Biomaterial-Associated Infection: Pathogenesis and Prevention

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1 The Clinical Problem

The use of medical devices, such as urinary stents, catheters, artificial heart valves, prosthetic joints and other implants, collectively often referred to as "biomaterials" has increased dramatically over the past century, and has become a major part of modern medicine and our daily life. With the aging society, the higher demand on these devices to restore function and quality of life, combined with the ever improving technology within the medical field, the problem of biomaterial-associated infection (BAI) is expected to increase.

Catheters, and orthopedic devices are among the most frequently used devices in human medicine [1, 2]. Catheters suspected for infection are replaced by a new catheter at a different location, since using the original location for re-implantation over a guide-wire is strongly discouraged because of the high reinfection risk [3]. Primary implantation of prosthetic joints like prosthetic hips, knees, elbows and ankles, is considered a so-called clean procedure [4], however, in 0.5–1% (hip or knee) to over 5% (elbow or ankle) of cases, infections occur [5, 6]. Revision surgery is associated with higher frequencies of infection, due to the compromised condition of the tissue, longer procedures and more extensive tissue damage during surgery.

The most common causative microorganisms in BAI are *Staphylococcus aureus*, a major pathogen in wound infections, and *Staphylococcus epidermidis*, the harmless skin commensal [6–8]. Depending on the type of device and location of application, other pathogens such as coagulase-negative staphylococci, enterococci, streptococci, *Propionibacterium acnes* and yeast can also cause BAI [9, 10].

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As early as in 1957, Elek and Conen studied the minimum infective dose of staphylococci for man in relation to suture infection [11]. In healthy volunteers, they estimated the minimum pus-forming dose of *S. aureus*—called *Staphylococcus pyogenes* in those days—on intradermal injections in absence of sutures to be 2–8 million bacteria, numbers which are improbable in case of a natural infection. However, the presence of a foreign body, a suture in this case, resulted in a dramatic reduction in the minimal inoculum required for pus production: a dose of 300 bacteria led to abscess formation. Higher inoculum doses even resulted in lesions with 'the size of an orange', caused fever and took over a week to resolve, in spite of penicillin therapy. Although this experiment clearly demonstrated the enhancing effect of the presence of a foreign body, but the authors stated that the outcome of the experiment "led to great difficulty in finding further volunteers". Nowadays, such an experimental set-up would not be easily approved by medical ethical committees, but it did provide crucial information on the pathogenesis of BAI. Thus, it has been recognized for at least 60 years that the presence of a foreign body predisposes for infection, and this has repeatedly been confirmed in animal studies [12–15]. In rabbits, for example, only 50 colony forming units (CFU) of *S. aureus* were sufficient for infection in the presence of a cemented hip implant, whereas 10,000 CFU were required in absence of the foreign body [16].

1.1 Biofilms

Bacterial biofilm formation is considered the major element in the pathogenesis of BAI [1, 10, 17]. Biofilm formation is initiated when planktonic bacterial cells attach to the surfaces of implants (Fig. 1). BAI are often caused by biofilm-forming bacterial strains able to cover the surface of the biomaterial, resulting in complex structures consisting of bacteria, extracellular polymeric substances (bacterial products like polysaccharides, proteins and DNA) and host proteins and cells [17]. Bacteria in biofilms behave differently from planktonic bacteria, particularly in response to antibiotic treatment [18]. The complex bacterial community of a biofilm is highly tolerant to antibiotics [19]. This is partly due to the complicated structure of the extracellular polymeric matrix of the biofilm, making the bacteria less accessible to many antibiotic agents [20]. As most antibiotics target active cell processes, the slow growth or starved state of the bacteria in a biofilm may also make them more tolerant. A subpopulation of these bacteria, the so-called persisters, reaches a dormant and drug-tolerant state. Such persisters are suggested to be largely responsible for the recalcitrance and recurrence of biofilm-associated infections [21]. Moreover, biofilm-entrapped bacteria are unreachable for the human immune system.

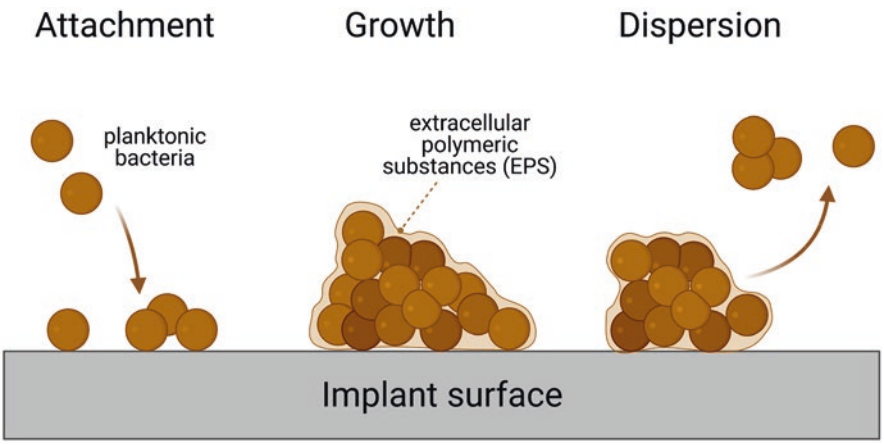

Fig. 1 Biofilm stages in biomaterials

1.2 Tissue Colonization

Next to biofilm formation, another important element in the pathogenesis of BAI is bacterial colonization of the tissue around implants (Fig. 2), due to dysregulation of the local immune response by the combined presence of bacteria and a foreign body [22–25]. Bacteria are inevitably introduced in the tissue wound during surgery, either originating from the patient's skin microflora or from the operation room [26]. Due to the implanted biomaterial, the efficacy of the host immune response is reduced. Already in the 1980s, Zimmerli *et al.* showed reduced neutrophil phagocytic activity in guinea pig tissue cage models infected with *S. aureus* [27]. When different challenge doses of *S. epidermidis* were injected along subcutaneously implanted catheter segments at the back of mice, the bacteria were more often found in the peri-implant tissue than on the biomaterial itself, and persisted for longer periods in the tissue than on the implant [28]. Moreover, *S. epidermidis* survives inside macrophages in tissue surrounding implants in mice (Fig. 2) [25, 28].

In a mouse subcutaneous BAI model, the possible routes of infection at the interface between implants and the surrounding tissue were studied [29]. In this study, *S. epidermidis* bacteria applied on the surface of titanium implants, both adhering and as a biofilm, relocate from the material to the surrounding tissue (Fig. 2), which is accordance with earlier studies with other types of materials [25, 28]. This suggests that it is a more general phenomenon occurring around implants manufactured from biomaterials as diverse as polymer and titanium, and with different bacterial species. In a study by Broekhuizen *et al.*, mice were treated with dexamethasone and BrdU, a nucleotide analogue that is incorporated into DNA of dividing cells and can be detected immunohistologically. Analysis of tissue samples collected at 14

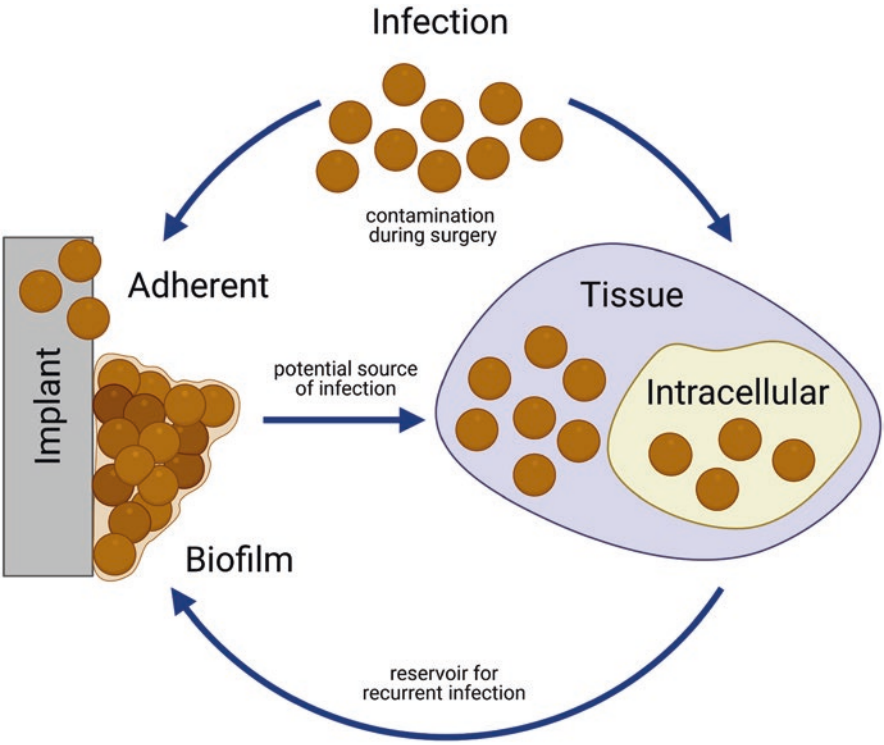

Fig. 2 Pathogenesis of the biomaterials associated infection

and 21 days after challenge with *S. epidermidis* showed regrowth of the bacteria with BrdU incorporated, which had apparently replicated between day 14 and 21, suggesting that tissue rather than the implant provides a hiding place for the bacteria [30]. Moreover, after incubation of peri-catheter tissue biopsies of deceased intensive care unit patients with BrdU, bacteria had incorporated BrdU *in situ*, proving that bacteria also reside and synthesize nucleic acids within tissue surrounding biomaterials in humans [30].

Bacteria colonizing the surface of a biomaterial not only are a focus of a localized biofilm infection, but can also be the source of tissue colonization (Fig. 2). Conversely, bacteria residing in the tissue can be a cause of infection after re-implantation, in experimental infection [31] as well as in patients [32].

Tissue-residing bacteria can be hard to eradicate by antibiotic treatment [33, 34]. For instance, when infected prosthetic joints are removed, patients usually require a prolonged regimen of systemic and local antibiotic treatment in order to reach and kill bacteria present in the tissue before re-implantation can be performed [6, 35]. In conclusion, next to the prevention of bacterial colonization of the implant and the subsequent biofilm formation, prevention of bacterial colonization of peri-implant tissue is of vital importance.

1.3 Intracellular Survival

In the subcutaneous mouse BAI model staphylococci predominantly co-localized with macrophages in the peri-implant tissue, even when the bacteria were present exclusively on the implant surface at the start of the experiment (Fig. 2) [29]. This interesting observation suggests that the bacteria were either removed from the implant by phagocytosis, or first detached and were subsequently phagocytosed. In this mouse model, both *S. epidermidis* [29] and *S. aureus* [36] were cultured in high numbers from the tissue and co-localized with macrophages in histology, particularly at 4 days after challenge, suggesting that these macrophages were not effectively killing the bacteria. Most likely, the local host immune response is impaired in presence of an implant, resulting in less or no clearance of bacteria. As mentioned before, neutrophils can have reduced phagocytic and bactericidal capacity in the vicinity of an implant [27, 37]. Moreover, the intracellular killing capacity of macrophages can be reduced due to altered cytokine tissue levels due to the presence of a biomaterial [25, 30, 37–39]. Staphylococci may even form small colony variants to adapt to this micro-environment, which are more resistant to antimicrobial compounds [40, 41]. Apparently, when bacteria are initially present near or on the surface of implants this results in ineffective eradication by phagocytes. This might lead to persistence of (intracellular) bacteria in the peri-implant tissue.

1.4 Antimicrobial Resistance

In addition to the difficulty of treating biofilm-encased or intracellularly residing bacteria with conventional antibiotic therapy, treating BAI is further hindered by the rising antibiotic resistance among pathogens. The World Health Organization recently endorsed a global action plan to tackle antibiotic resistance [42]. One of the key objectives of this plan is to develop novel antimicrobial drugs. The emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) pathogens, accelerated by the selective pressure exerted by extensive use and misuse of antimicrobials, further underscores the very pressing need for the discovery of novel treatment strategies to replace or complement the conventional antibiotics. Magiorakos *et al.* defined MDR bacteria as non-susceptible to at least one agent in three or more antimicrobial categories, XDR bacteria as non-susceptible to at least one agent in all but two or fewer antimicrobial categories, meaning bacterial isolates which remained susceptible to only one or two categories, and PDR bacteria as non-susceptible to all agents in all antimicrobial categories [43]. The occurrence of XDR and PDR strains illustrates the clinical challenges that we will be facing in the dark scenario of a possible “post-antibiotic era”. Antimicrobial resistance causing limited or no treatment options in critically ill patients, stresses the importance of the development of new agents that can be used

against drug-resistant bacteria. Clearly, it is vital that novel antimicrobial agents are also effective against drug-resistant Gram-negative bacteria belonging to the so-called ESKAPE panel (*Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species [44]), which cause the majority of US hospital infections [45] and are associated with high morbidity and mortality [46].

2 Preventive Strategies

As explained above, in addition to biofilm formation on the implant, colonization of peri-implant tissue is an important factor in the pathogenesis of BAI. Therefore, this niche needs to be taken into consideration when designing preventive strategies against BAI. Current strategies mainly focus on the development of four types of antimicrobial surfaces: (1) antifouling/anti-adhesive surfaces, (2) tissue-integrating surfaces, (3) contact-killing surfaces, and (4) surfaces which incorporate and release antimicrobials (Fig. 3) [47]. These approaches all have their benefits and limitations, which need to be taken into account when designing an antimicrobial strategy for a particular device [48].

2.1 Anti-adhesive

Implant surfaces are ideal substrates for opportunistic bacteria to attach to, colonize, and form biofilms on. Surface properties of the implant, like surface charges, hydrophobicity/hydrophilicity and surface chemistry play a major role in initial bacterial adhesion and proliferation. Already in 1987, Gristina suggested that tissue cell integration and bacterial adhesion compete for a spot on the implant's surface, summarized as the so-called 'race for the surface' concept [49]. In case the bacteria win this race, infection instead of tissue integration would be the end result. In addition,

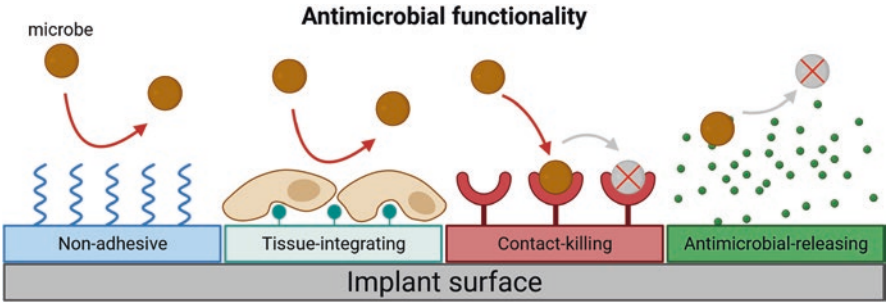

Fig. 3 Antimicrobial functionality in implant surface

Gristina also suggested that colonization of the tissue around implants was a possible mechanism of infection [49]. Bacterial adhesion and subsequent biofilm formation may be prevented by modifying the physicochemical surface properties of biomaterials, for instance by using hydrophilic polymer coatings, *e.g.* immobilized poly(ethylene glycol) (PEG), as applied on contact lenses, shunts, endotracheal tubes and urinary catheters [47, 50]. Functionalization of the surface with a dense layer of polymer chains commonly known as polymer brush coatings, is another approach [34, 51]. Large exclusion volumes of tethered polymer chains result in surfaces difficult to approach by proteins or bacteria, and these brush coating molecules may even possess antimicrobially active functional groups.

2.2 Antibiotics

In general, antibiotics are selected based on their capacity to prevent biofilm formation, but not on their ability to kill bacteria in the other niches relevant for BAI, like in peri-implant tissue and intracellularly in host cells [47]. Antibiotics often used in the treatment of BAI, such as vancomycin and gentamicin, have low or hardly any penetration into host cells, and are thereby not active against intracellular bacteria. On the other hand, rifampicin (against staphylococci) or fluoroquinolones (against Gram-negative bacilli) do target these intracellularly localized bacteria, but resistance develops rapidly against these antibiotics. The combination of vancomycin and rifampicin is often used to treat BAI, but—as vancomycin does not reach intracellular bacteria—this likely results in a high risk of resistance development towards rifampicin.

Coatings releasing antibiotic are widely used for medical devices, like in sutures and central venous and urinary tract catheters. These coatings have two major disadvantages: (1) a patient can be infected with a bacterium resistant to the released antibiotic, and (2) due to the local release a gradient of the antibiotic will be created near the implant, which increases the risk to select for resistant bacteria. In view of the increasing development of resistance, the use of antibiotics for medical device is discouraged by government regulatory agencies like the American Food and Drug Administration (FDA) [48, 52].

2.3 Antiseptics

As an alternative to antibiotics, commonly used antiseptics and disinfectants may be used, as they are less known to induce resistance and in general have a broader spectrum of activity than antibiotics. These biocides, such as alcohols, aldehydes and biguanides, are extensively used in hospitals and other health care settings, and also by the general public, as an essential part of infection control practices [53]. Probably the most widely used biocide in antiseptic products (*e.g.* hand wash and oral products) is chlorhexidine, owing to its broad spectrum activity, low toxicity

and good tolerability of soft tissue. Moreover, resistance development is extremely rare and chlorhexidine has been shown to prevent infection in animal models [36] and in patients [54]. It is used topically, for surgical site preparation, and also intracorporeally [55], and as dental irrigant fluid [56]. Chlorhexidine is currently FDA approved for coatings on intravenous catheters, and these catheters have been shown to be effective in decreasing catheter-related infection in humans [57, 58].

2.4 Antimicrobial Peptides

As discussed earlier, due to the major problems arising from resistance to conventional antibiotics, there is a strong need for antimicrobials not associated with resistance development. Antimicrobial peptides (AMPs) are innate defence molecules of animals, plants and microorganisms. These amphipathic, cationic peptides commonly have antimicrobial activity against a wide variety of pathogens, including bacteria, fungi and viruses, and low risk of resistance development [59, 60]. In addition, many AMPs have immune-modulatory and wound healing activities [61]. The low risk of resistance development is due to the fact that AMPs interact with microbial membranes, mostly resulting in membrane depolarisation, permeabilization and/or disruption leading to rapid cell death, or passing of the membrane to reach intracellular targets [62]. Naturally occurring human AMPs are considered excellent templates for the development of novel synthetic antimicrobials. Indeed, native AMPs have been used as design templates for a large variety of synthetic AMPs, some of which have now entered phase 2 and 3 clinical trials [63, 64].

For biomaterials, the predominant AMP-related antimicrobial strategies are coating by tethering AMPs to the surface, or to apply the peptides in controlled release coatings. Immobilisation of AMPs on surfaces has been performed with a variety of peptides, and with many different chemistries [65–68]. Peptides should retain the structural characteristics important for their antimicrobial activity after immobilisation, to be effective on a surface. Length, flexibility, and kind of spacer connecting the peptide to the surface, the AMP surface density and the orientation of the immobilised peptides are other decisive factors for success [69]. Interestingly, even short surface-attached peptides, which are unlikely to have a free interaction with the bacterial membrane, have antimicrobial activity [70], probably due to destabilisation of the membrane by displacement of positively charged counter-ions, changing bacterial surface electrostatics and activating autolytic enzymes or disrupting the ionic balance [70].

Surface attachment of peptides may have certain disadvantages. Firstly, chemical procedures of tethering AMPs to surfaces may cause strong decrease in their antimicrobial activity, or even their inactivation [71, 72] depending on the combination of peptides and immobilization technology. Secondly, proteins, blood platelets and dead bacteria may block the antimicrobial groups on the surface. Lastly, since the antimicrobial activity is restricted to the surface of the implant, there is a lack of antimicrobial impact on bacteria in the tissue surrounding the implant.

Incorporation of AMPs in controlled release coatings has not yet been extensively developed, although AMPs such as OP-145 [73], IB-367 (Iseganan) [74] and Omiganan [75] have already reached clinical phase 2 or 3 testing for infections not associated with biomaterials [64]. Application of AMPs in antimicrobial surface coatings is however a subject of increasing interest [65–67, 76, 77].

In addition to direct antimicrobial activity, AMPs can prevent excessive activation of pro-inflammatory responses by binding bacterial endotoxins such as lipopolysaccharide (LPS) of Gram-negative bacteria, and peptidoglycan (PG) and lipoteichoic acid (LTA) of Gram-positive bacteria, which leads to their neutralization. This way, AMPs combine the desired characteristics of both direct antimicrobial agents and immune-modulators. The immunomodulatory activity may be used to increase efficacy of clearance of bacterial biofilm infection [78, 79], and might help to prevent derangement of immune responses which increase susceptibility to infection [22, 80, 81].

3 Conclusions and Future Perspective

Prevention of BAI is a challenging problem, in particular due to the increased risk of resistance development associated with current antibiotic-based strategies. Here we showed the evidence of biofilms as a source for peri-implant tissue colonization, clearly showing the importance of preventive measures to be able to act both against implant and tissue colonization. Subsequently, we described different strategies to prevent BAI and other difficult-to-treat biofilm infections. Therefore we conclude that future research should focus on the development of combination devices with both anti-fouling or contact-killing capacities—to protect the implant—and controlled release of an antimicrobial agent to protect the surrounding tissue.

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