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Multiple and terminal grafting of linear polyglycidol for surfaces of reduced protein adsorption

Alicja Utrata-Wesołek [a, *], Wojciech Wałach [a], Jacek Anioł [b], Aleksander L. Sieroń [b], Andrzej Dworak [a]

[a] Centre of Polymer and Carbon Materials, Polish Academy of Sciences, M. Curie-Sklodowskiej 34, 41-819 Zabrze, Poland
[b] Department of General, Molecular Biology and Genetics, Medical University of Silesia, Medykow 18, 40-752 Katowice, Poland

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ABSTRACT

Hydrophilic surfaces based on linear polyglycidol (poly(2,3-epoxypropanol-1)) were prepared to examine protein-surface interactions. The grafting-to technique was applied to covalently attach the polyglycidol and its block copolymer with poly(ethylene glycol) to the silica wafers. Two types of surfaces with different polymer chains binding to the substrate were prepared and its ability to reduce the protein adsorption was compared. In the first case, a reaction between the hydroxyl groups of the linear polyglycidol (of M_n 8000 g/mol or 1.9×10^6 g/mol) and the anhydride groups of the surface was applied, causing multiple attachment of the polymer chain to the surface. In the second case, the living polyglycidol (M_n 8000 g/mol) or poly(glycidol-*co*-ethylene glycol) (M_n 6000 g/mol) chains were terminated by the chloropropyl groups of the surface leading to polymer brushes. Hydrophilic surfaces with polymer layer thickness between 1.5 and 140 nm were obtained. The morphology, affinity to water and layer thickness were influenced by the molar mass of the immobilized (co)polyglycidol and the type of binding with the surface. It was established that the fibrinogen adsorption was limited to 45–90% on the polyglycidol-coated surfaces compared to the bare silicon wafers. The ability to reduce fibrinogen adsorption was dependent on the molar mass of the polymer, polymer layer thickness, type of polymer bonding with the surface and presence of poly(ethylene glycol) on the outer surface layer.

1. Introduction

Materials in contact with biological fluids are exposed to protein adsorption and cell adhesion. This process, known as a fouling [1], is harmful and generates activation of the host defense mechanism, which leads to inflammatory response, thrombosis coagulation, fibrosis or infections [2]. Therefore, materials with antifouling properties have been the subject of increased interest in the last few years [3–6]. Antifouling behavior is usually achieved by coating the surface with a thin layer of protein-repelling macromolecules.

Next to natural polymers like dextrans [7] and carbohydrates [8], synthetic polymers are in most cases used. These materials can either be noncharged hydrophilic polymers or polyzwitterions. Most hydrophilic antifouling materials include poly(ethylene glycol) (PEG), oligo(ethylene glycol) (OEG), poly(oligo(ethylene glycol)) methacrylates (POEGMA), polyacrylamides, polyglycidols and polysaccharides. Polyzwitterions include polybetaines (derived from 2-methacryloyloxyethyl phosphorylcholine (MPC), sulfobetaine methacrylate (SBMA) and carboxybetaine methacrylate (CBMA) [5,9–11]) and polyampholytes [12]. Among these polymers, the best-known and most studied surfaces include those covered with poly(ethylene glycol) or its derivatives [13–17]. This is due to the fact that PEG is non-toxic, non-immunogenic, non-antigenic and biocompatible [18]. The ability to generate protein-repellent PEG surfaces is attributed to the fact that the polymer chains are well hydrated, neutral, highly mobile and flexible. Polyglycidol, a biocompatible polyether comparable to PEG, is non-cytotoxic, possesses free hydroxyl groups that are available for further functionalization, exhibits increased stability against oxidation compared to PEG and has high potential for biomedical and pharmaceutical application [19–24]. In spite of the apparent suitability of polyglycidol for fouling protection, relatively few data are available on such applicability of this polymer, and those present mostly concern dendritic or hyperbranched structures immobilized on gold, glass, titanium or polymer films [25–31].

* Corresponding author.
E-mail address: autrata@cmpw-pan.edu.pl (A. Utrata-Wesołek).

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Reported few polyglycidol coatings with antifouling properties were produced either via grafting-to technique or grafting-from process. The grafting-to procedure applied so far require modification of the linear polyglycidol (mostly oligomeric), hyperbranched or dendritic (with M_n from 1500 to 7500 g/mol) polyglycidol before surface immobilization. In case of linear polyglycidol the reactive function such as triethoxysilyl [32], amine [33] or thiol [33] has to be introduced at the polymer chain-end. The hyperbranched or dendritic polyglycidols are usually modified through reacting outer hydroxyl groups with compound introducing reactive functions such as thiols [20,31], amines [34], triethoxysilyl [32,35] or catechols [36,37]. The grafting-from approach whereas utilizes the anionic ring-opening polymerization of glycidol (initiated from the OH functions bound to glass, silicon, steel or aluminum surface followed by their deprotonation [27,30,38]), or the surface initiated radical polymerization of linear oligoglicydol macromonomers [26,29]. The antifouling properties of dendritic or hyperbranched polyglycidols are ascribed to the presence of highly flexible aliphatic polyether, hydrophilic groups present on the surface and highly branched architecture.

Numerous studies have shown that antifouling behavior is a highly complex process. Many factors such as the structure and molar mass of the polymer grafted to the surface, the thickness or grafting density of the polymer layer, conformation and flexibility of polymer chains and the affinity to water of the outer top layer appear to determine the type and strength of the protein–surface interactions [3,6,39]. Investigations of the protein-resistant surfaces have provided much valuable information, but many open questions still remain related to the polymer chain-protein or protein-surface interactions.

In this paper, we describe the application of hydrophilic, linear (co)polyglycidol layers immobilized on a solid support as antifouling surfaces for fibrinogen adsorption. The blood protein fibrinogen is known to have a high affinity to interfaces and thus serves as a model protein for investigation of adsorption in this work. The synthesis of the polyglycidol surfaces via the reaction between reactive hydroxyl groups of the linear polyglycidol and anhydride groups of the surface is demonstrated. The grafting-to technique through the surface induced termination (by the reactive chloropropyl groups) of the living polyether chains is also presented and provides a novel and alternative way to generate the polyglycidol brushes. The influence of the molar mass of the immobilized polyglycidol ($M_n = 8000$ or 1.9×10^6 g/mol), the type of its bonding with the surface, and the presence of poly(ethylene glycol) on the outer surface layer on the morphology, affinity to water and layer thickness is presented. The polyglycidol-coated surfaces were shown to reduce fibrinogen adsorption compared with bare silica supports. Materials with effective reduction of protein adsorption were obtained using the proper control over the structural parameters of the surface, such as molar mass of the immobilized polyglycidol, polymer layer thickness, density or type of polymer bonding with the surface and the presence of poly(-ethylene glycol) on the outer surface layer.

2. Materials and methods

2.1. Materials

Ethoxyethyl glycidyl ether (EEGE) was synthesized in an acid-catalyzed reaction of 2,3-epoxypropanol-1 (glycidol) (96%, Aldrich) with ethyl vinyl ether (Aldrich) according to the procedure described in Ref. [40]. Poly(ethylene-*alt*-maleic anhydride) (PEMA), typical $M_w = 100\,000$–$500\,000$ g/mol (Aldrich) was dissolved in acetone, precipitated in hexane and then dried at 120 °C for 24 h. Poly(ethylene glycol) monomethyl ether ($M_n = 350$ g/mol, PEG_{350}) (Aldrich), diethyl zinc (1 M solution in hexane, Aldrich), HCl (POCH, Poland), potassium *tert*-butoxide (99%, Fluka), oxalic acid (97%, Fluka), H_2SO_4 (95%, POCH, Poland), hydrogen peroxide (30%, Chempur, Poland), (3-aminopropyl)triethoxysilane (APTES) (99%, Aldrich), (3-chloropropyl)triethoxysilane (ChPTES) (95%, Aldrich), triethoxymethylsilane (TEMS) (99%, Aldrich), fibrinogen from human plasma Oregon Green®488 Conjugate (96%, Invitrogen), and sodium bicarbonate buffer of pH 8.3 (Aldrich) were used as received. Ethanol (99.8%, POCH, Poland), acetone (99.5%, POCH, Poland), THF (POCH, Poland) and methanol (POCH, Poland) were filtered prior to use. 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.

2.2. Polymer synthesis

2.2.1. Synthesis of high and low molar mass polyglycidol

The synthesis of high molar mass polyglycidol (LPG_H) has been previously described in Ref. [41]. Briefly, ethoxyethyl glycidyl ether (EEGE) was polymerized via a coordination process in bulk with $ZnEt_2/H_2O$ (1:0.8) for 24 h at 55 °C under inert gas atmosphere. Acidic hydrolysis with 3 M HCl removed the protective ethoxyethyl groups, resulting in linear polyglycidol. The synthesis of low molar mass polyglycidol was performed in reactors equipped with glass-Teflon valves and applying high vacuum technique according to the procedure described in Ref. [42]. Briefly, anionic polymerization of EEGE was performed in THF at 60 °C for 24 h using potassium *tert*-butoxide as an initiator. Afterwards, the THF was evaporated from a portion of the polymer solution, the polymer was hydrolyzed using oxalic acid, and the linear low molar mass polyglycidol was obtained (LPG_L). The other portion of the living polymer solution was poured on the modified wafers, leading to its termination, and surfaces with polymer bound via the chain end were obtained (LPG_{LT}). The detailed synthetic procedure is described in the following section.

2.2.2. Synthesis of copolymer of glycidol and ethylene glycol

The diblock copolymer of ethylene glycol and glycidol was synthesized using alcoholate of poly(ethylene glycol) monomethyl ether PEG_{350} as a macroinitiator. The synthesis was performed in reactor equipped with glass-Teflon valves and applying high vacuum technique according to the procedure described in Ref. [42]. Briefly, poly(ethylene glycol) was dissolved in bidistilled water, and CsOH in water (1.0 times the molar amount to the hydroxyl groups of the glycol) was added to the solution. After dissolution, benzene was added, and the mixture was refluxed. Water was removed by azeotropic distillation. The solution was transferred under dry argon into a polymerization reactor, and benzene was removed through liophilization. The calculated amount of ethoxyethyl glycidyl ether in THF was added. After dissolution of the macroinitiator, polymerization was performed at 65 °C for 72 h. Then, the solution of living diblock copolymer was poured on the modified wafers, leading to its termination and surfaces with polymer bound via the chain end were obtained ($L(EO\text{-}G)_{LT}$). The detailed synthetic procedure is described in the following section.

2.3. Synthesis of polyglycidol surfaces

2.3.1. Preparation of wafers

2.3.1.1. Cleaning and hydroxylation. The silica wafers were ultrasonically cleaned in freshly distilled water and ethanol. Then the wafers were immersed in a mixture of 30% hydrogen peroxide and 95% sulfuric acid (1:3) (piranha solution) for 2 h. Afterwards, they were rinsed with deionized water and held for 24 h at 120 °C in a dust-free atmosphere.