CHAPTER 5

General Discussion

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1. Introduction: Challenges on Bacterial Infections

Bacterial infection has been a challenge to human health ever since the beginning of human life. However, this challenge has become much more severe in recent decades because of the increasing prevalence of antimicrobial resistance.[1,2] There are three reasons for antimicrobial resistance: (1) The biofilm-mode of growth in which bacterial infections manifest themselves, providing opportunities for survival under reduced growth rates, options for horizontal gene transfer, a hard to penetrate matrix of extracellular polymeric substances, and an acidic, enzyme-rich interior;[3,4] (2) The abuse and overuse of existing antimicrobials both in medical field and agriculture accelerate the evolution of intrinsic antimicrobial resistance. Around 80% antibiotics sold in the US are used as growth supplement in livestock. Meanwhile, incorrect prescriptions have been reported in 30% to 50% of all human clinical cases;[5,6] (3) Under-investment by companies and governments impedes the development of new drugs to compete with antimicrobial resistance, which in turn declined the attractiveness and prestige of this field. It has been 30 years since a new class of antibiotics was last introduced.[7]

In principle, there are two strategies for combating bacterial infections: (1) Reduction of the unnecessary use of antimicrobials both in agriculture and human health care with the collaboration of the global public; (2) Promotion of the development of new antimicrobial drugs, which requires dedicated efforts from scientists with different backgrounds including pharmacology, biology and microbiology and last but not least nanotechnology.[8] Biomimetic antimicrobial strategies mimic natural strategies and are currently amongst the emerging new approaches.[9–11] In this thesis, two strategies are developed for combating bacterial infection using biomimetic self-assembly.

2.Merits and Shortcomings of the Biomimetic Strategies Developed in this Thesis

Biomimetic assembly uses biomimetic components and assembly methods to fabricate antimicrobial materials, such as cell mimetic nanoparticles and self-assembled hydrogels. First, we developed macrophage membrane coated nanoparticles (Me-ANPs) in **Chapter 2** with a self-assembled core formed by an amphiphilic conjugate (ANPs) composed of Triclosan and ciprofloxacin. The ANPs demonstrated better efficacy than ciprofloxacin in solution, indicating a rational conjugation and assembly with an existing antibiotic and enhanced antimicrobial efficacy, most notably towards staphylococci internalized in macrophages. The design may have to be adjusted to facilitate conjugation of other antibiotics. Me-ANPs could be selectively engulfed by infected macrophages, resulting in killing of staphylococci internalized in macrophages. Although the mechanism by which these Me-ANPs might be selectively engulfed by infected macrophages has been hypothesized upon, it is not fully understood. Particularly, questions on maintenance of membrane sidedness, fluidity and pathogen recognition need more solid experimental confirmation.

Then, in **Chapter 3**, we developed a self-assembly strategy to fabricate smart supramolecular hydrogels by mimicking natural G-quadruplex structures. This biomimetic G-quadruplex hydrogel demonstrated pH and glucose responsiveness and zero-order drug release properties, which are promising features in large scale production because of ease of fabrication and commercial availability of building blocks. The combination of a cascade reaction in the hydrogel yielded an antimicrobial hydrogel as a cascade reaction container

operating on a non-antibiotic basis (**Chapter 4**), without risk of producing antimicrobial resistance. Besides, the cascade reactions occurring in the hydrogel decreased the local glucose concentration, which is particularly helpful in the treatment of diabetic foot ulcers.

3. Other Biomimetic Strategies for Infection Control

Besides biomimetic assembly, two other biomimetic strategies also could be used to design antimicrobial materials, i.e. surface mimicry and biomimetic synthesis.[10] For completeness, these will be briefly discussed and compared with the current approaches described in this thesis.

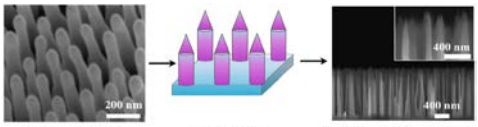
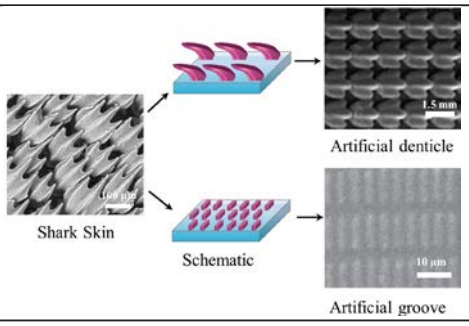
3.1 Surface mimicry

Surface mimicry implies modification of surfaces to mimic naturally-occurring antimicrobial surfaces. Most designs in this field are a mimicry of insect wings (such as cicadae, damselflies and dragonflies), shark skin, gecko feet and plant leaves (such as lotus leaves). Such natural surfaces with unique micro- or nano-structures, including nano-pillars, nano-grooves and nano-wrinkles, demonstrated favorable properties including self-clean, super-hydrophobicity and anti-biofouling. The topography of insect wings and shark skins and their mimicry are summarized in **Table 1**.

The advantage of surface biomimicry is that the use of antimicrobial drugs can be avoided or reduced. Macrophage membrane coated, nano-pillared surfaces for instance, have recently been described for use in extracorporeal bacterial capture devices for cleansing bacterially-infected blood.[12] Whether or not cleansing by extracorporeal devices based on surface biomimicry is truly sufficient or needs complementing with other antimicrobials, for instance our biomimetic ANPs, must still be established.

3.2 Biomimetic synthesis

Table 1. Strategies of building antimicrobial surfaces by mimicking insect wings and shark skins.

Natural surfaces	Nano-structure	Source surface and its mimicry	Antimicrobial mechanisms	References
Insect wings	-Nano-pillar; -Nano-wire;		-Physically disrupt bacterial or fungi cell membrane; -Reduce attachment of microorganisms due to hydrophobicity.	13,14
Shark skins	-Micro-denticle; -Nano-groove;		-Reduce colony size and inhibit bacterial migration to prevent biofilm formation.	15,16

Biomimetic synthesis fabricates new antimicrobials via chemical synthesis to mimic natural antimicrobials. Most biomimetically synthesized antimicrobials mimic naturally occurring antimicrobial peptides (AMPs). AMPs are mostly cationic and amphipathic molecules that attach through electrostatic double-layer attraction to negatively charged bacterial cell surfaces, leading to membrane disruption and perturbation of important membrane-associated processes, such as cell wall biosynthesis.[17]

By mimicking the amphiphilic properties of natural AMPs, peptide-like oligomers, synthetic polymers and amino acid-based short peptides have been developed. Peptide-like oligomers were designed using *β*-peptides and peptoids (N-alkyl glycine analogue) as a backbone to build helically structured motifs similar to some natural AMPs.[18] Several classes of peptide-like oligomers have been developed, such as PMX-10070,[19] AApeptides[20–22] and oligo-acyl-lysines (OAKs).[23] Synthetic polymers include poly(methacrylic acid) esters[24] and polynorbornene derivatives.[25] Amino acids like arginine, tryptophan, glycine, lysine, leucine and alanine are abundant in the native AMPs, which contribute to their antimicrobial properties. Utilizing this information, amino acids-based short peptides could be developed, as well as peptide dendrimers.[18]

4. Further Research and Outlook

Even though current biomimetic strategies yield promising new antimicrobials, there is room for optimization: (1) For biomimetic (self-)assembly of nanoparticles, their structural features and molecular components with respect to size distribution, molecular arrangement, sidedness, fluidity of cell membrane-coatings on nanoparticles, recognition and associated antimicrobial mechanisms should be better understood. (2) For surface mimicry, the geometry and fabrication techniques need to be better designed and improved, and their application inside the body is worth to exploring. (3) For biomimetic synthesis, synthesis, purification and scaling should be optimized.

Due to the ongoing increase in the occurrence of antimicrobial resistant infecting pathogens, also in infected wounds, antimicrobial hydrogels are expected to be used more frequently in the future. Since antimicrobial resistance to AMPs is rare, natural polymers with inherent antimicrobial activity, such as chitosan and AMPs, or their functionalized polymers might be considered as building blocks to fabricate antimicrobial hydrogels. For example, AMPs form fibrils through a variety of attractive non-covalent interactions, including hydrogen bonding, electrostatic forces, π-π stacking, hydrophobic interactions and Van der Waals forces. When the length and concentration of fibrils are sufficiently high, the fibrils can form hydrogels through branching, entanglement or association.[26,27] Alternatively, synthetic amphiphilic peptides mimicking natural AMPs can be applied to this end.[28]

Amphiphilic AMPs might also be considered to form ANPs nanoparticles through hydrophobic interactions to yield a hydrophobic core and hydrophilic domains on their surfaces. Similar as done in this thesis, these AMP nanoparticles could also be coated with cell membranes. It would be worthwhile to investigate whether membranes from other sources, such as red blood cells, white blood cells, platelets and other, are suitable for coating ANPs. Also, it would be clinically important to know whether membrane-coated ANPs have the ability to enter other cell types than only macrophages investigated in this study.

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