17 Bone-Implant Interface in Biofilm-Associated Bone and Joint Infections

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Introduction

Total hip and knee arthroplasties are considered the procedures of the twentieth century, with dramatic improvement to the overall quality of life for millions of patients around the globe. The application of fracture fixation implants and the replacement of the arthritic joints became a common practice in modern orthopedics, relieving hundreds of thousands of patients of pain and functional disability. With a share of 38 %, orthopedics and traumatology are the worldwide leading markets of implanted biomaterials, involving millions of new patients each year as an increasing trend [1]. Commonly used implants in orthopedics are mainly employed for the fixation or reconstruction of bones and joints or their parts and adjacent soft tissues (ligaments, tendons, menisci, etc.) and are made of biocompatible metals, polymers, ceramics, hydroxyapatite, and their combinations. The first requirement of a material's biocompatibility is that, whatever the desired function, the material should not induce any adverse effects in the patient, "just as the first principle of Hippocrates was that the doctor should do no harm" [2].

Although the clinical results are excellent, a number of complications, most of which present with signs and symptoms related to implant loosening, are associated with these procedures. The pathological processes that occur in bone-implant interface reflect pathogenetic mechanisms such as nonspecific macrophage response to wear particles (aseptic loosening), a specific hypersensitivity immune reaction to wear particles from the bearing surfaces, infection (septic loosening), primary joint-related pathology in revision arthroplasty tissues, and tumor formation in peri-implant tissues [3, 4].

Bacterial infections around implants of bones and joints represent the most devastating complication involving millions of citizens. The frequency of these infections varies with regard to the location. In the upper extremities, the rate of infection is reported to be higher for the elbow joint (7.7 %) than that for the wrist (2.39 %) or the shoulder (1.06 %) endoprostheses [5]. The overall rate of infection in primary major joint arthroplasty or fracture fixation implants ranges between 1 and 2 % and becomes much higher in patients with compromised immune response. The incidence increases with revision operations (e.g., 3.2 % in total hip replacement and 5.6 % in total knee replacement) [6–10]. Considering the hundreds of thousands of bone and joint implants applied every year around the world, the absolute number of patients needing costly reconstructive

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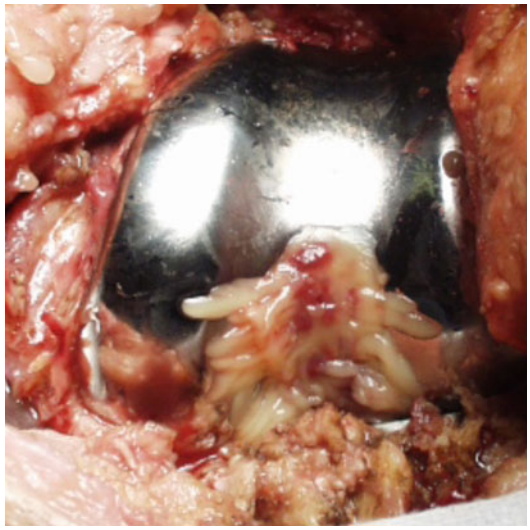

Fig. 17.1 Intraoperative picture showing the development of biofilm on the surface and around the femoral component of a total knee arthroplasty

surgery at multiple stages, as the only option, is rapidly increasing. In patients with osteosynthesis and particularly, after severe open fractures and open joint trauma with extensive soft tissue injury, infection rate is even higher [6–10]. In situations where an inert foreign material is implanted into the human body, a competition develops for the colonization of the implant surfaces between bacteria and the hosts' cells. Bacteria have some advantages over the immune system cells: they are of faster reproductive processes and are extremely flexible in adapting to the environment. Studies indicate that the procedures of implantation and the compromised local tissue environment from the presence of the prosthesis itself into a joint or at the site of a fracture may reduce the number of bacteria required to cause an infection by a factor of even 10,000 [11]. Infection into implanted bone and joint is directly related to the capability of the bacteria of establishing multilayered, highly structured biofilms on the artificial surfaces and the bare bone surfaces (Fig. 17.1). Indeed, implanted biomaterials are still known to be particularly susceptible to microbial colonization and able to favor the onset of infections [12]. Once biofilm is established, the infection becomes chronic and does not respond any longer to conventional systemic antibiotic therapy [13].

The high prevalence and the increasing social and financial burden of implant-related infections is mainly due to the (1) large number of surgical procedures (more than one million new total joint prosthesis performed annually in Europe), (2) expanding indications in the elderly and in patients with compromised immune defense, (3) frequent chronic and long-lasting behavior of bone and joint infections, (4) difficulty of eradicating the septic process and frequent relapses, (5) frequent occurrence (20–60 %) of multiresistant bacterial strains and mixed florae, and (6) variable incidence, from approximately 1 % after prosthetic surgery, in a normal host, to more than 25 % after osteosynthesis in contaminated fractures with local and/or systemic comorbidities or up to 40 % in bone tumor surgery, in spite of the best available surgical practice and antibiotic prophylaxis. Given the severe socioeconomic burden for the patient, his/her family, the treating physicians, as well as for the budget of the health-care system, it is imperative to devise efficient preventive and more effective treatment strategies. To improve the outcome in the management of biofilm infections around implants, it is necessary to combine the efforts of biologists, biochemists, engineers, microbiologists, and pathologists with those of the treating physicians for a better understanding of the interactions between the implant, the bacteria, and the host.

Herein, we present the current knowledge regarding the pathogenesis of implant-related biofilm infections, the histopathology of the bone-implant interface, and the mechanisms of tissue destruction resulting in osteolysis, which destroys the fixation of the fractures or the stability and function of the joint implants. We also discuss the current concepts in biofilm infection prevention and management.

Pathogenesis of Implant-Related Infections

The time span between trauma or surgery and the clinically apparent infection varies among patients. In some patients, the infection occurs either in the immediate postoperative period or within weeks after surgery, while in others,

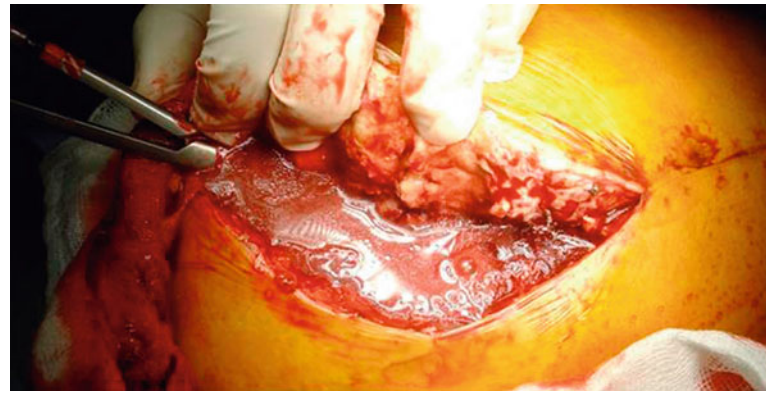

Fig. 17.2 Intraoperative picture showing pus evacuation during the initial stages of wound debridement in acute infection after a total hip arthroplasty

the infection becomes clinically apparent after years. An early acute infection may be attributed to direct intraoperative contamination by either exogenous or by endogenous bacteria (e.g., the skin-colonizing bacteria) (Fig. 17.2). In contrast, the late-onset infection usually results from bacteria contracted by the host at a later stage either through a hematogenous spreading or from a contiguous infected site. It is not uncommon, however, for "harmless" bacteria colonizing the skin or the epithelium (e.g., in the nose) to become invasive for reasons still remaining unknown [14–16].

The pathogenesis of infection has been extensively investigated in experimental studies. The microorganisms infecting the implants are either introduced during implantation of the prosthesis or derived from a temporary bacteremia. Then, they adhere to biomaterials establishing a bacterial colony and grow to form a biofilm. The trigger effect for the inflammatory response against infection is induced by the local release of chemokines (e.g., platelet-activating factor (PAF) or complement C5a) subsequently diffused into the adjacent intercellular space. At the nearby endothelial cells, upregulation of the specialized adhesion proteins make them "sticky," capturing thus the circulating leukocytes, predominantly the polymorphonuclear leukocytes (PMNs), from the peripheral blood, then becoming further activated and firmly attaching to the endothelial cells; they then actively migrate towards the source of infection. They start with phagocytosis of the individual floating bacteria, known as "planktonic" bacteria, followed by intracellular killing and apoptosis of PMNs. Triggering the Fcγ and the complement receptors provides the optimal signal for the phagocytosis and the killing by the PMNs [17]. In addition, Stroh et al. showed phagocytosis in *S. aureus* biofilms using human serum as a source of antibodies and as a complement for the "opsonization" [18]. The apoptotic PMNs in turn are phagocytosed by the macrophages [19–22]. This is a self-limiting process protecting from further spilling of the cytotoxic enzymes [23–25], and it results in the cleaning of the infected site, as prerequisite for healing and regeneration [22, 25, 26]. The study of natural ecosystems has demonstrated that "planktonic" bacteria are rare; instead, bacteria grow predominantly in biofilm formations. Biofilm formation is the result of a genetically driven process triggered by specific biochemical signals and resulting from the activation and expression of defined sets of genes, e.g., of those coding for adhesion proteins [27–30]. A structural examination of biofilm shows that about 15 % in volume is constituted by microbial cells, embedded in a matrix material in which channels carry bulk fluid into the bacterial community by convective flow. The physiological differentiation of sessile versus individual floating "planktonic" cells, as well as the complexity of the biofilm structure elaborated, suggests that bacterial communities forming biofilms are finely organized and necessarily regulated by signals analogous to the hormones and pheromones typical of multicellular communities of eukaryotic cells [31]. The formation of this community undergoes various stages, starting with an initial attachment of bacteria to an inert or a living surface. As they are multiplied they form microcolonies attached to the surface

and gradually differentiate into biofilm structure. Despite the many possible definitions, bacterial biofilms can simply be described as a structured consortium of bacteria encased in a self-produced matrix which are able to communicate by cell-to-cell signals. Depending on the bacterial species, strain type, and environmental conditions, the biofilm matrix consists of exo-polysaccharides, proteins, teichoic acids, and extracellular DNA (eDNA). The extracellular polysaccharide substance (EPS) produced in abundance by the overgrown number of bacteria is clinically visible even to the naked eye as "slime" or as a jelly film gluing together buds of bacteria on to the implant and tissue surface. eDNA has been up to now described in a variety of bacterial species, and its importance is recognized as a component which may contribute to structural solidity of biofilms and to their recalcitrance to antibiotics by inducing expression of antibiotic resistance genes [32]. Facilitated by the mobility within the liquid environment as in tissue or synovial fluid or blood, buds of bacteria in abundance may be released or tear off from the biofilm and subsequently form additional new colonies at adjacent or remote locations. Bacterial detachment and dispersion therefore characterize this final step of the bacterial life cycle, with many bacteria returning into a planktonic state.

In contrast to the single-living "planktonic" bacterial cells, the biofilms constitute a protected form of bacterial growth, allowing bacterial survival in a hostile environment, as they are resistant to antibiotics, disinfectants, and phagocytic components of the innate and adaptive immune system defense of the host [33–36]. Protective mechanisms include altered chemical microenvironment, slow-growing or non-multiplied biofilm cells, gradually developing resistant phenotypes as an adaptive response to stress, and incomplete biofilm penetration by antibiotics and antibodies [33, 37, 38]. Therefore, in a manner reminiscent of a vicious cycle, the protected bacteria within the biofilm could enhance host defense mechanisms and inflammation, and the sustained inflammation could further stimulate the development of resistant bacterial phenotypes. These properties could explain the persistent nature of chronic bacterial infections.

Bacterial Biofilms as the Cause of Tissue Destruction

The majority of biofilm infections presents with an insidious onset imposing diagnostic difficulties within the traditional microbiological methods [39]. Over the last decades, management with the administration of antibiotics has not provided any effective treatment against infections associated with implants [40–43] since the bacteria are growing, not as isolated microorganisms ("planktonic" phenotype) but as a distinct phenotype comprised of sessile microorganisms enclosed within a glycocalyx known as biofilm. The biofilms act as an impenetrable mechanical barrier against soluble agents, and multiresistant bacteria are often involved [44] and they persist, giving rise to a progressively destructive inflammatory process, with surrounding tissue damage and osteolysis, leading to septic loosening of the bone and joint implants [6, 7, 37, 45, 46]. Using scanning and transmission electron microscopy, Gristina and Costerton demonstrated the association of persistent bone and joint infections with biofilm formation on their surface [47]. More recent studies employing modern technologies such as confocal laser microscopy have demonstrated stable biofilm structures not only on biomaterials retrieved from patients with chronic bone or joint infections but also on the adjacent viable soft tissues [48, 49]. *Staphylococcus aureus* is the most common bacterium identified in periprosthetic infections. It binds to bone matrix with adhesion molecules and secrets toxins able to attract PMNs and macrophages in the very first hours after its formation, as the first line of cellular defense around the fresh biofilm. The examination of tissue samples from the infected site, during implant revision surgery, reveals pus composed of dead leukocytes, cellular debris, and serum. Using flow cytometry Wagner et al. have identified the infiltrated cells as polymorphonuclear neutrophils (PMNs comprising the 65–85 %), T-lymphocytes (5–15 %), natural

killer (NK) cells (5–15 %), B cells (<1 %), and monocytes (<1 %) [50, 51]. The cellular protagonists of the inflammatory response to biofilm infection and the specific role of PMNs and macrophages in the consequent tissue destruction are presented in the following paragraphs.

The Innate Immune Response

The innate immune system, also known as nonspecific immune system and the first line of defense, comprises the cells and mechanisms that provide the immediate defense of the host against infection in a generic, nonspecific manner [52, 53]. The cells of the innate system recognize and respond to pathogens by recruiting immune cells to site of infections, through the production of chemical factors, including specialized chemical mediators called cytokines. Other major functions of the vertebrate innate immune system include activation of the complement cascade to identify bacteria, activation of cells, and promotion of clearance of dead cells or antibody complexes, as well as the identification and removal of foreign substances present in organs, tissues, blood, and lymph by specialized white blood cells. In addition, the innate immune system contributes to the activation of an adaptive immune system through a process known as antigen presentation which acts as a physical and chemical barrier to infectious agents. The cellular component of the innate immune system include leukocytes (polymorphonuclear, B-, T-, and NK lymphocytes, basophils, eosinophils), monocytes/macrophages, mast cells, dendritic cells, and natural killer cells. The parts of the innate immune system have different specificity for different pathogens. In the case of extracellular bacteria such as staphylococcus, the certain strategy of defense is phagocytosis [54]. Although the host defense mechanisms against bacteria organized in biofilms are not completely understood and are still under investigation, previous studies have shown the essential role of innate immunity cells against staphylococcal biofilms, giving evidence that tissue degradation and (in bone) osteolysis are not direct effects caused by the infection per se [50, 51, 55].

The Role of Neutrophils Against Staphylococcus Biofilms

The PMNs are the first cells to arrive at the site of infection through chemical mediators, which are emitted at the infected site and act on the closeby endothelium. The endothelial cells upregulate adhesion proteins that capture the PMNs, which then bind to the endothelial cells and transmigrate between the endothelial cells towards the site of infection. Having reached the site, the PMNs exhibit upregulation of the surface receptors required for bacteria recognition and killing, such as high-affinity Fc-gamma receptor 1 (FcgR1, CD64), "lipopolysaccharide" receptor CD14, interleukin-8 (IL-8) which attracts more PMNs, the monocyte inflammatory proteins MIP-1a and MIP-1b, and the monocyte attractant MCP-1 [46, 50, 51]. Simultaneously in these PMNs the production of reactive oxygen species (ROS) is enhanced while they exhibit down-modulation of L-selectin (CD62L), which is required for the PMN emigration. Then, the PMNs take up and phagocytose the bacteria. The phagocytosis results in killing the bacteria and also induces the programmed cell death ("apoptosis") of the PMN. In addition to phagocytosis, other investigators have demonstrated that PMNs release lactoferrin and elastase upon contact with biofilm, and after prolonged contact, they also discharge DNA, which is involved in the formation of the so-called neutrophil extracellular traps (NETs), a further mechanism of bacterial killing [56]. It is of interest to note the differential behavior of PMNs towards the biofilm of *S. aureus* and of *S. epidermidis*, which has been documented in a previous study by employing time-lapse video microscopy [57]. In the case of *S. aureus* formed biofilm, the PMNs moving across were observed to scavenge bacteria along their path. Conversely, PMNs in contact with *S. epidermidis* biofilm were nearly immobile and only phagocytosed bacteria in close proximity [57]. Why biofilms of *S. aureus* appear more sensitive to a PMN attack compared to those produced by *S. epidermidis* is still not understood. Since killing of bacteria in biofilms is possible, the question remains, *why biofilms persist in patients and why biofilm-related*

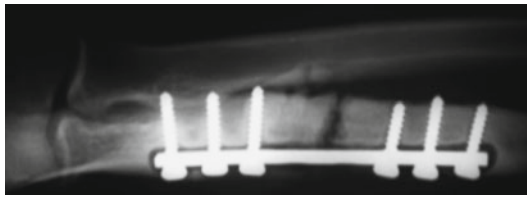

Fig. 17.3 Radiograph showing osteolysis and loosening of a plate fixation due to infection

implant infections become chronic? In cases with impaired local blood circulation, tissue scarring, and compromised immune response, infiltration of the infected site with PMNs is initially rather slow. Clinical studies suggest that lower local levels of PMN in the early surgical wound are directly related to the subsequent occurrence of septic complications, whereas higher early local leukocyte concentrations at the end of the surgical procedure do play a significant protective role against postoperative infection [58, 59]. However, since PMNs arrive at the infected site, they lose their migratory capacity, and thus, they cannot infiltrate the biofilm. Thus, PMNs surround the biofilm and become activated while they do not migrate into the biofilm, probably because of a lack of a chemotactic signal, as well as by the hindrance of migration into the "slimy" material. Although highly activated, the PMNs are not able to engulf the bacteria within the biofilm and to control the infection. Since living bacteria could still be isolated from the infected site inside the biofilm, an evasion of the local host immune defenses has been postulated [50]. Therefore, the rapidly established bacterial adhesions and biofilm formation on the implant surface is initially unchallenged. Consequently, phagocytosis and killing of the bacteria occur only on the surface, leaving the bulk of the biofilm unaffected.

What then is the fate of biofilm? The infection persists and progresses and the PMNs, in their attempt to kill bacteria, express their powerful cytotoxic (e.g., superoxides, ROS) and proteolytic armory to the point of damaging and even destroying the surrounding tissue. Further dire effects are osteolysis and resorption of bone, which usually result in implant loosening (Figs. 17.3 and 17.4). As a consequence, the implant has to be removed, and in the most severe cases, also extensive reconstruction of the bone has to be performed.

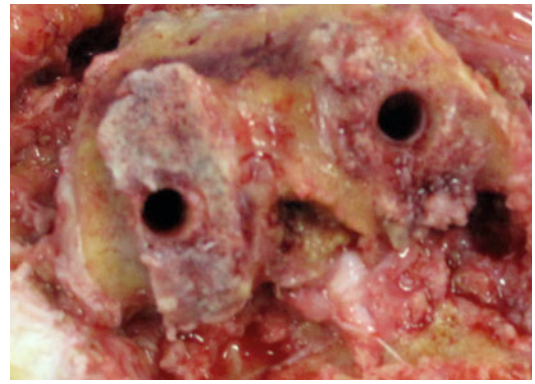

Fig. 17.4 Intraoperative picture showing osteolytic areas of the femoral condyles following the removal of the femoral component of an infected total knee arthroplasty

The Role of Macrophages Against Staphylococcus Biofilms

Macrophages are the most efficient phagocytes and can phagocytose substantial numbers of bacteria or other cells, foreign substances, and cellular debris. In tissues, organ-specific macrophages are differentiated from phagocytic cells present in the blood called monocytes. At the site of infection, the bacterial biofilm attracts monocytes from the peripheral blood [46, 50, 51] through the production of cytokines (e.g., IL-8, monocyte attractant MCP-1). The monocytes in tissues are differentiated to macrophages and to osteoclasts with bone-resorbing activity [60–63]. The macrophages clear the infected tissues from apoptotic PMNs, resulting in limiting of the biofilm-induced inflammatory process in a time and spatial manner; however, they exhibit downregulation of IL-1b, tumor necrosis factor (TNF) alpha, CXCL2, and CCL2 expression. They also exhibit reduced bacterial uptake, minimal iNOS expression, and consequent low efficiency in killing phagocytosed bacteria and a reduced induction of lymphocyte production of interferon-gamma. Thus, these scavenging cells appear able to migrate into the biofilm but cannot clear the site

from the pathogen causing the infection, as their bactericidal activity appears compromised [64]. On the other hand, the generation and activation of osteoclasts initiate a bone-resorbing activity, further enhancing tissue destruction and osteolysis. Osteoclasts originate from the differentiation of monocytes either following their interaction with T-lymphocytes or through a T-cell independent differentiation action of pro-inflammatory cytokines, such as TNF alpha, IL-1, IL-6, or IL-8, on the monocytes [65–68]. Since these cytokines are generated at the site of infection, the osteolysis is more pronounced adjacent to the implants, from osteoclasts with bone-resorbing activity [46]. Osteolysis is the hallmark of osteomyelitis. Although the link between bacterial infection and osteolysis has not been established yet and direct effects of bacteria cannot be ruled out, bone loss as a consequence of persistent inflammation is presumed [69–72], and the most likely mechanism is enhanced synthesis and/or activation of the bone-resorbing osteoclasts [46].

In conclusion, the "attempt without success" of the first line of defense causes the release of pro-inflammatory mediators from PMNs and of tissue-destroying substances. Moreover, additional bone resorption is further enhanced by osteoclastogenesis. All the above evidence indicates that staphylococcal biofilms evoke the persistent attack of activated leukocytes and so indirectly trigger tissue damage. At the same time sessile biofilm-encased bacteria escape the leukocyte-mediated bactericidal response through biofilm-mediated immune evasion mechanisms.

Septic Interface Pathology

The histological changes at the bone-implant interface reflect pathogenetic mechanisms that lead to complications of implant loosening and provide diagnostic information about the causes of failure [3]. Insertion of a joint implant component into the bone results in necrosis of the bone and bone marrow elements surrounding the implant [73]. Following necrosis, there is formation of granulation and cellular reparative fibrous tissue around the implant. The membrane itself is subsequently surrounded by reparative woven and lamellar bone that is remodeled along the lines of stress to which the bone is subjected. In a well-fixed stable implant, there is usually little intervening fibrous tissue between the implant and the surrounding cortical or cancellous bone; few or no macrophages are found in the pseudomembrane of a stable prosthesis since there is little generation of implant-derived wear particles [74]. In contrast, loose implants have a thick fibrous tissue membrane that often contains numerous implant-derived wear particles and a heavy foreign-body macrophage response. Active bone remodeling is also seen on the surface of the thickened bone at the bone-implant interface of a loose prosthesis.

The pathological changes in bone-implant interface membranes from cases of aseptic loosening represent reparative changes. There is granulation tissue with areas of hemorrhage and scattered lymphocytes and macrophages. It is of note that a few PMNs may be seen, but they are not as numerous as in cases of septic loosening, unless an inflammatory arthropathy such as rheumatoid arthritis is superimposed. Inside the pseudomembranes, there is deposition of numerous biomaterial wear particles which induce a heavy foreign-body macrophage reaction [75]. The cytokines produced by these foreign-body macrophages (e.g., IL-1, TNF alpha, IL-6) promote osteoclastogenesis and bone resorption. The fibroblasts within the pseudomembrane produce a macrophage colony-stimulating factor (M-CSF) and a receptor activator for the nuclear factor kappaB-ligand (RANKL), which are required for the differentiation of macrophages into bone-resorbing osteoclasts [62]. Interestingly, Krohmer et al. showed a similar immunohistochemical expression level of inflammatory factors in septic and aseptic interface membranes, suggesting that the pathological mechanisms of the progression of inflammation seem to be similar in both septic and aseptic interface membranes of wear particle type [76]. Histopathological examination of biopsy aspirates and specimens of periprosthetic tissues is commonly used to distinguish between septic and aseptic loosening [77]. Histological findings can be reported intraoperatively, to

give a guide as to whether a one- or a two-stage procedure needs to be carried out, or they may be used postoperatively to confirm the preoperative diagnosis of septic or aseptic loosening. Usually more than 5 PMNs per high-power (×400) field on average, after examination of at least 10 high-power fields, are found in cases of septic loosening. Only PMNs within peri-implant tissues and not on the surface of these tissues or in areas of hemorrhage should be counted. It is important that adequate sampling is undertaken because the focal PMN infiltrate may be present in only one of the sampled areas. According to Bori et al., the most accurate sample for histological diagnosis of prosthetic joint infections is the interface membrane [78]. In addition to a heavy infiltrate of PMNs, plasma cells and lymphocytes may also be seen in the samples.

It of crucial importance to provide the pathologist with information about the history as well as the clinical and operative findings of each case under investigation, since a heavy PMN infiltrate can be noted in the peri-implant tissues of patients with an inflammatory arthropathy such as rheumatoid arthritis. The histological and microbiological findings, as well as the clinical features, need to be carefully considered by the clinician and pathologist in making the diagnosis of septic loosening.

In conclusion, histological examination of bone-implant interface provides clues regarding the nature of the pathological processes that lead to the complications of implant-related joint disease and is required for diagnosis of infection-associated implant failure. Moreover, histological assessment is required for evaluation of the biological tissue response to biomaterials and other agents used in clinical trials, in order to evaluate the efficacy of these new therapeutic strategies.

Prevention of Biofilm Infections

The prevention of biofilm infections in bone and joint implants is an exciting new concept that can be pursued via elimination of organic debris from bone and joint implants, killing of planktonic bacteria prior to biofilm development, modulation and enhancement of local immune defense, and inhibition of bacterial cell communication that precedes biofilm formation.

Surface Cleaning of Orthopedic Implants

The presence of any residual matrices on the surface of an implant or even on a suture favors bacterial colonization and infection; therefore, polymeric or metal biomaterials must be perfectly clean and/or minimally exposed to air or to surgeons' gloves or "aseptic skin surface" prior to implantation. Data from the water industry have demonstrated that contamination of surfaces by organic materials (especially residual biofilm matrices) accelerates the process of planktonic cell adhesion and biofilm formation by at least tenfold [79]. Simple sterilization (e.g., ethylene oxide) of bone and joint implants kills the bacteria but fails to remove the residues, and thus, removal of these deposits is currently a standard preventive procedure for all implantable devices. Combination of enzymes and chemical agents (alkaline detergent and sodium hypochlorite solution) has been proven effective in eradicating biofilm both in vitro and in a clinically used dialysis machine [80].

Quorum Sensing Inhibition

Bacterial cell-to-cell signaling (quorum sensing) is a key feature inside biofilms. The discovery that the development of microbial biofilms is controlled by the quorum sensing process offers a new approach to the prevention of chronic biofilm infections. Bacteria produce and release chemical signaling molecules, the concentration of which increases as a function of cell density [31]. The signals that exercise this control are simple acyl-homoserine lactones (AHLs), in the case of gram-negative bacteria [81] and simple cyclic octapeptides in gram-positive bacteria [82]. When the concentration of these signaling molecules – and therefore the bacterial population – exceeds a threshold, distinct patterns of

gene expression are promoted and biofilm formation is initiated. It has been shown that natural and synthetic molecules that mimic these signals react with the cognitive signal receptor proteins and attenuate biofilm formation [83–86]. Biofilm formation by *Staphylococcus aureus* and virulence factor synthesis are controlled by a regulatory RNA molecule III [RNA-III], which is inhibited by the naturally occurring and synthetically available RNA-III-inhibiting peptide (RIP) [82]. Balaban et al. demonstrated that the RIP prevents biofilm formation by *Staphylococcus aureus* and *Staphylococcus epidermidis* [85, 87]. This inhibition of biofilm formation was shown in animal models of device-related infection, and the inhibitor was shown to be especially effective in infection control if it was combined with an antibiotic such as mupirocin [85]. The recently reported synergistic action of RIP with antibiotics may improve not only prevention but also treatment of staphylococcal infections [88].

Future Perspectives and Innovative Strategies to Combat Implant Infections: The Role of Biomaterial Science

The knowledge of the constituents and of the architecture of staphylococcal biofilms has allowed the development of strategies to disrupt biofilm, on which bacterial resistance to host defenses and therapeutic antibacterial measures mainly resides. While bacteria are hidden deep inside the biofilm and are thus protected against antibacterial agents, the biofilm matrix is instead accessible to the outside environment. In addition, the matrix is a porous network in which fluids run along channels. These features make the biofilm matrix a good target for antibiofilm therapies.

In order to achieve the development of an infection-resistant material, different strategies have been employed: (1) through modification of the biomaterial surface to give anti-adhesive properties, with adsorption of molecules conferring hydrophilic properties to the material surface and competing with the interaction between bacteria and host matrix proteins that film the implant. Heparin, with its strong hydrophilic properties, ascribed to the inhibition of the bacterium-fibronectin interaction, prevents adhesion of bacterial cells and is an excellent tool for an anti-adhesive coating [89–91]. A recent study showed how local activation of human leukocytes on a prosthetic surface, due to the use of tantalum metal, significantly increased local host defense [92], while others provide evidence that either coating an implant with granulocyte-stimulating factor [93, 94] or applying locally leukocytes or their stimulating factors to a wound [95–97] may significantly reduce the proliferation of bacteria and prevent or probably treat infections. (2) The second strategy is through doping the material with antimicrobial substances, such as the local delivery of antibiotics through carrier biomaterials. The use of coated materials that release conventional antimicrobial agents in order to kill planktonic bacteria before biofilm formation on the implant surface is an alternative concept. Elution of antibiotics from currently available local antibiotic delivery systems (e.g., PMMA cement) follows a biphasic pattern with an initial rapid phase in very high concentrations and a secondary slow phase with decreasing concentrations [98]. This may prevent colonization of implants during the early postoperative period; however, the subinhibitory antibiotic concentrations after the initial phase may favor the development of resistant strains of bacteria.

Newer technologies are tested for drug delivery through a ciprofloxacin-retaining polymer matrix coated with ordered methylene chains that form an ultrasound-responsive coating [99]. This system showed significant drug release when low-intensity ultrasound was applied and demonstrated significantly reduced accumulation of *Pseudomonas aeruginosa* biofilms, compared to biofilms grown in control experiments [99]. The future development of medical devices sensitive to external ultrasonic impulses and capable of preventing biofilm growth via "on-demand" release of antibiotics may be a useful addition to the orthopedic surgeon's armament. Besides antibiotics, chitosan, a natural cationic polysaccharide and weak polyelec-

trolyte, has proved effective as antimicrobial coating, and various sophisticated technologies have been studied for its grafting onto material surfaces [100]. It is also one of the most promising biopolymers for tissue engineering and has possible orthopedic applications since it enhances osteoblast functions. Quaternized chitosan-loaded PMMA has been shown to inhibit surface biofilm formation by antibiotic-resistant staphylococci, more strongly than PMMA alone, gentamicin-loaded PMMA, and chitosan-loaded PMMA [101]. *N*-acetylcysteine (NAC) is able to inhibit the production of biofilm polysaccharide and to promote the disruption of mature biofilms [102]. NAC could potentially be used, either alone or in combination with other antimicrobials, for prevention or treatment of biofilm-related implant infections [103]. (3) The 3rd strategy combining anti-adhesive and antimicrobial effects in the same coating is the most innovative. An example of an anti-adhesive and antibacterial biomaterial is the multilayer film constructed by assembling heparin and chitosan layer by layer which reduced bacterial adhesion and also killed the bacteria adhering to the surface [104]. Since the raising of antibiotic resistance is the major limit in the use of antibiotic-loaded biomaterials [105], recent interest has turned to cationic antimicrobial peptides against periprosthesis infections; perhaps they could be employed as such or could be immobilized on a biomaterial surface [106]. Some bacterial resistance to natural antimicrobial peptides has recently been reported [107]. Bagheri has reported examples of different biomaterials employed as surface supports for immobilizing cationic antimicrobial/peptides, such as resin beads, gold surfaces, polymer brushes, cellulose membranes, and block copolymers and iodine composites [108]. (4) With regard to the fourth strategy, in orthopedics, new biomaterials are being sought to resist the biofilm formation and, at the same time, to support bone repair. Hydroxyapatite coatings, besides their properties as infection-resistant material [109], have been proposed as a coating surface undergoing slow in vivo degradation and as a stable interface for osseointegration and bone fixation [110]. Hydrophobic polycationic coatings on stainless steel or titanium implants have proved to be effective in completely preventing biofilm formation and in supporting bone healing even in the presence of significant bacterial contamination [111]. Recently, bioglasses doped with gold nanoparticles, characterized by a very large surface area to volume ratio, were shown to integrate with living bone and to exert an antibacterial and antibiofilm activity [112]. Copper, zinc, and magnesium but especially silver and gold nanoparticles also display antibacterial activity [113]. The antimicrobial activity of titanium oxide (TiO_2) as a photocatalyst and of silver oxide (Ag_2O) nanoparticles can be enhanced by irradiation with visible light [114, 115].

Conclusion

The pathogenesis of biofilm-associated osteolysis includes a local inflammatory response, characterized by the infiltration of leukocytes, predominantly PMNs and T cells. The PMNs cannot phagocytose the biofilm efficiently, as they cannot migrate into the film under in vivo conditions. When the PMNs become activated, they will undergo cell death, resulting in release of their cytotoxic and proteolytic entities into the surrounding tissue, which will cause tissue damage. The escape from apoptosis is also associated with a synthesis of cytokines, e.g., IL-8, which, in turn, may attract more leukocytes but can also cause differentiation of monocytes to osteoclasts. Thus, the microenvironment created by the infiltrating leukocytes would, on one hand, perpetuate the inflammatory process and, on the other hand, promote osteolysis and tissue destruction. Immunological approaches blocking early bacterial adhesion and colonization, applications of enzymes able to interfere with biofilm synthesis or able to disrupt formed biofilms, and exploitation of quorum sensing inhibitors may have a role in preventing or treating these infections. The use of materials coated with immobilized antibacterial substances, particularly cationic antimicrobial peptides, appears very innovative and promising. Nanotechnologies and nanomaterials in

medical research have created new therapeutic horizons and are rapidly growing.

The substantial progress made over the last few years in understanding the functional and structural factors involved in biofilm formation and in the regulatory mechanisms controlling their expression, the advancements in molecular epidemiology [116], as well as improvements in the experimental models [117–119] and in diagnostic methods [120–124] are undoubtedly opening the way to new strategies to combat implant infections [120, 125]. Close collaboration between microbiologists, pathologists, and surgeons is essential to optimize management and maximize benefit to patients with chronic orthopedic infections.

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