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Self-organization of dipeptide-grafted polymeric nanoparticles film: A novel method for surface modification

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ABSTRACT

Novel dipeptide-grafted polymeric nanoparticles were prepared by grafting the dipeptide (Gly-Gly) to a block copolymer backbone, comprised of styrene-alt-(maleic anhydride) and styrene. In aqueous solution PSt_{130}-b-P(St-alt-MAn)$_{58}$-g-GlyGly$_{26}$ formed stable dispersed spherical aggregates of ca. 75 nm. The critical micelle concentration for the dipeptide-grafted block copolymer self-aggregates was 6.3×10^{-3} mg mL^{-1}. The zeta-potential of the aggregates was estimated experimentally. The dispersed polymer nanoparticles effectively self-organized to form stable nanoparticle thin films on hydrophobic solid surfaces, such as octadecyltrichlorosilane modified glass (OTS-G). As the ionic strength and temperature of the polymer suspension increased the surface coverage of the nanoparticle film increased and its hydrophobicity (water contact angle) decreased. Significantly less bovine serum albumin (BSA) adsorbed to nanoparticles modified surfaces with compared OTS-G surfaces. Diglycine grafted polymer nanoparticles have the potential to be used as a novel platform to study protein–protein interactions and to control fouling.

1. Introduction

A key challenge in the production of solid materials with functional properties is the formation of surface coatings, which have a well-organized nanostructure [1]. Layer-by-layer (LBL) techniques utilizing electrostatic and hydrogen-bonding interactions have been used to build multilayer thin films that possess different functionalities, structure and composition [2–5]. Within this class of films amphiphilic block copolymer micelles have frequently been used as molecular building blocks to allow the formation of nanostructured surface films [6,7]. Using LBL techniques polymeric micelles have been investigated for applications such as the production of nanocapsules [7,8] or anti-reflective films [9] and for producing nanostructured coating for surface modification [10]. To build LBL thin films through electrostatic interactions a charged substrate is necessary which can potentially limit film stability as disassembly can occur due to changes in pH or ionic strength [11,12]. The use of hydrophobic interactions presents an opportunity to produce defined thin film, on non-charged hydrophobic substrates, which have a high stability against changes in pH and ionic strength. Hydrophobic interactions are mainly responsible for the affinity between a hydrophobic solid surface and amphiphilic molecules, such as surfactants, in solution. This affinity has been used to prepare ordered molecular films of surfactants and polymers on hydrophobic solid surfaces at solid–solution interfaces [13,14]. For example, PEO-PPO-PEO block polymers have been shown to form stable films on hydrophobic surfaces [15,16]. Cationic and anionic surface charged polystyrene particle latexes in aqueous suspensions have also been used to form self-organized, dispersed-type and aggregated monolayers on hydrophobic solid substrates via hydrophobic interactions [17,18]. The adsorption kinetics and rearrangement of poly(*tert*-butylstyrene-b-sodium-4-styrenesulfonate) (PtBSNaPSS) diblock copolymer micelles at the solid–liquid interface was also reported [19].

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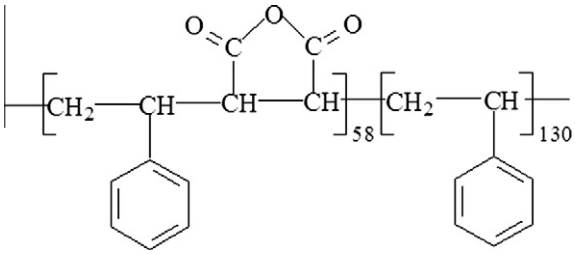
As proteins are the main functional components necessary for cell adhesion [20] there is great interest effort in designing protein or peptides grafted polymer surfaces to investigate specific cell–surface interactions. Recently modified surfaces of Arg-Gly-Asp- (RGD) and Arg-Asp-Gly- (RDG) grafted copolymer poly(L-lysine)-g-poly(ethylene glycol) (PLL-g-PEG) [21,22] were shown to efficiently block the adsorption of serum proteins to Nb_2O_5, TiO_x and tissue culture polystyrene while specifically supporting attachment and spreading of human dermal fibroblasts and to further reduce plasma protein adsorption. Further cell adhesion peptide-(YRGDS, YEILDV)-modified poly(ethylene glycol) grafted poly(acrylic acid) PEG-g-PA copolymers significantly reduced protein adsorption [23]. In order to develop novel materials to inhibit random protein fouling and bacterial adhesion, a Gly-Gly dimer was used as model peptide for the preparation of peptide modified PSt_{130}-b-P(St-alt-MAn)$_{58}$-g-$GlyGly_{26}$ copolymer coating. Gly-Gly was selected, as it is stable, commercially available, inexpensive, has a simple chemical composition and a well-characterized structure.

In this work, we present a new, simple route for the preparation of novel surface films of diglycine-grafted polymeric nanoparticles on hydrophobic solid surface via self-organization through hydrophobic interactions. The novel PSt_{130}-b-P(St-alt-MAn)$_{58}$-g-$GlyGly_{26}$ polymeric nanoparticles were produced by grafting, in a one-pot reaction, Gly-Gly dipeptides to a polymer backbone comprising of polystyrene and poly(styrene-alt-maleic anhydride) blocks (Scheme 1). In aqueous solution this grafted amphiphilic polymer self-assembled to form monodispersed micelle-like spherical nanoparticles which effectively self-organized onto a hydrophobic solid surface of octadecyltrichlorosilane modified glass (G-OTS), The nanoparticle modified G-OTS surface showed significantly reduced protein adsorption. The wettability, the surface properties and the morphologies of the resulting polymer nanoparticle thin films are reported and discussed.

2. Experimental

2.1. Materials

Polystyrene$_{130}$-b-poly(styrene-alt-maleic anhydride)$_{58}$ block copolymer (PSt_{130}-b-P(St-alt-MAn)$_{58}$, *Mn* = 25.3 kDa, polydispersity PDI = 1.33) was synthesized via radical addition fragmentation chain transfer (RAFT) copolymerization and characterized as described previously [24].



Scheme 1. Structure of P(St-alt-MAn)$_{58}$-b-PSt_{130} block copolymer.

Dipeptide Gly-Gly was obtained from Sigma, USA. Octadecyltrichlorosilane (OTS) and glass beads (d = 150–202 μm) were purchased from Sigma–Aldrich (Australia). Microscope glass slides (25 × 75 × 1 mm) were obtained from Biolab Scientific Ltd. (Australia). All the solvents used in this study were analytical or HPLC grade. Water used was deionised water ((Milli-Q®, 18.2 MΩ, TOC:<20 ppb, Millipore).

2.2. Synthesis of dipeptide-grafted polymeric nanoparticles

PSt_{130}-b-P(St-alt-MAn)$_{58}$-g-$GlyGly_{26}$ was prepared by the drop wise (about 0.01 mL every 10 s using a microsyringe) addition of 5.0 mL containing 0.218 g P(St-alt-MAn)$_{58}$-b-PSt_{130} copolymer (0.50 mmol MAn units) in DMF into 100 mL of Gly-Gly (0.33 g, 2.5 mmol) solution containing 5 mmol (0.68 mL) of triethylamine (TEA). The Gly-Gly solution was cooled at 0 °C in an ice bath prior and during the addition of polymer solution. The nanoparticle suspension was stirred intensively during copolymer addition and for a further 24 h, then dialyzed against water for 7 days. The nanoparticle suspension was freeze dried, the solids level determined and the nanoparticles stored until required for evaluation.

2.3. Preparation of alkylated glass substrate

Prior to silanization, glass substrates were submerged in a freshly prepared mixture of H_2SO_4 (98%) and H_2O_2 (30%) at a volume ratio of 7:3 for 1 h at 80 °C and then the samples were rinsed thoroughly with pure water and dried using a N_2 gas stream. The freshly cleaned glass substrates were dipped into 1% solution of silane coupling agents OTS in anhydrous toluene at room temperature for 24 h, followed by washing with toluene and drying under vacuum.

2.4. Self-organization of polymer particles on alkylated glass substrates

Self-organization of the polymeric nanoparticles onto alkylated glass substrates was carried out as follows: alkylated glass slides or beads were immersed into a polymer nanoparticle dispersion (pH 6.5) at a given concentration for 24 h, then removed from the dispersion and washed with water in an ultrasonic bath for 5 min to remove weakly adsorbed particles. The morphology of the polymer nanoparticle films was observed by SEM. Characterization of the modified hydrophobic glass surface was carried out by contact angle measurements of deionised water.

2.5. Characterization of Gly-Gly grafted copolymer

Infrared spectra were recorded using a DigiLab FTS 4000 spectrometer. ^{1}H NMR spectra were recorded on a 500 MHz Oxford 500 spectrometer and samples were prepared at concentrations of 10 mg mL^{-1} in DMSO-d_6. *Elemental analysis* was performed by the Campbell Microanalytical Laboratory, Department of Chemistry, University of Otago, using a Carlo Erba 1108 Elemental Analyzer.