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Regular Article

Design of hemocompatible and antifouling PET sheets with synergistic zwitterionic surfaces

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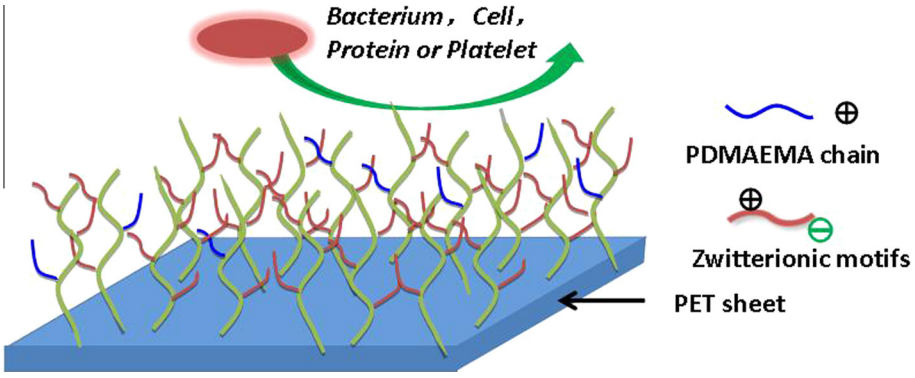
HIGHLIGHTS

- Synergistic zwitterionic polymer brushes were grafted from PET through two-step strategy.
- The resulting PET showed outstanding antifouling property and hemocompatibility.
- Synergistic surfaces with neutral zwitterions and weak cations are promising for biomedical applications.

GRAPHICAL ABSTRACT



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ABSTRACT

Zwitterionic surface has been proven to be a good candidate for improving hemocompatible and antibiofouling properties. However, it can only passively repel the adsorption of microbes and is unable to kill the adherent or trapped microbes. The purpose of our study is to develop a facile method based on synergy "repel and kill" strategy and prepare dual antifouling and antibacterial surface. Herein, the poly (2-(dimethylamino) ethyl methacrylate) (PDMAEMA) was first constructed *via* surface-initiated activators regenerated by electron transfer atom transfer radical polymerization (ARGET-ATRP) method, followed by partial quaternization in order to form polycarboxybetaine and polysulfobetaine. The conversion rates of PDMAEMA to polyzwitterions were evaluated by X-ray photoelectron spectroscopy analysis (XPS). Surface characterizations by ATR-FTIR, XPS, and AFM demonstrated that zwitterionic polymer brushes were successfully grafted. The remained PDMAEMA(weak cationic) and formed zwitterions(neutral) endowed the surface with the synergetic antibacterial and antifouling properties. The resulting PET sheets showed outstanding antifouling property featured by the reduced adhesion of 3T3 fibroblast cells and *E. coli*. Additionally, these sheets displayed excellent hemocompatibility such as non-cytotoxicity, repelled protein adsorption, reduced platelet adhesion, and prolonged blood blotting time. These synergistic surfaces with neutral zwitterions and weak cations are promising for biomedical applications.

1. Introduction

Two major complications generally occur in blood-contacting devices, namely thrombus formation and microbial infection [1]. Thrombus formation holds the risk of vascular occlusion and

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potential thrombus embolization, which may result in tissue damage or stroke [2]. Microbial invasion and infection of implanted device leads to device failure and associated adverse clinical reactions [3]. Therefore, improving the hemocompatible and antibiofouling properties of biomaterials and biodevices has become a very important avenue of research in the field of biomedical materials science.

A number of studies have focused on improving hemocompatible and antibiofouling properties in the development of novel biomaterials [4–7]. Ratner et al. pointed out that both poly(ethylene glycol) and zwitterionic surfaces showed promising interactions with blood [8]. Poly(ethylene glycol) can undergo oxidative degradation leading to chain scission as well as oxidation of chain termini when used for longer periods of time [9]. Zwitterionic surface has been proven to be a good candidate for improving hemocompatible and antibiofouling properties [10–20]. However, it can only repel the adsorption of microbes and is unable to kill the trapped them. Cheng et al. [21] have presented a novel switchable polymer surface which are antimicrobial and nonfouling. The cationic precursor of pCBMA is able to kill bacterial cells effectively and switches to a zwitterionic nonfouling surface and releases dead bacterial cells upon hydrolysis. Cao et al. [22] have synthesized two switchable carboxybetaine derivatives, which can reversibly switch between antifouling surface and antimicrobial surface adjusted by the pH conditions. The above approaches are based on "catch-and-kill" strategy. That is, the antibacterial surface kills bacteria first and then releases the dead bacteria. It is no doubt that the bacteria are numerous and the bacteria-killing activity of quaternary ammonium is limited. Recently, Onat et al. [23] have prepared substrates with dual function coatings, i.e. bacterial anti-adhesive and antibacterial agent releasing polymer films of zwitterionic block copolymer micelles. But even with these gains, it is still a great challenge to develop biocompatible materials that have dualantimicrobial and nonfouling capabilities.

The purpose of our study is to find a facile method based on synergy strategy to prepare dual antifouling and antibacterial surface. Comparing to Cheng and Cao's approaches, we adopt the "repel-and-kill" strategy. That is, repelling the bacteria first and then kills the trapped bacteria. Zwitterionic surface can reduce the initial attachment and delay colonization of microbes on surfaces. Meanwhile, the antibacterial surface can fatherly kill the trapped or contacted pathogenic microbes. As known, poly(2-(dimethylamino) ethyl methacrylate) (PDMAEMA) can form into polyzwitterions via ring-opening reaction. However, these conversions are incompletely and the yield rates are about 80%. Since the pKa of PDMAEMA is between 8.5 and 7.5 [24], the unconverted PDMAEMA would be partially protonated and has potential of bactericidal properties. PDMAEMA is known as non-viral gene carrier due to its electrostatic interaction with negative DNA [25]. Thus, the remained PDMAEMA (weak cationic) and zwitterions (anti-adhesive) can form dual antifouling and antibacterial surface, which is ideal for biomedical devices. Zwitterionic surface can reduce initial attachment and delay biofilm formation on surfaces, but they are not able to kill attached microorganisms. Thus, the PDMAEMA surface can kill the trapped and adherent pathogenic microbes. Combining the above features, the zwitterionic surface along with weak cationic charges should be promising for antifouling and antibacterial usage. In this respect, this synergetic surface is better than the single zwitterionic surface.

Poly(ethylene terephthalate) (PET) is used as substrate due to its excellent mechanical property and moderate inflammatory response. It has a wide range of medical applications including vascular prostheses, heart valve sewing cuffs, implantable sutures, and surgical mesh [26]. Previously, we have grafted zwitterionic polymer brushes via dopamine-initiated ATRP method based on synergy "repel and kill" strategy [27]. In the study, we aim to stepwise graft zwitterionic brushes of polycarboxybetaine and polysulfobetaine onto PET *via* a activator regenerated by electron transfer for atom transfer radical polymerization (ARGET-ATRP) process (Fig. 1). PET sheets were first alkaline hydrolyzed to yield hydroxyl groups, followed by the immobilization of a 2-bromoisobutyryl bromide initiator for living polymerization of 2-(dimethylamino) ethyl methacrylate (DMAEMA) monomer. Then, PDMAEMA chains were submitted to ring-opening with 1,3-propiolactone (PL) and 1,3-propanesultone (PS) to form polycarboxybetaine and polysulfobetaine brushes incompletely, respectively. The surface was characterized by attenuated total reflection Fourier transform infrared spectra (ATR-FTIR), water contact angle (WCA), atomic force microscope (AFM), and X-ray photoelectron spectroscopy (XPS). The conversion rates of PDMAEMA to polyzwitterions were evaluated by XPS analysis. The remained PDMAEMA (weak positive charged) and formed zwitterions(neutral charged) would form dual antifouling and antibacterial surface. Hemocompatibility was evaluated by platelet adhesion, bovine serum albumin (BSA) adsorption, and blood clotting time, as well as cytotoxicity. The antibiofouling property was measured by the adhesion of *E. coli* (ATCC 8739) and 3T3 fibroblast cell.

2. Materials and methods

2.1. Materials

PET sheets (1 mm in thickness) were provided by Hangzhou Dahua Plastic Industry Co., Ltd. (Hangzhou, China). The reagents 2-(dimethylamino) ethyl methacrylate (DMAEMA), 1,3-propanesultone (PS), and 1,3-propiolactone (PL) were obtained from Sigma-Aldrich. The chemicals 2-bromoisobutyryl bromide (BIBB, 98%), N,N,N′,N″,N″-pentamethyldiethylenetriamine (PMDETA, 99%), and 2-dimethylaminopryridine (DMAP, 99%) were obtained from Shanghai Aladdin Bio-Chem Technology Co., Ltd. Triethylamine (TEA, 99%), dichloromethane (CH_2Cl_2, AR), tetrahydrofuran (THF, AR), methanol (CH_3OH, AR), copper(II) bromide ($CuBr_2$, 99%), and ascorbic acid were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and purified before use. Platelet rich plasma (PRP) was provided by the Blood Center of Jiangsu Red Cross. An enhanced bicinchoninic acid (BCA) protein assay reagent kit was purchased from Thermo Scientific Inc., USA.

2.2. Alkaline hydrolysis of PET sheets to generate hydroxyl groups [28]

A simple two-step method is presented for the covalent immobilization of an ATRP initiator on a surface of PET. The first step consisted in the immersion of PET sheets (6×6 mm^2) in a mixture of sodium hydroxide (0.25 mol/L^{-1}) and acetonitrile at a 1:1 ratio (v:v) and shaking for 24 h at room temperature to generate the hydroxyl groups. The treated sheets were then thoroughly washed with water and dried in vacuum to afford the PET-OH sheets for the next step.

2.3. Immobilization of the ATRP initiator

The PET-OH sheets were introduced in a solution containing TEA (6.12 mL) and a catalytic amount of DMAP in THF (50 mL). BIBB (4.95 ml, 40 mmol) was added dropwise under stirring to the reaction mixture cooled with an ice bath. The reaction was allowed to proceed for 24 h at room temperature. Thereafter, the functionalized PET sheets (PET-Br) were thoroughly washed with dichloromethane and methanol under ultrasounds in order to remove residual reactants and by-products. The PET-Br substrates were finally dried in vacuum for the next step.