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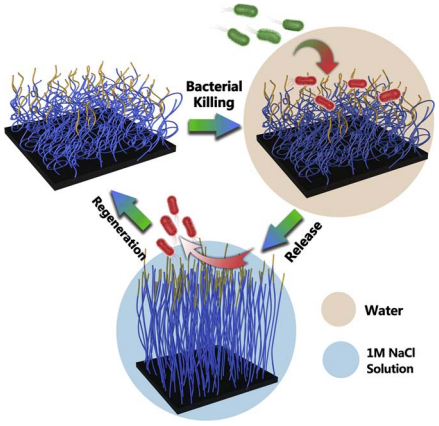
Bacteria killing and release of salt-responsive, regenerative, double-layered polyzwitterionic brushes

Lei Huang[a], Lixun Zhang[a], Shengwei Xiao[a], Yin Yang[a], Feng Chen[a], Ping Fan[a], Zhenping Zhao[b], Mingqiang Zhong[a], Jintao Yang[a,*]

[a] College of Materials Science & Engineering, Zhejiang University of Technology, Hangzhou 310014, PR China
[b] Zhijiang College, Zhejiang University of Technology, Hangzhou 310014, PR China

GRAPHICAL ABSTRACT



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ABSTRACT

Antibacterial surfaces that not only can kill bacteria but also can release the dead bacteria have become one of most promising antibacterial strategies, but the realization of high efficiency, rapid response and long-term reusability of such surfaces remains many challenges. Herein, we reported new antibacterial surfaces with high killing and release capabilities based on a salt-responsive brush recently developed by our group. These surfaces consist of two polymer brushes with two-layer architecture, where the upper-layer bactericidal brushes (poly [(trimethylamino) ethyl methacrylate chloride], polyMETAC or poly[2-(tert-butylamino)ethyl methacrylate], polyTA) were built on background layer of salt-responsive polyzwitterionic brush (poly(3-(dimethyl(4-vinylbenzyl) ammonio) propyl sulfonate), polyDVBAPS) via sequential surface initiated atom transfer radical polymerization. By the combination in this manner, the bacteria attach/release function of polyDVBAPS and bactericidal function of polyMETAC/polyTA were successfully integrated, resulting in the smart surfaces which can reversibly kill and release bacteria in response to the switch between water and salt solution. Both two systems in this study, i.e. poly (DVBAPS-b-METAC) and poly(DVBAPS-b-TA), exhibit high bactericidal activity by killing more than 93% and highly efficient regeneration capability by rapidly releasing ~90% of the attached bacteria. Specifically, such excellent antibacterial performance of poly(DVBAPS-b-TA) can be well retained even after four killing-release cycles, indicating the great potential in reusable biological materials and devices.

* Corresponding author.
E-mail address: yangjt@zjut.edu.cn (J. Yang).

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Furthermore, the correlation between physicochemical properties of the two-layer brushes with their bactericidal efficiency and regenerative capability was also investigated and discussed.

1. Introduction

Bacteria adhesion and proliferation on the surface is a common phenomenon in many fields, such as pharmaceutical, food packaging, and biomedical devices/implants [1–4]. This phenomenon which is also called as bacterial infection in biological community not only causes serious property loss, but also leads to enormous health consequences and even causes numerous deaths [5]. According to statistics, just in USA, more than 2 million patients are suffering from various kinds of nosocomial infections each year, resulting in more than 90,000 deaths, and adding an estimated $4.5–$5.7 billion of extra patient care [6,7]. Therefore, bacterial attachment prevention and infection control are critical for people's health and life safety.

In order to efficiently prevent the attachment of bacteria, formation of biofilm, and subsequent proliferation, many strategies have been developed, which can be categorized into three types: contact-killing, anti-adhesion/bacteria repelling, and killing-release. Contact-killing surfaces are functionalized with bactericide via covalent bonding, physical absorption or other coordination ways [8–10]. This surface can kill the attached bacteria by breaking the cell membrane of bacteria via penetration or charge disruption [11]. However, the accumulation of dead bacteria and debris will deactivate the bactericides [12,13]. Bacteria repelling surfaces, as another commonly used antibacterial strategy, can efficiently prevent bacteria adhesion by establishing steric repulsion through surface hydration or charge repelling [14–17]. Whereas, when these surfaces are employed for long-term applications, the eventual attachment of bacteria will deteriorate their repellent capacity and lead to the failure of function, albeit bactericides are sometimes included [18–20]. Killing-release is a newly developed antibacterial strategy [21–23]. As compared to aforementioned two strategies, this strategy realizes the reversible switch between functions of bacteria killing and dead bacteria release, which in turn induce the surface regeneration and result in significant advantages for long-term application [24]. To fabricate the smart surface of "killing-release" strategy, stimuli-responsive surfaces which show chemical or physical change in respond to environment change are usually used as platforms [25–28]. The first bacteria killing-release surface reported by Jiang's group was achieved by the reversible switch between cationic ring structure (bacteria killing) to zwitterionic structure (dead bacteria release) in response to change in pH [22]. Then, utilizations of other responsive polymers such as polyNIPAM, PMAA coordinating with bactericides to fabricate killing-release surfaces were reported [26,27]. These surfaces show highly efficient bacteria killing and release capability, but the function is usually triggered by the change of temperature or pH, limiting their applications to some extent. Recently, traditional antibacterial surfaces, i.e. polycationic brushes, were intelligently developed as killing-release surfaces by Chang's group [29]. In this system, the specific interaction between polycationic brushes and their counterions was used to modulate surface properties (surface hydration and charge characteristic), inducing the switching of surface functions between bacteria killing and release. This surface performs the functions effectively at a mild condition, but both bacteria release and antibacterial surface regeneration are realized by ion exchange, which is a time-consuming and complicate process. Thus, there is an urgent need for the development of killing-release surface with high efficiency, rapidity, and responsive sensitivity.

Zwitterionic polymers, in which cation and anion are in one molecule, have been one of most important polymers for antifouling and other biological purpose due to their high surface hydration capability and biocompatibility. Most zwitterionic polymers, such as polyCBMA, polySBMA, and polyMPC are highly hydrophilic, and presently used to prevent the fouling of non-specific protein, bacteria, and cells [30–33]. They also have been employed to integrate with bactericides for the fabrication of the surfaces with both antifouling and antibacterial properties [34,35]. However, the inevitably attached bacteria may lead to the failure of the functions, and additionally, the surfaces with these zwitterionic polymers cannot be regenerated and reused after the contamination, making them may inapplicable in some applications. Recently, by utilizing a kind of newly designed zwitterionic monomers (using vinylbenzyl as polymerizable group), we developed a salt-responsive polyzwitterionic surface which shows reversible change in surface characteristics in response to the switch between water and salt solutions. Differing from the traditional polyzwitterionic surfaces, although this surface absorbs bacteria in water, the attached bacteria can be efficiently removed by the treatment of salt solution. More importantly, such regeneration process shows highly reliable circulation [36]. The bacteria adhesion and release test indicated that this surface can absorb a large amount of bacteria in water and can release ~99% adhered bacteria by soaking in 1.0 M NaCl solution for 10 min, and two absorption/desorption cycles show the consistent bacteria release efficiency. In this work, such salt-responsive surface was further developed into bacteria killing-release surface by coordinating with bactericides, in which bactericidal polycationic and polyamine were covalently layered on salt-responsive polymer brushes by sequential surface initiated atom transfer radical polymerization (SI-ATRP). Physiochemical properties of this two-layer brush system, including thickness, surface morphology, composition, and charge characteristic were characterized by ellipsometer, atom force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), and ζ-potential measurements. *Escherichia coli* (*E. coli*, Gram-negative) and *Staphylococcus aureus* (*S. aureus*, Gram-positive) were used to challenge the surfaces to evaluate bactericidal effectiveness, and the surface with attached bacteria was soaked in 1.0 M NaCl to evaluate the bacteria release capability. Four cycles of such contact-killing and release process were used to examine the reliability of circulation. The relationship between the physiochemical properties and antibacterial effectiveness as well as surface regeneration was further discussed. Our study provides a new bacteria killing-release surface, and its mild-stimuli response, high efficiency, and environmental affinity show great potential in both biological-related and industrial applications.

2. Materials and methods

2.1. Materials

4-Vinlybenzyl chloride (VBC, 90%), dimethylamine solution (DMA, 40 wt% in H_2O), 1,3-propane sultone (PS, 98%), [2-(methacryloyloxy) ethyl]trimethylammonium chloride solution (METAC, 80 wt% in H_2O), 2-(tert-butylamino)ethyl methacrylate (TA, 97%), 2,2′-bipyridyl (bipy, 98%), N,N,N′,N″,N″-pentamethyldiethylenetriamine (PMDETA, 99%) and copper(I) bromide (CuBr, 98%), were purchased from Sigma-Aldrich Co. (Shanghai, China). Tris[2-(dimethylamino)-ethyl]amine (Me6TREN, 99%) was purchased from Tokyo Chemical Inc. (TCI). Methanol, 2,2,2-trifluoroethanol and isopropanol, as ATRP solvents, were purchased from Aladdin Co. (Shanghai, China). ATRP initiator that can covalently grafted on silica wafer, i.e., 3-(2-bromoisobutyramido) propyl-(trimethoxy)silane, was purchased from Gelest, Inc. (Morrisville, PA). Water used in these experiments was obtained from a Milli-Q water purification system. All other reagents and solvents were commercially obtained at extra-pure grade and were used as received.