease in the Debye length which results in a reduction in potential[23].

Attachment of E.coli and PS particles

The attachment result for *E. coli* on PSS-PEG, SA, PEG and OTS shown in Fig. 2A shows that all substrates had a relatively low attachment as compared to OTS ($\sim 33, 55, 30$ and 278 cells/ mm^2 for PSS-PEG, SA, PEG and OTS, respectively) at low ionic strength (i.e. $0.1\times$ PBS). The OTS surface was chosen as a control and a representation of hydrophobic surfaces such as most polymers. It was observed that PSS-PEG and SA as well as PSS-PEG

and PEG had no significant statistical difference ($p>0.05$) in terms of number of attachment. At higher ionic strength (i.e. $1\times$ PBS), the attachment on PSS-PEG and PEG increased to 54 and 45 cells/mm², respectively, although the increase for PEG was statistically insignificant ($p>0.05$). The attachment on OTS (~ 1320 cells/mm²) increased by approximately five times the amount observed at lower ionic strength whereas the attachment on SA decreased to 30 cells/mm², although statistically insignificant ($p>0.05$).

For the case of negatively charged PS particles, the overall attachment was lower than that of *E. coli* as shown in Fig. 2B. At low ionic strength, the number of attachment was ~ 26 , 13, 20 and 25 cells/mm² for PSS-PEG, SA, PEG and OTS, respectively, with no significant statistical difference ($p>0.05$) between PSS-PEG, PEG and OTS as well as between SA and PEG. At higher ionic strength, the number of attachment increased for PSS-PEG (31 cells/mm²) and decreased for SA and PEG (~ 12 and 14 cells/mm², respectively), although none of these changes were statistically significant ($p>0.05$). The dramatic change in the number of attachment on OTS that was observed for *E. coli* at high ionic strength was also observed when using negatively charged PS particles. The number of attachment increased dramatically at high ionic strength by approximately 6 times the amount at low ionic strength (~ 159 cells/mm²).

The extended *Derjaguin-Landau-Verwey-Overbeek* (XDLVO) theory was used in an attempt to explain the interaction behavior between *E. coli*/PS particles and the substrates.

The XDLVO theory considers that the total free energy of interaction ($\Delta G_{slc(d)}^{total}$) between two surfaces in an aqueous medium is the sum of Lifshitz-van der Waals (LW) interactions, Lewis acid-base interactions (AB) and electrostatic interactions (EL)[24]. The interactions can be represented mathematically by the following equations[3,25]:

$$\Delta G_{slc(d)}^{LW} = -\frac{AR}{6d} = 2\pi R d_0 \Delta G_{d_0}^{LW} \frac{d_0}{d} \quad (1)$$

$$\Delta G_{slc(d)}^{AB} = 2\pi R \lambda \Delta G_{d_0}^{AB} \exp\left(\frac{d_0 - d}{\lambda}\right) \quad (2)$$

$$\Delta G_{slc(d)}^{EL} = \pi R \varepsilon_r \varepsilon_0 \left(2\psi_c \psi_s \ln\left(\frac{1+e^{-\kappa d}}{1-e^{-\kappa d}}\right) + (\psi_c^2 + \psi_s^2) \ln(1 - e^{-2\kappa d}) \right) \quad (3)$$

The subscript s, l and c represent a substrate, the liquid medium and the colloid (bacteria or particle), respectively. A is the sphere-substrate Hamaker constant in water; R is the colloid radius; d is the separation distance between the colloid and the flat substrate; λ is the characteristic decay-length of AB interactions in water (taken to be ~ 0.6 nm)[3]; ε_r and ε_0 are the relative dielectric permittivity of medium and the permittivity in a vacuum, respectively; $1/\kappa$ is the Debye-Huckel length and κ (m⁻¹) is related to the ionic strength, I (M), of the medium by $\kappa = 3.28 \times 10^9 I^{1/2}$; and ψ_c and ψ_s are the surface potentials of the colloid and the flat substrate, respectively. The surface potential values were assumed to be equal to the zeta potential values in a similar manner that was followed by others[26–29].

$\Delta G_{slc,d_0}^{LW}$ and $\Delta G_{slc,d_0}^{AB}$ are the LW and AB interactions between a colloid and a flat substrate in a medium with a minimum equilibrium cut-off distance of d_0 (the critical distance below which the outer electron shells of adjoining molecules would overlap, which is approximately equal to 0.158 nm[30,31]), and they can be expressed as[3]:

$$\Delta G_{slc,d_0}^{LW} = -2 \left(\sqrt{\gamma_{sv}^{LW}} - \sqrt{\gamma_{lv}^{LW}} \right) \left(\sqrt{\gamma_{cv}^{LW}} - \sqrt{\gamma_{lv}^{LW}} \right) \quad (4)$$

and,

$$\Delta G_{slc,d_0}^{AB} = -2 \left(\sqrt{\gamma_{sv}^+} - \sqrt{\gamma_{lv}^+} \right) \left(\sqrt{\gamma_{sv}^-} - \sqrt{\gamma_{cv}^-} \right) - 2 \left(\sqrt{\gamma_{sv}^+} - \sqrt{\gamma_{lv}^+} \right) \left(\sqrt{\gamma_{sv}^-} - \sqrt{\gamma_{lv}^-} \right) - 2 \left(\sqrt{\gamma_{cv}^+} - \sqrt{\gamma_{lv}^+} \right) \left(\sqrt{\gamma_{cv}^-} - \sqrt{\gamma_{lv}^-} \right) \quad (5)$$

The subscript v represents vapor which is air in this case. γ^+ and γ^- are the electron-acceptor and electron-donor of the Lewis acid-base component of the surface energy.

The total free energy of interaction between *E. coli* and substrates in 0.1× PBS and 1× PBS are shown in Fig. 3A and Fig. 3B, respectively. At low ionic strength, the interaction energy showed a secondary minimum (See Table 2 for all minima values) for PEG, PSS-PEG, OTS and SA (-4.2, -4.1, -2.1 and -3.8 kT, respectively), but only a primary minimum for OTS. Additionally, an energy barrier (717 kT) for OTS could be seen at ~2 nm separation distance whereas the interaction energy for other substrates continued to increase and no specific energy barrier was observed for $d > d_0$.

These trends could qualitatively explain the behavior of *E. coli*'s attachment at low ionic strength. In other words, the presence of a secondary minimum indicates the possibility of *E. coli*'s attachment whereas the presence of a primary minimum (or lack of) could explain the severity of the number of attachment as was the case for OTS. At a higher ionic strength (Fig. 3B), again the interaction energy showed a secondary minimum for PEG, PSS-PEG and SA (-14.1, -14.0, -11.6 kT, respectively) with more negative values; whereas it only showed a primary minimum for OTS. The decrease in electrostatic interactions due to the compression of the electric double layer at higher ionic strength resulted in a secondary minimum that is more negative at smaller separation distances (see Table 2 for minima values and associated distances). The disappearance of the energy barrier for OTS at high ionic strength might explain the dramatic increase in the number of attachment of *E. coli* as compared to the attachment at lower ionic strength.

According to van Oss[30,32], a negative free energy of interaction $\Delta G_{ij,d_0}^{LW+AB}$ (the sum of $\Delta G_{ij,d_0}^{LW}$ and $\Delta G_{ij,d_0}^{AB}$) between solids i and j in water indicates that the net interfacial attraction between solids i and j is prevalent. In other words, solids i and j prefer interacting with each other as compared to interacting with water. This type of interaction is often

called “hydrophobic interaction”[30]. The attractive/repulsive hydrophobic interaction are almost entirely polar (AB) and are a consequence of hydrogen bonding of the surrounding water rather than the van der Waals attractions between the hydrophobic parts[30]. By looking at the values summarized in Table 3, OTS is the only substrate with a negative $\Delta G_{slc,d_0}^{AB}$ and a negative net free energy of interaction $\Delta G_{iwj,d_0}^{LW+AB}$ indicating a possible hydrophobic interaction between *E. coli*/PS particles and OTS in water. This type of interaction could explain the tendency of OTS to be fouled especially when the role of electrostatic interaction is diminished by increasing the ionic strength. For all other substrates, PEG, SA and PSS-PEG, the only negative term was the LW term, while the AB term was highly positive, i.e., not favoring attraction.

For the negatively charged PS particles, the lower number of attachment as compared to *E. coli* could possibly be explained by the ability of *E. coli* to move using their flagella[9] and as a result, overcoming the energy barrier whereas PS particles have no mobility mechanisms. The total interaction energy between PS particles and substrates at low ionic strength is shown in Fig. 3C. The interaction energy showed a secondary minimum of -7.6, -7.5 and -6.8 kT for PEG, PSS-PEG and SA, respectively. At higher ionic strength, the secondary minima decreased to a value of -27.7, -27.1, and -22.4 kT for PEG, PSS-PEG and SA, respectively. For these substrates, as the separation distance continued to decrease, the interaction energy increased dramatically, and no energy barrier was observed when *d* was greater than *d*₀, suggesting that the attachment occurred weakly (reversibly) at the secondary minimum for PEG, PSS-PEG and SA. In the case of OTS, similar behavior as that of *E. coli* – OTS was observed. In other words, OTS's interaction energy exhibited a secondary minimum (-3.8 kT), an energy barrier (1942 kT) and a primary minimum at low ionic strength; whereas it only exhibited a primary minimum at high ionic strength with no energy barrier. It was apparent that OTS's behavior seemed to depend on the ionic strength of the solution, as opposed to other substrates investigated in this study. For PSS-PEG, PEG and SA, the acid-base interaction seems to strongly favor the repulsion of colloidal attachment.

For demonstration purposes, the electrostatic and acid-base interaction energies between *E. coli* and the substrates at high ionic strength are shown in Fig. 4. The electrostatic interaction energy curves shown in Fig. 4A seem to indicate that all substrates apart from PEG have electrostatic repulsion towards *E. coli*. The hydrophobicity of OTS could cause hydroxyl ions to strongly adsorb on the surface resulting in a strong negative charge as described by Tian and Shen[33]. However, the negativity of OTS is not strong enough to overcome the hydrophobic attraction described by the acid-base interaction energy shown in Fig. 4B and as such, the AB term seemed to be the dominating factor in controlling the attraction/repulsion behaviors of bacteria/particle interacting with these four surfaces. This behavior could be attributed to the hydrophilicity (PEG, PSS-PEG and SA)/hydrophobicity (OTS) of these substrates as shown by the water contact angle in Table 1.

Attachment of mouse embryonic fibroblast cells and fibronectin adsorption

The attachment behaviors of mouse embryonic fibroblast NIH 3T3 cells on tissue culture plate (TCP), PSS-PEG, OTS and PEG are presented in Fig. 5A. The cells remained rounded and appeared to be un-able to spread after ~12 hours of incubation on OTS, PEG and PSS-

PEG as compared to the behavior that was observed on tissue culture polystyrene plate. The ability of adhesion-aiding proteins such as fibronectin and vitronectin to adsorb on surfaces plays a major role in cell adhesion[18–20]. To verify the cell attachment behaviors, fibronectin adsorption was carried out. The normalized coverage% of HiLyte 488-labeled fibronectin on OTS, SA, PEG and PSS-PEG after submersion in fibronectin-PBS solution for 1 hour and 7 days is shown in Fig. 5B along with the representative fluorescent images. Overall, the trend of the %coverage of fibronectin remained relatively the same (OTS>SA>PEG>PSS-PEG) regardless of submersion time.

After 1 hour of submersion, the PSS-PEG appeared to have the lowest coverage (~16% of that on 7days-OTS) followed by PEG (~25% of that on 7days-OTS) and SA (~37% of that on 7days-OTS) with OTS having the highest 1 hour coverage of all substrates (~78% of that on 7days-OTS). The 7 days adsorption results show that all substrates had an increase in protein coverage% compared to the 1 hour coverage% with PSS-PEG having the lowest coverage (~51% of that on 7days-OTS) followed by PEG (~65% of that on 7days-OTS) and SA (~76% of that on 7days-OTS).

Using the interaction energy in a similar manner that was used by van Oss for protein interaction in water[34], it is possible to correlate the adsorption results to the Lewis acid-base (AB) and Lifshitz-van der Waals interactions (LW) between fibronectin and substrates as shown in Table 3. The total interaction energy G_{slp}^{LW+AB} (s: substrate, l: liquid, p: protein) between OTS and fibronectin is negative indicating that the interaction is favorable and that fibronectin is likely to adsorb strongly on OTS. For PSS-PEG and PEG, the free energy of interaction is positive indicating an unfavorable interaction. This result is in agreement with the experimental observations and could explain the lack of cell spreading on PSS-PEG and PEG as the adsorption of such proteins is critical for cell adhesion[35]. The high coverage on OTS with the lack of cell spreading could be caused by the alteration of fibronectin's conformation and its ability to support cell adhesion when adsorbed on $-CH_3$ terminated alkane-thiols[36], which are structurally similar to OTS. Additionally, the presence of other proteins in the serum-containing cell medium such as albumin is known to reduce fibronectin adsorption on hydrophobic surfaces due to the strong preferential adsorption of such proteins and the resistance to be displaced by cell-adhesive proteins[35]. It is important to note that surface charge (protein/substrate) could play a major role in protein-substrate interaction although its effect is not very clear and is dependent on the conditions of the aqueous media such as pH and ionic strength[37]. It is not uncommon for proteins to adsorb on hydrophilic surfaces that carry a similar charge due to change in protein conformation, which results in an entropically favorable state[38–40].

Conclusions

In this paper, we assessed the possibility of applying a simple thermal curing method to generate a cross-linked PSS-PEG film and its potential application as a low fouling material under aqueous conditions. The low fouling behavior of cross-linked PSS-PEG films was compared to PEG, SA and OTS against attachment of *E. coli* and negatively charged PS particles on these substrates at two ionic strengths (1× PBS (165 mM) and 0.1× PBS (16.5 mM)) as well as the attachment of NIH 3T3 cells and the adsorption of fibronectin. The

bacterial attachment results showed that PSS-PEG, PEG and SA substrates had relatively low bacterial and particle attachment at both ionic strengths as compared to OTS, which had a dramatic increase in attachment at higher ionic strength. Additionally, the XDLVO theory was used in an attempt to explain the attachment behavior and the theory seemed to be in a qualitative agreement with the observed results.

The NIH 3T3 cells were unable to spread on PSS-PEG and remained rounded during the 12 hours of incubation as compared to the behavior observed on tissue culture plates. The cellular behaviors on PSS-PEG correlated to the ability of the surface to prevent the adsorption of fibronectin, a cellular adhesion protein. 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 applications subjected to an aqueous environment, 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, wound dressing, and membranes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Polystyrene sulfonic acid (PSS) and polyethylene glycol (PEG) were cross-linked
- The cross-linked film exhibited low fouling towards various foulants
- The low fouling was the result of negative charge and hydrophilicity
- The results agreed with the XDLVO and thermodynamics predictions

Fig. 1.

(A) The crosslinking reaction between Polystyrene Sulfonic Acid (PSS) and Polyethylene Glycol (PEG) under thermal conditions as well as the appearance of the spin-coated and dip-washed PSS-PEG films prepared using different PSS:PEG ratios and curing conditions. Enlarged optical microscope images are taken at the corners of the dip-washed sides of the films (the scale bar is 200 μm) and (B) XPS survey scan and the high resolution O1s spectra of the cured 55:45 PSS-PEG film on Si wafer.

Fig. 2.

The bacterial (A) and particle (B) attachment using the parallel flow chamber in 0.1× PBS (dashed bar) and 1× PBS (solid bar). The quantification was done by randomly selecting 10 locations and counting individual cells and particles. The number of attachment was normalized by the view area of the microscope. The error bars are the standard deviations ($n = 10$). Student's t-test was used to test for significance. Data sharing the same letter have significant statistical difference ($p < 0.05$).

Fig. 3. The XDLVO interaction energy between *E. coli* and substrates (PSS-PEG (solid-line), PEG (short dashed line), OTS (long dashed and dotted line) and SA (dotted line)) in 0.1× PBS (A) and 1× PBS (B) as well as the XDLVO interaction energy between polystyrene particles and substrates in 0.1× PBS (C) and 1× PBS (D).

Fig. 4. The electrostatic (**A**) and Acid-Base (**B**) interaction energies between *E. coli* and substrates (PSS-PEG (solid-line), PEG (short dashed line), OTS (long dashed and dotted line) and SA (dotted line)) in 1× PBS.

Fig. 5.

The phase contrast images (A) showing the attachment and spreading behavior of NIH 3T3 cells incubated for ~12 hours on PSS-PEG, tissue culture plate (TCP), PEG and OTS and the percent coverage of HiLyte 488 labeled Fibronectin (B) on PSS-PEG, SA, PEG and OTS obtained using the ImageJ's grayscale threshold feature of the green fluorescent microscopic images (shown correspondingly above each bar of the bar chart). The background noise was subtracted before normalizing by OTS-7days coverage (highest coverage). The error bars are the standard deviations (n = 8-10). The scale bars of the protein and cell images are 200 μ m.

Table 1

The contact angle, calculated surface energy and its components, and zeta potential (in 0.1× PBS and 1× PBS as the medium) for the colloids (PS particles and *E.coli*) and substrates (PSS-PEG, PEG, SA and OTS). The reported contact angle is the average of advancing and receding contact angles. The error bars are the standard deviations and n = 3.

Colloid/substrate	PS particles	<i>E. coli</i> ^a	PSS-PEG	SA	PEG	OTS
$\theta_w(^{\circ})$	14.1 ± 0.7	17.6 ± 1.1	20.4 ± 0.6	7.7 ± 0.9	33.4 ± 0.6	100 ± 4.5
$\theta_{\text{H}}(^{\circ})$	28.9 ± 1.4	46.3 ± 1.5	21.1 ± 3.1	33.0 ± 2.4	22.0 ± 0.4	48.2 ± 0.6
$\theta_{\text{EG}}(^{\circ})$	15.7 ± 0.8	17.6 ± 0.9	10.2 ± 0.8	6.0 ± 1.0	19.0 ± 1.1	68.9 ± 2.4
$\zeta(\text{mV})^b$	-93.4 ± 2.2	-48 ± 2.5	-49.3 ± 5.2	-25.3 ± 4.0	-38.4 ± 7.4	-66 ± 4.5
	-45.3 ± 1.2	-16.9 ± 0.6	-15.6 ± 3.3	-20.7 ± 4.9	-3.3 ± 3.9	-29.7 ± 2.3
R(μm)	0.5	0.39	-	-	-	-
γ	44.7	44.1	47.5	46.2	47.6	35.3
γ_{LW}	44.7	36.3	47.5	43.1	47.2	35.3
γ_{AB}	0.0	7.7	0.0	3.1	0.5	0.0
γ^+	0.0	0.2	0.0	0.0	0.0	0.0
γ^-	64.3	64.4	57.7	65.5	46.8	0.2

Note: the surface energy values are in mJ/m²

^a Contact angle and surface energy obtained from [1]

^b the zeta potential values measured in 0.1× PBS (upper row) and 1× PBS (lower row)

Table 2

The secondary minima of interaction energies, the separation distance at which the minima is found and the energy barriers of *E. coli*/PS-particles interacting with the substrates in $0.1 \times \text{PBS}$ and $1 \times \text{PBS}$.

Colloid	Substrate	Medium	G_{sm} (kT)	d_{sm} (nm)	Energy barrier (kT)
<i>E. Coli</i>	PSS-PEG	$0.1 \times \text{PBS}$	-4.1	19.4	**
		$1 \times \text{PBS}$	-13.9	5.8	**
	SA	$0.1 \times \text{PBS}$	-3.8	17.7	**
		$1 \times \text{PBS}$	-11.6	6.0	**
	PEG	$0.1 \times \text{PBS}$	-4.2	18.6	**
		$1 \times \text{PBS}$	-14.1	5.7	**
	OTS	$0.1 \times \text{PBS}$	-2.1	22.0	717 (at $d = 2 \text{ nm}$)
		$1 \times \text{PBS}$	*	-	**
	PSS-PEG	$0.1 \times \text{PBS}$	-7.5	20.2	**
		$1 \times \text{PBS}$	-27.1	5.6	**
	SA	$0.1 \times \text{PBS}$	-6.8	18.6	**
		$1 \times \text{PBS}$	-22.4	5.8	**
	PEG	$0.1 \times \text{PBS}$	-7.6	19.4	**
		$1 \times \text{PBS}$	-27.7	5.4	**
	OTS	$0.1 \times \text{PBS}$	-3.8	22.8	1942 (at $d = 1.8 \text{ nm}$)
		$1 \times \text{PBS}$	*	-	**

* indicates that no secondary minimum was observed

** indicates that no energy barrier was observed for $d > d_0$

Table 3

Lewis Acid-Base (AB) and Lifshitz-van der Waals (LW) interaction energy between *E. coli*/PS particles/protein and substrates immersed in water.

Colloid/protein	Substrate	$G_{\text{slc/p}}^{\text{LW}}$ (mJ/m ²)	$G_{\text{slc/p}}^{\text{AB}}$ (mJ/m ²)	$G_{\text{slc/p}}^{\text{LW+AB}}$ (mJ/m ²)
<i>E. coli</i>				
	PEG-PSS	-6.02	53.31	47.29
	SA	-5.14	56.71	51.57
	PEG	-5.97	46.21	40.24
	OTS	-3.45	-11.89	-15.34
PS Particles				
	PEG-PSS	-8.94	55.71	46.77
	SA	-7.63	59.59	51.96
	PEG	-8.86	47.88	39.02
	OTS	-5.12	-16.38	-21.50
Fibronectin ^a				
	PEG-PSS	-3.38	37.56	34.2
	SA	-2.89	39.78	36.9
	PEG	-3.35	32.77	29.4
	OTS	-1.94	-6.34	-8.3

^a surface tension properties of Fibronectin were obtained from [34]