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Antimicrobial functionalization of silicone surfaces with engineered short peptides having broad spectrum antimicrobial and salt-resistant properties

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ABSTRACT

Catheter-associated urinary tract infections (CAUTIs) are often preceded by pathogen colonization on catheter surfaces and are a major health threat facing hospitals worldwide. Antimicrobial peptides (AMPs) are a class of new antibiotics that hold promise in curbing CAUTIs caused by antibiotic-resistant pathogens. This study aims to systematically evaluate the feasibility of immobilizing two newly engineered arginine/lysine/tryptophan-rich AMPs with broad antimicrobial spectra and salt-tolerant properties on silicone surfaces to address CAUTIs. The peptides were successfully immobilized on polydimethylsiloxane and urinary catheter surfaces via an allyl glycidyl ether (AGE) polymer brush interlayer, as confirmed by X-ray photoelectron spectroscopy and water contact angle analyses. The peptide-coated silicone surfaces exhibited excellent microbial killing activity towards bacteria and fungi in urine and in phosphate-buffered saline. Although both the soluble and immobilized peptides demonstrated membrane disruption capabilities, the latter showed a slower rate of kill, presumably due to reduced diffusivity and flexibility resulting from conjugation to the polymer brush. The synergistic effects of the AGE polymer brush and AMPs prevented biofilm formation by repelling cell adhesion. The peptide-coated surface showed no toxicity towards smooth muscle cells. The findings of this study clearly indicate the potential for the development of AMP-based coating platforms to prevent CAUTIs.

1. Introduction

Infections associated with biomedical implants and devices are increasingly common and currently pose a major clinical threat that demands an urgent solution [1–3]. Such infections cause high morbidity and economic burden globally. In the United States alone, infections associated with cardiovascular and urological implants are estimated to cost up to $50,000 per patient and $250 million per year to treat [4,5]. Due to the widespread use of urinary catheterization, a significant fraction of nosocomial urinary tract infections is now associated with catheter usage [6–8]. For example, in intensive care units in the USA alone, 3.1–7.4 catheter-associated urinary tract infections (CAUTIs) per 1000 urinary catheter days are reported [9]. The majority of CAUTIs are preceded by microbial cell adhesion on the catheter surfaces, leading to the formation of colonies and subsequently biofilms [10]. Due to their matrix chemistry, biofilms are not easily destroyed and can facilitate horizontal gene transfer between resistant and non-resistant microbial strains, resulting in a much higher likelihood of antibiotic resistance development in biofilms compared to planktonic cells [11,12]. Therefore, biofilms function as reservoirs for pathogen proliferation, infections and resistance development. To eliminate CAUTIs, preventing biofilm formation is critical.

To date, the general coating strategy for preventing or reducing pathogen colonization and further biofilm formation has involved surface modification with antimicrobial compounds [13]. For urinary catheters, surface coating using antibiotics, silver, gendine (gentian violet and chlorhexidine) and nitric oxide have been reported [14–16], but these compounds are cytotoxic and are associated with the development of antibiotic resistance [17]. Antimicrobial peptides (AMPs) are ideal antimicrobial candidates for coating applications because they generally show broad

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spectrum antimicrobial activity and good biocompatibility [18,19]. Importantly, AMPs can combat some antibiotic-resistant pathogens and are less likely to evoke pathogen resistance because they disrupt cell membranes [20–23]. These advantages render AMPs an attractive catheter coating materials to curb CAUTIs. However, an important prerequisite for maximum efficacy is that peptide activity and stability must be retained under physiological conditions. Many AMPs succumb to salt inactivation at physiological salt concentrations [24–26], and salt-tolerant AMPs are needed in catheter coating for maximum efficacy.

This study reports the development of an AMP-based coating platform using two novel AMP candidates which were engineered for good broad antimicrobial spectrum and salt-tolerance properties: RK1 (RWKRWWRRKK) and RK2 (RKKRWWRRKK). An AMP immobilization chemistry based on allyl glycidyl ether (AGE) polymer brush tethering on a polydimethylsiloxane (PDMS) surface was developed, and successfully extended to silicone catheter surfaces. The AMP-coated surfaces showed excellent antimicrobial and anti-biofilm formation activities against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*, and did not demonstrate toxicity toward smooth muscle cells.

2. Materials and methods

2.1. Materials

Chemicals and reagents were purchased from Sigma Aldrich (Singapore), unless otherwise indicated. Antimicrobial activity and prevention of biofilm formation were investigated against three pathogens, i.e. (i) Gram-negative *E. coli* (ATCC 8739), (ii) Gram-positive *S. aureus* (ATCC 6538) and (iii) the fungus *C. albicans* (ATCC 10231). The peptides used in this study, i.e. RK1 and RK2, were chemically synthesized by GL Biochem Co., Ltd. (Shanghai, China) at 95% purity.

2.2. Antimicrobial activity determination of RK1 and RK2 in solution

The antimicrobial activities of RK1 and RK2 in solution were determined by performing broth microdilution tests against (i) *E. coli*, (ii) *S. aureus* and (iii) *C. albicans*. Minimum inhibitory concentrations (MICs) were determined as the lowest peptide concentration that inhibited cell growth by 100% after 12 h. Pathogens were grown overnight in Mueller Hinton Broth (MHB) and yeast mold (YM) medium for bacteria and yeast, respectively, then 1 vol.% of the cells were inoculated into fresh medium and grown at 37 °C (bacteria) or 28 °C (fungi) and 200 rpm until mid-log phase ($\sim OD_{600} = 0.8$) was attained. The antimicrobial assays were conducted in MHB or YM medium with and without 150 mM NaCl in 96-well plates. Lyophilized peptides were solubilized in distilled H_2O and filtered through a 0.22 μm filter (Minisart syringe filter, Sartorius, Singapore). Stock peptides were added to each well at different volumes to obtain final peptide concentrations ranging from 0 to 60 μM. A 10 μl aliquot of diluted cell culture (10^5 colony-forming units (CFU) ml^{-1}), which was diluted 10^3 times from a cell culture of $\sim OD_{600} = 0.8$ (10^8 CFU ml^{-1}), was added to each well to achieve a final cell concentration of 10^4 CFU ml^{-1}. After 12 h of growth, 10 μl cell culture was diluted to 10^4 CFU ml^{-1} for plating. The negative controls comprised the media and cell culture without peptides.

2.3. Preparation of PDMS and silicone urinary catheter for RK1 and RK2 immobilization

A flat PDMS slide was first employed as the model surface for peptide immobilization studies as it mimics the surface chemistry of silicone urinary catheters [27–30]. The PDMS pre-polymer and curing agent were obtained from the Sylgard 184 kit (Dow Corning Corporation, USA), and mixed at a 10:1 mass ratio before being subjected to polymerization reaction at 60 °C for 12 h. After polymerization, the PDMS was rinsed with acetone and dried in vacuum for 12 h, and used for further functionalization studies.

A urinary catheter segment (Foley catheter, Unomedical, Singapore) was sliced in two to expose the inner and outer surfaces (each having a surface area $\sim 1\ cm^2$) for surface functionalization studies.

Allyl glycidyl ether (AGE) polymer brush synthesis and peptide immobilization were performed on both the PDMS and silicone urinary catheter surfaces using the same method, as described in the following section.

2.4. AGE polymer brush synthesis on the PDMS and urinary catheter surfaces

AGE polymer brush was synthesized on the surface of PDMS by plasma ultraviolet (UV) induced surface grafting polymerization of AGE. The PDMS slide was pre-treated by radiofrequency (13.56 MHz) argon plasma (March PX-500, Nordson MARCH, Germany) at 300 W, 100 sccm for 5 min. The treated PDMS slide was immediately submerged in ethanol containing 10 vol.% AGE and exposed to UV (365 nm, 100 mW cm^{-2}) for 60 min. The polymer brush grafted slide (PDMS-AGE) was then rinsed in ethanol and water to remove any ungrafted oligomer or homopolymer.

2.5. RK1 and RK2 immobilization on PDMS and urinary catheter surfaces

RK1 and RK2 immobilization onto the AGE-grafted PDMS and urinary catheter surfaces was performed via reaction between the amine or amide groups of the peptides and the epoxy groups on the AGE-grafted surfaces. A PDMS-AGE slide ($\sim 1\ cm^2$ area) or urinary catheter segment ($\sim 1\ cm^2$ area) functionalized with AGE polymer brush was immersed in 2 ml peptide solutions containing 100 μM RK1 or RK2 in 50 mM phosphate buffer (pH 5.5) at 37 °C for 12 h. AMP concentrations on the surface of PDMS slides, i.e. PDMS-AGE-RK1 or PDMS-AGE-RK2, and AMP-immobilized urinary catheter segments, i.e. cat-AGE-RK1 or cat-AGE-RK2, were estimated by reverse-phase high-performance liquid crystallography (RP-HPLC) analysis, as described in Section 1.1 of the Supplementary materials.

2.6. Water contact angle measurement

Static water contact angle measurements were performed on the PDMS, PDMS-AGE, PDMS-AGE-RK1 and PDMS-AGE-RK2 surfaces using a goniometer (Firsttenangstroms (FTA32), ALV Technologies Pte Limited, Singapore). Digital images of a 2 μl water droplet on surface were captured and analyzed using the Fta32 Video software. Three different spots on the surface were tested for each sample and the average contact angle value was reported.

2.7. X-ray photoelectron spectroscopy (XPS) analysis

The elemental composition of PDMS-AGE-RK1 and PDMS-AGE-RK2 was analyzed by XPS using a Thetaprobe X-ray photoelectron spectrometer equipped with a monochromatic Al K_α X-ray source ($h\nu$) (1486.68 eV) and a hemispherical analyzer (Thermo Fisher Scientific, USA). The spectra were collected from 0 to 1100 eV with a pass energy of 200 eV and a 1 eV energy step size. The spectrometer was operated at a photoelectron take-off angle of 50 °C. Binding energies were calibrated with respect to $C1_s$ hydrocarbon bond at 285.0 eV.