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Optimizing the surface density of polyethylene glycol chains by grafting from binary solvent mixtures

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ABSTRACT

Polyethylene glycol (PEG) brushes are very effective at controlling non-specific deposition of biological material onto surfaces, which is of paramount importance to obtaining successful outcomes in biomaterials, tissue engineered scaffolds, biosensors, filtration membranes and drug delivery devices. We report on a simple 'grafting to' approach involving binary solvent mixtures that are chosen based on Hansen's solubility parameters to optimize the solubility of PEG thereby enabling control over the graft density. The PEG thiol-gold model system enabled a thorough characterization of PEG films formed, while studies on a PEG silane-silicon system examined the versatility to be applied to any substrate-head group system by choosing an appropriate solvent pair. The ability of PEG films to resist non-specific adsorption of proteins was quantitatively assessed by full serum exposure studies and the binary solvent strategy was found to produce PEG films with optimal graft density to efficiently resist protein adsorption.

1. Introduction

Non-fouling coatings are highly relevant to the success of various biomedical applications such as medical implants, drug delivery devices, tissue engineering scaffolds and biosensors [1]. Besides the field of biomedicine, non-fouling coatings are also important for the efficient function of surfaces related to water purification, food processing and marine industries [2]. In addition, biofilm formation on surfaces is believed to be initiated by the formation of a conditioning film which consists of a surface-adsorbed layer of organic molecules, such as proteins, from the surrounding environment [3]. The conditioning film facilitates the initial reversible physicochemical attachment of microbes, followed by irreversible adhesion and subsequent colonization of the surface [3]. One of the most important strategies in making non-fouling coatings involves preventing initial bio-adhesion by using a hydrophilic polymeric coating that acts as an interfacial steric barrier between the substrate and the conditioning film forming biomolecules [1,4]. Several molecules such as dextran, chitosan, alginate, hyaluronic acid, mannitol, polyacrylamide, oligo(ethylene glycol) (OEG), PEG and zwitterionic polymers have been demonstrated to resist fouling related processes such as protein adsorption or microbial adhesion [5]. Among the aforementioned molecules, PEG is one of the most widely used and well studied molecules related to non-fouling surfaces. Though PEG belongs to the chemical family of polyethers, it is exceptionally water soluble and possesses unique osmotic and elastic properties upon interaction with water, which is the main reason for its ability to resist non-specific adsorption [4]. The neutral charge and non-toxic nature of PEG makes it a perfect choice when it comes to biologically relevant or eco-friendly applications. The ability of PEG functionalized surfaces to resist non-specific adsorption depends on the conformation of the polymeric molecules, which in turn depends on the molecular weight and surface graft density of PEG chains [6]. It is known that, in the case of PEG coatings with high or low graft densities, PEG molecules generally exist either in the 'brush' or 'mushroom' conformation, respectively [7].

Surface bound PEG molecules resist non-specific adsorption more efficiently when they exist in brush conformation rather than in mushroom conformation [6c]. The nature of substrate-head group interaction is also an important property when it comes

Abbreviations: BSM, binary solvent mixture; PEG-SH, α-methoxy-ω-thiol poly(ethylene glycol), M_W 5 kDa; PEG-OEt_3, α-methoxy-ω-triethoxy poly(ethylene glycol), M_W 5 kDa.

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to designing long lasting non-fouling surfaces. For instance, Kingshott et al. demonstrated that covalently immobilized PEG coating had superior non-fouling properties when compared to a coating formed by electrostatic adsorption [8]. Approaches for functionalizing surfaces with PEG can be broadly classified into 'grafting to' and 'grafting from'. The 'grafting from' approach involves initiation of the polymer growth from the substrate through polymerization of monomers, whereas the 'grafting to' approach is formed by diffusion of polymer molecules, and subsequent attachment onto the substrate. Generally the 'grafting from' approach results in a polymer coating with higher graft density since the limiting factor is diffusion of monomer molecules onto the reactive ends of growing polymer molecules. On the other hand, the 'grafting to' approach is limited by the dependence on the diffusion and conformation of large polymer molecules onto the substrate [9]. Although the 'grafting to' approach results in low graft density polymer coatings, it is widely used due to its simplicity, versatility in terms of polymer synthesis and ability to form well-defined films [10]. Several strategies such as 'cloud point' grafting [11], grafting in homo-polymer solutions [12], grafting from polymeric melts [13], grafting at elevated temperature [14] and underbrush formation by backfilling with shorter molecules [15] have been used to overcome the low graft density limitation of PEG coatings made using the 'grafting to' approach. All the aforementioned strategies, except the backfilling strategy and elevated temperature strategy, work by minimizing the excluded volume interactions and subsequent inter-chain repulsion during attachment that is necessary to increase the final graft density.

Here, we demonstrate a simple versatile strategy to coat surfaces with PEG at high graft density by reducing the excluded volume interactions by using binary solvent mixtures (BSM). This is carried out by mixing a solvent having very low PEG solubility (poor solvent) with a good solvent for PEG, which decreases the PEG hydrodynamic radius and results in films that are thicker when compared to those formed from a high solubility condition. The 'good' and 'poor' solvent pair was chosen based on the solubility parameter distance for PEG and solvents used. The model study was performed initially by characterizing the PEG films formed by chemisorbing thiolated PEG molecules onto gold surfaces using the BSM strategy. In addition, silane functionalized PEG modification of silicon substrates was also studied to investigate the broader applicability of the BSM strategy. The protein resistance capability of the BSM grafted surfaces was examined against 100% fetal bovine serum (FBS).

2. Materials and methods

2.1. Chemicals

α-Methoxy-ω-thiol poly(ethylene glycol) (PEG-SH, M_W 5000 Da, 98% purity), α-methoxy-ω-triethoxy poly(ethylene glycol) (PEG-OEt_3, M_W 5000 Da, 98% purity) were purchased from Laysan Bio Inc. (Alabama, USA) and used in experiments as supplied. Phosphate buffered saline (PBS) buffer tablets, 25% ammonium hydroxide (NH_4OH), 30% hydrogen peroxide (H_2O_2), absolute ethanol, acetone, diethyl ether and fetal bovine serum (FBS) were purchased from Sigma–Aldrich (Aarhus, Denmark). Ultrapure Milli-Q (MQ) water with resistivity of 18.2 MΩ was used for making PBS buffer and the pH was adjusted to 7.4.

2.2. Substrate preparation and functionalization

Gold substrates used for PEG-SH functionalization were prepared by sputtering 3 nm titanium adhesion layer on silicon wafers followed by sputtering 50 nm gold layer. An RF sputtering system with Ti and Au targets of 10 cm diameter (2.54 W cm^{-2}) was used for sputtering and carried out inside a standard chamber maintained at an Ar pressure of 2×10^{-3} mbar. Just before the PEG-SH functionalization experiments, the gold surfaces were cleaned by UV/ozone treatment for 30 min, followed by a treatment with basic Piranha solution (H_2O:NH_4OH:H_2O_2 in ratio 4:1:1) at 70–80 °C for 5 min, and then rinsed with MQ water. The PEG-SH was dissolved in the different solvent mixtures by sonication for 30 min before immersion of the Au surfaces. Binary solvent mixture consisting of 'good' and 'poor' solvent pair (water and ethanol, respectively) was chosen based on the solubility parameter distance calculated from Hansen solubility parameters for PEG and solvents (Table 1) using Eq. (1) [16].

$$R_a^2 = 4(\delta_{D,p} - \delta_{D,s})^2 + (\delta_{P,p} - \delta_{P,s})^2 + (\delta_{H,p} - \delta_{H,s})^2 \quad (1)$$

where, R_a, $\delta_{D,p}$, $\delta_{P,p}$ and $\delta_{H,p}$ stand for the Hansen solubility parameters of the polymer, while $\delta_{D,s}$, $\delta_{P,s}$ and $\delta_{H,s}$ denote the solubility parameters of solvent.

Cleaned gold slides were immersed in 0.4 mM PEG-SH solution (mixtures of ethanol and acetone) for 1 h at room temperature followed by rinsing with MQ water to remove any physically adsorbed molecules. Preliminary kinetic studies were performed by following the film thickness and percentage of covalently bound thiol as a function of incubation time. The incubation time of 1 h was chosen after preliminary kinetics studies, which indicated that this incubation time was sufficient for monolayer formation (data not shown). In order to compare the results with a standard procedure known in the literature, grafting was done under the same high ionic strength buffer conditions used by Unsworth et al. [7]. The PEG-SH in high ionic strength buffer was sonicated for 30 min before the immersion of Au substrates. This grafting was performed at 30 °C for 1 h with an ionic strength of 1.9 M and PEG-SH concentration of 0.4 mM.

Silicon surfaces used for PEG-OEt_3 functionalization were cleaned using the same procedure used for the cleaning of gold surfaces as described above. The PEG-OEt_3 solvent mixture was sonicated for 30 min before the immersion of silicon surfaces. Silicon substrate functionalization unless otherwise mentioned was carried out by immersing the surfaces in 1 mM PEG-OEt_3 solution (mixtures of acetone and diethyl ether) for 15 h at room temperature, followed by thorough rinse with MQ water. Additionally a comparative standard grafting was done under high ionic strength conditions [7] and grafting was performed at 30 °C for 15 h with PEG-OEt_3 concentration of 1 mM.

2.3. Serum adsorption

All serum adsorption experiments were conducted by incubating the PEG functionalized surfaces in 100% FBS for 1 h at 37 °C, followed by rinsing with 100 mM PBS (pH 7.4) and then thoroughly rinsing with MQ water. The MQ rinsed surfaces were dried under a jet of nitrogen and subsequently the quantification of adsorbed protein was carried out using XPS.

2.4. X-ray photoelectron spectroscopy

XPS spectra were recorded using a Kratos Axis Ultra^{DLD} instrument (Kratos Ltd, Telford, UK) equipped with a monochromated $Al_{k\alpha}$ (1486 eV) source operating at a power of 150 W (15 kV and 10 mA). The spectra were measured at three areas on each sample and the photoelectron take-off angle with respect to the normal to the surface in all measurements was 0°. A hybrid lens mode was employed during analysis (electrostatic and magnetic). The pass energies for survey, high resolution C 1s and high resolution S 2p spectra were 160 eV, 20 eV and 80 eV, respectively. The measured binding energy positions were charge corrected with reference to