Biomaterial-associated infection: peri-implant tissue is an important niche for Staphylococcus epidermidis survival
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CHAPTER 1

General introduction
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Introduction

**Biomaterial-associated infection: magnitude of the problem.** Over the past decades, the use of medical devices such as catheters, artificial heart valves, prosthetic joints and other implants has grown significantly.

Despite the advancements in device design, surgical procedures, and surgical wound and catheter care, infection is still a frequent complication of the use of these devices. Such infections generally are difficult to treat, and treatment failure may lead to removal of the device. This obviously is very inconvenient for the patient and increases hospital costs. The costs for treating a device-associated infection can be 5-7 times the initial cost of the implantation. Table 1 gives an overview of device-associated infections.

<table>
<thead>
<tr>
<th>Implant or device</th>
<th>No. used or implanted</th>
<th>Incidence of infection (%)</th>
<th>Morbidity/mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravascular catheters: Peripheral</td>
<td>150-200 million/year</td>
<td>&lt; 0.1</td>
<td>15-20% mortality</td>
</tr>
<tr>
<td>Intravascular catheters: Central venous</td>
<td></td>
<td>3-7</td>
<td></td>
</tr>
<tr>
<td>Urinary catheters</td>
<td>4-5 million/year</td>
<td>5-10 daily risk</td>
<td>1-2% gram-negative sepsis</td>
</tr>
<tr>
<td>CNS shunts</td>
<td>&gt; 80,000/year</td>
<td>10-15</td>
<td></td>
</tr>
<tr>
<td>Hemodialysis grafts</td>
<td></td>
<td>10</td>
<td>28% mortality</td>
</tr>
<tr>
<td>Pacemaker leads</td>
<td>115,000-130,000/year</td>
<td>2-11</td>
<td>2% mortality</td>
</tr>
<tr>
<td>Vascular grafts</td>
<td>&gt; 60,000/year</td>
<td>0-3</td>
<td>40% mortality, 20-30% amputation</td>
</tr>
<tr>
<td>Prosthetic heart valves</td>
<td>&gt; 100,000/year</td>
<td>1-5</td>
<td>34% mortality, 25-30% Coagulase-negative staphylococcus complications</td>
</tr>
<tr>
<td>Total artificial heart</td>
<td>230 (1969-1991)</td>
<td>36</td>
<td>34% mortality</td>
</tr>
<tr>
<td>Genitourinary prosthesis</td>
<td>150,000 (until 2000)</td>
<td>5</td>
<td>72% permanent device removal</td>
</tr>
<tr>
<td>Total artificial hip</td>
<td>222,000/year</td>
<td>&lt; 1</td>
<td>7-63% mortality</td>
</tr>
<tr>
<td>Total knee arthroplasty</td>
<td>110,000/year</td>
<td>1-2</td>
<td>2.5% mortality, 80% non-functioning TKA</td>
</tr>
<tr>
<td>Dental implant</td>
<td>436,000/year</td>
<td>15 gingival</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Overview of device use and device-associated infections in the US; adapted from 2.

As devices are manufactured of biocompatible materials, they are often designated as “biomaterials”, and their infection as “biomaterial-associated infection”. Different
microorganisms have been isolated from infected biomaterials, but *Staphylococcus epidermidis* is the most frequently cultured species. Table 2 provides an overview of the most important microorganisms involved in biomaterial-associated infections.

<table>
<thead>
<tr>
<th>Type of microorganism</th>
<th>Proportion isolated from infected biomaterials</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus epidermidis</em> and other</td>
<td>40-75%</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci (CoNS)</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>10-20%</td>
</tr>
<tr>
<td>Yeasts</td>
<td>5-10%</td>
</tr>
<tr>
<td>Enterococci / streptococci</td>
<td>2-5%</td>
</tr>
<tr>
<td>Coryneform / difffroide species</td>
<td>2-5%</td>
</tr>
<tr>
<td>Gram negative species</td>
<td>2-5%</td>
</tr>
<tr>
<td>Miscellaneous organisms</td>
<td>1-3%</td>
</tr>
</tbody>
</table>

*Table 2: Microorganisms associated with biomaterial infections.*

Of all coagulase-negative staphylococci, *S. epidermidis* is by far the most often isolated species (80% of all CoNS). Therefore, the remainder of this review will focus on *S. epidermidis*.

**Pathogenesis of biomaterial-associated infection: the biofilm hypothesis.**

Experiments performed in the late 1950’s to investigate foreign body-associated infection caused by *Staphylococcus aureus*, showed that the dose of *S. aureus* required to produce purulent infection in healthy volunteers was much lower in the presence of a suture thread, than in the absence of any foreign material. Similarly, in the absence of a biomaterial implant the bacterial inoculum required to achieve an infection with *S. epidermidis* in animal models is very large, whereas in the presence of an implant much smaller inocula will cause infection.

Numerous studies over the past decades indicate that biomaterial-associated infections are caused by bacterial strains that form a biofilm which covers the surface of the biomaterial. Biofilms, complex aggregations of bacteria, bacterial products and host components, are formed in 2 steps. First, individual bacterial cells attach rapidly to the biomaterial surface; this is followed by a prolonged accumulation phase in which bacteria proliferate, adhere to each other and to the biomaterial, and produce extracellular components. The major extracellular component of staphylococcal biofilms has been identified as the polysaccharide intercellular adhesin (PIA). The genes responsible for the production of PIA are found in the intercellular adhesin (ica) locus, and comprise the icaR (regulator) and icaADBC (biosynthesis) genes.
Deletion of the *ica* locus abrogates the capacity to form PIA, and most strains without this locus do not form biofilms. The accumulation-associated protein (Aap) is (partly) responsible for the second phase in biofilm formation: the accumulation phase. In this phase the bacteria proliferate and accumulate, forming multilayered clusters of cells which are embedded in extracellular material. Several strains devoid of the *ica* locus are capable of forming biofilms through their Aap proteins.\(^{19}\)

Biomaterial-associated infections are studied in experimental animal models. In mice and rats the biomaterial is often implanted subcutaneously. Most studies concentrate on the biofilm as a virulence factor, and on the genes, proteins and polysaccharides involved in its formation. For example, studies comparing a fibrinogen-binding protein (Fbe)-deficient mutant\(^{20}\) and a polysaccharide intercellular adhesin/hemagglutinin (PIA/HA)-negative mutant\(^ {21}\) with the respective wild type bacterial strains, showed that the wild type strains cause bacteraemia and metastatic disease, whereas the infection rate is lower with mutant strains\(^ {20,21}\). In a recent study on an *agr* mutant of *S. epidermidis*, an opposite effect was noted. In a rabbit model, where medical tubing was implanted subcutaneously on the dorsum, this mutant had a higher capacity to colonize the implants than the wild type strain\(^ {22}\). Although not emphasized by the authors, the wild-type strain survived in higher numbers in the tissue surrounding the implant. Thus, the deletion of *agr* significantly increased success in the biofilm-associated colonization of indwelling devices, but the presence of *agr* seems to be important for tissue colonization.

Table 3 presents an overview of components of *Staphylococcus epidermidis* and their presumed functions in biofilm formation and in other ways to contribute to virulence and survival of the bacteria in the host (adapted from Queck and Otto\(^ {23}\)).

**Tissue as a possible niche for bacteria.** In addition to providing a surface for adherence and biofilm formation, the foreign body also reduces local efficacy of immune function. In guinea pigs with subcutaneously implanted tissue cages containing a biomaterial, local polymorphonuclear leukocytes (PMN’s) show impaired function, such as low phagocytic and bactericidal activities\(^ {24,25}\). This local impairment of the immune system contributes to bacterial survival. In previous studies from our laboratory in a mouse experimental BAI model it was shown that the biofilm indeed is not the only site where infecting bacteria are found.
In rabbit and mouse models of BAI, segments of non-coated and polyvinylpyrrolidone (pvp)-coated silicon elastomer (SE) catheters were subcutaneously implanted. In vitro, the pvp coating reduced adherence of *S. epidermidis*. Contrary to what was expected, abscess formation occurred in rabbits and mice carrying SEpvp catheter segments, whereas hardly any abscess formation was observed around SE catheters, even after challenge with very large inocula. Abscess formation was also observed when bacteria were pre-grown on the segments prior to insertion, and when a preparation of heat-killed bacteria or purified peptidoglycan was injected along the implant. Over time, the numbers of cfu in the tissue were significantly higher in the SEpvp-implanted group, and the bacteria persisted for up to 60 days. It appeared that around SEpvp a strong and protracted pro-inflammatory response occurred, which delayed the normal foreign body response and caused a reduced clearance of the infection.

Around another biomaterial, pvp-coated polyamide (PApvp) quite a different, relatively anti-inflammatory response was observed. In the tissue surrounding these implants *S. epidermidis* were seen intracellularly inside macrophages and the bacteria

<table>
<thead>
<tr>
<th>Bacterial component</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accumulation-associated protein: Aap</td>
<td>Biofilm accumulation</td>
</tr>
<tr>
<td>Autolysin: AltE</td>
<td>Autolysin/adhesin: attachment to polystyrene, vitronectin binding</td>
</tr>
<tr>
<td>Bap-homologous protein: Bhp</td>
<td>Biofilm formation</td>
</tr>
<tr>
<td>Fibronectin-binding protein: Embp</td>
<td>Fibronectin binding</td>
</tr>
<tr>
<td>Fibrinogen binding protein: Fbe</td>
<td>Fibrinogen binding, inhibition of phagocytosis</td>
</tr>
<tr>
<td>SD-repeat containing proteins: SdrG</td>
<td>Fibrinogen binding, inhibition of phagocytosis</td>
</tr>
<tr>
<td>Intercellular adhesin gene cluster: icaADBC</td>
<td>PIA production</td>
</tr>
<tr>
<td>Lipases: gehC, gehD</td>
<td>Persistence in fatty secretions</td>
</tr>
<tr>
<td>Poly-γ-D-glutamic acid: PGA</td>
<td>Immune evasion, osmoprotection</td>
</tr>
<tr>
<td>Polysaccharide intercellular adhesin: PIA</td>
<td>Biofilm exopolysaccharide: cell-cell adhesion, haemagglutination, inflammatory effects, evasion of host immune system</td>
</tr>
<tr>
<td>Staphylococcal surface proteins: SSP-1, SSP-2</td>
<td>Biofilm initiation: attachment to polystyrene</td>
</tr>
<tr>
<td>Phenol-soluble modulins (PSM)</td>
<td>A pro-inflammatory staphylococcal product that triggers human innate immune responses</td>
</tr>
</tbody>
</table>

Table 3: Bacterial components of *S. epidermidis* and their presumed functions.
increased in numbers over time, whereas around SEpvp lower levels of intracellular persistence were observed \(^{27}\). These studies indicate that in the presence of a foreign body, the peri-implant tissue becomes susceptible for bacterial colonization.

Since the tissue around SEpvp showed a protracted pro-inflammatory response with persistently elevated IL-1 levels, wild type and IL-1 receptor knock-out mice were compared to assess whether the elevated levels of IL-1 were causally related to the increased susceptibility to infection. The wild type mice were more susceptible to abscess formation and contained significantly higher numbers of \(S. \text{epidermidis}\) cfu in the tissue after 14 days \(^{14}\). IL-1 receptor knockout mice had no abscess formation and a reduced susceptibility to \(S. \text{epidermidis}\) infections. Therefore, in the case of “pro-inflammatory” biomaterials, inhibition of the local IL-1 activity may be beneficial to the outcome of BAI. Interestingly, like in the wild type mice, the bacteria that survived in the infected IL-1 receptor knockout mice were found more often in the peri-implant tissue than on the implant.

Mice carrying PApvp, which showed many bacteria surviving in macrophages in the peri-implant tissue, did not have increased IFN-\(\gamma\) levels after infection. To assess whether this lack of IFN-\(\gamma\) caused the intracellular survival, mice were treated with IFN-\(\gamma\). This resulted in decreased intracellular and extracellular persistence of bacteria around the implants, indicating that IFN-\(\gamma\) might be beneficial for prevention of biomaterial-associated infection around materials which provoke a relatively anti-inflammatory response in presence of bacteria \(^{28}\). Most likely IFN-\(\gamma\) acted by activating the macrophages, which is important to kill and remove the bacteria from the tissue.

In addition to the studies in animal models, several studies focussing on patient materials have pointed to the surrounding tissue as a possible niche for infecting bacteria. In a study on failed breast implants, the tissue surrounding the implants was culture positive whereas the implants themselves yielded negative cultures \(^{29}\). Also in orthopaedic joint infection peri-implant tissue may contain bacteria. Routine hospital culturing of samples from tissue surrounding failed implants showed bacterial growth in 41% of 22 cases. After prolonged culturing of tissue samples (incubated for 7 days at 37°C aerobically and anaerobically) the frequency of positive cultures increased to 64% \(^{30}\). These results combined with the extensive animal experimental data from our laboratory suggest that the tissue surrounding implanted devices is an as yet not well recognized niche for infecting bacteria, and warrants further investigation.
Antibiotic treatment of biomaterial-associated infection. Biomaterial-associated infections are notoriously difficult to treat, and require high dosage and prolonged treatment periods. This has been attributed to the local impaired immune response and to the relatively poor penetration of antibiotics into the biofilms on the biomaterial, and to a dormant state of the bacteria. The biofilm appears to increase the resistance of the bacteria to antibiotics. A second possibility is that bacteria reside in the surrounding tissue, in for example macrophages, and that this may contribute to the low effectivity of the antibiotics. Therefore, it would be very interesting to investigate the antibiotic activity against the bacteria residing in tissue.

Antibiotics to treat infections by biofilm bacteria. Up to now, antibiotic strategies to prevent or treat biomaterial-associated infection are generally based on the assumption that the infecting bacteria are present in biofilms. The antibiotics to treat these infections are specifically chosen for their propensity to penetrate in the biofilm and reach the bacteria herein.

Rifampicin for instance, has a low molecular weight and is only slightly soluble in water. It penetrates well and has good bactericidal activity in biofilms. Vancomycin has a higher molecular weight and is highly water soluble, and for optimal killing activity of vancomycin, bacterial growth is required. Rifampicin and vancomycin are often used in combination in the treatment of biomaterial-associated infections.

However, in vitro the combination of vancomycin and rifampicin failed to eradicate S. epidermidis in biofilms after 72 h of incubation. In rabbits implanted with polyethylene discs covered with a biofilm of S. epidermidis, systemic vancomycin reduced the numbers of viable bacteria on the disks within 48 h. There was no further reduction after 72 h with vancomycin only. Combinations of rifampicin, vancomycin and fusidic acid were most active against staphylococcal biofilms. Both linezolid and vancomycin, used as single agent, effectively reduced the numbers of S. epidermidis present on catheters as compared to controls in an in vitro pharmacodynamic model of gram-positive catheter-related bacteraemia; however neither was able to completely sterilize the colonized catheters. In S. epidermidis infected vascular grafts tested in vitro, rifampicin was the most effective antibiotic, at concentrations of 4 times the MIC (minimal inhibitory concentration). At concentration higher than 4 times the MIC, S. epidermidis rapidly developed resistance. Thus, the use of
rifampicin as single agent should be avoided and for effective treatment of bacteria in biofilms combination with another antibiotic is advised.

**Antibiotics to combat tissue-residing bacteria.** As infecting bacteria may reside in tissue surrounding implants, even inside macrophages, antibiotics used to treat biomaterial-associated infection should penetrate the tissue and act intracellularly. Vancomycin is frequently used in experimental studies investigating biomaterial-associated infection, and is commonly used in the clinic. In a BAI model rats were implanted with a vascular graft on the dorsal side which was infected with *S. epidermidis*. In one group vancomycin was delivered locally from glycerylmonostearate implants containing vancomycin, and another group received intramuscular vancomycin injections. None of the animals in the implant group, who had vancomycin delivered locally during the entire period of the experiment showed evidence of infection by *S. epidermidis*, but intramuscular injections of vancomycin failed to clear infection in 4/6 rats. Since vancomycin does not penetrate cells, the antibiotic presumably acted on the bacteria before they were able to “find shelter” within host cells in the tissue. This apparently was only possible when vancomycin was delivered locally and for prolonged periods, and not when systemic injections were given. In a rat study, the animals were implanted subcutaneously with either sterile Dacron or ePTFE grafts and received local or systemic antibiotic prophylaxis. Only a combination of topical rifampicin and systemic vancomycin inhibited *S. epidermidis* growth completely. In a rabbit model where a stainless steel screw was placed into the femur, vancomycin nor minocycline was effective when used as single agent in eradicating an established *S. epidermidis* infection of this orthopaedic device; however, a combination of rifampicin and vancomycin was significantly more effective than vancomycin alone in eradicating the infection. As vancomycin does not penetrate intracellularly, vancomycin may not have reached bacteria present within cells in the tissue surrounding the implants, whereas it did kill bacteria present between cells in the tissue. A combination of antibiotics which kill bacteria in biofilms as well as within tissue and intracellularly may therefore be required for effective treatment of biomaterial-associated infection.

Rifampicin shows excellent penetration into tissue. A 90% cure rate has been reported in patients with prosthetic valve endocarditis caused by methicillin-resistant *S. epidermidis* when rifampicin was combined with vancomycin compared to a
cure rate of 50% when treated with vancomycin alone. Zimmerli et al. have used antibiotic regimens of ciprofloxacin with or without rifampicin to treat infections of hip prostheses. A combination with rifampicin increased the cure-rate significantly. It would be interesting to test this regime in experimental biomaterial-infection models, and to assess whether bacteria on the implants as well as in the peri-implant tissue are eradicated.

**Antibodies to prevent or treat biomaterial-associated infection.** As antibiotic therapy of biomaterial-associated infections is hampered by biofilm formation and tissue colonization by the bacteria, and as antibiotic resistance is an increasing problem, methods for prevention of these infection other than by antibiotic prophylaxis are highly desired. The use of anti-staphylococcal antibodies might be such a method. Currently available antibodies are predominantly directed against polysaccharide antigens. Table 4 presents an overview of targeted antigens of *S. epidermidis* and the activity of their respective antibodies *in vitro* and *in vivo*.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Activity of antibodies <em>in vitro</em></th>
<th>Activity of antibodies <em>in animal models</em></th>
<th>Antigen expressed in patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fbe</td>
<td>Inhibition of adherence to fibrinogen coated microtiter plates / increase macrophage phagocytosis</td>
<td>Lowering the levels of bacterial proliferation</td>
<td>Yes 45, 47, 50</td>
</tr>
<tr>
<td>Aap</td>
<td>Inhibit biofilm formation to microtiter plates</td>
<td></td>
<td>Yes 47, 50</td>
</tr>
<tr>
<td>AlIE</td>
<td>Increase macrophage phagocytosis</td>
<td></td>
<td>Yes 46, 50, 52</td>
</tr>
<tr>
<td>LTA</td>
<td>Reduction in adherence to fibrin platelet clots</td>
<td></td>
<td>Yes 54</td>
</tr>
<tr>
<td>PNAG</td>
<td>Opsonic</td>
<td>Protection against bacteremia</td>
<td>Yes 56, 58</td>
</tr>
<tr>
<td>20kDa PS PS/A</td>
<td>Opsonic</td>
<td>Protects against bacteremia and endocarditis</td>
<td>Yes 52</td>
</tr>
</tbody>
</table>

*Table 4: Overview of targeted antigens of *S. epidermidis*.**
Below, the major antigens used for active and passive immunization are discussed in more detail.

**20kDa PS polysaccharide and capsular polysaccharide adhesin; 20 kDa PS and PS/A.** Biofilm formation consists of two important steps, adherence and accumulation. During the accumulation phase multilayered cell clusters are formed which are surrounded by a slimy matrix. This slimy matrix is thought to play a crucial role in biomaterial-associated infections. The 20kDa PS (polysaccharide) is part of this matrix, and is organized in such a way that it is exposed at the surface of the cell, accessible to antibodies. Polyclonal antisera against this 20-kDa polysaccharide show high specificity in recognizing the major surface antigenic determinants of slime-producing *S. epidermidis* strains, and antibodies against this major polysaccharide are also found in human serum.

Active and passive immunization in a rabbit model of endocarditis with the capsular polysaccharide adhesin (PS/A), or with anti-PS/A, respectively, provides protection against bacteraemia and endocarditis. In a bacterial keratitis model, rabbits actively immunized with 20-kDa PS or passively immunized with antibodies against 20-kDa PS show less corneal damage, and are significantly better protected against *S. epidermidis* keratitis.

**Poly-N-acetylglucosamine; PNAG.** Poly-N-acetylglucosamine (PNAG) is involved in intercellular adhesion and therefore is also referred to as polysaccharide intercellular adhesin. It is the main component of extracellular slime. High molecular weight isoforms of PNAG are highly immunogenic when injected into mice and rabbits, and anti-PNAG antibodies can mediate opsonophagocytic killing of *S. epidermidis* and *S. aureus*. PNAG is produced within the biofilm matrix and must be anchored to the bacterial cell to contribute to biofilm formation. As PNAG is also secreted from the bacterial cell surface, it may act as a decoy by binding potentially opsonic antibodies away from the cell.

In a guinea pig model of BAI, the anti-PNAG IgG antibody titres in sera of the animals challenged with *S. epidermidis* were significantly higher than those in sera of the control group. Therefore PNAG apparently is produced by the bacteria *in vivo*, and is accessible for host immune recognition. Indeed, in animal models IgG antibody titers against PNAG are correlated with protection against infection. However,
no studies have been performed in which the influence of passive immunization with PNAG antibodies on biomaterial infection, particular with respect to peri-implant tissue colonization, was investigated.

**Accumulation-associated protein; Aap.** Biofilm formation by a polysaccharide-independent mechanism of *S. epidermidis*, mediated by the accumulation-associated protein (Aap) has been identified. Aap is present on the cell wall of *S. epidermidis* grown in suspension and in biofilms. The proteolytic processing of Aap is responsible for a biofilm-positive phenotype. *In vitro*, monoclonal antibodies raised against *S. epidermidis* Aap specifically bind Aap and partially inhibit biofilm formation. Banner et al. describe the presence of localized tufts of fibrillar appendages on a subpopulation of cells of *S. epidermidis* and report that the fibrils are comprised of Aap. These data indicate that Aap is present on the cell surface and may be an interesting target for antibodies.

**Fibrinogen binding protein; Fbe.** The fibrinogen binding protein (Fbe) is a 119-kDa protein present on the surface of *S. epidermidis*. Anti-Fbe antibodies opsonize and increase phagocytosis of *S. epidermidis in vitro*. Pretreatment of *S. epidermidis* with antibodies against Fbe also significantly reduced their adherence to catheters which had a natural coating of host plasma proteins and other components, since they were either retrieved from rats at 24 h after subcutaneous implantation, or from patients who had undergone intravenous injections through these catheters. The adherence-blocking effect was more pronounced for catheters coated only by fibrinogen *in vitro*, indicating that fibrinogen absorption to the surfaces might be crucial in biomaterial-associated infections and that antibodies inhibiting binding of bacteria to fibrinogen might help prevent such infections.

**Lipoteichoic acid; LTA.** The teichoic acids and lipoteichoic acid (LTA), along with peptidoglycan are major components of the cell wall of gram-positive bacteria. LTA is a major membrane-associated amphiphilic molecule and can be considered a virulence factor that has an important role in infections and in postinfectious sequelae caused by gram-positive bacteria. The lipid moiety of LTA has a central role in the adherence of *S. epidermidis* to fibrin-platelet clots. *In vitro*, pretreatment of *S. epidermidis* with anti-LTA antibodies reduces adherence of these bacteria to fibrin-
platelet clots. Healthy individuals have low serum levels of IgG against lipid S, a short chain length form of LTA. Higher levels of IgG against lipid S have been detected in serum from patients with central venous catheter-related sepsis and infection of orthopaedic prostheses. 

Anti-LTA antibodies have not yet been tested in animal models of foreign body infection. It would be very interesting to investigate whether anti-LTA is able to reduce adherence of *S. epidermidis* to implanted biomaterials and to prevent infection of the biomaterials as well as of the peri-implant tissue.

*Intravenous immunoglobulin; IVIG.* Intravenous immunoglobulin (IVIG) preparations contain a wide variety of antibodies to pathogens and foreign antigens, and are therefore used to treat patients with autoimmune and systemic inflammatory disorders. The stability of IVIG preparations and their IgG titers vary, depending on the number of donors (up to 11-fold difference in titer for certain pathogens between different preparations). Preparations with high titers of opsonic antibodies offer better protective effects in animal models of infection. IVIG with ≥ 90% opsonic activity promotes *S. epidermidis* clearance from blood in rats, and significantly enhances survival of the rats when compared with lots with ≤ 50% opsonic activity.

The major staphylococcal antigen targeted by the antibodies in IVIG preparations is considered to be the 20 kDa PS. The amount of 20-kDa PS specific antibodies in different IVIG preparations varies, and this may be associated with variation in protective activity. Despite this variation, several studies in humans did show a protective effect of IVIG preparations. In low birth weight preterm neonates who received high titer (> 200 antibody units/ml of the 20-kDaPS) IVIG preparations as a prophylactic agent, the rate of bacteraemia due to slime-producing *S. epidermidis* was considerably lower compared to the control group who did not receive treatment. In patients with primary immunodeficiencies, different IVIG preparations containing different immunoglobulin subclasses were tested, and provided the patients with sufficient amounts of antibodies to protect them against gram positive and gram negative bacterial infection. IVIG preparations thus seem to have perspective in preventing infections in patients with primary or secondary immunodeficiencies associated with defective antibody production or in low birth weight preterm neonates.
Commercial development. Different companies are currently in the process of developing vaccines and immunoglobulin preparations for prevention and treatment of staphylococcal infections and some products have reached phase III clinical trials. StaphVAX (a *Staphylococcus aureus* polysaccharide conjugate vaccine) and INH-A21 (an intravenous immunoglobulin preparation from donors with high titers of antistaphylococcal antibodies) were unsuccessful in phase III trials. In response to the failure of StaphVAX, the production of Altastaph (an anti-*Staphylococcus aureus* immunoglobulin targeting the capsular polysaccharide) was suspended. Due to these disappointing results, it remains to be seen whether novel antibody preparations will be marketed. This demonstrates the difficulty in preparing an effective antistaphylococcal preparation for passive or active vaccination.

Aim of the thesis. As biomaterial-associated infections are a major problem in modern medicine, and *S. epidermidis* is usually associated with these infections, it is important to gain more insight in the pathogenesis of these infections and to assess the efficacy of different antimicrobial agents to prevent or treat these infections. The aim of this thesis is to investigate the pathogenesis of biomaterial-associated infections, with emphasis on the presence and role of *S. epidermidis* in the peri-implant tissue. In the second chapter we study the kinetics of colonization of implants, as well as peri-implant tissue, with two *S. epidermidis* strains, two biomaterials, and two mouse strains, C57BL/6 and BALB/c. The third chapter describes the influence of an antibiotic regime consisting of vancomycin / rifampicin on biomaterial as well as tissue colonization in the mouse BAI model. To our knowledge, antibodies have not been tested *in vivo* in presence of a biomaterial. Therefore, in the fourth chapter the mouse model is used to assess the effect of two monoclonal antibodies on biomaterial-associated infections. The fifth chapter describes a new technique to demonstrate the presence and location of viable bacteria in peri-implant tissue. The technique is based on application of 5-bromo-2-deoxyuridine (BrdU), a synthetic thymidine analogue which is incorporated into newly synthesized DNA during replication and DNA repair. In chapter 6 we investigate whether also in humans tissue surrounding a foreign body is a reservoir for bacteria associated with biomaterial-associated infection. To this purpose, we examined catheters and peri-catheter tissue obtained from deceased patients at autopsy. In chapter 7, finally, the general implications of our findings are discussed.
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