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Protein repellent properties of covalently attached PEG coatings on nanostructured SiO_2-based interfaces

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Abstract

In this study, we report the systematic comparison of different poly(ethylene glycol) (PEG) self-assembled monolayers on glass with respect to their protein adsorption and cell adhesion resistance. Combining PEGylation with micellar nanolithography allowed the formation of gold nanoparticle arrays on glass and selective coverage of the free glass area by PEG. The gold nanoparticles serve as anchor points for the attachment of individual proteins and peptides such as the cell–matrix adhesion promoting cyclic RGDfK motif or the kinesin motor protein Eg5. The capability of the motor protein to bind microtubules remained unaffected by the immobilization. It was shown that the film thickness of a water swollen PEG layer is crucial to maximize the interaction between proteins and peptides with the nanostructures. Non-specific interaction between cells or microtubules and the surface was minimized. The optimum PEG layer thickness correlated with the size of gold nanoparticles which was approximately 5 nm.

Keywords: Poly(ethylene glycol) (PEG); Cell adhesion; Protein adsorption; Quartz crystal microbalance (QCM); Nanostructures; Biofunctionalization

1. Introduction

Immobilized biomolecules at solid–liquid interfaces serve as a versatile tool to address biophysical and biochemical questions [1,2]. Thin films of poly(ethylene glycols) (PEGs) were successfully employed to minimize unspecific interaction between proteins and inorganic surfaces. Several approaches to prepare such surface coatings have been reported including self-assembly [3,4], polymer grafting on activated surfaces [5–8], physisorption [9–11], and surface polymerization [12–14]. In order to get biomolecules embedded in a protein repellent PEG film, scores of possibilities have already been published, ranging from statistical functionalization of PEG coated surfaces [14,15] to micro- and nanostructures embedded into PEG matrices [16,17].

Gold nanopattern surrounded by PEG proved to be a powerful tool for this kind of applications [17–20]. These nanoparticles on glass offer the advantage of combining two chemically very different materials which allow both the specific functionalization of gold via thiol chemistry and glass via silane chemistry [21–23]. The density of the gold particles can be varied to control the surface concentration of biomolecules [17–19]. The particles are small enough (<8 nm) to prevent protein clustering on the adhesive area itself [17]. It is crucial to focus not only on the protein resistant properties of the coating but also on its swelling behavior under experimental conditions to obtain optimum protein repellence in the interparticle area without blocking the nanoparticles.

In this work, we compare systematically different PEG systems with respect to their film formation, cell and protein repellent properties on gold nanoparticle structured

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SiO_2-wafers. It is the aim to describe optimal surface modification procedures which provide an inert background to which proteins or cells do not respond. Then, such an inert background is equipped with signal molecules or proteins, e.g. cyclic RGDfK peptides or Eg5 molecular motors, in order to investigate responses of biological objects, e.g. cells or microtubule (MT), entirely due to the presentation of signal molecules or proteins on a nanometer scale. The gold nanoparticle density was kept constant while varying the PEG matrix in between. We could show that the swelling behavior of different PEG derivatives estimated by the de Gennes model [24] is crucial to optimize the accessibility of ligands or proteins located on the gold nanoparticles for cells and proteins and to minimize non-specific interaction in areas between gold nanoparticles.

2. Experimental

2.1. Materials

All solvents used for functionalization and synthesis were dried over molecular sieve 3 Å (Carl Roth GmbH&Co., Karlsruhe, Germany) for at least 24 h under nitrogen atmosphere (Messer Griesheim, Krefeld, Germany). All other chemicals were used as received. Deionized water was purified with a MilliQ plus system (Millipore™, Eschborn, Germany) and used throughout the whole study.

Cyclohexane was purchased from NeoLab (Heidelberg, Germany), dioxane, glycidyloxypropyltriethoxysilane (GOPTES), 3-isocyanatopropyltriethoxysilane (3-ITPS), methoxy-terminated PEG750 (mPEG750), and mPEG2000 were from Fluka (Neu-Ulm, Germany) and mPEG-amines (mPEGA750, −2000, −5000) from Rapp Polymere (Tübingen, Germany), PEG-6000 was bought from Acros (Geel, Belgium). Bovine serum albumin (BSA), 1,4-diazabicyclo[2,2,2]octan, fibronectin, paclitaxel and tetrachloroauric acid were obtained from Sigma-Aldrich (Schnelldorf, Germany). Dimethylformamide (DMF), ethyl acetate, methanol and toluene were from Merck (Darmstadt, Germany) and triethylamine from Riedel-De-Häen (Neu-Ulm, Germany). Glass coverslips ($20 \times 20\,mm^2$, $24 \times 24\,mm^2$) were acquired from Carl Roth GmbH & Co., silicon(1 0 0) wafer from Crysteco (Allen, TX, USA) and quartz crystals (QSX 303) from Q-sense (Västra Frölunda, Sweden). Phosphate buffered saline (PBS), fetal calf serum (FCS), Dulbecco's modified essential medium (DMEM) were from Gibco (Karlsruhe, Germany). PS-PVP-diblock copolymer was from Polymer Source Inc. (Montreal, Canada). The cyclic RGDfk was synthesized in the group of Prof Kessler, TU Munich (Germany).

2.2. Synthesis of the mPEG-silane derivatives

The mPEG750- **(1)** and mPEG2000-carbamate **(2)** (Scheme 2) were prepared by adding one equivalent of 3-ITPS to a dry solution of methoxy-terminated PEG (mPEG) in 140 ml dioxane (3 mmol mPEG750, 1 mmol mPEG2000) in the presence of 99.6 mg (0.89 mmol) 1,4-diazabicyclo[2,2,2]octan and refluxed for 24 h. Afterwards, the solvent was evaporated and the raw products were purified by recrystallization from toluene/cyclohexane 1:4 to yield **(1)** as a white waxen solid (89%) and **(2)** as white powder (94%).

2.2.1. mPEG750-carbamate (1)

^{1}H-NMR (300 MHz; $CDCl_3$): $\delta = 0.54$ (m, 2H, Si-CH_2), 1.15 (t, $^3J = 7.0$ Hz, 9 H, O-CH_2-CH_3), 1.54 (m, 2H, Si-CH_2-CH_2), 3.08 (m, 2H, CH_2-N), 3.30 (s, 3 H, O-CH_3), 3.55-3.70 (m, 60H, O-CH_2-CH_2-O), 3.74 (q, $^3J = 7.0$ Hz, 6H, O-CH_2-CH_3), 4.13 (m, 2H, CO-O-CH_2), 5.05 (br s, 1H, *NH*). **^{13}C-NMR** (75 MHz; $CDCl_3$): $\delta = 7.3$ (Si-CH_2), 18.0 (Si-O-CH_2-CH_3), 23.0 (Si-CH_2-CH_2), 43.1 (CH_2-NH), 58.1 (Si-O-CH_2-CH_3), 58.7 (O-CH_3), 63.4 (CO-O-CH_2), 68.3 (CO-O-CH_2-CH_2), 69.3-72.1 (O-CH_2-CH_2-O), 156.0 (C = O). Molecular weights determined by **MALDI**: M_n(number average) = 904; M_w(weight average) = 932; $M_w/M_n = 1.03$.

2.2.2. mPEG2000-carbamate (2)

^{1}H-NMR (300 MHz; $CDCl_3$): $\delta = 0.54$ (m, 2H, Si-CH_2), 1.14 (m, 9 H, O-CH_2-CH_3), 1.53 (m, 2H, Si-CH_2-CH_2), 3.08 (m, 2H, CH_2-N), 3.30 (s, 3H, O-CH_3), 3.55–3.70 (m, 180 H, O-CH_2-CH_2-O), 3.74 (m, 6H, O-CH_2-CH_3), 4.13 (m, 2 H, CO-O-CH_2), 5.05 (br s, 1H, *NH*). **^{13}C-NMR** (75 MHz; $CDCl_3$) $\delta = 7.4$ (Si-CH_2), 18.1 (Si-O-CH_2-CH_3), 23.1 (Si-CH_2-CH_2), 43.2 (CH_2-NH), 58.2 (Si-O-CH_2-CH_3), 58.7 (O-CH_3), 61.9 (CO-O-CH_2), 63.6 (CO-O-CH_2-CH_2), 69.4–72.1 (O-CH_2-CH_2-O), 156.1 (C = O). **MALDI**: $M_n = 2252$; $M_w = 2269$; $M_w/M_n = 1.01$.

For the mPEG-urea derivatives (Scheme 2) one equivalent of 3-IPTS was added to a dry solution of mPEG-amine (3 mmol mPEGA-750; 1 mmol mPEGA-2000; 0.5 mmol mPEGA-5000) in 15 ml dimethylformamide at room temperature under nitrogen atmosphere and stirred for 72 h. Afterwards, the solvent was removed by destillation at room temperature and the products were purified by recrystallization from toluene/cyclohexane 1:4 to yield **(3)** as a white waxen solid (85%), **(4)** as slightly beige (93%), and **(5)** as white powder (95%).

2.2.3. mPEG750-urea (3)

^{1}H-NMR (300 MHz; $CDCl_3$): $\delta = 0.59$ (m, 2H, Si-CH_2), 1.18 (t, $^3J = 7.0$ Hz, 9H, O-CH_2-CH_3), 1.55 (m, 2H, Si-CH_2-CH_2), 3.11 (m, 2H, CH_2-CH_2-CH_2-N), 3.34 (s, 3H, O-CH_3), 3.55–3.70 (m, 60H, O-CH_2-CH_2-O), 3.79 (q, $^3J = 7.0$ Hz, 6H, O-CH_2-CH_3), 5.01-5.07 (br s, 2H, *NH*). **^{13}C-NMR** (75 MHz; $CDCl_3$): $\delta = 7.7$ (Si-CH_2), 18.3 (Si-O-CH_2-CH_3), 23.8 (Si-CH_2-CH_2), 40.3 (CH_2-CH_2-CH_2-NH), 42.9 (NH-CH_2-CH_2-O), 58.3 (Si-O-CH_2-CH_3), 59.0 (O-CH_3), 68.3 (CO-NH-CH_2-CH_2), 70.1-71.9 (O-CH_2-CH_2-O), 158.5 (C = O). **MALDI**: $M_n = 951$; $M_w = 961$, $M_w/M_n = 1.01$.

2.2.4. mPEG2000-urea (4)

^{1}H-NMR (300 MHz; $CDCl_3$): $\delta = 0.57$ (m, 2H, Si-CH_2), 1.17 (t, $^3J = 7.0$ Hz, 9 H, O-CH_2-CH_3), 1.53 (m, 2H, Si-CH_2-CH_2), 3.10 (m, 2H, NH-CH_2-CH_2-CH_2), 3.33 (s, 3H, O-CH_3), 3.37 (m, 2H, N-CH_2-CH_2-O), 3.50 (m, 2H, N-CH_2-CH_2-O), 3.55–3.70 (m, 172 H, O-CH_2-CH_2-O), 3.77 (m, 6 H, Si-O-CH_2-CH_3), 5.03 (br s, 2 H, *NH*).**^{13}C-NMR** (75 MHz; $CDCl_3$): $\delta = 7.6$ (Si-CH_2), 18.2 (Si-O-CH_2-CH_3), 23.7 (Si-CH_2-CH_2), 40.2 (CH_2-CH_2-CH_2-NH), 42.8 (NH-CH_2-CH_2-O), 58.3 (Si-O-CH_2-CH_3), 58.9 (O-CH_3), 70.0 (CO-NH-CH_2-CH_2), 70.2-71.9 (O-CH_2-CH_2-O), 158.5 (C = O). **MALDI**: $M_n = 2160$; $M_w = 2176$, $M_w/M_n = 1.01$.

2.2.5. mPEG5000-urea (5)

^{1}H-NMR (300 MHz; $CDCl_3$): $\delta = 0.58$ (m, 2 H, Si-CH_2), 1.16 (t, $^3J = 7.0$ Hz, 9 H, O-CH_2-CH_3), 1.55 (m, 2 H, Si-CH_2-CH_2), 3.12 (m, 2H, N-CH_2-CH_2-O), 3.35 (s, 3H, O-CH_3), 3.37 (m, 2 H, N-CH_2-CH_2-O), 3.55–3.72 (m, 440 H, O-CH_2-CH_2-O), 3.78 (m, 6 H, Si-O-CH_2-CH_3), 5.03 (br s, 2H, *NH*).**^{13}C-NMR** (75 MHz; $CDCl_3$): $\delta = 7.6$ (Si-CH_2), 18.2 (Si-O-CH_2-CH_3), 224.2 (Si-CH_2-CH_2), 40.0 (CH_2-CH_2-CH_2-NH), 42.7 (NH-CH_2-CH_2-O), 58.1 (Si-O-CH_2-CH_3), 58.8 (O-CH_3), 70.2–71.9 (CH_2-O-CH_2), 155.5 (C = O). **MALDI**: $M_n = 5038$; $M_w = 5078$, $M_w/M_n = 1.01$.

2.3. PEGylation of SiO_2 surfaces

Glass coverslips and silicon(1 0 0) samples were first cleaned and chemically activated by immersing them into a freshly prepared piranha solution for 30 min, rinsed with water, sonicated for 3 min, rinsed with water again and finally blown dry with nitrogen. The activated surfaces were directly used for the grafting of PEG.

We used a two-step coupling mechanism where the epoxydation of the activated surface with GOPTES was followed by a nucleophilic