Synthesis of quaternary ammonium coated surfaces

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Chapter 8

General discussion

8.1 Introduction

The average life expectancy in the Western-world is increasing and with an aging population the need for biomaterial implants and devices to maintain the quality of life at an acceptable level is growing as well. The number one cause of failure of biomaterial implants and devices is biomaterial-associated infection (BAI). Failure rates due to infection depend strongly on the implant site, but are generally in the range of 0.5 – 5%.[1] Besides healthcare costs, BAIs have severe clinical and societal consequences. Often BAI results in disabilities or psychological trauma. In the case of cardiovascular implants, BAI even has a high mortality risk,[2] while the mortality associated with some forms of BAI exceeds the one of several cancers.[3]

In this thesis we have explored the preparation of a coating that kills bacteria upon contact and *in vitro* evaluated the resistance of such coatings against inherent biofilm formation. Our study did not only involve the ability of the coatings to facilitate bacterial contact-killing, but also encompassed phagocytosis of contact-killed bacteria, as a layer of dead bacteria may interfere with the contact-killing abilities of such coatings.

8.2 Development of a contact-killing coating

In chapter 3, we have explored the possibilities of a template coating based on an elegant one-pot synthesis to obtain functionalizable hyperbranched polymers from AB_2 monomers. These AB_2 monomers can be spin-coated on an activated surface to form a shape-adaptive coating (chapter 4) which is able to kill bacteria on contact after tethering quaternary ammonium compounds (QUATs) onto this template. As an amine source, we used polyethyleneimine (PEI) which was alkylated to create QUATs (see Figure 8.1). By using a 20 wt% PEI solution, good bacterial killing was observed, but fibroblasts were not spreading on such coatings (chapter 4). This coating may therefore only be suitable for medical implants where no tissue integration is necessary, but BAI constitutes a risk. If bacterial killing needs to be combined with tissue integration, a lower wt% PEI can be applied, which is one of the reasons why we used 15 wt% in chapter 6. Another option to improve tissue integration could be to add, for example, RGD moieties on periphery of the hyperbranched polymers. Hyperbranched polymers comprise reactive groups at the end of each branch and are therefore ideal for creating multi-functional coatings which can promote tissue integration, while still demonstrating bacterial contact-killing. The homogeneity of the hyperbranched coating layer was substantially improved (chapter 5) by pre-polymerizing the AB_2 monomers to a molecular weight of about 2000 g/mol prior to spin-coating. In a pilot experiment, it was demonstrated that the coating can be re-used and sterilized with 70% ethanol or autoclaving, while keeping the same bacterial killing efficacy.

Before our coating system can be used in commercial applications, the production process should be further optimized. The preparation and application of the coating takes now up to 2 weeks. Extensive washing steps and careful anchoring reactions were performed, in order to be absolutely sure there is no leaching of antibacterial compounds from the coating which consumes a major part of the production process. Optimization of these steps will shorten the production time by a factor of 2.

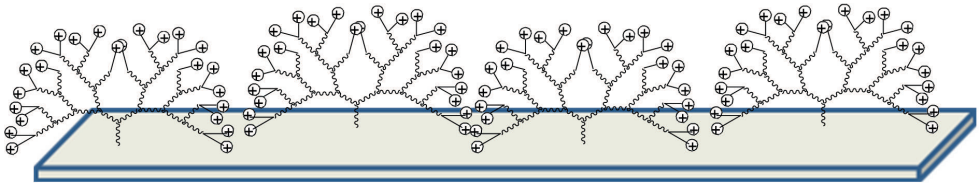

Figure 8.1 Schematic presentation of a hyperbranched coating consisting of alkylated polyethyleneimine to create QUATs on a surface (taken from chapter 5).

8.3 Translation to clinical application

For a downward translation to clinical application, it is important to have reliable *in vitro* evaluation of newly developed coatings. To investigate how the QUAT-coating performs in clinical situations, additional evaluation methods should be performed to mimic the conditions in the human body. We have made some steps in that direction by including serum (chapter 6) and macrophages (chapter 7) in our studies. Nevertheless, this still needs further elaboration. For example, more Gram-positive and Gram-negative bacteria as well as yeast strains need to be investigated in order to ensure that this coating is acceptable for clinical application. Some preliminary tests however, indicate a broad activity. Although the coating was firmly immobilized, the stability of this coating in physiological solutions still need to be studied. Depending on the application, it may be desirable to study the influence of proteins on the coatings, like serum and salivary proteins. After different microorganisms have been tested, co-cultures with tissue cells, tri-cultures with tissue cells and immune cells with microorganisms will be the next challenge before animal experiments can be performed.

8.4 Conclusions and future research

The number one cause of failure of biomaterial implants and devices is biomaterial associated infection (BAI). Replacement of existing biomedical implants and devices by ones prepared from intrinsic antimicrobial materials is difficult and laborious, because different implants and devices require different mechanical and physico-chemical properties. Immobilizing antimicrobial coatings on existing biomedical implants and devices is possibly a better and simpler solution. Moreover, the same type of coating can be used on various devices. Since we can, in principle, tether any (bio)active compound onto the branches of the hyperbranched polymers, it would be interesting to study dual or multiple-functional coatings, combining the bacterial killing potential of a QUAT-coating with, for instance, antifouling properties of PEG brushes. This antifouling and bacterial killing coating could, in addition, be combined with cell adhesion molecules, like RGD moieties.

The ultimate goal of creating a surface that is completely antibacterial, while having good interaction with the host is still far from accomplished. However, with our research presented here we believe that we made steps in the right direction. It is worthwhile to mention that QUAT coatings can also be very usable in other disciplines than in biomedical applications. For example, beads with a QUAT-coating can be used to make filters for gas or liquid (drinking water)

sterilization, coatings for the food industry or even on toys for children. The potential of this hyperbranched platform is only limited by our imagination.

References

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