SUMMARY

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Antimicrobial-resistant infections are becoming an increasingly serious health care problem and threaten to be the main cause of death by the year 2050. In **Chapter 1,** the current situation and expert opinions in infection control are summarized. Development of new antibiotics with ever 'better' bacterial killing has long been considered the appropriate response to the growing threat of antimicrobial-resistant infections. However, the time-period between the introduction of a new antibiotic and the appearance of resistance amongst bacterial pathogens is getting shorter and shorter. This suggests that alternative, non-antibiotic based pathways to eradicate bacterial infection should be taken.

Nanotechnology offers many novel infection-control strategies that may help prevent and treat antimicrobial-resistant bacterial infections. In particular, photothermal nanoparticles can be used to generate local heat upon near infrared (NIR) irradiation and are under consideration for tumor treatment. Therefore, the aim of this thesis is to explore and evaluate the advantages and disadvantages of photothermal nanoparticles for the control of bacterial infection. In **Chapter 2**, we synthesized polydopamine, photothermal nanoparticles (PDA-NPs) without further surface-functionalization to evaluate their potential with respect to biofilm-control. Most ESKAPE-panel pathogens in suspension with photothermal nanoparticles showed three- to four log-unit reductions upon NIR-irradiation, but for enterococci only less than two log-unit reduction was observed. Exposure of existing *Staphylococcus aureus* biofilms to photothermal nanoparticles followed by NIR-irradiation did not significantly kill biofilm-inhabitants. This indicates that the biofilm mode of growth poses a barrier to penetration of photothermal nanoparticles, yielding dissipation of heat to the biofilm-surrounding rather than in its interior. Staphylococcal biofilm growth in the presence of photothermal nanoparticles could be significantly prevented after NIR-irradiation because PDA-NPs were incorporated in the biofilm and heat dissipated inside it. Thus, unmodified photothermal nanoparticles have potential for prophylactic infection-control, but data also constitute a warning for possible development of thermo-resistance in infectious pathogens.

Clinical application of photothermal nanoparticles initially involved tumor treatment. Application towards much smaller, micrometer-sized bacterial infections however, bears the risk of collateral damage by heat dissipating into tissue surrounding an infection site. This can become a complication, e.g. when photothermal nanoparticle coatings are clinically applied on biomaterial surfaces requiring tissue integration, such as for instance titanium-made, bone-anchored dental implants. Dental implants can fail due to infection in the pocket formed between the implant screw and surrounding soft tissue ("peri-implantitis"). In **Chapter 3**, we addressed the hitherto neglected potential complication of collateral tissue damage by evaluating photothermal, PDA-NP-coatings on titanium surfaces in different co-culture models. NIR-irradiation of PDA-NP-coated (200 $\mu g/cm^2$) titanium surfaces with adhering *S. aureus* killed staphylococci within an irradiation time window of around 3 min. Moreover, when covered with human gingival fibroblasts, this irradiation-time window maintained surface coverage by fibroblasts. Contaminating staphylococci on PDA-NP-coated titanium surfaces, as can be per-operatively introduced, reduced surface coverage by fibroblasts and this could be prevented by NIR-irradiation for 5 min or longer prior to allowing fibroblasts to adhere and grow. Negative impacts of early post-operative staphylococcal challenges to an existing fibroblast layer covering a coated surface were maximally prevented by 3 min NIR irradiation. Longer irradiation times

caused collateral fibroblast damage. Late postoperative staphylococcal challenges to a protective keratinocyte layer covering a fibroblast layer, required 10 min NIR irradiation for adverting a staphylococcal challenge. This was longer than foreseen from monoculture studies, because of additional heat uptake by the keratinocyte layer. Summarizing, photothermal treatment of biomaterial-associated infection requires precise timing of NIR irradiation to prevent collateral damage to tissue surrounding the infection site.

The lack of bacterial killing efficacy of photothermal nanoparticles towards an existing biofilm (**Chapter 2**) combined with the potential of collateral heat damage to surrounding tissue (**Chapter 3**), suggests that eradication of existing infectious biofilms requires modification of the nanoparticles to allow their penetration and accumulation in a biofilm. To this end, in **Chapter 4,** we encapsulated photothermal PDA-NPs in pH-responsive, mixed shell polymeric micelles, composed of stealth poly-ethylene glycol (PEG) and pH-sensitive poly (*β*-amino ester) (PAE). In order to allow encapsulation, PDA-NPs were made hydrophobic by coupling of indocyanine green (ICG). Coupling of ICG also enhanced the photothermal conversion efficacy of PDA-NPs from 33% to 47%. Photothermal conversion was not affected by micellar encapsulation. *In vitro* experiments demonstrated absence of cell toxicity and hemolytic effects of PEG-PAE encapsulated PDA-ICG-NPs and showed good penetration and accumulation in a *S. aureus* biofilm. Penetration and accumulation were absent when nanoparticles were encapsulated in PEG-micelles without a pH responsive moiety. PDA-ICG-NPs encapsulated in PEG-PAE-micelles found their way through the blood circulation to a sub-cutaneous infection site after tail-vein injection in mice to yield faster eradication of infection upon NIR irradiation than could be achieved after encapsulation in PEG-micelles. Moreover, also staphylococcal counts in surrounding tissue were reduced facilitating faster wound healing. The combined effect of targeting and localized NIR irradiation prevented collateral tissue damage. Based on these results, encapsulated photothermal nanoparticles in pH-responsive micelles have been brought an important step closer to clinical application.

In **Chapter 5**, the advantages and disadvantages of photothermal nanoparticles as a non-antibiotic based infection-control strategy are discussed in depth in view of the findings presented in this thesis. First of all, it is concluded that pH-responsive, micellar encapsulation and other means to facilitate penetration and accumulation of photothermal nanoparticles into an existing infectious biofilms are promising for clinical application, provided properly dosed and NIR irradiated at the right power and for the right duration in order to prevent collateral tissue damage. Further insight into the possible development of thermo-resistance amongst human pathogens is required however, before human clinical translation of photothermal infection control is warranted. Clinical translation should also be preceded by animal experiments, other than in infected-wound healing models in mice in order to determine whether deeper infections can benefit from photothermal eradication.