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Surface Modified with a Host Defense Peptide-Mimicking β-Peptide Polymer Kills Bacteria on Contact with High Efficacy

Yuxin Qian,[†,‡,§,∥] Fan Qi,[†,‡,§,∥] Qi Chen,[†,‡,§,∥] Qiang Zhang,[†,‡,§,∥] Zhongqian Qiao,[†,‡,§,∥] Si Zhang,[†,‡,§,∥] Ting Wei,[⊥] Qian Yu,[⊥] Shan Yu,[#] Zhengwei Mao,[#] Changyou Gao,[#] Yiru Ding,[††] Yanyong Cheng,[¶] Chenyu Jin,[¶] Hexin Xie,[†,∇,○] and Runhui Liu*[,†,‡,§,∥]

[†]State Key Laboratory of Bioreactor Engineering, [‡]Key Laboratory for Ultrafine Materials of Ministry of Education, [§]Research Center for Biomedical Materials of Ministry of Education, and [∥]School of Materials Science and Technology, [∇]Shanghai Key Laboratory of New Drug Design and [○]School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China

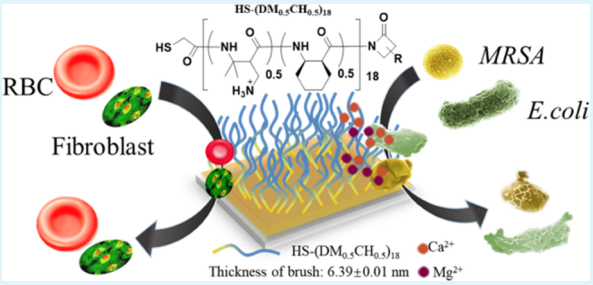
[⊥]State and Local Joint Engineering Laboratory for Novel Functional Polymeric Materials, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, China

[#]MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, China

[¶]Department of Anesthesiology, Shanghai Ninth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200011, China

Supporting Information

ABSTRACT: Methicillin-resistant *Staphylococcus aureus* (MRSA) has been one of the major nosocomial pathogens to cause frequent and serious infections that are associated with various biomedical surfaces. This study demonstrated that surface modified with host defense peptide-mimicking β-peptide polymer, has surprisingly high bactericidal activities against *Escherichia coli* (*E. coli*) and MRSA. As surface-tethered β-peptide polymers cannot move freely to adopt the collaborative interactions with bacterial membrane and are too short to penetrate the cell envelop, we proposed a mode of action by diffusing away the cell membrane-stabilizing divalent ions, Ca^{2+} and Mg^{2+}. This hypothesis was supported by our study that Ca^{2+} and Mg^{2+} supplementation in the assay medium causes up to 80% loss of bacterial killing efficacy and that the addition of divalent ion chelating ethylenediaminetetraacetic acid into the above assay medium leads to significant recovery of the bacterial killing efficacy. In addition to its potent bacterial killing efficacy, the surface-tethered β-peptide polymer also demonstrated excellent biocompatibility by displaying no hemolysis and supporting mammalian cell adhesion and growth. In conclusion, this study demonstrated the potential of β-peptide polymer-modified surface in addressing nosocomial infections that are associated with various surfaces in biomedical applications.



KEYWORDS: *β-peptide polymer, graft to, antimicrobial surface, divalent ion, membrane destabilization, biocompatible surface, antimicrobial resistance, MRSA*

INTRODUCTION

Hospital-acquired microbial infections are grand challenges to human health. A large proportion of norsocomial infections are associated with the surfaces of facilities, surgical tools, and implanted biomaterials and devices. To prevent such infections, antibiotics, amine (majorly quaternary ammonium)-containing small molecules and polymers, and silver and singlet oxygen-generating compounds have been used or studied either in solution or as surface coatings.[1−28] The quick emergence of antimicrobial drug-resistant bacteria implies far less and even no available antibiotics for effective treatment.[29] Quaternary ammonium antimicrobials have been extensively used over years as efficient antimicrobial agents but are limited to topical applications because of the cytotoxicity of quaternary ammonium groups in general. Therefore, huge amount of efforts have been devoted to reduce the cytotoxicity by introducing biocompatible poly(ethylene glycol) and polysaccharides into the structures of quaternary ammonium antimicrobial agents.[2,30]

To address the antimicrobial resistance challenge, especially for conventional antibiotics, host defense peptides (HDPs) and their polymeric mimics were actively studied.[30−44] Biocompatible β-peptide polymers have also been explored as membrane-active mimics of HDPs to display low cytotoxicity and potent activities against drug-resistant microbes.[45−52] However, up to date, the potent antimicrobial activity of β-peptide polymers is

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only demonstrated in solution. Mechanism study indicated the translocation of β-peptide polymers through the outer membrane of *Escherichia coli* (*E. coli*), followed by disruption of the inner membrane to kill the bacteria.[53] Obviously, surface-tethered β-peptide polymers are unable to move away from the substrate and will not follow the above-mentioned antimicrobial mechanism. Therefore, it is unknown whether surface-tethered β-peptide polymers can also display desired antimicrobial activity. The contribution of this study is we demonstrated, for the first time, that surface-tethered HDP mimicking β-peptide polymers have excellent biocompatibility and potent antimicrobial activity against both Gram -negative *E. coli* and Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA). This demonstration implies a broad application of these types of polymers in antimicrobial surfaces, especially against drug-resistant super bugs.

RESULTS AND DISCUSSION

To explore the antibacterial property of the β-peptide polymer-modified surface, a thiol-terminated 18 mer heterochiral β-peptide polymer, 1:1 DM-CH, was synthesized and covalently attached to the gold surface using well-defined Au−thiol chemistry (Figure 1).[54] A C-terminal cysteine-modified

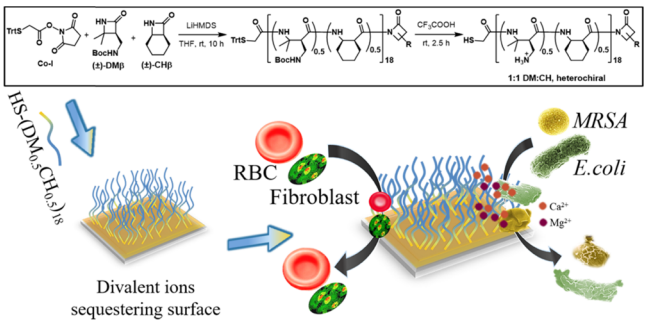

Figure 1. Preparation of the antimicrobial β-peptide polymer and polymer-modified surface.

magainin 2 peptide was used as a comparison in this study for several considerations. First, magainin 2 is a representative and extensively studied HDP.[31] Second, magainin has 24 amino acid residues that give a chain length close to that of the 18 mer β-peptide polymer. Finally, the cysteine residue was placed at the C-termini of the magainin chain to present a higher density of positive charges at the end away from the thiol group. This design was carefully chosen to have a close comparison between magainin and the polymer because this β-peptide polymer has slightly higher density of positive charge at the end away from the thiol-functionalized termini due to the slightly biased subunit distribution along the polymer chain.[55] To obtain antimicrobial surfaces with a high grafting density, a large excess of the thiol-terminated polymer or magainin was used to modify the gold surface.[54,56,57] The X-ray photoelectron spectroscopy spectrum of the β-peptide polymer and magainin-modified Au surface showed signals of C, N, and O from the β-peptide polymer or magainin (Figure S1). Ellipsometry characterization on a dry sample indicated an average polymer thickness of 2.22 ± 0.05 nm. Because the interactions between the bacteria and polymer-modified surface are within the solution, we also did ellipsometry characterization on a wet surface in solution and obtained an average polymer thickness of 6.39 ± 0.01 nm and a surface polymer density of 1.17 chain/nm^2 (see Supporting Information). Although this characterization in the wet state is technically more challenging, the data provides critical information of surface-tethered polymer chains at the condition where they interact with bacterial cells. The characterization on wet sample underpins the argument of our proposed mode of action for the polymer-modified antimicrobial surface.

The antibacterial activities of the β-peptide polymer-modified surface were evaluated by incubating the bacterial suspension with the surface and examining the killing efficacy against bacteria.[58] As summarized in Figure 2, surfaces modified with magainin and β-peptide polymer both effectively killed Gram-negative *E. coli*, with β-peptide polymer-modified surface performing better and having a complete killing of *E. coli*. For Gram-positive MRSA, a super bug, it is noteworthy and surprising to find that the β-peptide polymer-modified surface kills MRSA completely though the magainin-modified surface has almost no activity. A separate experiment examined the supernatant that was incubated with the β-peptide polymer-modified surface for different times and found no bacterial killing effect at all (Figure S2). This result supported that the strong bactericidal activity observed above was truly from the surface killing on contact.

The bacterial cell morphology before and after cell incubation with the β-peptide polymer-modified surface was characterized by field emission scanning electron microscopy (SEM) as shown in Figure 3. Both *E. coli* and MRSA cells in the control groups have intact and smooth cell membrane. After bacterial cells were incubated with the β-peptide polymer-modified surface, rough cell membrane and even total disruption of cell membrane were observed.

The possible hemolytic effect of β-peptide polymer- and magainin-modified surfaces toward human red blood cell

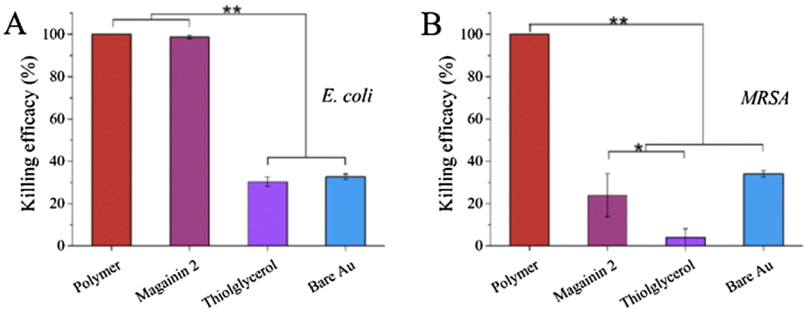

Figure 2. Antibacterial activity of the β-peptide polymer-modified surface. Thioglycerol-modified surface and bare gold surface were used as controls. (A) *E. coli* and (B) MRSA. $^{**}p < 0.01$ and $^{*}p < 0.05$.

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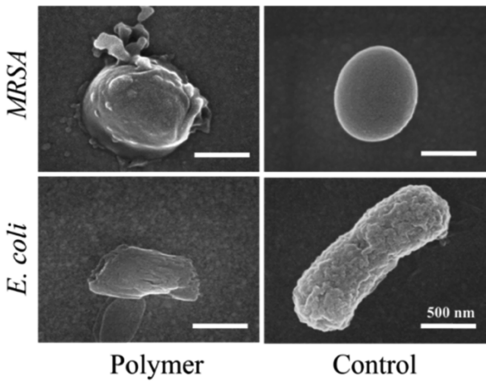

Figure 3. SEM characterization of bacterial cells before and after incubation with the β-peptide polymer-modified surface.

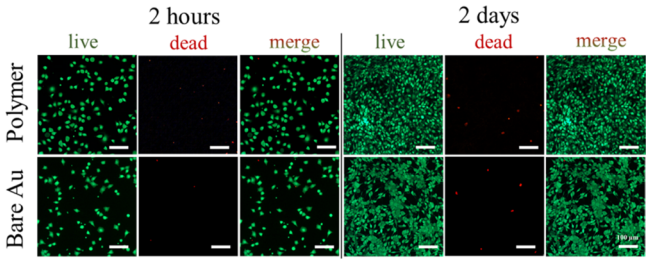

Figure 5. Characterization of mammalian cell adhesion and culture on the β-peptide polymer-modified surface using live/dead staining. The images were taken after cells were seeded on the surface for 2 h and 2 days, respectively, with live cells stained with green fluorescence and dead cells stained with red fluorescence.

(hRBC) was also examined (Figure 4a). The magainin-modified surface showed a moderate (about 7%) hemolytic effect, but the β-peptide polymer-modified surface displayed no observable hemolytic effect. SEM characterization (Figure 4b) showed that hRBCs still retain healthy cell morphology after incubation with β-peptide polymer-modified surface.

The biocompatibility of the β-peptide polymer-modified surface was examined using NIH 3T3 fibroblast cells. The cells displayed normal adhesion and spreading on the β-peptide polymer-modified surface 2 h post cell seeding. After 2 day culture, the cell number increased significantly and live cells covered the whole surface evenly as demonstrated using live/dead staining (Figure 5).

Previous mode-of-action study on antimicrobial β-peptide polymer indicated that the free polymer in solution targets on the inner membrane of *E. coli* and that the polymer passed through the outer membrane quite easily without causing leakage or damage on the outer membrane.[53] On the basis of this, it is surprising to find that the surface-tethered β-peptide polymer also kills bacteria in high efficacy because the β-peptide polymer in this case is unable to move away from the surface and thus unable to follow the antimicrobial mechanism as a free antimicrobial agent in solution. According to the preceding literature, surface-tethered polymers may kill bacteria via two distinct mode of actions: penetrating the bacterial envelop to induce the leaking of cytoplasmic contents or depriving Ca^{2+} and Mg^{2+} ions from the bacterial membrane to destabilize the membrane.[59,60] The cell envelop is about 46 and 40 nm, respectively, for Gram-negative *E. coli* and Gram-positive *S. aureus*,[61,62] which means that surface-tethered polymer chains should be at least longer than the aforementioned bacterial envelop to follow the cell penetration mode of action.[59,60,63] Considering that the 18 mer β-peptide polymer (1:1 DM-CH) only has a length of 6.39 nm when tethered to the Au surface, the polymer is too short to penetrate the cell envelop. Alternatively, we hypothesized that the highly charged surface could deprive divalent ions, majorly Ca^{2+} and Mg^{2+}, which are important for stabilizing the bacterial cell membrane as studied or reviewed in the precedent literature.[11,63−67] To examine this hypothesis, 20 mM Ca^{2+} or 35 mM Mg^{2+} was supplemented, respectively, into the medium for bactericidal test to quench or block the ability of the polymer surface to diffuse divalent ions away from the bacterial cell membrane. As summarized in Figure 6a, a profound reduction (up to over 80% reduction) in the bactericidal efficacy was observed for both *E. coli* and MRSA when divalent ions were added to the medium, with *E. coli* being more sensitive to Ca^{2+} and MRSA being more sensitive to Mg^{2+}. In a further examination, as summarized in Figure 6b, divalent ion chelating agent ethylenediaminetetraacetic acid (EDTA) tetrasodium salt was added into the medium of the above conditions at a final concentration of 0.9 mM. At such an EDTA concentration in the presence of preadded Ca^{2+} or Mg^{2+} ions, no direct damage of bacterial cells by EDTA was observed. After addition of EDTA, a significant increase of bacterial killing efficacy was observed in all conditions. It is noteworthy that the addition of EDTA had stronger effect on *E. coli* in the Ca^{2+} supplemental condition and stronger effect on MRSA in the Mg^{2+} supplemental condition, which was consistent with the above observation that *E. coli* was more sensitive to Ca^{2+} and MRSA was more sensitive to Mg^{2+}. The partial regain of bacterial killing efficacy upon addition of EDTA into the

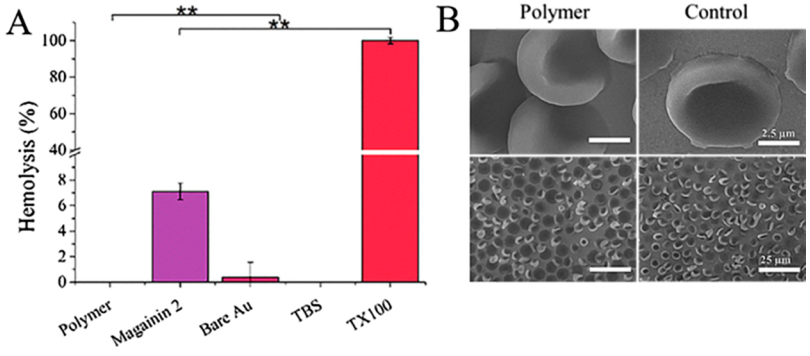

Figure 4. Hemolytic study. (A) Hemolysis of the β-peptide polymer-modified surface toward hRBC. $^{**}p < 0.01$. (B) SEM characterization of hRBCs before and after incubation with the β-peptide polymer-modified surface.

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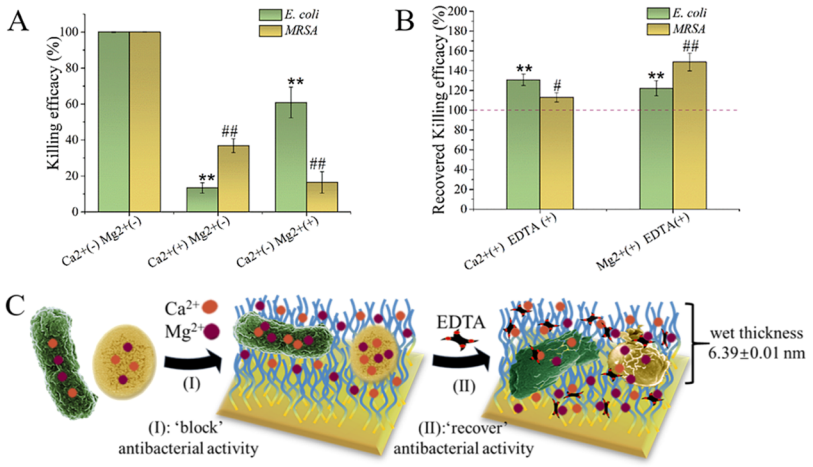

Figure 6. Study of the interactions between bacteria and β-peptide polymer-modified surface: (A) reduction of killing efficacy for surface-tethered polymer upon supplementation of Ca^{2+} or Mg^{2+} ions; (B) recovered killing efficacy upon addition of EDTA to the system, with EDTA free condition normalized to 100%; and (C) cartoon to illustrate the reversible process of bacterial killing by surface tethered β-peptide polymer. $^{**}p < 0.01$ and $^{\#\#}p < 0.01$ compared to no divalent ion supplementation condition in (A); $^{**}p < 0.01$, $^{\#\#}p < 0.01$ and $^{\#}p < 0.05$ compared to the condition with Ca^{2+} or Mg^{2+} supplementation but without EDTA in (B).

medium could come from the deprival of Ca^{2+} and Mg^{2+} by EDTA to partially recycle the divalent ion diffusion capability of the surface-tethered β-peptide polymer. These results altogether supported that divalent ion diffusion away from the bacterial membrane by the surface-tethered β-peptide polymer played a critical role in bacterial killing on contact within this study. Our finding that this antimicrobial surface likely kills bacteria by depriving Ca^{2+} and Mg^{2+} ions is reasonable because a similar effect was proposed in the precedent literature that EDTA and antimicrobial peptides and polymers in solution and surface-tethered polymers can destabilize the bacterial membrane by depleting Ca^{2+} and Mg^{2+} from the membrane.[11,12,59,60,63,65−67] Moreover, in the process when this β-peptide polymer-modified surface deprives Ca^{2+} and Mg^{2+} ions from the bacterial cell membrane, surface-tethered β-peptide polymers may also interact with the membrane and result in negative curvature on the membrane as discussed in the precedent literature.[64,68−70]

CONCLUSIONS

In conclusion, we have established the foundation of an alternative and important application for the HDP-mimicking β-peptide polymer as effective bactericidal surface modification. The excellent bactericidal activity on contact against both Gram-negative *E. coli* and Gram-positive MRSA, excellent biocompatibility, structural diversity, and easy preparation altogether imply potentially broad applications of β-peptide polymers in antimicrobial surface modification for self-sterilizing surface of biomaterials, surgical devices, and biosensors. The short length of the polymer layer (6.39 nm) rules out the possible membrane penetration mechanism. However, the divalent ions and subsequent EDTA supplementation tests support the hypothesis that the surface-tethered β-peptide polymer kills bacteria on contact by diffusing divalent ions away from the bacterial membrane.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.8b01117.

Bioassay, compound and surface preparation, and characterization (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: rliu@ecust.edu.cn.

ORCID

Qian Yu: 0000-0003-3612-6951

Changyou Gao: 0000-0001-5084-7208

Hexin Xie: 0000-0003-1033-9211

Runhui Liu: 0000-0002-7699-086X

Present Address

[††]Summer visiting student from School of Pharmacy, China Pharmaceutical University, Nanjing 210009, China (Y.D.)

Notes

The authors declare no competing financial interest.

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