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Bacterial anti-adhesive and pH-induced antibacterial agent releasing ultra-thin films of zwitterionic copolymer micelles ☆

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ABSTRACT

We report on preparation of substrates with dual function coatings, i.e. bacterial anti-adhesive and antibacterial agent releasing polymer films of zwitterionic block copolymer micelles (BCMs). BCMs were obtained by pH-induced self-assembly of poly[3-dimethyl (methacryloyloxyethyl) ammonium propane sulfonate-*b*-2-(diisopropylamino)ethyl methacrylate] (βPDMA-*b*-PDPA), resulting in BCMs with zwitterionic βPDMA-coronae and pH-responsive PDPA-core. These zwitterionic BCMs were then used as building blocks to construct mono- and multi-layer films. We found that the number of layers in the film was critical for the anti-adhesive property and 3-layer films were the most anti-adhesive against a model Gram-positive bacterium, *Staphylococcus aureus*. Antibacterial activity could be introduced to the films by loading Triclosan into βPDMA-*b*-PDPA micelles. Triclosan containing films were effective against Triclosan-sensitive *Staphylococcus aureus* specifically at moderately acidic conditions due to pH-induced disintegration of the micellar core blocks and release of Triclosan from the surface. Three-layer films also exhibited anti-adhesive property at physiological pH against a model Gram-negative bacterium, *Escherichia coli*. At moderately acidic pH, the coatings showed a contact antibacterial effect against an isolate of *Escherichia coli* with low sensitivity to Triclosan only when micellar cores were loaded with Triclosan. Such dual function films can be promising to combat biofouling at the non-homogeneous and/or defective parts of an anti-adhesive coating. Moreover, considering the moderately acidic conditions around an infection site, these multilayers can be advantageous due to their property of pH-induced antibacterial agent release.

Statement of Significance

This study presents preparation of substrates with dual function ultra-thin coatings of zwitterionic block copolymer micelles which show bacterial anti-adhesive properties against a Gram-positive and a Gram-negative bacterium. Such coatings are also capable of releasing antibacterial compounds in response to pH changes. Films were prepared by self-assembly of polymers at the surface. Our findings showed that zwitterionic micellar coronae introduced bacterial anti-adhesive property to the films, whereas pH-responsive micellar cores enabled release of an antibacterial agent from the surface at acidic pH. Considering the moderately acidic conditions around an infection site, such multilayers can be promising for the coating of implants/medical devices.

1. Introduction

Hospital-acquired pathogenic bacteria that may cause severe illness and mortality have been a major medical concern both in developed and developing countries causing extra healthcare-related economic burden. In the United States alone, ~1.7 million Hospital Acquired Infections (HAIs) were observed in 2002,

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including the bacteria-derived ones [1]. Antibiotics and some antibacterial agents have been very commonly used on patients for the treatment of bacterial infections with limited success due to the development of resistance against these drugs. Bacteria that develop resistance against multiple drugs are known as Multiple Drug Resistant (MDR) bacteria. They are all observed as isolates of Gram-positive and Gram-negative bacteria, and are the most common reasons for the onset of hospital-acquired infections [2]. There is no standard-of-care therapy for some MDR *Staphylococcus aureus* infections. For instance, one significant Gram-positive MDR bacterium is Methicillin-resistant *S. aureus* (MRSA), which is encountered predominantly in the intensive care units of hospitals, with no globally known antibiotic treatment [3]. Gram-negative MDR isolates of *Escherichia coli* cause community and hospital-acquired bloodstream or urinary tract infections [4]. For example, MDR *E. coli* ST131, which produces extended-spectrum β-lactamase is currently the dominant extraintestinal pathogenic *E. coli* worldwide [5]. Both Gram-positive *S. aureus* and Gram-negative *E. coli* could cause biofouling through biofilm development. Bacteria develop biofilms in a two-step process: adherence on the surface and maturation. If adherence is delayed or prevented, biofilm formation could either be delayed or completely eliminated [6]. Biofilm-associated bacteria are 100–1000 times more resistant to antibiotics than the planktonic bacteria [7], so the elimination of biofilm development would lead to greater success with antibiotic treatment.

Polymers have been used extensively to modify surfaces to prevent biofilm formation. Polymer coated substrates kill the bacteria either on contact [8] or by releasing antibiotics and other antibacterial compounds [9,10] (antibacterial coatings) or polymer coated substrates intrinsically possess bacterial anti-adhesive properties [11] due to their chemical nature (anti-adhesive coatings). Recently, polymer nanostructures that intrinsically show antibacterial property due to their chemical nature or possess antibacterial activity without loaded antibacterial agents have been reported to show great efficacy against bacteria [12–16]. Both antibacterial and anti-adhesive coatings prevent biofilm formation on medical devices. The major concern with releasing bactericidal agents from surfaces is the long term elimination of the biofilm formation due to depletion of the active agent. As an alternative approach, surfaces were modified by covalent attachment of antibiotics [17], however, limitations include efficacy against antibiotic-sensitive bacteria, biofilm formation on layers of dead bacteria on the surface and development of resistance to the drug molecules. Coating surfaces with anti-infective peptides have shown efficacy against antibiotic resistant bacteria [18,19], though they are disadvantageous in being sensitive to degradation by proteases in the serum.

To date, despite the recent progress in the development of antibacterial surfaces using stimuli-responsive polymers, only few of them have achieved long-term elimination of biofilm formation [19–21]. The drawbacks of drug-releasing and contact-killing surfaces have increased the need for development of bacterial anti-adhesive surfaces. Using polymers to prepare bacterial anti-adhesive surfaces has specifically become a promising approach in recent years due to a wide range of functional chemical groups that polymers provide to modify the properties of a surface. Poly (ethylene glycol) (PEG) has been extensively used to modify surfaces due to its biocompatibility, low toxicity, low immunogenicity [22,23] and anti-adhesive properties. However, PEG, especially when its molar mass is below 400 Da, has the disadvantage of being prone to oxidative degradation into toxic diacid and hydroxyacid metabolites by alcohol and aldehyde dehydrogenases in the body [24].

Recently, zwitterionic polymers have proven to be anti-adhesive against protein adsorption, platelet adhesion, and bacteria adhesion [25–27]. Zwitterionic polymers endue their anti-adhesive property by interacting with water molecules through ionic solvation and H-bonding [28] and formation of a network of water molecules at the film-water interface. In contrast to ordinary polyelectrolytes, only small disruptions of the network of hydrogen-bonded water molecules at the film-water interface were observed on polyzwitterion coated films, which was reported to be the reason for their anti-adhesive property [29]. Betainized polymers such as poly(phosphobetaine methacrylate) (pPBMA), p oly(carboxybetainemethacrylate) (pCBMA), and poly(sulfobetaine methacrylate) (pSBMA), possess biocompatibility [30] and have been used to prepare bacterial anti-adhesive biointerfaces [31–33]. Recently, our group has reported on the bacterial anti-adhesive properties of monolayer films of zwitterionic micelles with polysulfobetain coronal chains [34].

Layer-by-layer (LbL) self-assembly of polymers at surfaces is a powerful technique for modification and functionalization of surfaces. Layer-by-layer deposition of polymers has found application in developing films with antibacterial properties [35–45]. Silver nanoparticles have also been incorporated into polymer multilayers to impart antibacterial properties to LbL films [35,46,47]. LbL films with both anti-adhesive and antibacterial properties have also been reported [48–50].

In this study, we developed substrates with dual function ultrathin polymer coatings which show bacterial anti-adhesive properties as well as release hydrophobic antibacterial compounds in response to pH changes. Different from our previous work on bacterial anti-adhesive properties of a monolayer of zwitterionic βPDMA-*b*-PDPA micelles [34], this study examined the effect of number of layers on the bacterial anti-adhesive properties of the films and also reports on the preparation of dual function ultrathin coatings of zwitterionic BCMs, i.e. bacterial anti-adhesive and pH-induced antibacterial properties. This study is the first, demonstrating the use of zwitterionic BCMs with pH-responsive cores as building blocks in the construction of dual function surfaces. Such films hold promise to control the bacterial adhesion on the surface of medical implants/devices.

2. Experimental part

2.1. Materials

Sodium dihydrogen phosphate dehydrate and Luria Bertani (LB) broth (MILLER) were purchased from Merck Chemicals (Darmstadt, Germany). Pharmaceutical secondary standard 5-Chloro-2-(2,4-di chlorophenoxy)phenol (Triclosan), poly(sodium 4-styrene sulfonate) (PSS) (M_W 70,000), Mueller-Hinton (MH) broth, Phosphate Buffered Saline (PBS), Gram Staining Kit and Bovine Serum Albumin (BSA) were purchased from Sigma-Aldrich (USA). Agar bacteriological (Agar No. 1, Oxoid) and Micro BCA protein assay kit were purchased from Thermo Scientific (USA). Sterile PTFE syringe filters (0.22 μm and 0.45 μm) were purchased from Sartorius AG (Goettingen, Germany). Cell culture plates were purchased from Sarstedt (Nibrecht, Germany). C_{12}-Resazurin was purchased from Life Sciences (USA). The deionized (DI) H_2O was purified by passage through a Milli-Q system (Millipore). *Staphylococcus aureus* ATCC 29213 strain and *Escherichia coli* ATCC 8739 were kindly provided by Dr. Emel Uzunoglu (Microbiology Laboratory, Giresun Medical Faculty) and Prof. Dr. Aysegul Cetin Gozen (Department of Biology, Middle East Technical University), respectively.

2.2. Synthesis of poly[3-dimethyl (methacryloyloxyethyl) ammonium propane sulfonate]-block-poly[2-(diisopropylamino) ethyl methacrylate] (βPDMA-b-PDPA)

Poly[2-(dimethylamino)ethyl methacrylate]-*block*-poly[2-(diisopropylamino)ethyl methacrylate] (PDMA-*b*-PDPA) with 61 mol%