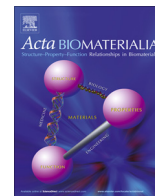




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# The fabrication of superlow protein absorption zwitterionic coating by electrochemically mediated atom transfer radical polymerization and its application

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## ABSTRACT

A well-controllable electrochemically mediated surface-initiated atom transfer radical polymerization (e-siATRP) method for the fabrication of superlow protein absorption zwitterionic hydrogel coatings based on poly(sulbetaine methacrylate) (pSBMA) was developed in this work. The effects of the electric condition on polymerization as well as its antifouling performances both in vitro and in vivo were also investigated. Different potentials (−0.08 V, −0.15 V and −0.22 V) and polymerization times (from 8 to 48 h) were chosen to study the polymerization procedure. X-ray photoelectron spectroscopy, atomic force microscopy and ellipsometry measurements were used to characterize the properties of the polymer layers. Ellipsometry measurements showed that a higher potential provided faster polymerization and thicker polymer layers; however, the protein absorption experiments showed that the best polymerization condition was under a constant potential of −0.15 V and 32 h, under which the protein absorption was 0.8% in an enzyme-linked immunosorbent assay (compared to a bare gold electrode). The electrodes with a pSBMA coating effectively deduced the current sensitivity decay both in undiluted serum and in vivo. The usage of the commercially available polymerization monomer of SBMA, the simple convenient synthesis process regardless of the presence of oxygen and the excellent controllability of e-siATRP make it a very promising and universal technique in the preparation of zwitterionic polymer coatings, especially in the development of biocompatible material for implantable devices such as neural and biosensor electrodes.

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## 1. Introduction

The need for implantable medical devices has kept on growing in recent years, with many new devices having been brought to market. Even so, some fundamental obstacles hinder the use of implantable devices in clinical practice, especially with regard to their long-term reliability. Among those obstacles, non-specific protein absorption, also called biofouling, plays a key role [1]. Biofouling begins in the very early stages of implantation. For electronic devices, such as biosensors and neural electrodes, it causes rapid decay in sensitivity. Generally, the procedure of biofouling occurs in the following order: protein adsorption, complement activation, inflammatory cell adhesion. Proteins from plasma, such

as albumin, fibrinogen, complement, fibronectin and  $\gamma$  globulin, are involved [2]. Some molecular-level characteristics of surface determined protein adsorption include hydrophilicity, hydrogen-bond acceptors or donors and neutral electrical charge [3]. Therefore, the modification of the surfaces of those electrodes is important. Efforts have been put into anti-biofouling research. Hydrophilic polymers, such as poly(ethylene glycol) (PEG) [4,5] derivatives, polyethylene oxide (PEO) [6], oligo(ethylene glycol) (OEG) [7] and phosphorylcholine (PC) [8], are widely used to reduce non-specific protein absorption. Research from Shen et al. showed that absorption of fibrinogen to plasma deposited PEO was less than 10 ng cm<sup>−2</sup>, a 20-fold decrease compared to tissue culture polystyrene [9]. But evidence suggested that PEG could decompose in the presence of superoxide anions of H<sub>2</sub>O<sub>2</sub>, which lead to fouling in the long term [10].

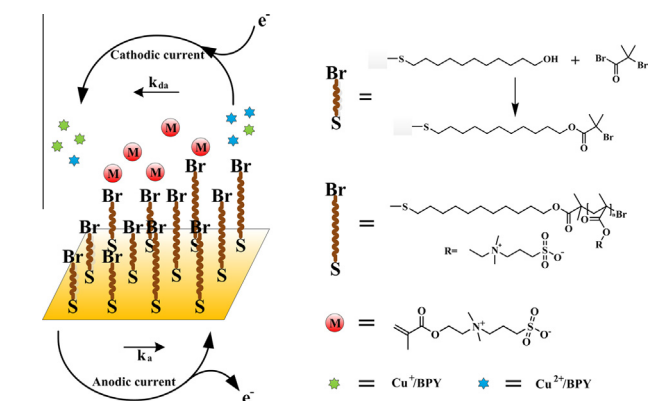
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In the past few years, zwitterionic hydrogels containing both cationic and anionic groups on the same monomer residue, synthesized by atom transfer radical polymerization (ATRP), showed great properties in repelling protein [11–13], anti-cell adhesion [14,15] and biosensing [16,17]. In Yang et al.'s research, surfaces with 62 nm poly(sulfobetaine methacrylate) (pSBMA) brushes presented the best nonfouling character with protein adsorption of  $6 \text{ ng cm}^{-2}$  [18]. Chang et al. enhanced this to less than  $2 \text{ ng cm}^{-2}$  in diluted plasma solution [19], while in the experiment of Li et al., pSBMA coated surface had very low nonspecific protein adsorption levels of  $0.8 \text{ ng cm}^{-2}$  from 100% human serum [20]. Furthermore, the well-defined copolymerization of poly(SBMA) with poly(propylene oxide) (PPO) reduced protein adsorption to  $3 \text{ ng cm}^{-2}$  [21]. Poly(carboxybetaine methacrylate) (polyCBMA) reduced single protein adsorption to  $0.3 \text{ ng cm}^{-2}$  [22]. The data from Vaisocherová et al. showed that a thin layer of poly(carboxybetaine acrylamide) (pCBAA) on gold-coated substrates achieved almost no non-specific protein adsorption in undiluted human blood serum [23]. Recently, functional polycarboxybetaine acrylamide (pCB), which created high polymer packing densities on the surface, was found to have a super low adsorption of  $<0.3 \text{ ng cm}^{-2}$  [24]. Moreover, zwitterionic gel showed excellent bacteria-repellent properties as well [25–27].

ATRP is conducted by an active equilibrium between lower and higher oxidation states of a transit metal complex. These complexes combine metal ions and corresponding ligands, which provide radicals, then promote monomer addition. The speed of polymerization of monomers is effectively controlled by the concentration of radicals, which is regulated by the ratio of the lower state to the higher state of metal activators. Then the higher state oxidation deactivator terminates propagation. ATRP provides highly uniform nano-brush-like surfaces, giving those polymers particular features. Most of those experiments based on ATRP require careful handling: an  $\text{O}_2$ -free environment, anhydrous operation, and so on. All of those inconveniences restrain the wide application of zwitterionic hydrogels, especially in the development of biomedical devices, such as enzyme electrodes.

In recent years, a new kind of ATRP, electrochemically mediated ATRP (eATRP), was introduced [28] and comprehensively inspected. Applied potential becomes a key force that promotes polymerization [29]. Soon after, electrochemically induced surface-initiated ATRP (e-siATRP) was developed [30]. Scheme 1 shows the mechanism of polymer brush growth using e-siATRP. The reaction was carried out in an electrochemical cell with a two- or three-electrode system. A constant potential was applied to generate the  $\text{Cu}^{\text{I}}$  complex through one-electron reduction of  $\text{Cu}^{\text{II}}$ /bipyridine (bpy) in the vicinity of the initiator-decorated gold surface to initiate the



**Scheme 1.** Mechanism of electrochemically induced si-ATRP at a cathodic current to generate the  $\text{Cu}^{\text{I}}/\text{BPY}$  complex that triggers the polymerization. ( $k_a$  and  $k_{da}$  represent the rate constants of activation and deactivation propagation, respectively).

polymerization. e-siATRP is a powerful method which could control the whole procedure of polymerization by precisely manipulating the ratio of  $\text{Cu}^{\text{I}}/\text{Cu}^{\text{II}}$  through tuning applied potentials and the distance of catalysis complex diffusion [31]. As a universal and convenient ATRP method, e-siATRP of zwitterionic gel shows a broad prospect in functional biomaterials preparation.

In the previous research, most of the zwitterionic gels showing great antifouling performance were synthesized by conventional ATRP. However, those monomers such as CBMA, CBAA, and CB are not commercially available. In this work, in order to demonstrate a universal approach to prepare antifouling coating, a commercially available zwitterionic monomer, SBMA, was chosen as the model monomer. For the first time, e-siATRP was applied to polymerize zwitterionic gel on gold electrodes potentially used as implantable devices. The effects of electric condition to polymerization were investigated and the thicknesses of pSBMA coating on Au-covered silicon wafers were measured by ellipsometry to discover the relationship between anti-biofouling performance and the thickness of the polymer layers under different electrical conditions. The antifouling performances both in vitro and in vivo were also investigated. Compared with traditional material, such as PU, pSBMA coating showed better performance.

## 2. Experimental methods

### 2.1. Materials and instruments

N-(3-sulfopropyl)-N-(methacryloxyethyl)-N, N-dimethyl ammonium betaine (SBMA, 97%), bromoisobutyrate (BIBB, 98%), 11-mercapto-1-undecanol (97%), 2,2'-bipyridine (bpy, 99%) were purchased from Sigma-Aldrich (Milwaukee, USA). Benzyltri-n-butylammonium chloride (BBAC, 98%) was purchased from Alfa Aesar (Tianjin, China). Copper chloride (AP), methanol (AP) and tetrahydrofuran (THF, HPLC grade) were purchased from Aladdin (Shanghai, China). Biotin-labeled goat anti-mouse IgG (H + L), HRP-labeled streptavidin and TMB horseradish peroxidase color development solution were purchased from Beyotime (Shanghai, China). Gold wire (99.999%) was purchased from New-Metal (Beijing, China). Other regular chemicals used were of analytical reagent grade, purchased from Sinopharm (Shanghai, China).

Electropolymerization and electrochemical measurements were performed with an electrochemical workstation ( $\mu\text{Autolab III}$ , Metrohm, Switzerland). Scanning electron microscopy images were collected on a thermal field emission scanning electron microscope (FEI SIRION-100, The Netherlands). All electrochemical experiments were carried out at room temperature.

### 2.2. Synthesis of initiator

$\omega$ -Mercaptoundecyl bromoisobutyrate (Br-thiol) was synthesized through reaction of BIBB and 11-mercapto-1-undecanol using a method published previously [11,32]. The product after purification was a colorless viscous liquid ( $0.79 \text{ g ml}^{-1}$ ). Nuclear magnetic resonance (NMR) was recorded using deuterated methanol as the solvent. (Proton nuclear magnetic resonance ( $^1\text{H NMR}$ ) (400 MHz,  $\text{CDCl}_3$ ): 4.15 (t,  $J = 6.9$ , 2H,  $\text{OCH}_2$ ), 2.51 (q,  $J = 7.5$ , 2H,  $\text{SCH}_2$ ), 1.92 (s, 6H,  $\text{CH}_3$ ), 1.57–1.72 (m, 4H,  $\text{CH}_2$ ) and 1.24–1.40 (m, 16H,  $\text{CH}_2$ )).

### 2.3. Preparation of Au-Si working electrodes

Double-sided polished Si wafers were cut into sticks of  $1 \times 20 \times 0.4 \text{ mm}$  (width  $\times$  length  $\times$  thickness). After thorough rinsing with acetone and anhydrous ethyl alcohol, the sticks were put in a customized Teflon fixture closely side by side, exposing

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