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Antiadhesive zwitterionic poly-(sulphobetaine methacrylate) brush coating functionalized with triclosan for high-efficiency antibacterial performance

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ABSTRACT

In this study, a high-efficiency antibacterial surface with both antimicrobial and antiadhesive functionality was fabricated on a silicon substrate by combining a zwitterionic poly-(sulphobetaine methacrylate) (PSBMA) brush and triclosan (TCS). The uniform PSBMA polymer brush on the substrate was successfully prepared using surface-initiated atom transfer radical polymerization (SI-ATRP). A series grafting ratios of TCS was covalently conjugated to the polymer brush. The surface's antibacterial performance was evaluated using the *Actinomyces naeslundii* and *Escherichia coli* attachment tests. Bacterial adhesion to the surface was significantly reduced following PSBMA brush modification, but the remaining bacteria on the surface were not necessarily killed. After coupling the TCS to the PSBMA brush, the surface had a high-efficiency antibacterial performance from the combination of resisting bacterial adhesion and possessing bactericidal activity.

1. Introduction

Bacterial infection is a critical problem in biomaterial applications, including implant materials, medical devices, and so on [1]. The adherent bacteria and subsequent formation of a biofilm on the material's surface can cause pathogenic infections [2]. In recent years, a variety of antibacterial coatings have been fabricated onto various surfaces to prevent bacterial infection. Among them, the polymer brush has received significant attention due to its robust mechanical stability, controllable brush thickness, and further modification potential [3]. Antibacterial surfaces coated with a polymer brush can be grouped into two categories [4]. One category includes passive antibacterial surfaces, which aim to reduce bacterial adhesion without killing the bacteria. The other includes surfaces that are designed to kill the bacteria or prevent their growth; these are active antibacterial surfaces.

Passive antibacterial surfaces are fabricated by immobilizing the polymers on the surface, and they possess the ability to resist the protein and bacterial adhesion [5,6]. The polymer used for antibacterial coating includes hydrophilic polymers, zwitterion polymers and amphiphilic coating. Among these, the hydrophilic polymer brush possesses antiadhesive properties because of the steric barrier created by the hydration layer [7]. Polyethylene glycol (PEG) is commonly used as an antibacterial material to prevent bacterial adhesion [8,9]. However, PEG loses its resistance performance in the presence of oxygen and transition metal ions or at temperatures above 35 °C. As an alternative, zwitterionic polymers, such as poly-(2-methacryloyloxyethyl phosphorylcholine) (PMPC), poly-(carboxybetaine methacrylate) (PCBMA) and poly-(sulphobetaine methacrylate) (PSBMA), have attracted increasing attention because they efficiently reduce bacterial attachment and improve the materials' biocompatibility [10–15]. For example, Jiang and co-workers have developed many carboxybetaine and sulphobetaine based zwitterionic polymers that have ultralow fouling in different media [16–19]. The amphiphilic copolymers with alternating hydrophilic and hydrophobic domains also resist fouling [20,21]. However, a simple polymer brush coating cannot completely prevent bacterial adhesion. This means that a few bacteria might remain attached on the surface. Unfortunately, the few

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adhering bacteria may form a mature biofilm, resulting in fatal consequences [22].

The active antibacterial surface can kill adherent bacteria. Antibacterial polymers like poly (*N*,*N*-dimethylaminoethyl methacrylate) (PDMAEMA) [23,24] and poly (4-vinyl pyridine) (P4VP) [25] are widely used as antibacterial materials to kill attached bacteria. However, accumulated dead bacteria that coat the surface would dramatically decrease the antibacterial efficiency in most cases. In addition, the antimicrobial agents are incorporated into the polymer brush through physisorption or are covalently tethered to the surface [26–29]. For example, Ag nanoparticles [30–32] and Cu cations [33,34] were physically adsorbed to the polymer brush systems. However, release of the antimicrobial regent may negatively affect human health and the environment [35]. Decorating the surface with antibiotics improves the antibacterial performance of the materials [36,37]. Antimicrobial peptides (AMPs) [38–40] and lysozyme [41,42] were also conjugated to the antiadhesive brush. The peptide and protein activities are easily affected by their environment and altered molecular conformation after immobilization. It is challenging to fabricate antibacterial surfaces that possess high efficiency, stability, and long-term functionality.

To achieve high-efficiency antibacterial performance on the surface, we conjugated a zwitterionic PSBMA polymer and antimicrobial triclosan (TCS), which have broad-spectrum antibacterial effects. The PSBMA brush shows ultralow fouling performance because of its extreme hydrophilicity. It also possesses appropriate biocompatibility for biomedical applications [15,18,43]. Moreover, the PSBMA brush exhibits adequate cell antifouling until it is incubated in cell culture medium for up to 4 months [44]. The use of a surface-tethered hydrophobic poly(methyl methacrylate) or polystyrene blocks before PSBMA polymerization could improve brush stability in an artificial marine environment [45]. TCS has been a widely used broad-spectrum antibacterial agent that poses little risk to health; it is commonly used for daily necessities and can be detected in human body fluids [46–49]. The schematic illustration of the surface modifications on the substrate is shown in Fig. 1. The PSBMA brush was prepared using surface-initiated atom transfer radical polymerization (SI-ATRP) with the aim of generating a high-density, uniform polymer brush [50,51]. To achieve stable, long-term killing, TCS was covalently conjugated to the PSBMA brush via a sulphonic ester. Moreover, to investigate the influence of the TCS grafting ratio on the antibacterial efficiency, we prepared a series of TCS-conjugated PSBMA brushes with different TCS grafting ratios. The antibacterial property of the modified surfaces was evaluated using the *Actinomyces naeslundii* and *Escherichia coli* attachment tests. The covalent conjugation of a PSBMA brush and TCS onto the substrate results in a robust, chemically stable, long-term antibacterial coating.

2. Experimental section

2.1. Chemicals and materials

Silicon wafers [n-doped, (100)-oriented, 0.56-mm thick, 100-mm diameter, one side polished] were obtained from Wacker Chemtronics (Germany). *N*-(3-sulphopropyl)-*N*-(methacryloxyethyl)-*N*, *N*-dimethylammonium betaine (SBMA, 98%) was purchased from Aldrich. Triclosan (TCS, 97%) was obtained from Shanghai Kayon Biological Technology Co., Ltd (China). Bromoisobutyryl bromide (BIBB, 98%); 2, 2′-bipyridine (Bpy, 99%); ethyl 2-bromoisobutyrate (EBIB, 99%); and 3-aminopropyltriethoxysilane (APTES) were obtained from Aladdin Chemistry Co., Ltd. The above chemicals were used as received. Copper (I) bromide (CuBr, 98%), which was obtained from Tianjin Dalu Chemical Reagent Co., Ltd. (China), was purified via overnight stirring in glacial acetic. Then, it was washed six times with ethanol and dried in a vacuum oven. Dichloromethane and toluene were dehydrated by stirring with calcium hydride; then, the sample was distilled. Triethylamine (TEA) was dehydrated using soaking with a molecular sieve. A Live/Dead Bacterial Viability Kit (KGA502) was purchased from Nanjing KeyGEN Biotech. Co., Ltd. (China). Phosphate buffered saline (PBS, pH, 7.2-7.4; ion strength, 0.01 M) was purchased from Betcton Dickison Co., Ltd. and used as received. Double distilled water was purified using a Millipore water purification system. Other chemicals were analytical-grade reagents and were used as received.

2.2. Surface modification

2.2.1. Preparation of the amino-functionalized substrate

The silicon wafers were treated with a hot "piranha" solution (a mix of H_2O_2 and H_2SO_4 at a volume ratio of 3:7), which was followed by extensive rinsing with water. Then, the wafers were dried at 80 °C in an oven to obtain a clean hydroxyl-terminated substrate (Si-OH). The freshly prepared Si-OH substrate was immersed in a toluene solution of ATPES (3 vol%) at room temperature for 24 h to acquire an amino-functionalized surface (Si-NH_2). The resulting substrate was then ultrasonically washed successively with toluene, toluene/ethanol (1/1), and ethanol. Finally, the substrates were dried with argon flow.

2.2.2. Preparation of the initiator-immobilized substrate

The Si-NH_2 substrate was immersed in a TEA solution (5 vol%) within dried dichloromethane. After the sample was cooled to 0 °C in an ice bath, BIBB (0.5 mL, 4.04 mmol) was drop-wise added into the solution and maintained in an ice bath for 2 h. The reaction was left at room temperature for another 24 h. Afterwards, the obtained initiator-functionalized silicon wafers (Si-initiator) were extensively rinsed with dichloromethane, ethanol, and water [52].

2.2.3. Preparation of PSBMA brush

The PSBMA brush was grafted from the Si-initiator surface via SI-ATRP. The procedure is described in previously published papers [53]. A typical procedure is defined as follows. Si-initiator substrate, SBMA monomer (1.2 g, 8.6 mmol), EBIB (8 μL, 0.06 mmol) and Byp (38 mg, 0.24 mmol) were dissolved in 20 mL of methanol and water (volume ratio 4:1). The mixture was degassed twice using a freeze-pump-thaw cycle. Then, CuBr (16 mg, 0.11 mmol) was added to the mixture and degassing was performed another two times. Polymerization was performed for 24 h in the oil bath at 30 °C with stirring. After polymerization, the substrate was ultrasonically washed with double distilled water three times and then dried with argon steam.

2.2.4. Preparation of TCS-Conjugated substrate

Conjugation of TCS to the surface was realized by reacting the sulphonic groups from the PSBMA brush with the phenolic hydroxyl group in TCS. First, the Si-PSBMA substrate was refluxed in thionyl chloride for 3 h to activate the sulphonic groups. The activated surface was then immediately placed into a solution of TCS with anhydrous dichloromethane and left to react for 1 h at 30 °C. We prepared a series of Si-PSBMA-TCS surfaces with different TCS grafting ratios by changing TCS concentration (0.01, 0.05, and 0.1 mol/L) during the reaction. The obtained surfaces from the three different TCS concentrations were called surface Si-PSBMA-TCS-1, Si-PSBMA-TCS-2 and Si-PSBMA-TCS-3, respectively. Si-PSBMA-TCS-2 is presented as Si-PSBMA-TCS in the remaining text. Afterwards, the substrate was ultrasonically washed in succession with dichloromethane, ethanol, and water and for 15 min each.