Chapter 6

General Discussion
Magnetic nanoparticles (MNPs) have been successfully used to enhance the killing efficacy of biofilm bacteria by antibiotics through promoting their full-depth penetration [1,2]. Different from previous work, here MNPs were used as “diggers” instead of “drug carriers” to destroy biofilm structures, making them more vulnerable and penetrable to antibiotics. The use of MNPs for the treatment of biofilm infections was simplified step by step. It started from antimicrobials loading, which highly relied on complicated chemical synthesis and sophisticated magnetic targeting systems, but still showed limited therapeutic efficacy (Chapter 2). Here, MNP use is advanced to artificial channel digging by magnetically-propelled movement of MNPs, which showed significant improvement of treating-efficacy without the need of precise magnetic targeting to biofilm-associated infections, regardless of whether biomaterials-implants associated or not (Chapter 3, 4 and 5). Those steps effectively promoted use of MNPs from an entirely new perspective, presenting great application potential.

The choice of magnetic targeting

Targeted drug delivery is a newly, emerging drug delivery method, showing promising drug administration effects by targeted transport of drugs into an aimed site [3]. Compared with other methods, targeted drug delivery can increase local drug concentrations to improve treating efficacy while decreasing side effects, such as biotoxicity to the human body [4]. Magnetic targeting was chosen here to treat biofilm-related infections mainly because of its strong controllability under an applied external magnetic field, which is the most effective way to defeat the EPS matrix that enwraps and protects biofilm inhabitants. E.g. electrostatic double-layer attraction can actively transport antimicrobials into the depth of a biofilm [5], biofilm structure can be destructed by sonication [6] or decomposing chemicals (nitric oxide [7], d-amino acids [8]). Yet, all these methods lack a strong driving force, and on-demand availability that can be achieved by magnetic targeting. Moreover, most targeting methods need complicated chemical synthesis which limits clinical application. As a physical method, magnetic targeting has shown incomparable potential to slow down the generation of antimicrobial resistance, that remains to be clinically proven however.
General Discussion

**Smart and advanced magnetic targeting systems**

Although magnetic targeting has exhibited good performance in *in vitro* biofilm treatment, there is still a long way to go before it can treat an *in vivo* biofilm-related infection in the clinic. One of the biggest obstacles is that we do not have a magnetic targeting system available to direct drug-loaded MNPs to the right, micron-sized place, i.e. an infectious biofilm. Neither do we have an imaging system available to see where and when the particles arrive at a target side in the human body in real-time, nor a driving system to propel the particles across tissue-barriers in a controllable way [9]. Therefore, in order to accelerate the steps of magnetic targeting from laboratory toward clinical application, on the one hand, imaging and targeting technologies should be improved. On the other hand, we should try to avoid those obstacles. Channel digging is an innovative and effective method, not requiring complicated magnetic targeting systems, no need for time-consuming chemical synthesis, no limitations to the choice of antimicrobials and applicability regardless of the pathogenic strain involved.

**Magnetic tools for EPS destruction: open questions and suggestions for future research**

Magnetic tools have shown promising effects to destruct the protective EPS structure of a biofilm upon application of an external magnetic field. In this work, we used spherical or cubic, nano-sized magnetic nanoparticles with or without a polydopamine coating as tools to dig channels. As time is always a limiting factor, this PhD thesis still leaves several points for consideration and future research:

- What if we use other shaped magnetic nanoparticles, such as rodlike or urchin-like? It has been reported that sharp edges of nanoparticles can cut bacterial membranes, causing leakage and death without using antibiotics. Could this yield synergistic killing with antimicrobials?
- What if we use micro-sized or larger magnetic particles to dig channels? Will they yield increased penetrability or will the stability of the channels decrease below an unacceptable limit?
What if we coat polydopamine in different thickness on MNPs or what if we use different polymers to coat MNPs (different surface functional groups and charge)? Surface coatings significantly affected the interactions between particles and bacteria and thus affected channels digging and biofilm-killing by antimicrobials. Further exploration and development of more advanced tools for channel digging is worthwhile.

Does the propulsion of MNPs do collateral damage to tissue cells?

Would bacterial dispersal from a biofilm into a planktonic mode followed by antibiotic treatment be more efficacious than channel digging or would this yield increased risk of spreading dispersed, infecting pathogens to cause infections elsewhere in the body?

The year 2050 may appear like far away in the future, but it is coming. 2050 is the year in which it is predicted that multidrug-resistant bacterial infections will become the number one cause of death [10]. Concerted action and targeted research and development is required now. Based on my thesis, I believe that magnetically-targeted treatment of infectious biofilms will show its superiority above other alternatives as time going on.

References