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Synthesis and characterization of well-defined ligand-terminated block copolymer brushes for multifunctional biointerfaces

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ABSTRACT

Polymer brush-functionalized surfaces promise to provide access to absolute control over the biointerface functionality. To enable in this context systematic studies of cell attachment and proliferation, we investigated the synthesis of well-defined block copolymer brushes of poly(acrylamide) (PAAm), poly(oligo(ethylene glycol)methylether methacrylate) (POEGMA) and poly(acrylic acid) (PAA) by surface-initiated atom transfer radical polymerization (SI-ATRP) and subsequently the introduction of exposed ligands for cell adhesion. Detailed X-ray photoelectron spectroscopy (XPS), Fourier transform infrared (FTIR) spectroscopy, time of flight secondary ion mass spectrometry (ToF-SIMS) and ellipsometry measurements unraveled the progress and the livingness of the homo- and copolymerization of the three monomers. In particular, the correlation of the temporal evolution of the ellipsometric thickness with the decrease in bromine content pointed to a progressive loss of active chain ends, which limits the attainable maximum brush thickness. Non-fouling brushes of POEGMA and PAAm exhibited long term stability and full functionality in cell medium for more than 1 month. Block copolymer brushes with defined thickness comprising a non-fouling POEGMA or PAAm block and a short terminal PAA block were functionalized with a peptide containing the arginine-glycine-aspartic acid (RGD) sequence. Cell attachment assays with fibroblasts revealed facilitated cell adhesion on RGD modified block copolymer brushes. The specific signaling was further confirmed with inhibited cell adhesion on arginine-alanine-aspartic acid (RAD) functionalized brushes (negative control). Hence on the basis of the unraveled synthesis behavior of block copolymer brushes and side chain modification a versatile strategy to fabricate biologically inert surfaces that facilitate integrin-specific cell adhesion has been developed.

1. Introduction

In recent years, the interest in the development of biologically active surfaces has grown, due to the indispensability in many fields [1]. Examples of applications include medical implants that replace or improve vital body functions in humans, biosensors for biomedical diagnostics and non-biofouling surfaces for food industry. All these processes require an understanding of the interactions that occur at the interface between a solid material surface and the biological environment [2]. For the creation of materials used in many biotechnological applications, interfaces are required which have both enhanced specific binding and reduced nonspecific binding.

Recently polymer brushes have attracted considerable attention for the creation of bioactive surfaces due to their intriguing physicochemical surface properties, versatile architectures and ease of processing. Due to the non-fouling properties and the chemical inertness of the polymer brushes, as known for PAAm and POEGMA brushes, it is generally necessary to properly activate or modify them prior to the introduction of bioactive molecules, which can be achieved by side chain modification and chain end modification [3–5]. For this reason it is necessary to extend the brushes with a second block, presenting side chains with high binding capacity (e.g. esters or carbonates), to an inert first layer, which can be activated and reacted with groups available on biomolecules [6]. In this context, PAA brushes have received considerable interest due to their rich content of terminal carboxyl groups and protein immobilization via EDC/NHS activation as previously reported by Wang and Dai et al. [7–9].

Polymer brushes synthesized by a “grafting-from”

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polymerization technique as for example surface initiated atom transfer radical polymerization (SI-ATRP) lead to narrow-polydispersity polymer films with controlled architecture at the molecular level [10–12]. SI-ATRP produces an end-functional polymer that can be used as a macroinitiator for the polymerization of a second monomer resulting in a block copolymer brush. With the use of these preferences Brittain and group members have grown AB and ABA type block copolymer brushes of poly(styrene) (PS) and poly(methyl methacrylate) (PMMA) on planar silica substrates by ATRP using SAMs of a bromoisobutyrate initiator [13,14]. Also Huck and coworkers prepared triblock copolymer brushes containing poly(methacrylic acid) (PMAA) by aqueous ATRP [15]. For the synthesis of surface tethered block copolymer brushes good control over the thickness of each block of the copolymer is required.

Many extracellular matrix proteins (like fibronectin, fibrinogen, collagen, vitronectn and laminin) exhibit the tripeptide RGD, which is an important ligand for cell adhesion [16]. For example Tugulu et al. prepared poly(2-hydroxyethyl methacrylate) (PHEMA) brushes functionalized with a RGD peptide (GG**RGD**S) and observed the adhesion of HUVEC cells [17]. Based on these observations, the group of Klok synthesized mixed polymer brushes of HEMA, poly(ethylene glycol) methacrylate (PEGMA) and 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA) with varied RGD peptide concentrations for density controlled adhesion of fibroblast cells [18]. Also Gao and co-workers prepared RGD terminated PNIPAAm-b-PAA gradients to regulate cell adhesion and detachment [19]. However, to date there has been no report on the use of diblock copolymer brushes of PAAm and POEGMA containing PAA as the outer block for the fabrication of bioactive cell substrates.

Herein, we present a strategy for the synthesis of bioactive surfaces based on the side chain biofunctionalization of PAA terminated PAAm and POEGMA (block co-)polymer brushes. First, SI-ATRP was used to synthesize PAAm, POEGMA and PAA brushes on gold by using a previously reported procedure [20–22]. In particular, we focused on the polymerization kinetics and its potential impact on the use of these brushes as a macroinitiator. Later on, the synthesis of block copolymer brushes was accomplished by a sequential grafting process by SI-ATRP and provided additional insight in the “livingness” of the polymerization procedure. The homo polymer brushes of OEGMA and AAm have shown to prevent cell adhesion. Finally, an RGD binding sequence (G**RGD**S peptide, Gly-Arg-Gly-Asp-Ser, pentapeptide) was covalently attached onto the PAA brush modified surfaces by forming amide bonds via an NHS ester intermediate and this enabled the adhesion of NIH 3T3 fibroblasts.

The combination of SI-ATRP and postmodification by EDC/NHS chemistry provides a well-controlled and efficient process for the synthesis of a wide variety of new biomaterials. The joined process allows to covalently immobilize biomolecules such as RGD peptides on polymer brushes with desired mechanical and chemical properties of the first block. The mechanical properties of polymer brushes can be varied due to the introduction of a cross-linker during polymerization, as demonstrated in an earlier study [20].

2. Materials and methods/experimental

2.1. Materials

Microscopy glass slides were purchased from Thermo scientific, Menzel-Gläser (ISO 8037/1; 76 mm × 26 mm), Gold from Allgemeine Gold- und Silberscheideanstalt AG (Pforzheim); 99.99% (granules), Titanium from Chempur; 99.998% (pieces: 1–6 mm), HCl: purchased from Sigma Aldrich (37%), Ethanol: purchased from Fischer Scientific, 97%, denaturated, Methanol: purchased from J.T. Baker, Aluminum oxide: purchased from Macherey–Nagel, neutral for column chromatography, Cu(I)Br: synthesized according to the literature [23]; 1,1,4,7,7, PMDETA [pentamethyldiethylene triamine]: purchased from Sigma Aldrich and BiPy [Bipyridine], 99%: purchased from Alfa Aesar; OEGMA [oligo(ethylene glycol)methylether methacrylate] (M_n = 300 g/mol): purchased from Sigma Aldrich, was purified by passing through a column of aluminum oxide, AAm [acrylamide] (99.9%) and NaA [sodium acrylate] (97%) purchased from Sigma Aldrich, were used as received for the polymer brush synthesis. The initiator, MUBiB [ω-mercaptoundecyl bromoisobutyrate], was synthesized according to Jones et al. [24] EDC [*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride]; 98% and NHS [*N* –hydroxysuccinimide]; 98% purchased from Sigma Aldrich were used as received for biofunctionalization. The peptides, G**RGD**S (Gly-Arg-Gly-Asp-Ser) and G**RAD**S (Gly-Arg-Ala-Asp-Ser), were purchased from the PolyPeptide group (Torrance, USA). Milli-Q water drawn from a Millipore Direct Q8 system (Millipore Advantage A 10 system, Schwalbach, with Millimark Express 40 filter, Merck, Germany) with a resistivity of 18.0 MΩcm was used throughout the whole study.

2.2. Formation of self-assembled monolayers (SAMs) of ω-mercaptoundecyl bromoisobutyrate (MUBiB) on gold

The gold substrates were prepared by coating an adhesion promoting titanium layer (5 nm) and a gold layer (100 nm) onto previously cleaned glass substrates by electron beam evaporation under high vacuum (Edwards E306 coating system, Moorfield, UK). For cell adhesion studies transparent gold substrates were prepared with a thinner gold layer (20 nm) [25]. Before further use, the gold substrates were cleaned by irradiation in an UV/ozone cleaner (Pro Cleaner, BioForce, Nanosciences, Ames, USA) for 30 min and then rinsed with Milli-Q water, ethanol and transferred into a 1 mM ethanolic solution of the initiator overnight at ambient temperature. The substrates were then thoroughly rinsed with ethanol and Milli-Q water before use.

2.3. Preparation of AAm, OEGMA and NaA brushes via SI-ATRP

Separate reaction solutions for each monomer, were prepared as followed. For PAAm brushes PMDETA (0.28 mL, 1.34 mmol), 20 mL of Milli-Q water and methanol (3:7), AAm (2.00 g, 28.15 mmol) and CuBr (64 mg, 0.45 mmol) [20,21], for POEGMA brushes BiPy (312 mg, 2.0 mmol), Milli-Q water and methanol (1:4), OEGMA (4.77 mL, 16.7 mmol) and CuBr (143 mg, 1.0 mmol) [22] and for PAA brush films PMDETA (0.42 mL, 2 mmol), Milli-Q water (12 mL), NaA (2.17 g, 23.1 mmol) and CuBr (0.144 mg, 1 mmol) according to Dong et al. was used [26]. Each time the chelating agent (PMDETA, BiPy) was first dissolved in a degassed mixture of water and methanol. The mixture was stirred, while the monomer was added under argon gas flow. Later on, CuBr was added and the solution was stirred until all CuBr was dissolved. The functionalized gold substrates were placed in the reaction flask and flushed with argon. Then the reaction solution was transferred via a syringe into the flask under argon. After the reaction, the substrates were rinsed and washed with Milli-Q water and ethanol and dried in a flow of nitrogen. PAA brush films were additionally incubated in 0.1 mM HCl (90 min) for protonation. For the study of the polymerization kinetics, polymerizations were carried out for different times at 23 °C.

2.4. Preparation of block copolymer brushes

Block copolymerizations were performed in the same manner as the typical ATRP procedures for PAAm, POEGMA and PAA brush