Biomimetic approaches toward the control of bacterial infections

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SUMMARY

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Bacterial infection continues to be a growing health problem worldwide, mainly due to increasing prevalence of antimicrobial resistant bacteria. New strategies and materials are therefore needed in order to improve the treatment efficacy of the available antimicrobials and develop novel strategies to deal with the growing problem of eradicating antimicrobial resistant infections. Biomimetic strategies mimic natural strategies. In **Chapter 1**, two different biomimetic strategies are discussed to create biomimetic, antimicrobial cell membrane-coated nanoparticles and a new hydrogel-based cascade reactor container that employs natural substrates in the human body to generate reactive oxygen species. In **Chapter 1.1**, we summarize the properties of natural cell membranes relevant for infection-control, possible ways to maintain membrane sidedness and fluidity upon encapsulation of antimicrobial nanoparticles and mechanisms of selective or non-selective pathogen targeting. Finally, an overview is provided with biomimetic, cell membrane-coated nano-antimicrobials for combating bacterial infections.

Currently, many novel antimicrobials are non-antibiotic based. Cascade reactions integrate two or more reactions, of which each subsequent reaction can only start when the previous reaction is completed. Employing natural substrates in the human body such as glucose and oxygen, cascade reactions can generate reactive oxygen species (ROS) for bacterial infection-control. Applications and perspectives of cascade reactions in bacterial infection control are discussed in **Chapter 1.2**. Main advantages for infection-control cascade reaction strategies include the fact that they are non-antibiotic based and induction of ROS resistance is unlikely. However, the amount of ROS generated is generally low and antimicrobial efficacies reported are less than 3 to 4 log units necessary for clinical efficacy. Increasing the amounts of ROS generated by adding more substrate, bears the risk of collateral damage to tissue surrounding an infection site. Collateral tissue damage upon increasing substrate concentrations may be prevented by locally increasing substrate concentrations, for instance using smart nanocarriers. Smart, pH responsive nanocarriers can self-target and accumulate in infectious biofilms from the blood circulation to confine ROS production inside the biofilm. Increasing bacterial killing efficacies using cascade reaction components containing nanocarriers constitutes a first, major challenge in the development of infection-control cascade reactions. Nevertheless, their use in combination with clinical antibiotic treatment may already yield synergistic effects, but this remains to be established for cascade reactions. Furthermore, specific patient groups possessing elevated levels of endogenous substrate (for instance diabetic or cancer patients) may benefit from the use of cascade reaction components containing nanocarriers.

Inspired by the natural versatility of cell membranes, we developed a macrophage membrane coated nanoparticle to treat the infection caused by *Staphylococcus aureus* internalized in macrophages. Internalization of *Staphylococcus aureus* by macrophages can inactivate bacterial killing mechanisms, allowing intracellular residence and dissemination of infection. Concurrently, these staphylococci can evade antibiotics that are frequently unable to pass mammalian cell membranes. In **Chapter 2**, a binary, amphiphilic conjugate composed of Triclosan and ciprofloxacin was synthesized that self-assembled through micelle formation into antimicrobial nanoparticles (ANPs). These novel ANPs were stabilized through encapsulation in macrophage membranes, providing membrane-

encapsulated, antimicrobial-conjugated NPs (Me-ANPs) with similar protein activity, Toll-like receptor expression and negative surface charge as their precursor murine macrophage/human monocyte cell lines. The combination of Toll-like receptors and negative surface charge allowed uptake of Me-ANPs by infected macrophages/monocytes through positively-charged, lysozyme-rich membrane scars created during staphylococcal engulfment. Me-ANPs were not engulfed by more negatively-charged sterile cells possessing less lysozyme at their surface. The Me-ANPs killed staphylococci internalized in macrophages *in vitro*. Me-ANPs likewise killed staphylococci more effectively than ANPs without membrane-encapsulation or clinically-used ciprofloxacin in a mouse peritoneal infection model. Similarly, organ infections in mice created by dissemination of infected macrophages through circulation in the blood were better eradicated by Me-ANPs than by ciprofloxacin. These unique antimicrobial properties of macrophage-monocyte Me-ANPs provided a promising direction for human clinical application to combat persistent infections.

Another biomimetic strategy applied in this thesis involved the self-assembly of biomimetic, antimicrobial hydrogels. In **Chapter 3**, we describe the fabrication of a new supramolecular guanosine (G)-quadruplex hydrogel by multi-component self-assembly of endogenous guanosine (G), 2-formylboronic acid (2-FPBA), and tris(2-aminoethyl) amine (TAEA) in the presence of KCl. The G-quadruplex hydrogel possessed (1) versatility due to commercial availability of building blocks with different functions, (2) dynamic iminoboronate bonds with pH and glucose-responsiveness, and (3) zero-order drug release because of the superficial peel-off of the hydrogel in response to stimuli.

On the basis of G-quadruplex hydrogel formation described in **Chapter 3**, we designed guanosine-quadruplex (G_4-)hydrogels composed of guanosine, 2-formylphenylboronic acid and putrescine as a cascade reaction container. In **Chapter 4**, G_4-hydrogels were loaded with glucose-oxidase and hemin. The first cascade reaction transformed glucose and O_2 into gluconic acid and H_2O_2, assisted by glucose-oxidase, followed by hemin-assisted transformation of H_2O_2 into ROS. *In vitro*, the first cascade reaction was most influential on killing planktonic *Staphylococcus aureus* or *Pseudomonas aeruginosa*, while the second reaction enhanced killing of biofilm bacteria through diffusion of hemin and production of ROS in the biofilm. Healing of infected wounds in diabetic mice proceeded faster upon wound coverage by our G_4-hydrogels than by ciprofloxacin irrigation. Moreover, local glucose concentrations around infected wounds decreased. Based on these results, a G_4-hydrogel loaded with glucose oxidase (GOx) and hemin was a good candidate for antibacterial wound dressings particularly in diabetic patients.

There are still great challenges for the effective treatment of infectious diseases, especially when antimicrobial resistance arises. In **Chapter 5**, we discuss both biomimetic strategies developed in the thesis in view of each other merits and shortcomings. Also, we summarize other possible biomimetic approaches and their application for infection control. Suggestions for further research are given.

Summarizing, nature is a treasure-house from which we can learn new concepts and methods amongst which the biomimetic strategies applied in this thesis. Biomimetic antimicrobial strategies have great potential and versatility in combating antimicrobial resistant bacterial infections.