Switchable Polymer Surfaces

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A Switchable Biocompatible Polymer Surface with Self-Sterilizing and Nonfouling Capabilities**

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Microbial adhesion onto implanted biomaterials and the subsequent formation of biofilms is one of the major causes of biomedical device failure. The use of antimicrobial and nonfouling coatings are two strategies for the prevention of the attachment and spreading of microorganisms on the surfaces of implantable materials. Antimicrobial surfaces containing covalently linked quaternary ammonium compounds (QACs) have proved to be able to efficiently kill a variety of microorganisms.[1–7] A major problem with QAC surfaces is the attachment of dead microorganisms remaining on antimicrobial coatings, which can trigger an immune response and inflammation, and block its antimicrobial functional groups. In addition, such antimicrobial coatings can not fulfill the requirements of nonfouling[8] and biocompatibility as implantable biomaterials. Poly(ethylene glycol) (PEG) derivatives[9–14] or zwitterionic polymers[15–18] have been extensively used as nonfouling materials to reduce bacterial attachment and biofilm formation. However, the susceptibility of PEG to oxidation damage has limited its long-term application in complex media.[11,19] We recently showed that zwitterionic materials such as poly(sulfobetaine methacrylate) (pSBMA) were able to dramatically reduce bacterial attachment and biofilm formation[18] and were highly resistant to nonspecific protein adsorption, even from undiluted blood plasma and serum.[20–24] Although zwitterionic coatings can reduce the initial attachment and delay colonization of microbes on surfaces, there is a possibility of introducing pathogenic microbes into the patient during implantation operations and catheter insertions, which results in the failure of implanted devices; the use of antimicrobial agents will then be necessary to eliminate these microbes. Surface-responsive materials have been developed for a broad spectrum of applications,[25] but it is still a great challenge to develop biocompatible materials that have both antimicrobial and nonfouling capabilities. To the best of our knowledge, no such materials have been reported to date.

Herein we report a new switchable polymer surface coating, which combines the advantages of both nonfouling and cationic antimicrobial materials and overcomes their disadvantages (Figure 1). In this system, poly(*N*,*N*-dimethyl-*N*-(ethoxycarbonylmethyl)-*N*-[2′-(methacryloyloxy)ethyl]-ammonium bromide) (pCBMA-1 C2, cationic precursor) on a surface can kill greater than 99.9% of *Escherichia coli* K12 in one hour, and 98% of the dead bacterial cells can be released when the cationic derivatives are hydrolyzed to nonfouling zwitterionic polymers. pCBMA-1 C2 and control coatings were grafted by surface-initiated atom transfer radical polymerization (ATRP) onto a gold surface covered with initiators. The thicknesses of the obtained polymer coatings, as measured by atomic force microscopy (AFM),[26] were 26–32 nm (Table 1).

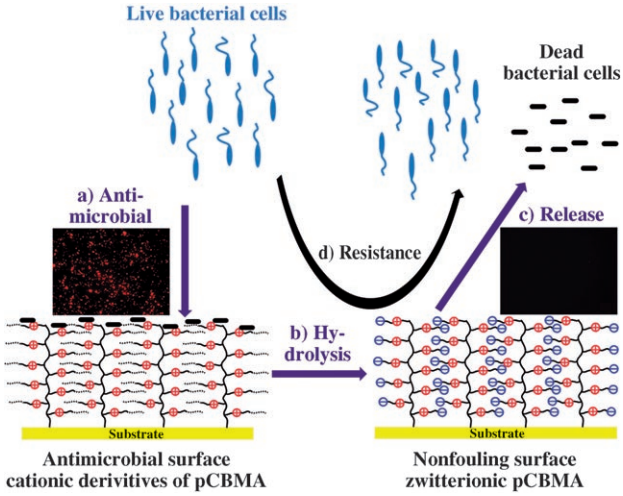

Figure 1. A surface that switches from an antibacterial surface to a nonfouling surface upon hydrolysis. a) Antimicrobial cationic pCBMA-1 C2 effectively kills bacteria, b) pCBMA-1 C2 is converted into nonfouling zwitterionic pCBMA-1 upon hydrolysis, and c) dead bacteria remaining on the surface are released from nonfouling zwitterionic pCBMA-1. d) Zwitterionic pCBMA-1 itself is highly resistant to bacterial adhesion.

The bactericidal activity of pCBMA-1 C2 surfaces was determined using *E. coli* K12, according to a modified literature procedure.[2] The permanently cationic poly(methacryloyloxyethyldimethyloctylammonium bromide) (pC8NMA, cationic control) and the zwitterionic poly(2-carboxy-*N*,*N*-dimethyl-*N*-[2′-(methacryloyloxy)ethyl]ethanaminium) (pCBMA-2, zwitterionic control) were used as the positive and the negative control surfaces, respectively (Scheme 1). The antimicrobial efficiency was defined as the amount of live cells on the tested surfaces relative to those on the pCBMA-2 surface. Figure 2 shows that pCBMA-1 C2 and

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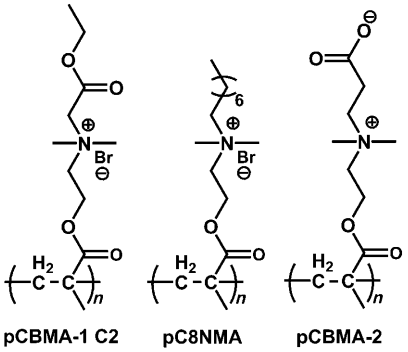
Table 1: Film thicknesses (av ± std dev.) of pCBMA-1 C2, pC8NMA, and pCBMA-2 grafted onto gold-coated glass slides by ATRP and fibrinogen adsorption on these surfaces measured by SPR before and after hydrolysis under different conditions.

	pCBMA-1 C2	pC8NMA	pCBMA-2
polymer brush thickness [nm]	(31.2±2.4)	(27.8±2.8)	(26.1±2.5)
	protein adsorption [ng cm^{-2}]		
0 h	229.2	243.4	1.5
24 h H_20	189.9	–	–
24 h CHES [pH 9.0]	114.9	–	–
24 h CAPS [pH 10.0]	0	285.1	0.7



Scheme 1. Chemical structures of switchable pCBMA-1 C2, antimicrobial cationic pC8NMA, and nonfouling zwitterionic pCBMA-2.

pC8NMA surfaces kill greater than 99.9% and 99.6%, respectively, of the *E. coli* in one hour relative to pCBMA-2 surfaces. The total number of live bacterial cells on the gold surface, which was also used as a negative-control surface, is similar to that on the pCBMA-2 surface.

The attachment and release of *E. coli* K12 were tested on the pCBMA-1 C2 surfaces before and after hydrolysis. Cationic pC8NMA and zwitterionic pCBMA-2 were used as the negative and the positive nonfouling control surfaces, respectively, and as the positive and the negative antimicrobial control surfaces, respectively. Figure 3 shows that a large amount of bacteria were attached to the cationic pCBMA-1 C2 and pC8NMA surfaces before hydrolysis, whereas very few bacterial cells were attached to the zwitterionic pCBMA-2 surface. In contrast to pC8NMA, pCBMA-1 C2 released the majority of cells after hydrolysis while pCBMA-2 remained nonfouling. Figure 4 shows quantitative data for the amount of bacterial cells remaining on all three polymer surfaces before and after hydrolysis. There were similar amounts of bacterial residues on both cationic pCBMA-1 C2 and

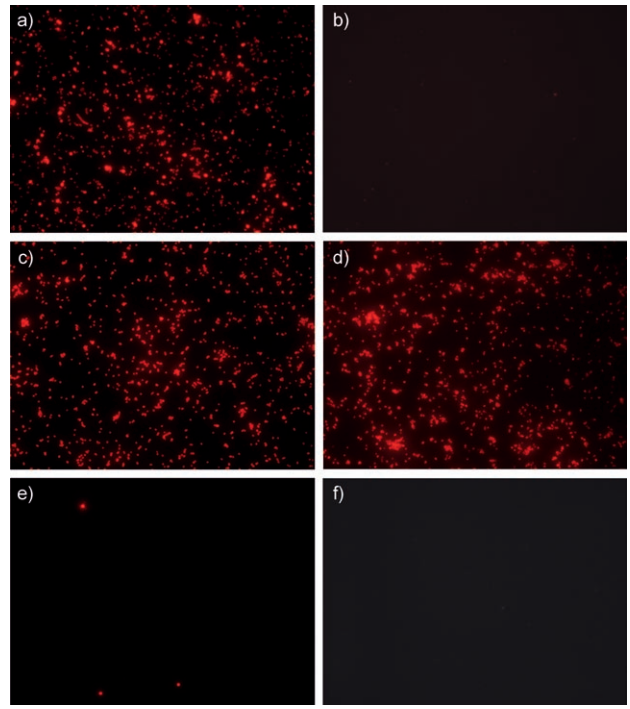


Figure 3. Representative fluorescence microscopy images of attached *E. coli* K12 cells (red) from a suspension of 10^{10} cells mL^{-1} after exposure to the surfaces covered with various polymers for one hour. Images (a), (c), and (e) show surfaces pCBMA-1 C2, pC8NMA, and pCBMA-2, respectively, before hydrolysis; images (b), (d), and (f) show the same systems after hydrolysis for eight days with CAPS (10 mM, pH 10.0).

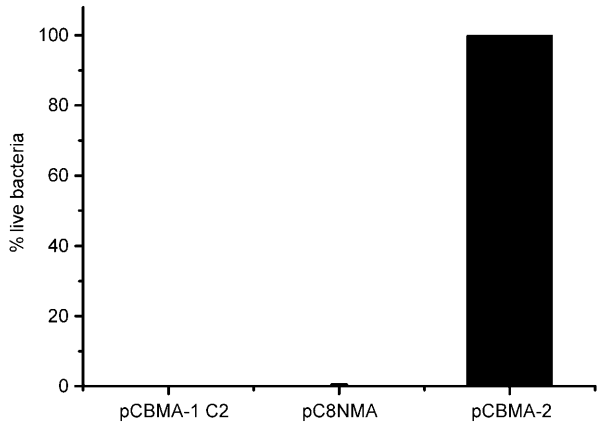


Figure 2. Bactericidal activity of pCBMA-1 C2 and pC8NMA against *E. coli* K12. The percentage of live *E. coli* K12 colonies growing on the surfaces coated with antimicrobial polymers is calculated relative to the number of colonies growing on the pCBMA-2 control ($n = 3$).

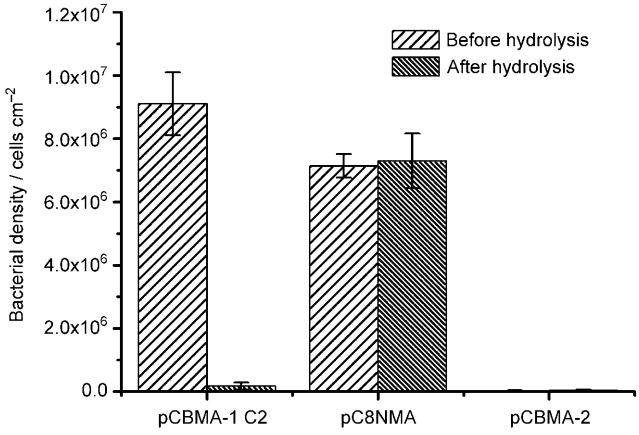


Figure 4. Bacterial cell density of *E. coli* K12 from a suspension of 10^{10} cells mL^{-1} after exposure to pCBMA-1 C2, pC8NMA, and pCBMA-2 for one hour, before and after hydrolysis ($n = 3$).

pC8NMA surfaces before hydrolysis, while the amount of attached cells on the pCBMA-2 surface is less than 0.3% of that on both cationic pCBMA-1 C2 and pC8NMA surfaces. To test the release of bacterial residues, the three surfaces were incubated in *N*-cyclohexyl-3-aminopropanesulfonic acid (CAPS) buffer (10 mM, pH 10.0) at 37°C for 8 days. The pCBMA-1 C2 surfaces were hydrolyzed to poly(*N*-(carboxymethyl)-*N*,*N*-dimethyl-2-[(2-methyl-1-oxo-2-propen-1-yl)-oxy]-ethanaminium) (pCBMA-1) and 98% of the dead bacterial cells were released. In contrast, no release of the dead cells was observed on pC8NMA surfaces ($p > 0.1$) while pCBMA-2 surfaces retained very low bacterial adhesion.

The release of the attached bacterial cells is dependent on the conversion of cationic pCBMA-1 C2 into zwitterionic pCBMA-1. It is known that the hydrolysis rate of betaine esters is influenced by several factors, such as the length of the spacer between the quaternary amine and the carboxyl groups,[27] the nature of the hydrolyzable group, temperature,[28] and pH value. The majority of polymer chains of the ester group used in this work were hydrolyzed. The hydrolysis rate of the betaine esters is also slower after bacterial cells and proteins are attached to the surface.[28,29] In this work, pCBMA-1 C2, which has one methylene spacer, was chosen and the experimental temperature was set at 37°C to achieve a fast hydrolysis rate and to provide a physiologically relevant temperature. The protein adsorption study (Table 1) showed that the clean, cationic pCBMA-1 C2 surface was hydrolyzed into a nonfouling zwitterionic surface after only 24 h at 37°C and pH 10.0, while it took 48 h to form a nonfouling surface and release bacterial residues after the attachment of bacteria from an *E. coli* K12 suspension of 10^7 cells mL^{-1}. When bacterial cells were attached to the pCBMA-1 C2 surface from a suspension of 10^{10} cells mL^{-1}, the release of attached bacteria took eight days under the same hydrolysis conditions.

Nonspecific protein adsorption on various surfaces was measured by a surface plasmon resonance (SPR) sensor to determine the nonfouling characteristics of the surfaces (Table 1). Hydrolysis conditions for pCBMA-1 C2 and control surfaces were investigated in situ in the SPR sensor. Figure 5a,b shows representative SPR sensorgrams for fibrinogen adsorption on pCBMA-1 C2 and control surfaces over time. The fibrinogen adsorption on pCBMA-1 C2 before hydrolysis was 229.2 ng cm^{-2}. After 24 h of incubation with CAPS buffer (pH 10.0), there was no measurable protein adsorption on the pCBMA-1 C2 surface, which indicated that pCBMA-1 C2 was completely hydrolyzed to nonfouling zwitterionic pCBMA-1. In contrast, hydrolysis of pCBMA-1 C2 was not complete after 24 h incubation in either H_2O or *N*-cyclohexyl-2-aminoethanesulfonic acid (CEHS) buffer (pH 9.0). As shown in Figure 5b, high fibrinogen adsorption was observed on the pC8NMA surface before and after the surface was incubated with CAPS buffer (pH 10.0) for 24 h at 37°C. However, under identical conditions, the pCBMA-2 surface still exhibited excellent nonfouling properties, with less than 2 ng cm^{-2} fibrinogen absorption. This result indicates that the obtained zwitterionic surfaces are highly resistant to protein adsorption and are qualified as ultralow fouling surfaces,[30] which are required for the surface coatings of implantable medical devices.

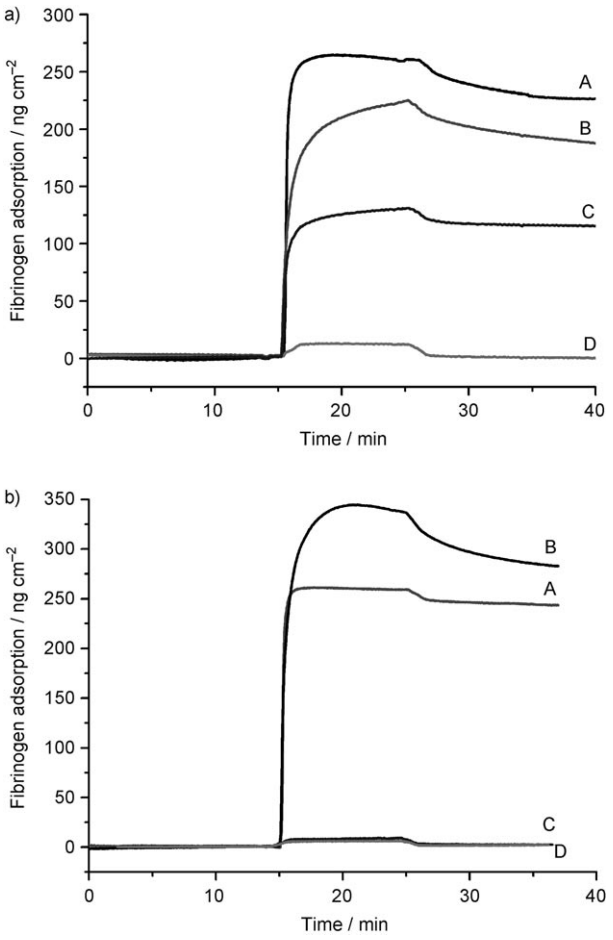


Figure 5. a) Representative SPR sensorgrams showing the adsorption of fibrinogen (1 mg mL^{-1}) in PBS buffer on the surfaces grafted with pCBMA-1 C2 by ATRP A) before hydrolysis and after hydrolysis for 24 h with B) H_2O, C) CEHS (10 mM, pH 9.0), and D) CAPS (10 mM, pH 10.0). b) Representative SPR sensorgrams showing the adsorption of 1 mg mL^{-1} fibrinogen in PBS buffer on the surfaces grafted with pC8NMA A) before and B) after incubation for 24 h with CAPS (10 mM, pH 10.0), and on surfaces grafted with pCBMA-2 C) before hydrolysis and D) after hydrolysis for 24 h with CAPS (10 mM, pH 10.0).

In conclusion, a novel switchable polymer surface that integrates antimicrobial and nonfouling properties and is biocompatible has been presented. 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. Moreover, the resulting nonfouling zwitterionic surface can further prevent the attachment of proteins and microorganisms and reduce the formation of a biofilm on the surface. The switchable process from antimicrobial to nonfouling surfaces can be finely tuned through adjusting the hydrolysis rate of these polymers for specific requirements of applications. This new switchable polymer surface coating has great potential for applications in the field of implantable medical devices.

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