Antimicrobial and nanoparticle penetration and killing in infectious biofilms

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Chapter 1

General introduction

Initial bacterial adhesion to surfaces in the human body can result in biofilm formation, which plays a critical role in bacterial infections. It is estimated that approximately 60% of all bacterial infections are caused by microbial biofilms[1]. In a biofilm, bacteria embed themselves in a matrix of extracellular polymeric substances (EPS), acting as 'the house of the biofilm cells[2]. EPS consists of water, polysaccharides, proteins, extracellular DNA (eDNA) and other molecules and protects the biofilm from the human immune system, mechanical forces, penetration of antimicrobials, and desiccation[3,4]. One of the problems with biofilm infections is that they can be up to 1000 times more recalcitrant to antimicrobials than planktonic bacteria[5].

Biofilm recalcitrance to antimicrobials is dependent on the biofilm structure, biofilm composition[6], and the phenotype (metabolic processes) of the bacteria. Biofilm structure and biofilm composition are closely related and affect penetration of antimicrobials and therewith the killing of bacteria in the biofilm. Recently, also viscoelastic properties of biofilms were related to penetration of antimicrobials in *in vitro* and *in situ* oral biofilms[7]. Killing of bacteria in a biofilm is a complex process, since it is amongst others dependent on biofilm thickness, antimicrobial concentration, duration of antimicrobial treatment, antimicrobial characteristics like charge and size, and components in the EPS which can interact with the antimicrobial agent[8]. Negatively charged components like alginate and eDNA in EPS can interact with positively charged antimicrobials[9,10] and therewith block the penetration of the antimicrobials and protect the bacteria in the deeper layers of the biofilm.

Increasing numbers of drug resistant bacteria have been reported since the discovery of antibiotics and a huge increase in multi-drug resistant bacteria the last couple of years which is a main problem affecting modern health care[11]. If no new antimicrobials or new strategies to deliver antimicrobials to bacterial infections are developed, an era is faced in which there might be no treatment for bacterial infections[12] with even the possibility that in 2050 microbial infections will become the number one cause of death[13]. Antimicrobial peptides (AMPs) have been mentioned to battle antimicrobial resistance[14,15]. AMPs are available in the innate immune system, and play an essential role in the first reaction against microbial infections[14]. AMPs exist of 5-150 amino acids and are generally positively charged amphipathic molecules. AMPs adhere to the bacterial cell membrane by electrostatic interactions[12], resulting in pore formation and disruption of the membrane, causing leakage and finally in bacterial cell death[15,16]. Other AMPs act on intracellular processes[15,16] like protein, DNA and RNA syntheses, folding of proteins and cell wall synthesis[12]. It has been hypothesized that bacterial resistance against AMPs is improbable, since the bacteria need to alter their cell wall to obtain resistance[14]. However, several cases of AMP resistance have been observed *in vitro*[16]. Most of the AMPs have not made it to the clinic so far because of their salt sensitivity and sensitivity to proteolysis[17,18]. Therefore, nanocarriers to encapsulate and deliver AMPs to the infection site might protect AMPs from

degradation or chelation and increase their effectivity. Loading of antimicrobials into nanocarriers has shown improved efficacy of the antimicrobials compared to the administration of antimicrobials alone[19,20]. Other advantages of using nanocarriers include improved antimicrobial solubility[21], improved longevity in the circular system, sustained and controlled release, and drug targeting[22,23].

The aim of this thesis is to investigate the penetration and killing of AMPs and nanocarriers in infectious biofilms *in vitro* and *in vivo*. In addition, the relation between penetration of antimicrobials and biofilm characteristics has been explored.

References

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