Materials Science and Engineering C 36 (2014) 57–64

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Fabricating antigen recognition and anti-bioadhesion polymeric surface via a photografting polymerization strategy

Lingjie Song [a,b], Jie Zhao [a], Jing Jin [a], Jiao Ma [a], Jingchuan Liu [a], Shifang Luan [a,*], Jinghua Yin [a,*]

[a] State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China

ARTICLE INFO

Article history:
Received 16 July 2013
Received in revised form 8 November 2013
Accepted 28 November 2013
Available online 10 December 2013

Keywords:
Photografting polymerization
Anti-bioadhesion
Antibody immobilization

ABSTRACT

A polymeric platform for immunodiagnostic bioassay was constructed based on biostable polymeric support and two functional monomers, low-fouling methacryloyloxyethyl phosphorylcholine (MPC) and acrylic acid (AAc), by a photografting polymerization approach. Covalent binding of antibody to pAAc chains was achieved by activating carboxyl with NHS/EDC partner. The resultant surface showed obvious suppression of nonspecific protein adsorption and platelet adhesion relative to the control sample, exhibiting good anti-bioadhesion performances. Based on the polymer-supported matrix, a highly sensitive antibody–antigen specific recognition was confirmed in both native plasma and diluted human plasma due to the enhanced antibody loading capacity and lowered bioadhesion as compared to the reference.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Interfacial processes play a vital role in determining the fate of materials in numerous biomedical applications [1], such as artificial implant [2–4], drug/gene delivery [5,6] and biosensor [7–10]. The primary failure for the artificial implant and the poor targeting efficiency of diagnostics is generally because of the nonspecific protein adsorption induced by their nature hydrophobicity. Substrate wettability could be adjusted via various chemical immobilization of hydrophilic substances on their surfaces. Nowadays, photografting polymerization strategy is widely used due to its advantages of high grafting density, low cost, easy operation and mild reaction conditions [11–16]. Regarding the issue of constructing a low-fouling surface, poly(ethylene glycol) (PEG) and its derivatives with good hydrophilicity and high configurational mobility have been recognized as one kind of low nonspecific bioadhesion materials [17–23]. Furthermore, a relatively hydrophilic environment provided by PEG chains is beneficial to the bioactivity maintenance of some immobilized biomolecules [24,25]. Therefore, PEG has been widely adopted as a low-fouling/spacer polymer for the immobilization of biomolecules [26,27]. However, the susceptibility of PEG to oxidative degradation and cleavage of ethylene oxide units in biological systems, especially in the presence of transition metal ions, deteriorates the protein resistance and limits its long-term application [28]. Recently, zwitterionic-based materials which contain carboxybetaine (CB) [29–32], sulfobetaine (SB) [33–35], and phosphorylcholine (PC) moieties [36–40], were widely used as alternatives to PEG substrates due to their high resistance to nonspecific protein adsorption, stability, and biocompatibility. For example, Yang et al. modified microporous polypropylene membrane (MPPM) by the UV-induced grafting of polysulfobetaine methacrylate (pSBMA); they found that the modified MPPM showed strong resistance to protein fouling, and significant prohibition of the initial bacterial adhesion [41]. Ishihara et al. reported that polymer nanoparticles covered with phosphorylcholine and active ester group for antibody immobilization presented strong potential in highly selective separation of proteins via the biological affinities [42]. As for diagnostic bioassay based on specific antibody–antigen affinity, an important issue is to fabricate a surface that can prohibit nonspecific bioadhesion for reducing background noise and false results [43]. Meanwhile, the activity of specifically-immobilized bioactive species will be highly possessed. Typically, the physical adsorption methods for anchoring antibody to a surface, which usually combines antibody through noncovalent bonds, present poor adsorption durability under the subsequent treatments and low sensitivity arising from the hydrophobic interaction between antibodies and material surfaces [44]. In addition, the relatively fussy immobilization process and higher consumption limit the application of bio-affinity immobilization such as protein A/G in sandwich assay [45,46]. On the other hand, chemical conjugation method represents a powerful approach that has been explored for fabricating the “ready-to-use” commercially available surfaces without significantly diminishing the antigen-binding ability [46–51]. For example, Gubala et al. found that the simple plasma treatment facilitated the formation of readily reactive species on the thermoplastic surface, which could be used directly for the specific immobilization of biomolecules through carboxyl or aldehyde/ketone interactions. It was useful for fast attachment of biorecognition elements without sophisticated surface treatments [52]. Gan et al. reported

* Corresponding authors. Tel./fax: +86 431 85262109.
E-mail addresses: sfluan@ciac.ac.cn (S. Luan), yinjh@ciac.ac.cn (J. Yin).

0928-4931/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.msec.2013.11.044

that 3-aminopropyltriethoxysilane (APTES) silanization had been successfully anchored on an inert organic surface containing pure alkyl C–H bonds by the pre-activation via protocontrolled hydroxylation. After further aldehyde modification through the reaction between aminated surface and glutaraldehyde (GA), the proteins were effectively immobilized on the surface, revealing a potential application in diagnostics [53]. Of particular note is that the mobility of bound antibodies can be suppressed by the steric hindrances originating from the direct antibody immobilization, while antibody-binding via a long and flexible linker can overcome this weakness and enhance antibody sensitivity [46].

In this study, we reported a simple and time-efficient method to prepare antigen recognition and anti-bioadhesion surface on a polymeric platform (polypropylene film) for immunodiagnostic bioassay. Two functional monomers, methacryloyloxyethyl phosphorylcholine (MPC) and acrylic acid (AAc) were sequentially fixed on a biostable polymer substrate by a facile photografting polymerization, followed by the NHS/EDC activation of the carboxyl group in the AAc chains to chemically immobilize antibody. Both the nonspecific anti-bioadhesion behaviors and the antigen specific recognition of the tailored platform were investigated.

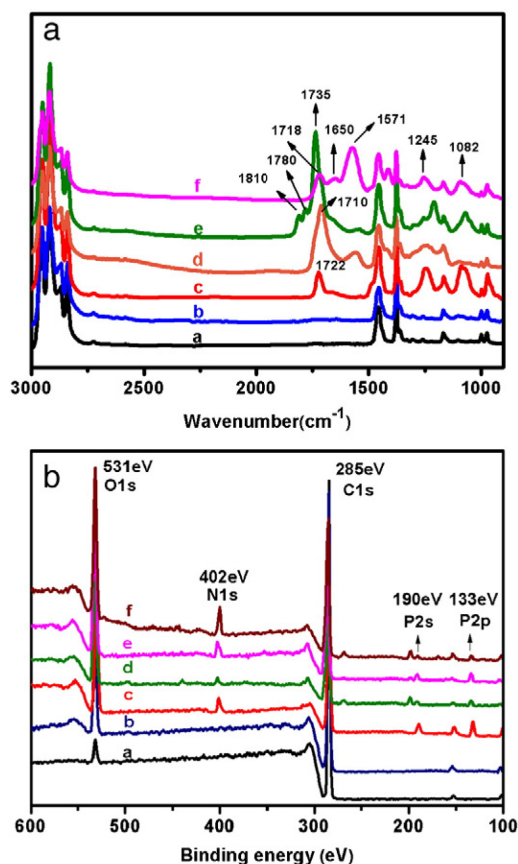

Fig. 1. FTIR-ATR (A) and XPS (B) spectra of the samples. (a) Virgin PP, (b) plasma-treated PP, (c) PP-*g*-MPC, (d) PP-*g*-MPC-*g*-AAc, (e) PP-*g*-MPC-*g*-AAc-Suc, and (f) PP-*g*-MPC-*g*-AAc-IgG.

2. Experimental

2.1. Materials and reagents

2-Methacryloyloxyethyl phosphorylcholine (MPC) was purchased from Letianyuan Science and Technology Research Center (China). Acrylic acid (AAc) was provided by Sigma. PP (GM 1600E) was obtained from Shanghai Petrochemical Company. Benzophenone (BP) was obtained from Peking Ruichen Chemicals (China). Bovine serum albumin (BSA, pI = 4.8), lysozyme (LYS, pI = 11.8), bovine fibrinogen (FIB, pI = 5.8), phosphate buffered solution (PBS, pH = 7.4), sodium dodecyl sulfate (SDS) and human IgG were purchased from Dingguo Bio-technology (China). Micro BCA™ protein assay reagent kit (AR1110) was purchased from Boster Biological Technology (China). Hepatitis B surface antibody (HBsAb, 2 mg mL^{-1}), patient plasma containing hepatitis B surface antigen and second HBsAb labeled with horseradish peroxidase were kindly provided by WEGO Group (China). N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 3,3′,5,5′-tetramethylbenzidine (TMB) were obtained from Alfa Aesar. Other reagents were AR grade and used without further purification.

2.2. Surface modification of the polymeric support

The hot-pressed PP films were cleaned and subjected to argon plasma (DT-03 plasma apparatus, Suzhou Omega Technology) at a pressure of approximately 15 Pa for 90 s. After being exposed in air for 30 min to form the peroxide groups, the pre-treated films were placed between two quartz plates and uniformly coated with 12% (w/v) degassed MPC aqueous solution. Then the sandwiched system (shown in Fig. 1) was exposed to UV light (high-pressure mercury lamp, 400 W, main wavelength 380 nm) for a desired time. All the films were washed with deionized water and ethanol to remove residual monomers and homopolymers, followed by drying in a vacuum oven to obtain the pMPC-modified samples (denoted as PP-*g*-MPC). Subsequently, the PP-*g*-MPC sample was immersed in the acetone solution of BP (0.5% (w/v)) for 30 min, and dried at room temperature. Similar to the above procedures of photografting polymerization and the corresponding post-treatment, the PP-*g*-MPC samples were subjected to another surface modification procedure of AAc (aqueous solution, 3% (w/v)) to prepare the pMPC/pAAc-modified samples (denoted as PP-*g*-MPC-*g*-AAc).

2.3. Carboxyl group activation on the polymeric support and antibody immobilization

The carboxyl groups on the PP-*g*-MPC-*g*-AAc surface were activated with a mixture of NHS (0.05 mg mL^{-1}) and EDC (0.05 mg mL^{-1}) in deionized water at room temperature for 2 h (the activated sample was denoted as PP-*g*-MPC-*g*-AAc-Suc). After being rinsed with deionized water and dried under argon stream, the obtained samples were respectively reacted with human IgG and hepatitis B surface antibody (HBsAb) (200 μg mL^{-1}) in PBS buffer at 37 °C for 2 h (corresponding samples were denoted as PP-*g*-MPC-*g*-AAc-IgG and PP-*g*-MPC-*g*-AAc-HBsAb, respectively). Finally, these samples were rinsed with PBS buffer to remove the physically adsorbed antibodies and residual NHS-esters, dried under argon stream and stored at 4 °C for further use.

2.4. Characterization of surface chemistry

ATR-FTIR spectra of the samples were obtained from a Fourier transform infrared spectrometer (FTIR, BRUKER Vertex 70) with a resolution of 4 cm^{-1} in absorbance mode.

Surface elemental compositions of the samples were determined via an X-ray photoelectron spectroscopy (XPS, VG Scientific ESCA MK II Thermo Avantage V 3.20 analyzer) with Al/Kα ($h\nu = 1486.6$ eV) anode mono-X-ray source at the detection angle of 90°. The spectra were collected over a range of 0–1200 eV and high-resolution spectra of C_{1s}, N_{1s}, and P_{2p} regions were collected. The atomic concentrations of the elements were determined by the peak-area ratios.

Download English Version:

https://daneshyari.com/en/article/1429221

Download Persian Version:

https://daneshyari.com/article/1429221

Daneshyari.com