Biosensors and Bioelectronics 26 (2011) 3967–3972

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Short communication

Toward immunoassay chips: Facile immobilization of antibodies on cyclic olefin copolymer substrates through pre-activated polymer adlayers

Daekyung Sung[a], Dong Ho Shin[c], Sangyong Jon[a,b,*]

[a] Graduate Program of Medical System Engineering (GMSE), School of Life Sciences, Gwangju Institute of Science and Technology (GIST), 261 Cheomdan-gwagiro, Buk-gu, Gwangju 500-712, Republic of Korea
[b] Cell Dynamics Research Center, School of Life Sciences, Gwangju Institute of Science and Technology (GIST), 261 Cheomdan-gwagiro, Buk-gu, Gwangju 500-712, Republic of Korea
[c] Biomed Research Team, BT Convergence Technology Research Department, IT Convergence Technology Research Laboratory, Electronics and Telecommunications Research Institute (ETRI), 138 Gajeongno, Yuseong-gu, Daejeon 305-700, Republic of Korea

ARTICLE INFO

Article history:
Received 10 November 2010
Received in revised form 17 February 2011
Accepted 8 March 2011
Available online 16 March 2011

Keywords:
Amphiphilic polymers
Cyclic olefin copolymer
Polymer adlayers
Anti-biofouling
Antibody immobilization
Immunoassay

ABSTRACT

Our research efforts have focused on the surface modification and the potential as a biochip material of commercially available cyclic olefin copolymer (COC) substrates. For the surface modification of hydrophobic COC substrates we synthesized two types of amphiphilic polymers having three important functions: hydrophobic (dodecyl or benzyl) groups, serving to anchor the COC substrate; a PEG component, which acts as a repellent of non-specific biomolecules; and an NHS ester group for conjugation of biomolecules. Formation of the polymer adlayers on COC surface was confirmed using a contact angle analyzer. The anti-biofouling property of the polymer-coated COC surface was examined by measuring the extent of nonspecific adsorption of immunoglobulin G (IgG), resulting in a very low level of the protein adsorption compared to uncoated COC surfaces (control). In addition, antibodies, used as representative biomolecules for immunoassay, could be selectively immobilized on the polymer-coated COC surface. Sandwich immunoassay using anti-rabbit IgG immobilized COC surface showed linear response to rabbit IgG of model analyte over a range from 1 to 1000 ng/mL with detection limit of 1–10 ng/mL. We also fabricated a Lab-On-a Chip type of COC biochip, which could detect a cardiac marker protein, troponin I (TnI), with detection limit of 10 ng/mL.

1. Introduction

The construction of biosensors and biochips for diagnostic applications requires efficient attachment of biologically active molecules on the surface of solid substrate materials while minimizing non-specific adsorption. (Delamarche et al., 1997; Jakob, 1994; Pirrung, 2002; Mitchell, 2002; Bernard et al., 1998; Siqueira et al., 2010; Peterbauer et al., 2006; Ostuni et al., 2000; Schweitzer et al., 2002) To date, inorganic materials such as silicon oxide and gold have been widely used as substrates for various biodetection assays and devices. (Kanan et al., 2002; Manning and Redmond, 2005; Ulman, 1996; Love et al., 2005) Surface modification methods used to prepare such inorganic substrates include the use of siloxane-containing chemicals or polymers for modifying silicon oxide substrates and sulfhydryl-containing molecules for gold substrates. Cyclic olefin copolymers (COCs) have attracted considerable recent attention as a substrate for biochips because of their unique physico-chemical features: low density, resistance to many solvents, high transparency, low autofluorescence, and high flowability. (Becker and Gartner, 2008; Henares et al., 2008) COCs have been used for DNA immobilization, microarrays, nucleic acid purification, and immunoassays. (Liu et al., 2007; Diaz-Quijada et al., 2007; Bhattacharyya and Klapperich, 2007; Jönsson et al., 2008) Although the properties of COCs are excellent for many applications, it is difficult to modify the surface of COCs because they are made of pure hydrocarbons with no native groups amenable to covalent functionalization. A simple way to immobilize biomolecules on plastic surfaces is to physically adsorb them, either through van der Waals interactions between hydrophobic protein residues and the plastic surfaces (Kai et al., 2003) or through electrostatic interactions between negatively charged proteins and poly(L-lysine)-coated plastics. (Azze et al., 1999) However, because the immobilization process is uncontrollable, such physical adsorption methods often lack reproducibility in terms of spatial density of adsorbed biomolecules. Another way to attach biological substances on a COC surface is oxidation of the non-reactive COC surface using oxygen plasma treatment, producing

* Corresponding author at: Cell Dynamics Research Center, Graduate Program of Medical System Engineering (GMSE), School of Life Sciences, Gwangju Institute of Science and Technology (GIST), 261 Cheomdan-gwagiro, Buk-gu, Gwangju 500-712, Republic of Korea. Tel.: +82 62 715 2504; fax: +82 62 715 2484.
E-mail address: syjon@gist.ac.kr (S. Jon).

0956-5663/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2011.03.005

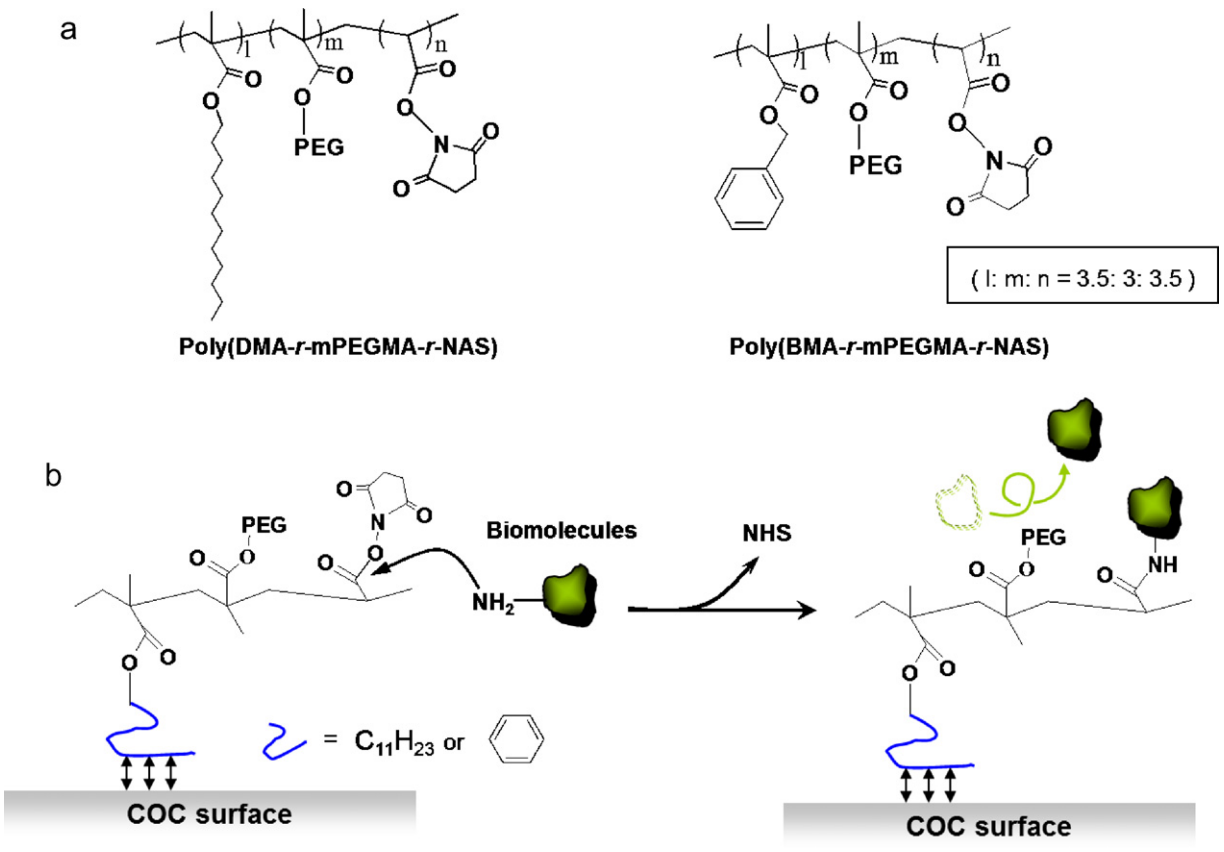

Fig. 1. (a) Chemical structures of the amphiphilic polymers designed in this study. (b) Schematic representation of the procedure for immobilizing biomolecules onto a polymer-modified COC surface with anti-biofouling properties.

a functional group (hydroxyl) that facilitates the immobilization of NH_2-terminated silanol compounds, such as aminopropyl triethoxysilane (APTES), (3-aminopropyl)dimethylethoxysilane, and (3-aminopropyl)trimethoxysilane; biomolecules are then attached using a suitable linker molecule. (Kanan et al., 2002; Vianello et al., 2000) However, the intrinsically hydrophobic nature of COCs causes nonspecific binding of target biomolecules, resulting in strong background signals during immunoassay procedures. (Rebeski et al., 1999) To overcome this problem, researchers have attempted to alter the hydrophilic COC surface in several ways, including photografting, ozonolysis and oxygen plasma treatment. (Stachowiak et al., 2007; Laib and MacCraith, 2007; Larsson et al., 2007; Larsson and Liedberg, 2007; Raj et al., 2009) Although these methods effectively create a hydrophilic surface, unfortunately they fail to block nonspecific adsorption of proteins during assays; thus, a novel approach that departs from conventional strategies is needed.

In our previous work, we demonstrated that rationally designed amphiphilic polymers composed of surface anchoring hydrophobic moieties, an anti-biofouling polyethylene glycol (PEG) component, and tertiary carboxylic acid for functionalization could be used for surface modification of polystyrene surfaces, forming stable polymer adlayers in aqueous medium on which biomolecules could be selectively immobilized. (Sung et al., 2009; Park et al., 2008, 2009) However, there are limitations to the use of these previously described amphiphilic polymers: (i) it is difficult to directly conjugate a large protein molecule, such as an antibody, to the sterically hindered tertiary carboxyl group [$-C(R)(CH_3)-CO_2H$] in the polymers; and (ii) an additional surface treatment step, involving treatment of coupling reagents to convert the carboxylic acid of the polymer layers into an activated ester such as N-hydroxysuccinimide (NHS), is required before conjugating biomolecules.

To overcome drawbacks of previous polymers, we designed and synthesized a second generation of amphiphilic polymers referred to as poly(DMA-*r*-mPEGMA-*r*-NAS) and poly(BMA-*r*-mPEGMA-*r*-NAS) that contain activated secondary carboxylic acid with higher reactivity and less steric hindrance than the tertiary one. The chemical structures of these polymers are shown in Fig. 1a. Using these second-generation amphiphilic polymers, we here report the facile formation of pre-activated polymer layers on a COC surface and subsequent selective immobilization of proteins (i.e., antibodies) on the surface. To demonstrate the usefulness of these amphiphilic polymers, we carried out a sandwich immunoassay using antibody immobilized COC biochips.

2. Materials and methods

2.1. Synthesis of polymers

Prior to polymerization, neat mPEGMA was passed over an inhibitor-removal column (Sigma–Aldrich, Milwaukee, WI, USA). DMA or BMA (3.5 mmol, 3.5 equiv), mPEGMA (3 mmol, 1.425 g, 3 equiv), and NAS (3.5 mmol, 0.592 g, 3.5 equiv) were placed in a vial and dissolved in 10 mL of tetrahydrofuran (anhydrous, 99.9%, inhibitor-free). This mixture was degassed for 10 min by bubbling with a stream of N_2 gas. After adding 0.1 mmol AIBN (16.5 mg, 0.1 equiv) as a radical initiator, the vial was sealed with a Teflon-lined screw cap. The polymerization reaction was carried out at 70 °C for 24 h. The final product solution was cooled to room temperature and stored at 4 °C until use. ^{1}H NMR (400 MHz, $CDCl_3$): poly(DMA-*r*-mPEGMA-*r*-NAS), δ = 4.14 (br, 2H,