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Pathogenesis and prevention of biomaterial centered infections

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One of the major drawbacks in the use of biomedical materials is the occurrence of biomaterials centered infections. After implantation, the host interacts with a biomaterial by forming a conditioning film on its surface and an immune reaction towards the foreign material. When microorganisms can reach the biomaterials surface they can adhere to it. Adhesion of microorganisms to an implant is mediated by their physico-chemical surface properties and the properties of the biomaterials surface itself. Subsequent surface growth of the microorganisms will lead to a mature biofilm and infection, which is difficult to eradicate by antibiotics. The purpose of this review is to give an overview of the mechanisms involved in biomaterials centered infection and the possible methods to prevent these infections.

1. Introduction

Foreign materials are used more and more in modern medicine after trauma, oncological surgery or wear to replace, support or restore human body function, for example in hip prostheses, prosthetic heart valves or catheters. The extended use of these materials, usually referred to as biomaterials, has some major drawbacks. One of these is the possible occurrence of biomaterials centered infections (BCI) [1]. The incidence of this type of infections varies from 4% for hip prostheses [2] to 100% for urinary tract catheters after 3 weeks use [3] (see Table I). In BCI microorganisms are present in close association with the biomaterials surface forming a so-called biofilm. BCI can cause severe problems, from disfunctioning of the implanted device to lethal sepsis of the patient. Furthermore, treatment of BCI is complicated, as microorganisms in a biofilm are more resistant to antibiotics [4] than their planktonic counterparts [5]. As a consequence and if possible, the only remedy is removal of the infected implant at the expense of considerable costs and patient's suffering. A more convenient way to deal with this problem is to prevent the development of an infectious biofilm on the biomaterials surface. To achieve this, a thorough understanding of how these biofilms develop is necessary.

2. Host–biomaterials interactions

2.1. Conditioning films

The interaction of biomaterials with the body of the host depends on the place where the implant or device is situated, for example in the oropharyngeal cavity, the urinary tract, different body tissues or in the circulatory system. When a biomaterial is inserted, first a so-called conditioning film from organic matter present in the surrounding fluid is deposited on the biomaterials surface. Depending on the body site the surrounding fluid can be saliva, urine, tear fluid, tissue fluid, serum, or blood, and the conditioning film will mostly consist of adsorbed proteins, which for serum are mainly albumin, immunoglobulin, fibrinogen, and fibronectin. The composition of the conditioning film depends on the physico-chemical properties, i.e. chemical composition, hydrophobicity and charge, of the biomaterials surface. For example, on polyethylene hydrophobicity gradients exposed to blood serum it was found that at the hydrophobic end less proteins were adsorbed with relatively more fibrinogen, while on the hydrophilic end more albumin was present [6]. In time, smaller proteins like albumin are usually replaced by higher molecular weight proteins, like fibrinogen and fibronectin. With blood contacting biomaterials, also blood cells will adhere to the biomaterials surface. Especially adhesion of blood platelets to artificial vascular grafts can initiate the blood coagulase cascade, causing thrombosis, a frequent complication in these applications. Another unwanted phenomenon at the biomaterials surface is calcification of the implant, which can decrease the necessary flexibility of, for example, prosthetic heart valves. Ideally host derived cells will colonize the implanted biomaterials, forming a thin capsule around the implant.

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TABLE I Incidences of infection of different biomedical implants and devices adapted from Dankert *et al*. [2] and arranged according to body site

Body site	Implant or device	Incidence (%)
Urinary tract	UT catheters	10–20
Percutaneous	CV catheters	4–12
	Temporary pacemaker	4
	Short indwelling catheters	0.5–3
	Peritoneal dialysis catheters	3–5
Subcutaneous	Cardiac pacemaker	1
Soft tissue	Mammary prosthesis	1–7
	Intraocular lenses	0.13
Circulatory system	Prosthetic heart valve	1.88
	Multiple heart valve	3.6
	Vascular graft	1.5
	Artificial heart*	40
Bones	Prosthetic hip	2.6–4.0
	Total knee	3.5–4

*From experiments in calves and sheep.

2.2. Immune reactions

The host will also actively interact with the biomaterials surface if it is invasively implanted, as this is a normal reaction to any foreign body that enters the host. The coagulation cascade and complement system are activated, leading to formation of a fibrin network and opsonization of the biomaterial [7, 8]. These processes will attract and activate the innate immune system, i.e. macrophages and polymorphonuclear cells, leading to inflammation [9, 10]. This immune response can disappear when the wound is healed and the biomaterial is encapsulated. However, in many cases the host–biomaterials interface remains in a state of chronic inflammation, as few metals and plastics are chemically inert in the warm, wet, oxygenated environment of living tissues, causing the release of inflammatory compounds from the biomaterial, like corrosion products, plasticizers, and monomers [1, 11]. Chronic inflammation impairs host cell growth on the implant [12] and can cause chronic pain.

3. Pathogenesis of biomaterials centered infections

The presence of a foreign material significantly compromises the host to cope with microorganisms. In a classical study in man it was shown that the presence of a subcutaneous suture reduced the required inoculum to produce infection with *Staphylococcus aureus*, an infamous virulent pathogen, from 10^6 to only 200 bacteria [13]. Furthermore, the relatively avirulent *Staphylococcus epidermidis*, normally not capable of establishing infection, is the most common infecting organism in BCI [14].

3.1. Inoculation

Probably the most important factor determining the occurrence of BCI is the chance that microorganisms will reach the biomaterials surface. Biomaterials in contact with the outer part of the body, for example intravenous catheters, peritoneal dialysis catheters, or urinary tract catheters are readily reached by microorganisms and consequently have a higher incidence of BCI than fully implanted biomaterials (0.5–100% vs. 0.1–7%) [2]. Microorganisms can reach a biomaterials implant in several ways at several time points, which determines the properties of the biomaterials surface they will meet. Airborne microorganisms, which can be present in the operating theater, can reach the surface as early as before the implantation [15, 16] and interact with a bare substratum surface, not even covered with a conditioning film. Also during insertion of the biomaterial, microorganisms from the skin can be pushed towards the implant surface. Furthermore, microorganisms from the skin can contaminate the operation wound and reach the implant surface through diffusion, active movement or hematogenous transport. Perioperative contamination is believed to be the most common cause of BCI [17].

Generally, it is also assumed that microorganisms can reach the implant via the hematogenous route at any time after implantation, causing so-called hematogenous infections. As shown in Table II skin infections, surgical or dental interventions, pneumonia, abscesses, or bacteriuria can cause temporal or chronic bacteremia resulting in infections [18]. In addition, microorganisms can translocate from the gastro-intestinal tract to other body parts [19]. When the microorganisms survive in the bloodstream, they can be transported to the biomaterials surface, establishing an infection. In this respect it is especially interesting to note that it has been proposed that macrophages play a role in transporting microorganisms to the biomaterials surface [20] as some strains are capable to survive within macrophages [21]. As the biomaterials surface elicits a foreign body reaction in the first weeks after implantation, and in the case of chronic inflammation also hereafter, macrophages are specifically attracted to the biomaterials surface thus potentially transporting microorganisms to the biomaterials implant or device [22]. As hematogenous infection can happen anytime, biomaterials implants are sometimes called ''microbial time bombs''.

The etiology of BCI can provide information about the origin of the infecting organisms. Table III shows the organisms found in examples of studies with vascular grafts, hip and knee arthroplasties. *S. epidermidis* and *S. aureus*, primarily skin inhabitants, are the predominant infecting organisms [14], followed by Gram-negative bacilli like *Escherichia coli* and *Pseudomonas aeruginosa*, primarily present in the gastro-intestinal and urinary tract, streptococci from the mouth and pneumococci from the respiratory tract [18, 23]. Interestingly, except in the case of airborne microorganisms, the

TABLE II Distant infectious foci of hematogeneously infected hip [17] and knee arthroplasties [18]

Distant foci	Hip ($n = 27$) (%)	Knee ($n = 72$) (%)
Cutaneous region	19	39
Urinary tract	15	19
Respiratory tract	15	14
Oral cavity	30	7
Gastrointestinal tract	4	6
Septic arthritis	0	3
Abdominal abscess	0	1
Unknown	19	11

TABLE III Microbial etiology of vascular graft [23] and hematogenous orthopedic implant [17, 18] infections

Germ	Vascular grafts (%)			Orthopedic implants (%)	
	Abdominal ($n=17$)	Inguinal ($n=60$)	Popliteal ($n=8$)	Hip ($n=27$)	Knee ($n=72$)
S. aureus	14	40	33	52	52
S. epidermidis	7	13	17	4	4
Streptococci	14	8	25	22	14
Pneumococci	n.d.	n.d.	n.d.	7	6
E. coli	42	9	0	7	11
Proteus species	3	11	0	4	4
other Gr. neg. bacilli	0	8	17	4	4
Other bacteria	10	5	0	0	1
Candida species	3	1	0	0	0
Unknown	7	5	8	0	0

n.d. = not determined. Values for n indicate number of patients.

infecting organisms usually originate from the hosts microflora. There is evidence that the host might be immuno-tolerant to some of these microorganisms, which has been especially investigated for the intestinal microflora [24–26]. This might be an overlooked virulence factor for causing BCI. As immuno-tolerated microorganisms can survive longer in the blood without being attacked by the hosts immune system, they have a bigger chance to reach an implanted biomaterial.

3.2. Microbial adhesion

When microorganisms have reached the biomaterials surface, initial microbial adhesion can occur. Microbial adhesion is mediated by specific interactions between cell surface structures and specific molecular groups on the substratum surface [14], or when viewed from an overall, physico-chemical view-point by non-specific interaction forces, including Lifshitz–Van der Waals forces, electrostatic forces, acid–base interactions, and Brownian motion forces [27]. Specific interactions are in fact non-specific forces acting on highly localized regions of the interacting surfaces over distances smaller than 5 nm, while non-specific interaction forces have a long-range character and originate from the entire body of the interacting surfaces, as is shown in Fig. 1 [28]. Upon approach of a surface, organisms will be attracted or repelled by the surface, depending on the resultant of the different non-specific interaction forces. Thus the physico-chemical surface properties of the biomaterial, with or without conditioning film or epithelial cells, and microorganisms play a major role in this process.

The conditioning film on the biomaterials surface (and on the bacterial cell surface) plays an important role, as it changes the physico-chemical properties of the interacting surfaces. Most proteins are capable of reducing the adhesion of microorganisms. Albumin is a strong adhesion inhibitor, for unknown reasons, although changes in hydrophobicity and sterical hindrance are proposed mechanisms [14]. Fibronectin and fibrinogen have been shown to promote the adhesion of *S. aureus* and certain *S. epidermidis* strains, which is mediated by specific adhesive cell structures directed to these proteins [14, 29].

The adhesion mechanism of late hematogenously transported microorganisms is unclear, as by that time the biomaterial will be covered with host derived cells, which will decrease the adhesion probability of microorganisms [1]. Hematogenous infections are, besides with intravascular prostheses, almost exclusively reported with orthopedic implants [17, 18]. Orthopedic implants are usually not completely integrated within host tissue, as they consist often of metal parts, which are not easily colonized by host tissue cells. The uncovered metal surface can be colonized readily by microorganisms. Another explanation could be that the repeated hinging of orthopedic implants, for example in knee prostheses, can cause cell damage, providing adhesive sites for microorganisms [1].

When the microorganisms have adhered to a biomaterials surface they are protected against phagocytosis, as the microorganism and biomaterial together are too large to ingest. Furthermore Zimmerli and coworkers reported that the activity of phagocytes and polymorphonuclear leukocytes is decreased in the presence of a biomaterial [30, 31]. After adhesion to biomaterials most microorganisms start secreting slime and embed themselves in a slime layer, the glycocalix, which is an important virulence factor for BCI and which explains the extraordinary prevalence of slime producing *S. epidermidis* in BCI [14]. The glycocalix provides protection against humoral and excreted cellular immune components, as these cannot readily diffuse through the slime layer [4] and once a glycocalix has formed a BCI with all its complications, including ultimately removal of the implant, seems almost inevitable. However, to do real damage the adhering microorganisms first have to grow.

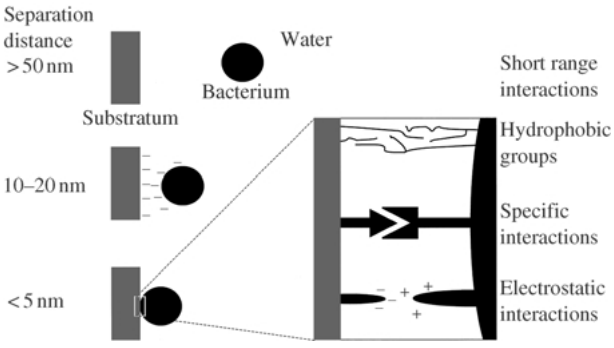

Figure 1 At separation distances of >50 nm, only attractive Van der Waals forces occur. At 10–20 nm, Van der Waals and repulsive electrostatic interactions influence adhesion. At <5 nm, short-range interactions can occur, irreversibly binding a bacterium to a surface. Adapted from Busscher and Weerkamp [27].

3.3. Microbial growth

Initial microbial growth, i.e. the growth in the first hours after microbial adhesion, is another important factor for the outcome of BCI, as biofilm organisms become more resistant to the immune system while the biofilm matures. Growth of sessile microorganisms has been mainly studied *in vitro* [32]. Barton *et al*. [33] found that growth of sessile *P. aeruginosa, S. epidermidis* and *E. coli* depended on the biomaterial involved. Only for *P. aeruginosa* they could find a correlation between physico-chemical properties of the biomaterials surface and initial growth rates. Also proteins in the conditioning film can influence the growth rates of adhering microorganisms. Poelstra *et al*. found that growth rates of *P. aeruginosa* decreased in the presence of pooled immunoglobulin G [34].

Van Loosdrecht *et al*. [35] concluded that adhesion of bacteria does not directly influence their metabolism and growth yield. Changes in growth rate due to adhesion of bacteria were suggested to be mainly the result of changes in nutrient availability. Depending on the amount of adsorbed nutrients and whether adsorption is easily reversed, growth rates of adhering bacteria can be decreased or increased with respect to the growth of their planktonic counterparts. Another mechanism, causing a decrease in the growth rates of *E. coli* after adhesion was proposed to be the strong attraction to positively charged biomaterials surfaces [36].

There is little information about microbial growth *in vivo*. Barth *et al*. followed the number of bacteria for 48 h on subcutaneously implanted polymeric and metal biomaterials in rabbits, after inoculation at the time of implantation [37]. They found, as is shown in Fig. 2, that the number of non-slime producing *S. epidermidis* decreased directly after implantation, probably due to action of the host immune system, while the number of slime producing *S. epidermidis* and *S. aureus* increased in the first 8–12 h to a maximum, whereafter their number decreased again. Here also materials differences played a role. *S. aureus* grew faster on the metal, while *S. epidermidis* grew faster on polymeric biomaterials. An important difference between *in vitro* and *in vivo* experiments is the presence of the immune system. Although unprotected bacteria are eradicated by the immune system, it has been reported that those microorganisms that do survive accelerate their growth rates under influence of cytokines excreted by macrophages [38].

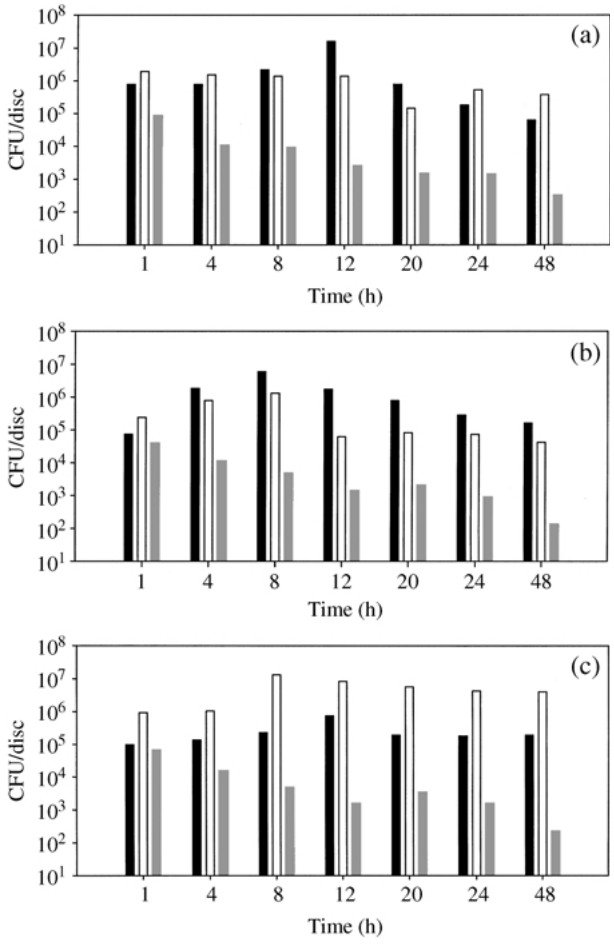

Figure 2 The numbers of colony forming units per disk of (a) PMMA, (b) UHMW-PE and (c) titanium vanadium aluminum alloy after different *in vivo* implantation times. SE-360 (black) is a slime producing and SP-2 (gray) is a non-slime producing strain of *S. epidermidis*. White bars represent *S. aureus*. Note the decline in adhering non-slime producing *S. epidermidis* in time. Adapted from Barth *et al*. [36].

3.4. Consequences of BCI

The presence of a microbial biofilm on biomaterials impairs the function of the implant or device and/or worsens the clinical state of the patient. Examples with non-implanted devices are voice prostheses, which are situated between the trachea and the upper digestive tract, as is shown in Fig. 3, or urinary tract catheters. The action of the voice prosthesis is impaired by biofilm formation, because microorganisms block the valve mechanism, or cause leakage of food into the trachea [39]. As a consequence the prosthesis has to be replaced every 4 months on average [40]. Urinary tract catheters rarely escape colonization by microorganisms causing blockage or, more seriously, bacteriuria [41]. Infections of indwelling catheters, like for example central venous catheters, often results in bacteremia which can cause sepsis and endocarditis. Totally implanted prostheses have lower rates of infections, but the consequences are often more serious. Especially infections of implants in the circulatory system, i.e. prosthetic valves and vascular grafts, yield a high mortality (70% and 50% respectively [42]). Infection of deep tissue implants, for example

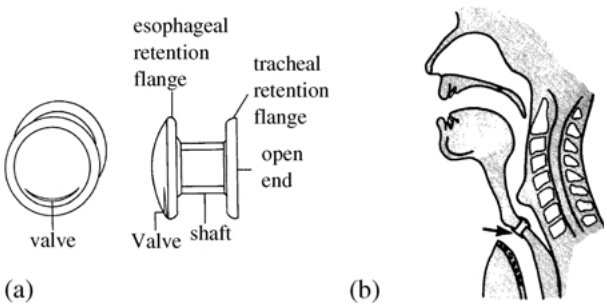

Figure 3 The voice prosthesis (a) and anatomy after total laryngectomy (b). The voice prosthesis is placed in the tracheo-esophageal shunt, an area which is heavily inhabited by microorganisms.

orthopedic implants or mammary prostheses, will usually result in less serious complications like pain, swelling and loosening of the implant, although mortalities up to 20% are reported with orthopedic implants [18, 43, 44]. The clinical signs of deep implant infections are reported to appear up to a year after microbial seeding [45]. Apparently the infectious biofilm can stay silent for a long period, and probably a significant part of the infections is never recognized. Recent investigations at our laboratory revealed that the so-called aseptic loosening, i.e. loosening of an orthopedic implant without microorganisms present, is often diagnosed falsely as aseptic. As standard microbiological techniques are used to test for the presence of infectious microorganisms, slow growing biofilm organisms often remain undetected. Similarly, it has been reported that five of 28 removed scleral explants were covered with a biofilm, while clinical signs of infection were only present in one out of these five cases [46]. Thus the incidence of problems associated with BCI is possibly higher than generally assumed.

4. Treatment and prevention of BCI

4.1. Treatment

Treatment of an established BCI is difficult, as the minimal inhibitory concentration (MIC) of antimicrobial agents, necessary to kill the microorganisms, is significantly higher for microorganisms in a biofilm than for planktonic ones [4, 5]. As antibiotics have little effect on BCI, the standard procedure for infected orthopedic prostheses is the removal of the implant and implantation of an antibiotic releasing device at the implant site. A new prosthesis is inserted when the implant site is free of microorganisms, usually six months later. For many implants, especially those in the circulatory system, removal of the implant is dangerous, and a high mortality is associated with these infections. Much research has been done to make biofilm bacteria more susceptible to antibiotics. Ultrasonic treatment of the infecting biofilm, for example, has been shown to enhance the action of antibiotics towards these biofilm bacteria [47]. Also application of an electrical field yields enhanced effects of antibiotic treatment, the so-called bioelectric effect [48]. The application of these techniques in patients could facilitate the treatment of BCI with antibiotics in the future.

4.2. Prevention

Surgeons take considerable effort in preventing the contamination of implants with microorganisms during implantation. Although application of prophylactic antibiotics and better operation hygiene has reduced the incidence of BCI the last four decades, still a significant number of patients suffer from these infections.

Different strategies seem useful to prevent BCI. In general it is aimed to reduce the attractive force between bacteria and biomaterials surface by optimizing the physico-chemical surface properties of the biomaterial. Bacterial adhesion is low, for example, on extremely hydrophobic surfaces [49, 50], while also more negatively charged biomaterials attract less bacteria [51]. Albumin and heparin coatings have shown to decrease the adhesiveness of biomaterials [52].

However, microorganisms always seem to be able to adhere to some extent to solid materials. Moreover, when proteins are present they can cover an anti-adhesive biomaterial, and be anchors for microorganisms to adhere to. Another approach to prevent biofilm formation is to prevent the growth of adhering microorganisms. This can be achieved by application of antimicrobial agents near the biomaterials surface. One way to do this is the design of antibiotic releasing biomaterials. Examples are gentamicin-loaded bone cement and silver-loaded catheters [53, 54]. A disadvantage of these applications is that they usually only work for a few days to weeks, as the amount of antibiotic that is actually released is extremely limited and does not exceed 15% of the total amount incorporated [55]. A more dangerous problem with antibiotic releasing materials and the low dose actually released is the development of antibiotic resistant microbial strains [56]. A better approach would be to couple the antimicrobial agent covalently onto the biomaterials surface, while maintaining its activity. As in this approach the antimicrobial agent can only reach the outside of the microbial cells, it can only be employed with antibiotics working at the level of the cell wall or membrane. Polymers with incorporated quaternary ammonium groups have shown such antimicrobial activity *in vitro* [57, 58], thus these compounds might have the required properties.

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