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Branched polyglycidol and its derivatives grafted-from poly(ethylene terephthalate) and silica as surfaces that reduce protein fouling

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ABSTRACT

Hyperbranched polyglycidol-coated silica or poly(ethylene terephthalate) surfaces were obtained as materials with reduced protein adsorption properties. To perform polymer grafting, the wafers were first functionalized: silica (Si) with 3-[bis(2-hydroxyethyl)amino]propyltriethoxysilane, and plasma treatment was applied for poly (ethylene terephthalate) (PET). The grafting-from method, using initiation of the anionic-ring opening polymerization of glycidol by the deprotonated alkoxide groups located on the wafers, was applied to immobilize the polymer on these two kinds of wafers. The grafting-from reactions carried out in solution allowed high molar masses of the tethered polyglycidol to be obtained. The polymerization was performed in the presence of a "free initiator"; thus, the information about molar mass and the structure of grafted polymers was estimated. The hydroxyl groups of Si-immobilized polyglycidol were modified with hydrophilic poly(ethylene glycol) or hydrophobic ethyl carbamate groups. The affinity of the polymer layers to water was controlled by the grafting density and type of polymer modification applied. All hyperbranched polyglycidol-based surfaces were able to reduce the protein adsorption to a high level.

1. Introduction

Non-specific protein adsorption on a material surface, regardless of whether the biomaterial is in contact with body fluids in an intra- or extracorporeal manner, causes a deterioration of the functioning of implants, biosensors or external medical devices [1,2]. Therefore, the prevention of such adsorption on biomaterials has been extensively studied [3–5]. This was achieved by a biomaterial surface modification with polymeric coatings of poly(ethylene glycol) (PEG), oligo(ethylene glycol) (OEG), poly(oligo(ethylene glycol)) methacrylates (POEGMA), polyacrylamides, polysaccharides or polyzwitterions [6–8]. Among them, oligomers or polymers of ethylene glycol were the most commonly studied polymers as they possess highly hydrophilic, flexible, non-immunogenic, non-toxic and biocompatible properties [9–12]. Despite their wide use, PEGs present some drawbacks like susceptibility to oxidative degradation [13], limitations in regard to polymer architecture or limited functionality.

Polyglycidol is a biocompatible, non-cytotoxic polymer that has hydroxyl functional groups which are capable of further functionalization [14,15]. A variety of different architectures ranging from linear, graft and hyperbranched to dendritic polyglycidols [16–18] are accessible, which implies its high potential for application in biology and pharmacy [19]. As an alternative to PEG, the use of polyglycidol as a coating material to reduce non-specific protein adsorption has been proposed in recent years [20–28].

The preparation of surfaces of linear (LPG) or branched (HPG) polyglycidol, which reduce protein adsorption, was mainly focused on the grafting-to procedure [21,22,24–27,29–31]. For that purpose, the modification of polyglycidol with reactive function such as triethoxysilyls [25,26], amines [30,31], thiols [24,30] or catechols [22,27] was applied. Unmodified polyglycidol was also directly bound to the surface through the surface-induced termination of living chains (by the reactive chloropropyl groups) or via the reaction between hydroxyl groups of the linear polyglycidol and anhydride groups of the surface [20]. All of the obtained polyglycidol surface were generally able to sufficiently reduce protein adsorption on a level that is comparable to PEG. Nevertheless, in some cases, difficulties with the uniformity of surface coverage affected the antifouling behavior [31].

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Although well-defined polymers were immobilized on a surface in the grafting-to procedure, this technique led to polymer layers of low thickness and grafting density, which affects protein adsorption [32,33]. Higher polymer grafting density and layer thickness can be achieved by using the grafting-from technique, involving the initiation of polymerization by the initiator bound to the surface of the wafer [34]. The use of this technique to immobilize hyperbranched polyglycidol for antifouling applications of the resultant materials was previously described only in [28,35,36]. In these cases, the anionic ring-opening polymerization of glycidol was performed in bulk and was initiated by the deprotonated silanol functionalities of the glass or silicon.

In this work, we describe the synthesis of hyperbranched polyglycidol coatings on a medically relevant material, poly(ethylene terephthalate) (PET), and on silica (Si) using the grafting-from procedure. To estimate the molar masses of immobilized polyglycidol, two procedures were verified: polymerization from Si wafers in the presence of a "free initiator" and grafting-from the porous silica gel (Si_{gel}). For the synthesis of polymer coatings, a new approach was applied. The initiation sites, reactive alkoxides, were generated on the surface of wafers, followed by the anionic ring-opening polymerization of glycidol performed in solution. High molar masses of the grafted polymer were obtained, which improved the surface coverage. The outer layer of polyglycidol immobilized on silica was modified with hydrophilic poly (ethylene glycol) or the more hydrophobic ethyl carbamate groups in order to study the influence of peripheral groups on protein adsorption. The polyglycidol-coated surfaces with optimized properties were obtained and tested against protein adsorption.

2. Experimental

2.1. Materials

2,3-epoxypropanol-1 (glycidol) (96%, Aldrich) was dried over molecular sieves and distilled under reduced pressure. Ethyl isocyanate was distilled under a dry nitrogen atmosphere. Tetrahydrofuran (THF) was refluxed over Na/K alloy while dimethyl sulfoxide (DMSO) was dried over CaH_2, distilled, dried over BaO and fractionated under reduced pressure. *N,N′*-dimethylformamide (DMF) was dried over CaH_2 and distilled under reduced pressure. Ethanol (99.8%, POCH, Poland) was filtered prior to use. Poly(ethylene glycol) monomethyl ether (M_n = 350 g/mol, PEG_{350}) (Aldrich), potassium *tert*-butoxide (99%, Fluka), H_2SO_4 (95%, POCH, Poland), hydrogen peroxide (30%, Chempur, Poland), 3-[bis(2-hydroxyethyl)amino]propyltriethoxysilane (HAPTES) (65% solution in ethanol, Aldrich), hexyltriethoxysilane (HETES) (97%, TCI), dibutyltin dilaurate (DBTL) (95%, Aldrich), *N,N′*-carbonyldiimidazole (CDI) (> 97%, Aldrich), fibrinogen from human plasma, Oregon Green®488 Conjugate (96%, Invitrogen) and sodium bicarbonate buffer (Aldrich) were used as received.

Polished prime silica wafers (Cemat Silicon S.A, Poland) with a thickness of 500–550 μm and ϕ = 100 mm were cut into 1 × 1 cm pieces. Poly(ethylene terephthalate) sheets (Goodfellow) with a thickness of 1 mm were cut into approx. 1 × 1 cm pieces. Silica gel (pore size 60 Å, 35–60 mesh particle size, 480 m^2/g) was obtained from Aldrich.

2.2. Synthesis of polyglycidol surfaces

2.2.1. Preparation of silica or PET wafers and silica gel

The Si wafers were ultrasonically cleaned in distilled water and ethanol. The wafers were hydroxylated using a mixture of 30% hydrogen peroxide and 95% sulfuric acid (1:3) (piranha solution), followed by rinsing with deionized water and drying for 24 h at 120 °C in a dust-free vacuum atmosphere. To obtain the different density of hydroxyl groups on the surface, the wafers were immersed in a 3% solution of HAPTES or in a mixture of HAPTES and HETES (1:1 mol/mol; 1:9 mol/mol) in ethanol for 2 h. Subsequently, they were rinsed with ethanol and annealed for 24 h at 120 °C. Then, they were rinsed again with ethanol for 15 min to remove any residual contaminants and annealed again. The prepared wafers were stored in a vacuum desiccator.

The PET wafers were cleaned with ethanol 3 times for 15 min and then dried in a dust-free vacuum atmosphere for 1 h. They were then exposed to oxygen plasma using the FEMTO plasma system (40 kHz, 100 W) from Diener Electronic for 3 min. Prior to polymerization, wafers were left in contact with ambient air for about 30 min.

Si_{gel} was rinsed with deionized water and dried at 100 °C. Then, Si_{gel} was hydroxylated with a mixture of 30% hydrogen peroxide and 95% sulfuric acid (1:3) (piranha solution) for 2 h, followed by rinsing several times with deionized water. Then, it was dried in a dust-free oven at 100 °C for several hours and transferred to an ampoule with the glass-Teflon valve and dried again. Dry silica gel was next reacted with HAPTES in anhydrous ethanol for several hours. Si_{gel} was then filtered and rinsed with ethanol and with tetrahydrofuran. Finally, modified silica gel was dried over a high vacuum.

2.2.2. Surface-initiated polymerization of glycidol from Si and PET wafers

The hydroxylated Si or PET wafers were placed separately in glass holders and transferred into reactors under an argon atmosphere.

Then, 60 mL of a solution of potassium *tert*-butoxide in THF (0.85 g/L) was poured into a reactor containing Si wafers. The deprotonation reaction was carried out for 1 h at 45 °C. Afterwards, freshly distilled glycidol (6 mL) was added under an argon atmosphere and polymerization was performed at 50 °C for 48 h.

In the case of PET wafers, a solution of potassium *tert*-butoxide in DMSO (0.85 g/L, 60 mL) was applied for deprotonation. The reaction was carried out for 1 h at 45 °C. Afterwards, DMSO solution with an initiator was removed from the flask, the wafers were rinsed with fresh DMSO and then glycidol in DMSO solution (7 vol%) was added. The polymerization was performed at 50 °C for 48 h.

After polymerization, the modified Si and PET wafers were removed from the reactors and washed intensively several times with water and dried under an argon stream. Then, they were stored in a vacuum desiccator prior to further reactions or characterization.

During the grafting of glycidol from silica wafer, "free" hyperbranched polyglycidol was formed in solution simultaneously to that on surface. Therefore, after polymerization, the THF was evaporated and the water was added to dissolve the non-bonded polyglycidol. The water was evaporated and the polymer was collected and subjected to analysis.

2.2.3. Surface-initiated polymerization of glycidol from Si_{gel} and the polymer detachment

Modified Si_{gel} (3.80 g) was transferred to the ampoule equipped with the glass-Teflon valve and dried at 120 °C under vacuum (1×10^{-5} mbar) for 3 days. After cooling to room temperature, a solution of potassium *tert*-butoxide (0.106 g) in THF (30 mL) was added and the ampoule was shaken for one hour. Then, 6 mL (6.65 g) of glycidol was added and the polymerization was carried out at 50 °C for 48 h. The content of the ampoule was removed and the filtered silica gel was washed with water and dried at 60 °C under atmospheric pressure. Finally, 4.57 g of Si_{gel} with grafted polyglycidol was obtained.

To detach the polymer, the silica gel with grafted polyglycidol was placed in a 5% solution of HF in water. The mixture was stirred for about 4 h at 50 °C until the dissolution of the gel was completed. Then, the aqueous solution of sodium carbonate was added. Afterwards, the polymer was desalinated using ion-exchange columns, the water was evaporated and the polymer was dried and subjected to analysis.

2.2.4. Modification of hyperbranched polyglycidol on Si wafers

Modification with ethyl isocyanate. The Si wafers covered with hyperbranched polyglycidol were transferred to the reactor and the solution of DBTL in DMF (50 mL) was added. After a few minutes, the ethyl isocyanate was added drop-wise. The molar ratio [ethyl