Applied Surface Science 258 (2012) 3750–3756

Contents lists available at SciVerse ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Synthesis and conformational characterization of functional di-block copolymer brushes for microarray technology

Gabriele Di Carlo[a], Francesco Damin[a], Lidia Armelao[b], Chiara Maccato[c], Selim Unlu[d,e], Philipp S. Spuhler[e], Marcella Chiari[a,*]

[a] Institute of Chemistry of Molecular Recognition, National Research Council of Italy, Via M. Bianco 9, 20131 Milano, Italy
[b] ISTM-CNR and INSTM, Department of Chemistry, University of Padova, Via F. Marzolo 1, 35131 Padova, Italy
[c] Department of Chemistry and INSTM, University of Padova, Via F. Marzolo 1, 35131 Padova, Italy
[d] Department of Electrical and Computer Engineering, Boston University, St. Mary Street 8, Boston, MA 02215, United States
[e] Department of Biomedical Engineering, Boston University, St. Mary Street 8, Boston, MA 02215, United States

ARTICLE INFO

Article history:
Received 16 June 2011
Received in revised form
20 September 2011
Accepted 3 December 2011
Available online 13 December 2011

Keywords:
Surface initiated polymerization
Reversible addition–fragmentation chain transfer polymerization (RAFT)
Block copolymers
Polymer brushes
DNA microarray

ABSTRACT

Surface initiated polymerization (SIP) coupled with reversible addition-fragmentation chain transfer polymerization (RAFT) was used to functionalize microarray glass slides with block polymer brushes. N,N-dimethylacrylamide (DMA) and N-acryloyloxysuccinimide (NAS) (*graft*-poly[DMA-*b*-(DMA-*co*-NAS)]) brushes, with di-block architecture, were prepared from a novel RAFT chain transfer agent bearing a silanating moiety (RAFT silane) directly anchored onto the glass surfaces. Conformational characterization of the coatings was performed by Self Spectral Interference Fluorescence Microscopy (SSFM), an innovative technique that describes the location of a fluorescent DNA molecule relative to a surface with sub-nanometer accuracy. X-ray Photoelectron Spectroscopy (XPS) and Scanning Electron Microscopy (SEM) were used to characterize the coatings composition and morphology.

1. Introduction

Many of the methods used in genomics or proteomics are based on solid phase hybridization or complexation reactions between surface immobilized probes and free solution targets. Protein and DNA microarrays are examples of technologies for the simultaneous detection and quantification of a large number of biomolecules based on this principle. Chemical or biochemical reactions between free fluid components and immobilized probes require that an adequate number of molecules, with appropriate conformation, are immobilized on the surface. The most commonly used immobilization methods on glass surfaces involve the deposition of reactive silane films with terminal functional groups that react with biomolecules, either directly or through a subsequent modification. However, steric hindrance may significantly reduce the reaction between surface functional groups and probe molecules on these types of monodimensional coatings, leading to poor grafting density. A more advantageous method of immobilization implies the formation of a chemically reactive polymer film on the glass surface [1–5]. A polymeric coating is needed to control the local chemical environment so as to retain the native conformation of proteins [6]. In addition, polymer coatings provide a three dimensional binding scaffold, which leads to a substantial increase in the density of probes per unit area.

Homo- and block-copolymers brushes bearing chemical functionalities for the covalent attachment of biomolecules can be obtained by "grafting-onto" or "grafting-from" methods [7–13].

In this work, by using a "grafting from" approach we have functionalized glass substrates with brushes obtained by surface initiated (SI) controlled radical polymerization (CRP) [14]. CRP techniques, including nitroxide-mediated radical polymerization (NMP) [15], atom transfer radical polymerization (ATRP) [16] and reversible addition–fragmentation chain transfer polymerization (RAFT) [17], are excellent methods for the preparation of well-defined polymer structures such as block copolymers, star shape polymers, and interpenetrating polymer networks [18]. In particular, RAFT has recently emerged as a promising controlled radical polymerization technique due to its versatility and simplicity. A major advantage of RAFT polymerization over other processes for living/controlled free-radical polymerization is its compatibility with a wide range of monomers including functional monomers.

* Corresponding author. Tel.: +39 02 28500035.
E-mail address: marcella.chiari@icrm.cnr.it (M. Chiari).

0169-4332/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsusc.2011.12.019

There is an increasing number of SI block brushes produced by CRP techniques but only few of them incorporate chemically reactive monomers in the external block [19]. In this work, a method to prepared a bioreactive coating made of N,N-dimethylacrylamide (DMA) and N-(acryloyloxy)succinimide (NAS) was devised and the "diblock" structure of the coating was confirmed by functional experiments, based on solid phase DNA hybridization, and by direct evaluation of the film conformation.

2. Experimental

2.1. Chemicals

N-hydroxysuccinimide (99%, NHS), acryloyl chloride (97%), triethylamine (≥99.5%, TEA), dichloromethane (≥99.5%; H_2O ≤0.005%), (3-aminopropyl)-trimethoxysilane (97%, APS), γ-mercaptopropyl trimethoxysilane (>95%, γMPS), 2-bromopropionylbromide (97%), phenylmagnesium bromide (1.0 M in THF), carbon disulfide (99.9%), tetrahydrofuran anhydrous (99.9%, inhibitor-free, THF), sodium sulfate (≥99%), S-(thiobenzoyl)thioglycolic acid (99%), 2-methyl-2-propanethiol (99%), hydrogen peroxide (30% in H_2O), ammonium hydroxide solution (28% in H_2O), octyl-trimethoxysilane (96%, nOS), sodium hydroxide (97%), N,N-dimethylacrylamide (99%, DMA), molecular sieves (4 Å, beads, 8–12 mesh), and microscope glass slides (75 mm × 25 mm) were purchased from Sigma–Aldrich. N,N-dimethylformamide (>99.8%, DMF), α,α′-azobisisobutyronitrile (≥98%, AIBN), aluminium oxide were purchased from Fluka. Diethyl ether (99.9%) was purchased from VWR. Toluene (>99.7%) was purchased from Riedel-de Haën. Davisil 663XW (500 Å pore size, 78–85 m^2/g surface area) was purchased from Supelco (Bellefonte, PA). The monomer N-(acryloyloxy)succinimide (NAS) [20] and the free chain transfer agent *tert*-butyl dithiobenzoate (tBDB) [21] were synthesized as previously reported.

2.2. Synthesis of 1-oxo-1-(3-(trimethoxysilyl)propylamino)propan-2-yl benzodithioate (RAFT silane, **2**) (Scheme 1)

At 0 °C a solution of 2-bromo propionyl bromide (2.83 mL, 0.027 mol) in dichloromethane (5 mL) was added dropwise to a stirred solution of (3-aminopropyl)-trimethoxysilane (APS; 4.86 mL, 0.027 mol) and triethylamine (TEA; 3.77 mL, 0.027 mol) in dichloromethane (30 mL). The solution was stirred at room temperature under nitrogen pressure for 2 h, then it was filtered and evaporated under vacuum. The crude product was purified by bulb-to-bulb distillation (175 °C, 1 mBar) to form the bromine derivative (**1**) as a colorless oil (7.6 g, 0.024 mol, 89% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.60 (1H, NH), 4.40 (q, 1H, −CHBr), 3.55 (s, 9H, OCH_3), 3.25 (m, 2H, $-CH_2N$), 1.85 (d, 3H, $-CH_3$), 1.65 (m, 2H, $-CH_2-$), 0.65 (m, 2H, $Si-CH_2-$).

In a second step, CS_2 (2.9 mL, 0.048 mol) was slowly added at 0 °C to a stirred solution of phenylmagnesium bromide (1 M in THF, 25.4 mL, 0.025 mol). The solution was stirred at room temperature under nitrogen pressure for 1.5 h and then cooled at 0 °C. A solution of **1** (7.6 g, 0.024 mol) in THF (10 mL) was added dropwise. The mixture was then allowed to react overnight at room temperature, and the solvent was evaporated under vacuum. The resulting residue was dissolved in CH_2Cl_2 (15 mL), washed with water and brine and dried with Na_2SO_4. The RAFT silane (**2**) was obtained as a red oil (6.6 g, 0.017 mol, 69% yield) and used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.15–8.10 (m, 5H, phenyl), 6.60 (1H, NH), 4.75 (q, 1H, −CHBr), 3.55 (s, 9H, OCH_3), 3.25 (m, 2H, $-CH_2N$), 1.85 (d, 3H, $-CH_3$), 1.65 (m, 2H, $-CH_2-$), 0.65 (m, 2H, $Si-CH_2-$).

2.3. Silica surfaces functionalization

Microscope glass slides pretreatment. Glass slides were cleaned and activated in a Harrick Plasma Cleaner for 15 min at high radio frequency level by oxygen-plasma.

RAFT silane functionalization. Glass slides were dipped in a solution of RAFT silane and octyltrimethoxysilane (nOS) in toluene for 4 h at room temperature under nitrogen atmosphere. The ratios between RAFT silane and nOS are reported in Table 1. After the reaction was completed, the slides were washed with fresh toluene and THF and then dried in a vacuum oven at room temperature for 30 min.

2.4. Synthesis of graft-poly(DMA) via RAFT polymerization (Scheme 2)

A 100 mL solution of DMA (45.95 mL, 0.446 mol, filtered on aluminium oxide to remove inhibitors), tBDB (0.262 g, 0.125×10^{-2} mol) and AIBN (0.041 g, 0.025×10^{-2} mol) in dry toluene (dried on molecular sieves) was degassed under argon at 0 °C for 1 h. Silanized slides (see Table 1) were immersed overnight in the monomer solution brought to 80 °C under nitrogen atmosphere, then Soxhlet extracted with THF for 12 h. The slides were finally dried under N_2 flow.

2.5. Synthesis of graft-poly[DMA-b-(DMA-co-NAS)] via free radical polymerization (Scheme 2)

The extension of poly(DMA) to form the active block of poly[DMA-*b*-(DMA-*co*-NAS)] as reported in Scheme 2, was carried out by polymerizing a mixture DMA (9.27 mL, 0.09 mol, filtered on aluminium oxide to remove inhibitors), NAS (1.69 g, 0.01 mol) and AIBN (9.2 mg, 0.056×10^{-3} mol) in 100 mL of DMF (dried on molecular sieves), degassed under argon at 0 °C for 1 h. Slides coated with the first block of poly(DMA) were immersed overnight in the reaction mixture at 80 °C under nitrogen atmosphere, Soxhlet extracted with THF for 12 h and dried in a vacuum oven at room temperature for 2 h. To the same solution, slides derivatized with RAFT silane/nOS 1:1 were also added to produce *graft*-Poly(DMA-*co*-NAS) coated slides in which the functional copolymer was directly attached to the surface without the intermediate layer of poly(DMA) (monoblock coating).

2.6. DNA–DNA hybridization test

A SciFlexArray spotter (Scienion, Berlin, Germany) was used to pattern a subarray of a 23-mer oligonucleotide of sequence 5′-NH_2-$(CH_2)_6$-GCC CAC CTA TAA GGT AAA AGT GA). The oligonucleotide was dissolved at 10 μM concentration in sodium phosphate buffer (150 mM, 0.01% Triton, pH 8.5). The volume of spotted drops was 400 pL. In each printed array (100 replicated spots), the diameter of the spots was ∼100 μm. After probe deposition, the slides were placed in an uncovered storage box placed in a sealed chamber, saturated with NaCl, and incubated at room temperature overnight.

Unreacted sites on the slides were blocked with a 50 mM solution of ethanolamine in 0.1 M Tris–HCl at pH 9 for 15–20 min at 50 °C, rinsed with water and then washed in 4× saline-sodium citrate (SSC) buffer/0.1% w/v sodium dodecilsulfate (SDS) for 15–20 min at 50 °C. An oligonucleotide target (23-mer Cy3-oligonucleotide labelled at the 5′ terminus) complementary to the sequence of the immobilized probe was dissolved in the hybridization buffer (5× SSC/0.1% w/v SDS/0.02% w/v bovine serum albumine-BSA) and immediately applied to the microrrays. After hybridization (2 h, 65 °C), the slides were first washed in 2× SSC/0.1% SDS buffer (10 min, 65 °C) and rinsed with 0.2 × SSC (1 min), followed by a rinse with 0.1× SSC (1 min). Scanning for