Applied Surface Science 363 (2016) 619–626

Contents lists available at ScienceDirect

Applied Surface Science

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

Novel antifouling surface with improved hemocompatibility by immobilization of polyzwitterions onto silicon via click chemistry

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ARTICLE INFO

Article history:
Received 21 September 2015
Received in revised form 1 December 2015
Accepted 11 December 2015
Available online 14 December 2015

Keywords:
Zwitterionic polymer
Click chemistry
Antifouling
Hemocompatibility
Silicon membrane
Artificial kidney

ABSTRACT

A novel procedure is presented to develop an antifouling silicon surface with improved hemocompatibility by using a zwitterionic polymer, poly(sulfobetaine methacrylate) (polySBMA). Functionalization of the silicon surface with polySBMA involved the following three steps: (1) an alkyne terminated polySBMA was synthesized by RAFT polymerization; (2) a self-assembled monolayer with bromine end groups was constructed on the silicon surface, and then the bromine end groups were replaced by azide groups; and (3) the polySBMA was attached to the silicon surface by azide–alkyne cycloaddition click reaction. Membrane characterization confirmed a successful silicon surface modification with almost 100% coverage by polySBMA and an extremely hydrophilic surface after such modification. The polySBMA-modified silicon surface was found to have excellent anti-nonspecific adsorption properties for both bovine serum albumin (BSA) protein and model bacterial cells. Whole blood adsorption experiments showed that the polySBMA-modified silicon surface exhibited excellent hemocompatibility and effective anti-adhesion to blood cells. Silicon membranes with such antifouling and hemocompatible surfaces can be advantageously used to drastically extend the service life of implantable medical devices such as artificial kidney devices.

1. Introduction

Kidney not only serves as a biological filter of the human body to remove wastes from blood, but also helps regulate the body electrolyte balance, acid–base balance, blood pressure, and hormone secretion. However, about 26 million American adults suffer from chronic kidney diseases, many of which have developed to the end-stage renal disease (ESRD) and thus the function of kidney is completely lost [1]. Although dialysis is considered useful for treating ESRD, the survival rate at three years after the start of the therapy is only 50% [2]. In addition, a routine thrice-per-week dialysis treatment can be very costly and time-consuming, let alone seriously deteriorate the patients' life quality. Although kidney transplantation may achieve a much higher survival rate (91%) at three years and significantly improve the patients' life quality [2,3], such alternative treatment has been severely limited by the shortage of donor organs. Therefore, implantable artificial kidneys, which use a manmade membrane filtration system to substitute the function of human kidney, are highly promising for ESRD treatment [4,5].

In recent years, significant progress has been made in the development of implantable artificial kidneys [4,5]. As the core component of an artificial kidney, membranes function as a filter to remove toxic compounds and wastes while retaining biomacromolecules in blood. In order to achieve such precise separation, membrane pore sizes must be fine-tuned to allow for only an extremely narrow distribution. Conventional polymeric membranes, which typically have an irregular structure and wide pore size distribution, are thus not suitable for this purpose. Nanostructured silicon membranes with a uniform geometry and precise pore sizes have been recently developed and shown excellent separation performance in an implantable artificial kidney device [2]. However, silicon membranes are susceptible to nonspecific adsorption of biomacromolecules (e.g., platelets, proteins) from blood, thereby leading to full-scale platelet adhesion/activation and ultimately the formation of thrombosis/embolism that can be fatal to patients [6]. In addition, the adhesion of biomacromolecules can also clog membrane pores, reduce membrane permeability and selectivity, and hence severely shorten the lifetime of an artificial kidney device.

Surface functionalization is an extremely useful approach to prevent nonspecific adsorption and improve hemocompatibility of

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http://dx.doi.org/10.1016/j.apsusc.2015.12.081
0169-4332/

silicon membranes. Poly(ethylene glycol) (PEG) has been widely used and proven very efficient to reduce nonspecific adsorption on surfaces [7–11]. Unfortunately, the autoxidation of PEG in the presence of oxygen and transition metal ions alters the chemical structure of the polymer and eliminates its ability to repel nonspecific adsorption [12,13]. For this reason, the application of PEG in blood-contacting environment where various metal ions, organic compounds, and oxygen exist, is very limited [14].

As a new antifouling material, zwitterions have received growing attention over recent years due to their excellent anti-adhesive property against protein adsorption and bio-attachment [15–18]. It has been reported that zwitterionic monomers or polymers are effective, stable materials that can prohibit protein fouling and have an excellent anti-biofouling property against bacterial attachment in a biological system. The anti-adhesive nature of polyzwitterions can be attributed to their high hydration capability and conformational structure. Zwitterion is a molecule containing both positively and negatively charged functional groups, which neutralize each other and thus maintain an overall neutrality. Zwitterionic polymers are made by polymerization of zwitterions, thus containing localized charges within every repeated side chain while the overall charge neutrality is maintained. Compared with PEG, the zwitterionic polymer is able to strongly bind to water molecules with localized charges. Such electrostatically induced hydration can greatly inhibit the attachment of biomacromolecules to a polymer surface due to the physical swelling of the polymer. Overall, zwitterions are very hydrophilic, non-cytotoxic, and at an acceptable endotoxin level, making them an ideal antifouling material for surface modification of silicon membranes used in an artificial kidney.

Depending on different charged functional groups carried by the polymers, zwitterionic polymers used for generating antifouling surfaces are typically classified as sulfobetaine, carboxybetaine, and phosphobetaine. Phosphobetaine, a mimic of the phospholipids of the outer surface of cell membranes, is the very first type of zwitterions ever studied [13,19]. Recently, sulfobetaine and carboxybetaine have attracted an intense interest due to their superior antifouling properties compared with phosphobetaine, and they have been used to functionalize various surfaces such as polymeric membranes [20–27], silicon/silica [14,28–30], glass [29,31,32], gold [33–35], and steel [36,37]. Studies have shown that carboxybetaine may exhibit better anti-adhesion properties than sulfobetaine [38]. When fouling is a primary concern, however, sulfobetaine is more often used to prevent/mitigate nonspecific adsorption. This is because the charged functional groups (of a "strong-strong" type) of sulfobetaine maintain much more stable localized charge properties under different conditions. In a wide pH range (e.g., 3–10), a sulfobetaine polymer can still retain its zwitterionic property, while carboxybetaine with charges of a "strong-weak" type loses the localized negative charge from carboxyl groups under acidic conditions [39]. As a result, the zwitterionic property of the nanobrush is impaired and the polymer exhibits overall positive charge instead. This positive charge may adsorb biomacromolecules with negative charges and thus lead to severe fouling of the surface.

In this study, we employed a zwitterionic polymer, poly(sulfobetaine methacrylate) (polySBMA), to improve the antifouling properties of silicon surfaces. The polySBMA was immobilized onto the silicon surface using a highly efficient azide–alkyne click reaction, following a three-step procedure: (1) synthesis of alkyne terminated polySBMA by a reversible addition-fragmentation chain-transfer (RAFT) mediated radical polymerization, (2) functionalization of silicon by self-assembled monolayer (SAM) with bromine end groups and conversion of bromine groups to azide groups by nucleophilic substitution, and (3) immobilization of alkyne terminated polySBMA to the silicon surface by azide–alkyne click reaction. Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS) and water contact angle measurement were carried out to characterize and confirm the functionalization of silicon surfaces. The anti-nonspecific adsorption ability of the modified samples was evaluated by protein adsorption using quartz crystal microbalance with dissipation (QCM-D) and bacterial cell adhesion experiments. The hemocompatibility of the modified surface was evaluated by whole blood contact experiments.

2. Materials and methods

2.1. Polyzwitterion synthesis

All chemicals were analytical grade or higher and obtained from Sigma–Aldrich (St. Louis, MO). The deionized (DI) water was used in all experiments. PolySBMA was synthesized in our lab by a RAFT mediated radical polymerization [27]. Briefly speaking, azodiisobutyronitrile (AIBN) was used as an initiator, and alkyne-terminated S-1-dodecyl-S′-(R,R′-dimethyl-R″-acetic acid)trithiocarbonate (alkyne-SDDAT) as a chain transfer agent. First, 5 g SBMA ([2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl) ammonium hydroxide), 0.2 g alkyne-SDDAT, and 50 mg AIBN were mixed with 100 mL pure ethanol in a single-necked flask. Nitrogen was then bubbled through the solution for 30 min to remove oxygen from the flask. Next, RAFT polymerization was performed at 55 °C for 24 h to obtain alkyne–polySBMA, followed by cooling the reactor in ice water. The synthesized polymer was then collected by filtering the solution through a vacuum filter (Cole Parmer, Vernon Hills, IL), rinsing with ethanol, and subsequently drying overnight in oven at 65 °C. The collected alkyne–polySBMA was stored in desiccator prior to use in the grafting experiments.

2.2. Silicon surface functionalization

The overall modification procedure for silicon surface is illustrated in Fig. 1. Before modification, a silicon wafer was first cleaned in UV/Ozone chamber for 30 min, rinsed by DI water and ethanol, and then dried with nitrogen gas. The silicon wafer was then brominated by immersing it in (3-bromopropyl)trichlorosilane (BPTS) (0.005% v/v) anhydrous toluene solution to form an SAM on the surface. After reacting in a sealed vessel placed in a desiccator for 24 h, the wafer was taken out, thoroughly rinsed with toluene, DI water, and pure ethanol, and finally dried with nitrogen gas. Next, the bromine groups were converted to azide groups by placing the wafer in 10 g/L sodium azide solution in a small vessel, and the reaction continued at room temperature for 24 h. Finally, the wafer was washed with DI water and subsequently dried with nitrogen gas.

PolySBMA was immobilized onto the silicon surface by click chemistry through azide–alkyne Huisgen cycloaddition. The polymer solution for the click reaction was prepared by dissolving 0.50 g alkyne–polySBMA and 0.011 g $CuSO_4$ in 50 mL DI water. The polymer solution was degased by nitrogen for 30 min and then azide-functionalized silicon wafer was immersed in the solution. Afterwards, 0.024 g sodium ascorbate was added into the solution to initiate the click reaction. The flask was placed on a shaking table for 24 h to complete the click reaction. The polySBMA immobilized silicon wafers were taken out and thoroughly washed with DI water for 24 h on a shaker by changing water frequently, followed by rinsing with ethanol and drying with nitrogen gas.

2.3. Surface characterization

FTIR measurement was carried out on a Thermo Nicolet Nexus 670 spectrometer with a Smart Golden Gate accessory and a