Biomaterial-associated infection: peri-implant tissue is an important niche for Staphylococcus epidermidis survival

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CHAPTER

General discussion
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**Overall conclusion.** The formation of a biofilm by bacteria on biomaterial implants is generally regarded as the pivotal element in the pathogenesis of biomaterial-associated infections (BAI). Other important factors involved in BAI are the dysregulation of the local immune system due to the presence of a foreign body and bacteria and bacterial components. A major issue is the difficult treatment of these infections. The aim of this thesis was to obtain more insight in the pathogenesis of BAI, by investigating the relative importance of colonization of the biomaterial and the surrounding tissue. The results provide strong evidence that the tissue surrounding implants is a very (maybe even more) important niche for bacteria to reside and to “hide” from the host immune system, and protects bacteria from being reached by antibiotics and antibodies.

Biomaterial-associated infections are studied in *in vitro* and *in vivo* models. The advantage of *in vivo* models is the presence of a complete humoral and cellular immune system. In case of BAI, the local immune system is dysregulated, facilitating the establishment of an infection around the implants. Opportunistic pathogens, such as *Staphylococcus epidermidis*, may take advantage of the impaired host defence around the implanted biomaterial. In our studies, catheter segments were implanted on the back of C57Bl/6 mice and alongside the implants different challenge doses of *S. epidermidis* were injected. We observed that the bacteria were more often found in the tissue surrounding the implant; bacteria colonized the implant itself only when larger inocula were used. Bacteria persisted in the tissue for longer periods than on the implant itself. This was observed in different mouse strains, with different biomaterials and with different strains of *S. epidermidis* \(^1\). Treating mice with dexamethasone and/or BrdU between 14 and 21 days after challenge showed a relapse in the infection, indicating that bacteria were still present at 21 days after challenge, even though after 14 days they were almost undetectable. As more bacteria were retrieved from the peri-implant tissue than from the implant itself, it is likely that tissue rather than the implant, was the bacterial “hiding place” and therefore the source of the relapse of the infection \(^2\).

In order to assess whether the findings in the mouse model were representative for the situation in humans, catheters and surrounding tissue obtained from deceased patients of the ICU in the Amsterdam Medical Centre were studied. Indeed, also in
these human samples bacteria (predominantly *S. epidermidis* and / or *E. faecalis*) were retrieved in higher numbers from the peri-implant tissue than from the catheters. To our knowledge this is the first study in which the tissue surrounding biomaterials is shown to be a niche for bacteria in BAI.

**Antibiotic treatment of BAI.** Treatment of biomaterial-associated infections is often aimed at the biofilms, however it is not known whether this treatment is also effective against bacteria residing in the peri-implant tissue or even intracellularly within macrophages. When mice were given an antibiotic regime of vancomycin / rifampicin for 8 days, *S. epidermidis* were still cultured from 44% of the tissue biopsies, whereas almost all cultures of the biomaterials were negative. The antibiotics were used in concentrations shown to reduce bacterial counts in studies of *S. aureus* BAI. Possibly higher concentrations or a longer treatment period are required to also eradicate the bacteria from the tissue.

Mermel *et al.* described vancomycin to be the preferred antimicrobial agent against methicillin-resistant coagulase-negative staphylococci. However, as vancomycin does not reach bacteria residing intracellularly in the tissue, an additional antibiotic which is able to penetrate cells, such as for instance rifampicin, should be used. When a regime of vancomycin / rifampicin is applied, local bacterial resistance to rifampicin could occur as vancomycin does not reach intracellular bacteria, resulting in a monotherapy of rifampicin. A point mutation would be sufficient for the bacteria to become resistant to rifampicin. In our model, no rifampicin resistance was detected, indicating that the inability of the antibiotics to clear all bacteria from the tissue was not due to resistance, but more likely to lack of sufficiently high local (intracellular) concentrations. New antibiotics should therefore fulfil several criteria: they should be able to penetrate and kill bacteria in biofilms, but at least equally important, they should be able to kill bacteria present in the tissue, both intracellularly and intercellularly. Quinupristin / dalfopristin and linezolid are able to penetrate into macrophages, and quinupristin / dalfopristin, ranbezolid and daptomycin are active against biofilms. Daptomycin may be a potential alternative drug therapy for multidrug-resistant gram-positive organisms but this antimicrobial agent has been shown to be less active against bacteria adherent to biomaterial surfaces. Similarly, linezolid does not completely eradicate bacterial colonization of catheters. In single patient case studies, linezolid is effective against prosthetic
knee infection \(^{24}\) and complicated ventriculo-peritoneal (VP) shunt infection caused by \(S.\) \textit{epidermidis} \(^{25,26}\). Linezolid therefore might to be an effective antibiotic against coagulase-negative staphylococci in BAI. However, linezolid-resistant strains are emerging, and this has implications for the use of linezolid as a therapeutic agent \(^{27-29}\). In the studies mentioned above, the tissue surrounding the implanted devices was not investigated. As shown in this thesis, the tissue is an important niche for bacteria associated with biomaterial infections. We propose that in future studies, the efficacy of novel antibiotics in clearing bacteria not only from the biomaterial, but also from the surrounding tissues should be investigated.

**Prevention with antibodies.** Prevention of biomaterial-associated infections might be achieved by means of vaccination or passive immunisation. In chapter 4, we studied the effect of antibodies that were raised against \(S.\) \textit{epidermidis} major surface protein antigens \(^{30}\). Accumulation-associated protein (Aap) was identified as a highly immunodominant antigen. Monoclonal antibodies against Aap and surface-exposed lipoteichoic acid (LTA) were used for passive immunization of the mice in experimental BAI. No opsonophagocytosis was observed \textit{in vitro}, and no decrease in frequency of culture positive peri-implant tissue samples, or in numbers of cfu was recorded when antibodies were used in the mouse model. Possibly, the antibodies stimulated macrophages to ingest the bacteria, but these cells were unable to kill the bacteria due to the presence of the implant. Thus, the antibodies may actually have contributed to a more rapid transport of \(S.\) \textit{epidermidis} into a ‘safe heaven’ \(^{31}\). Surprisingly, an increase in binding of bacteria to the implants was seen when anti-LTA had been administered, whereas such enhanced binding was not observed \textit{in vitro}, not even in the presence of serum. Therefore the increased adherence \textit{in vivo} most likely was not due to the presence of components which were present in serum. Another possibility could be that the bacteria / antibody complexes bound to Fc-receptors on blood platelets or on other cellular components attached to the implant, leading to increased adherence. A monoclonal antibody to LTA has been shown to enhance phagocytosis and killing of \(S.\) \textit{epidermidis}, and to increase survival of passively immunized suckling rats \(^{32}\). This antibody was however not tested in presence of a biomaterial. To our knowledge, no antibodies have been tested in animal models in the presence of a biomaterial implant before; therefore our results indicate a possible risk associated with the use of antibodies to prevent
or treat biomaterial-associated infections.

Recently, a patent describing a novel vaccine based on the *S. epidermidis* PS1 conjugate was released. The *S. epidermidis* PS1 conjugate vaccine is an investigational vaccine in clinical development for the prevention of *S. epidermidis* infections. Opsonic antibodies were generated following PS1-rEPA vaccination. *In vitro* these antibodies induced ≥ 70% opsonic killing of *S. epidermidis* which express the PS1 antigen. Furthermore, PS1-specific IgG is effective in clearing *S. epidermidis* induced bacteremia by passive immunization, as is vaccination with PS1-rEPA. This vaccine was tested in a mouse bacteremia model, where high titres of *S. epidermidis* were administered. The model did not include biomaterial implants, and therefore it is not known whether this vaccine will also have a positive effect on biomaterial-associated infections caused by *S. epidermidis*, or whether an increase in bacterial binding to the implant or tissue survival will result after administration of this vaccine.

**Implications of tissue colonization in biomaterial infections.** Biomedical devices can roughly be separated into two categories: permanent implants and removable implants, such as catheters. Permanent implants need to be placed under maximally axenic conditions, to avoid introduction of bacteria during surgery. Infection of implants may have severe consequences, as this may lead to the need of their replacement. In case transcutaneous devices such as catheters, it is very important to prevent extraluminal infection. As shown in Chapter 6, bacteria are present on the skin, and also in high numbers in the tissue surrounding the implant. Replacement of a catheter over a guidewire might lead to a new infection as bacteria from the tissue could re-colonize the newly inserted catheters. In addition, measures to reduce colonization of the skin surrounding the catheter should be taken. Dressings containing chlorhexidine gluconate have proven effective in decreasing infection rates. Also, dressings with medical grade honey may offer great perspective as there is no risk of resistance development. A study in healthy volunteers showed that after 2 days of application of honey, the extent of forearm skin colonization was reduced 100-fold. It is therefore interesting to investigate whether medical grade honey will also be effective to prevent catheter-related infections.

The biomaterial used in our mouse model, polyvinylpyrrolidone-coated silicon elastomer (SEpvp) studies has anti-adhesive properties for bacteria *in vitro*. 
This could partly explain the high bacterial numbers retrieved from the tissue surrounding this biomaterial. However, the anti-adhesive properties of SEpvp per se are not responsible for the survival of *S. epidermidis* in the peri-implant tissue, since no tissue survival was observed when the same material was tested in an IL-1 R-/- mouse strain. In addition, survival of *S. epidermidis* in peri-implant tissue is not unique to SEpvp. In general, the effects of anti-adhesive coatings on colonization of biomaterials and surrounding tissue *in vivo* are unknown. In fact, tissue survival could be a problem for materials with different physicochemical characteristics. Therefore, the influence of these biomaterial characteristics on tissue survival should be considered when creating novel anti-adhesive or other biomaterials.

In the postmortem patient study described in chapter 6, 37% of the catheters were coated with an antimicrobial agent. The non-coated catheters and their surrounding tissue were more often culture-positive than the coated catheters (36% versus 15%, NS). In literature, antimicrobially coated catheters have been found to have reduced binding of bacteria. However, presence of bacteria in the surrounding tissue was not investigated. Due to the limited number of patients investigated in our study, we can only speculate that a reduction in the culture positivity of both the catheter and surrounding tissue was due to the antimicrobial coating on the catheters. As we have shown, the peri-implant tissue is an important niche for bacteria. Therefore, an effective antimicrobial coating for catheters or other devices should ideally not only prevent surface adherence of bacteria, but also their survival in the surrounding tissue.

In conclusion, the tissue surrounding biomaterial implants was proven to be an important niche for bacteria in biomaterial-associated infections. Novel antibiotic strategies, antibody-based strategies and biomaterial technology aimed at development of anti-adhesive and / or bactericidal properties of the biomaterial should therefore also be studied with respect to their preventive effect on tissue colonization.
REFERENCES

1. Chapter 2 of this thesis.

2. Chapter 5 of this thesis.

3. Chapter 6 of this thesis.

4. Chapter 3 of this thesis.


30. Chapter 4 of this thesis.


