Colloids and Surfaces B: Biointerfaces 103 (2013) 52–58

Contents lists available at SciVerse ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Grafting of carboxybetaine brush onto cellulose membranes via surface-initiated ARGET-ATRP for improving blood compatibility

Miao Wang[a], Jiang Yuan[a,*], Xiaobo Huang[a], Xianmei Cai[a], Li Li[a], Jian Shen[a,b]

[a] Jiangsu Key laboratory of Biofunctional Materials, College of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210023, PR China
[b] Jiangsu Engineering Research Center for Biomedical Function Materials, Nanjing Normal University, Nanjing 210046, PR China

ARTICLE INFO

Article history:
Received 2 August 2012
Received in revised form 10 October 2012
Accepted 11 October 2012
Available online 29 October 2012

Keywords:
Carboxybetaine
Zwitterions
ARGET-ATRP
Blood compatibility
Cellulose

ABSTRACT

Grafting-from has proven to be a very effective way to create high grafting densities and well-controlled polymer chains on different kinds of surfaces. In this work, we aim to graft zwitterionic brush from cellulose membrane (CM) via ARGET-ATRP (Activator Regenerated by Electron Transfer ATRP) method indirectly for blood compatibility improvement. Characterization of the CM substrates before and after modification was carried out by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), water contact angle measurements, X-ray photoelectron spectroscopy analysis, and atomic force microscopy, respectively. The results demonstrated zwitterionic brushes were successfully grafted on the CM surfaces, and the content of the grafted layer increased gradually with the polymerization time. The platelet adhesion, hemolytic test and plasma protein adsorption results indicated the cellulose membrane had significantly excellent blood compatibility featured on lower platelet adhesion and protein adsorption without causing hemolysis. The functionalized cellulose substrate could have a great potential usage for biomedical applications.

1. Introduction

Cellulose has been widely used, as not only it is the most abundant polymer in the world but also an inexpensive and renewable biomedical material. However, the blood compatibility of the raw cellulose is inadequate and needs to be improved before use. Various conventional polymerization techniques have been attempted quite extensively for grafting polymers onto cellulose [1,2]. In recent years, zwitterionic materials containing both anionic and cationic units in the same molecule have been attractive as a class of materials with excellent blood compatibility and antifouling property [3–8]. Zwitterionic monomers generally refer to phosphbetaine such as 2-(methacryloyloxy)ethyl phosphorylcholine (MPC) [9–12], sulfobetaine-based monomers [13–17], and carboxybetaine-based monomers [18–22]. Zwitterionic materials are known to reduce bacterial adhesion dramatically and therefore biofilm formation [23,24]. It is also proved that the zwitterionic materials could potentially be used for biomaterials [25], biosensors [26], marine coatings [27] and so on.

Comparatively, carboxybetaine has superior antifouling functionality as compared to sulfobetaine [8,28]. The carboxybetaine monomer could be synthesized via two reaction routes, i.e. tertiary amine ring-opening reaction with beta-propiolactone (PL) to yield carboxybetaine via one-step method; protection of ammonium bromides with ester group and then hydrolysis to obtain the carboxybetaine via two-step method [29]. The former method is not economic due to the expensive beta-propiolactone and the latter is much more complex. It is noted the former method can yield a high-rate production.

Polymer brushes have become a widely-used means for modifying surface properties. To construct polymer brush, both "grafting to" and "grafting from" approaches have been employed. "Grafting to" involves tethering of preformed end-functionalized polymer chains onto surfaces by one end. However, it is difficult to obtain dense brushes. Currently, various novel routes based on "graft to" and "grafted from" strategy are developed to prepare zwitterionic-based antifouling surfaces [4,30,31]. Grafting-from method has proven to be a very effective way to create high grafting densities and well-controlled polymer structures on different kinds of surfaces [32]. "Grafting from" consists of two steps, attachment of initiators onto surfaces and subsequently, polymer growth from initiator sites monomer by monomer via covalent bonding. This can lead to much stronger anchoring and the polymer conformation can be easily tuned via varying the initiator surface density and the polymerization time.

* Corresponding author. Tel.: +86 25 85891536; fax: +86 25 83599188.
E-mail addresses: jyuan@njnu.edu.cn (J. Yuan), lili3@njnu.edu.cn (L. Li).

0927-7765/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2012.10.025

The atom transfer radical polymerization (ATRP) process developed at Carnegie Mellon by Professor Krzysztof Matyjaszewski in 1994 is effective and most widely used methods of conducting a controlled radical polymerization (CRP). Activator Regenerated by Electron Transfer ATRP (ARGET-ATRP) is a new ATRP initiating system developed to facilitate solution and emulsion ATRP in aqueous media [33]. It uses excess reducing agent such as ascorbic acid to ensure Cu(II) can be reduced to Cu(I) rapidly in the polymerization system, and then catalytic polymerization ATRP monomer. In addition, ARGET-ATRP can tolerate a large excess of the reducing agent and, as a result, the reaction can be conducted in the presence of limited amounts of air. To the best of our knowledge, grafting zwitterionic brush from cellulose membrane via ARGET-ATRP has not yet been reported.

In this study, we aim to graft carboxybetaine brush from cellulose membrane (CM) via ARGET-ATRP (Fig. 1) to improve blood compatibility and antifouling property. The ATRP initiator was first immobilized covalently onto CM surfaces followed by living polymerization of 2-(dimethylamino) ethyl methacrylate (DMAEMA) monomer. Finally, the poly(DMEMA) chain was ring-opening reacted with beta-propiolactone to yield carboxybetaine brush. The surface structure, wettability, morphology and thermal stability of CM were characterized by attenuated total reflectance Fourier transform infrared spectra (ATR-FTIR), water contact angle (WCA), atomic force microscopy (AFM), and X-ray photoelectron spectroscopy (XPS), respectively. The blood compatibility of the CM was evaluated by protein adsorption and platelet adhesion tests in vitro. The effects of polymerization time on the properties of CM substrates, as well as the relationship between protein adsorption and platelet adhesion were studied.

2. Experimental

2.1. Materials

Cellulose membrane (CM) was purchased from Sigma–Aldrich and cut into circular pieces. 2-Bromoisobutyryl bromide (BIBB, 98%), 2-dimethylaminopryridine (DMAP, 99%), and N,N,N′,N″,N″-pentamethyldiethylenetriamine (PMDETA, 99%) were obtained from Aladdin. Beta-propiolactone (PL, 95%) and 2-(dimethylamino) ethyl methacrylate (DMAEMA) was acquired from Sigma–Aldrich. Triethylamine (TEA, 99%), chloroform (CH_2Cl_2, AR), tetrahydrofuran (THF, AR), methyl alcohol (CH_3OH, AR), copper(II) bromide ($CuBr_2$, 99%) and ascorbic acid were purchased from Sinopharm Chemical Reagent Co., Ltd. and purified before use. Platelet rich plasma (PRP) was provided by Blood Center of Jiangsu Red Cross. An enhanced BCA (Bicichoninic acid) protein assay reagent kit and sodium dodecylsulfate (SDS, 10 wt% in PBS) were purchased from Sunshine Biotechnology, China.

2.2. Immobilization of initiator on CM

Prior to the immobilization of the initiator, CMs (6 mm × 6 mm) were washed with ethanol and dried in vacuum. The available hydroxyl groups on the surface were converted into ARGET-ATRP initiators by immersing CM substrates in a solution containing TEA (4.44 g, 44 mmol) and a catalytic amount of DMAP in THF (50 mL). The reaction mixture was stirred under an ice bath and BIBB (9.2 g, 40 mmol) was then added into the mixture dropwise. The reaction was allowed to proceed for 24 h at room temperature on a stirring device. Thereafter, the initiator functionalized CMs (hereinafter refer to CM-Br) were thoroughly washed in dichloromethane and ethanol ultrasonically to remove residual reactants and byproduct. The CM-Br substrates were finally dried in a vacuum for further grafting.

2.3. ARGET-ATRP initiated polymerization of DMAEMA from CM-Br

The CM-Br substrates and $CuBr_2$ (80 mg, 1.0 mmol) were added to a dry Schlenk flask with a magnetic stirring bar, and the flask was evacuated and flushed with argon. Degassed solution (methanol and distilled water in 1:1 volume ratio) containing DMAEMA (0.65 g, 4.0 mmol) and PMDETA (0.35 g, 2.0 mmol) were then added into the flask. The mixture was stirred for 10 min and then degassed by three freeze-pump thaw cycles. The flask was placed in a water bath maintained at 25 °C. Finally, ascorbic acid (70 mg, 1.0 mmol) was added. The ARGET-ATRP reaction was preceded at 25 °C for predetermined time period. After the reaction, the functionalized CMs (hereinafter refer to as CMG-*t*, where *t* means the grafting polymerization time, min) were removed from the solution, thoroughly washed with PBS and distilled water ultrasonically and dried under vacuum.

2.4. Polycarboxybetaine brush formation via ring-opening reaction

Under an argon protection, the CMG-*t* (6 mm × 6 mm) was immersed into a flask containing methanol (5 mL), beta-propiolactone (1.0 g) at 55 °C for 24 h. After the reaction, the functionalized CMs (hereinafter referred to as CMP-*t*, where *t* means the grafting polymerization time) were removed from the solution, thoroughly washed with methanol and distilled water ultrasonically, and dried under vacuum.

2.5. Membrane characterization

ATR-FTIR study was performed on a Nicolet 170 sx FTIR equipped with an Omni sampler over 32 scans. The spectra were recorded with a resolution of 4 cm^{-1}. WCA was performed on a Dynamic/Static contact angle instrument manufactured at 25 °C and 60% relative humidity, using the sessile drop method with 3 μL water droplets by Kino industrial Company Ltd. The XPS measurements were performed on ESCA Lab MK II (V.G. Scientific Co. Ltd., U.K.) equipped with an Mg Kα radiation source (12 kV and 20 mA at the anode). The takeoff angle of the photoelectron was kept at 45°. The binding energy was referenced by setting the C1s hydrocarbon peak to be 285.0 eV. The topography of the modified cellulose surfaces were studied by AFM, under dry conditions, using a tapping mode at a scan rate of 0.5 Hz over an area of 5 μm × 5 μm. The morphology of the pristine CM, materials adhered with platelets and materials adhered with bacteria were observed on scanning electron microscope ((JSM-5610 SEM, JEOL, Japan).

2.6. Hemolysis assay [34]

Fresh anticoagulated blood from human volunteers (2 ml) was diluted with 2.5 ml of normal saline. The diluted blood (0.2 ml) was added in test samples. The mixture was kept at 37 °C for 60 min and then centrifuged at 1500 rpm for 10 min. The supernatant was transferred to a 96-well plate where the absorbance was measured at 545 nm using a BioTek synergy 2 Multi-Mode Microplate Reader. The mean value of three measurements was calculated. Positive controls consisted 0.2 ml diluted blood in 10 ml deionized water while negative controls consisted 0.2 ml diluted blood in 10 ml normal saline. Hemolysis degree was calculated as follows:

$$\text{Hemolysis\%} = \frac{D_t - D_{nc}}{D_{pc} - D_{nc}} \times 100\%$$

where D_t is the absorbance of sample, D_{nc} is the absorbance of the negative control, D_{pc} is the absorbance of the positive control.