New trends in diagnosis and control strategies for implant infections

Carla Renata Arciola¹,², Lucio Montanaro¹,², John William Costerton³

¹Research Unit on Implant Infections, Rizzoli Orthopaedic Institute, Bologna
²Department of Experimental Pathology, University of Bologna, Bologna - Italy
³Center for Genomic Sciences, Allegheny-Singer Research Institute, Pittsburgh, Pennsylvania - USA

ABSTRACT

In implant infections, a quick and reliable identification of the etiological agent is crucial to realizing efficacious therapies. Among molecular methods, automated ribotyping has proven to be an accurate and rapid technique. More recently, MALDI-TOF/MS and PCR-electrospray ionization (ESI)/MS have been applied successfully to microbiological diagnosis. In implant infections, biofilm is still the major problem for bacterial persistence and recalcitrance to antibiotic therapy. Among biofilm-disrupting agents, enzymes promise the greatest therapeutic possibilities. DNase I degrades biofilm extracellular DNA and has been shown to sensitize biofilm to various biocides and anionic detergents, while dispersin B acts on biofilm exopolysaccharide and, combined with antiseptic, gives a broad-spectrum antibiofilm and antimicrobial activity. The novel antimicrobial approach based on photodynamic treatment (PDT) applies, in combination with antibiotics, to the implant or medical devices reachable by optical fibers. Better progress could be gained by the development of infection-resistant biomaterials able to both inhibit bacterial adhesion and promote tissue integration. New knowledge regarding the fibronectin-mediated internalization of Staphylococcus aureus by osteoblasts, and on its role in the pathogenesis of implant-related osteomyelitis, paves the way for the development of vaccines against staphylococcal adhesins, to prevent both adhesion on biomaterials and bacterial invasion of bone cells.

KEY WORDS: Implant infections, Biofilm, Microbiological diagnosis, Antibiofilm agents, Infection resistant materials, Extracellular DNA

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Control strategies for implant infections

Classically, methods used to diagnose prosthesis-related infections start with the in vitro culture of biopptic samples taken from periprosthetic tissues, to ascertain any bacterial growth.

In the field of biofilm-centered implant infections, these classical culture methods, developed for acute infective diseases caused by planktonic bacteria, have met with rising skepticism. As recently reviewed by Costerton et al (2), etiological diagnosis for biofilm infections encounters serious limits owing to the frequent failures in detaching and collecting biofilm cells from infected tissues and in culturing them on agar, since planktonic bacteria produce colonies on agar, whereas biofilm-forming bacteria do not. The failure to isolate the causative bacterium of an implant infection often leads to the greatly abused diagnostic conclusion of “aseptic loosening”, even in cases in which clinical signs of infection clearly exist, thus leading to the grave consequence that any rational basis for the therapy is missing.

In recent years, a variety of new technologies have been proposed that allow more rapid microbiological analyses. The new microbiological testing technologies provide many benefits in comparison with conventional methods, in terms of precision, sensitivity, objectivity and quickness of response, often offering real-time results. In the field of implant infections, these new technologies offer valuable support and promise real advancements toward possible solutions, in particular for etiological diagnosis (3). Identification of the etiological agent may be attained by molecular methods, which, in the case of nucleic acid–based methods, require the extraction of DNA and/or RNA from samples (tissue, swab, or needle aspirate) to be analyzed. As outlined by Costerton et al in their review (2), the presence of biofilm and the need for bacteria digestion are critical steps requiring appropriate procedures.

Moreover, molecular methods based on DNA amplification require the selection of appropriate primers. If the 16S rDNA, universally present in prokaryotes, is searched, the primer selection is obvious, but if species- or subspecies-related genes are to be investigated, the selection of the appropriate primers has to be decided a priori. In the case of orthopedic implant infections, epidemiological knowledge of the most frequent etiological agents may help in specifying the DNA amplification procedures and the choice of primers.

*Staphylococcus aureus*, *Staphylococcus epidermidis*, and other coagulase-negative staphylococci are the main etiological agents in implant orthopedic infections (4-8). The identification of staphylococci in a diagnostic laboratory may be problematic if, besides a primary distinction between *S. aureus* and coagulase-negative staphylococci (CoNS), a more careful and fine distinction among the different species of CoNS is also required. Therefore, many clinical microbiology laboratories still limit their diagnostic responses on staphylococcal clinical isolates to only the coagulase positivity or negativity report. However, various species belonging to the *Staphylococcus* genus, other than *S. aureus* and *S. epidermidis*, exhibit great abilities as opportunistic pathogens (5, 7, 9). Consequently, the availability of methods for an accurate identification of these non-*Sau*/*non-Sep* species is crucial to assessing the pathogenic characteristics of individual species. Among the molecular methods available for a correct identification at species and subspecies levels, ribotyping is one of the most reliable techniques. In our experience, automated ribotyping, even if still subject to improvements, has proven to be a rapid and accurate technique able to recognize the newly emerging pathogens in implant-related orthopedic infections (10, 11). Some drawbacks of automated ribotyping, by means of the RiboPrinter® apparatus, are the high cost of the instrument and consumables, together with the need for a previous bacteriological culture of the pathological sample to obtain one single colony to be processed. On the other hand, a scientific advantage is given by the possibility to assign each clinical isolate to a ribogroup and, thus, to identify epidemic or sporadic clones of pathogens (12). This is valuable in conducting both molecular epidemiology studies, aimed at depicting the virulence profiles of the most epidemic clones (13), and hospital microbiological surveillance programs.

A technique recently applied successfully to microbiological diagnosis of staphylococcal prosthetic joint infections is matrix-assisted laser desorption ionization coupled with time of flight analysis mass spectrometry (MALDI-TOF/MS) (14). This technique is based on a soft laser ionization of intact bacteria, or the bacterial extract, or even of the pathological sample, to detect peptide and protein ions of the bacterial cell surfaces through the spectra deriving from their relative masses and charges. Since every bacterial species produces a different spectrum, the comparison of the spectrum of a single clinical isolate with all the bacterial spectra in the database leads to a rapid identification (15). Using MALDI-TOF/MS improved by dedicated software,
Harris et al. were successful in identifying 158 characterized staphylococcal isolates from prosthetic joint infections, including 36 isolates of *S. aureus*, 100 of *S. epidermidis*, 10 of *Staphylococcus capitis*, 8 of *Staphylococcus lugdunensis*, 2 of *Staphylococcus warneri*, and 2 of *Staphylococcus haemolyticus* (14).

MALDI-TOF/MS also has the potential of differentiating within a species, since it is able to give a subtyping by improving the algorithm exploited for comparing different spectra (16). Dhiman et al. analyzing performance and costs of different conventional and molecular identification techniques, demonstrated that MALDI-TOF/MS is the most convenient technique in terms of cost (US $0.50 per specimen for MALDI-TOF, in comparison with US $6.86 for API-Staph or US $20.02 for sequencing) and in terms of time (0.64 hours total turnaround time per specimen with MALDI-TOF, in comparison with 48–72 hours for API-Staph or 3.5 hours for sequencing) (17).

The goal of optimally replacing the obsolete culture methods with more rapid and informative molecular methods diagnosing bacterial diseases is achieved, according to the review by Costerton et al. (2), by the new Ibis technique, which, as it is founded on nucleotide base ratios (not base sequences), appears to be the molecular system with the greatest promise for fulfilling the requirements of routine diagnosis in orthopedic surgery.

The new advanced technologies for rapid bacteriological identification demonstrate a shift from the traditional biochemical and molecular testing methods toward those using mass spectrometry (MS) for the semiquantitative analysis of microbial proteins and genetic elements. The two most recent technologies are PCR-electrospray ionization (ESI)/MS, on which Ibis is based, and the above-mentioned MALDI-TOF/MS. Their diagnostic accuracy, as well as their feasibility and cost-effectiveness, is the subject of the up-to-date research of Kaleta et al. (18).

Besides the advances in etiological diagnosis of implant infections by means of methods more and more rapid, accurate, and economical, research is also proceeding to find highly sensitive and specific biochemical and hematological markers, which can be applied to both serum and joint fluid aspirate for early diagnosis of infection. In this special issue of the *International Journal of Artificial Organs, Focus on Implant Infections*, Parvizi et al. scrutinize current research efforts in the field of these markers, to evaluate their features and their positive or negative predictive values in diagnosing implant infections (19).

Biofilm and antibiofilm agents

With regard to the treatment of implant infections, biofilm-centered infections give rise to major concerns regarding their ability to resist medical therapies and surgical curetages (20, 21). Therefore, the search for strategies for controlling biofilm-centered infections arouses lively interest in the scientific world, together with full attention toward finding new means to eradicate them.

Given the resistance of biofilms to conventional antimicrobial agents and to host defenses, most implant-associated infections have a chronic course. Frequently, surgical removal of the prosthesis, debridement of the site, and reimplantation of the prosthesis are the only recourse. Alternatively, biofilm detaching/disaggregating agents may represent a potentially useful clinical strategy for eradicating biofilm-related prosthesis infections. Among many agents taken into consideration for staphylococcal biofilm disruption, enzymes able to attack biofilm components appear the most rational and promising. Two well-studied biofilm-dispersing enzymes are deoxyribonuclease I (DNase I) and dispersin B (DspB) (22). DNase I degrades extracellular DNA (eDNA), a newly highlighted structural component conferring firmness and stability to the biofilm (see below), and DspB hydrolyzes the biofilm poly-(β-1,6)-N-acetylglycosamine exopolysaccharide, also known as polysaccharide intercellular adhesin (PIA). DNase I is able to inhibit biofilm formation when present in the culture medium, at the time of bacterial seeding, but when biofilm has grown in the absence of DNase I, the enzyme appears unable to disrupt or detach the formed biofilm. Rather than inhibiting biofilm formation and detaching established biofilms, DNase I has been shown to sensitize biofilm bacteria to killing by various biocides and also to detachment by anionic detergents. Therefore, according to Kaplan, DNase I-mediated biofilm detachment may occur rapidly and at clinically achievable concentrations of the enzyme (22). DspB from the periodontal pathogen *Aggregatibacter actinomycetemcomitans* is a β-hexosaminidase exhibiting biofilm detachment ability. Kaplan et al. first reported that DspB is able to disperse and detach biofilm produced by *S. epidermidis* and suggested that the enzyme could be used as an antibiofilm agent to remove *S. epidermidis* biofilm from medical devices (22). Darouiche et al. observed that a combination of DspB with antisepsics (triclosan or chlorhexidine) gave a synergistic broad-
spectrum antibiotic and antimicrobial activity against *S. aureus*, *S. epidermidis*, and *Escherichia coli*; moreover, the antibiotic activity of DspB was shown to be efficacious also in vivo (23, 24). Mack's research group (25), starting from the reexamination of an ancient therapy, the maggot or larvae therapy, in which the intentional introduction of live, disinfected fly larvae into nonhealing skin and soft tissue wounds of humans or animals had the effect of cleaning out the necrotic tissues with a wound debridement and disinfection, studied the effects of the excretions/secretions of *Lucilia sericata* larvae and observed that the nascent biofilm formation of *S. epidermidis* was inhibited and the preformed biofilms disrupted. The authors propose that, in a purified form, these larvae secretions may have a general applicability for the treatment or prevention of biofilm infections caused by staphylococci (25).

The studies on ways and possibilities of disrupting biofilm appear strictly focused on biofilm structural features. Studies on the structure of biofilm in implant infections are now steering toward analyzing the composition of extracellular polymeric substances (EPS) in depth. In staphylococci, the ica-dependent PIA has been found to be not strictly necessary for biofilm formation. In fact, biofilm-forming mechanisms which are alternatives or even complementary to PIA have been reported for *S. aureus* as well as for *S. epidermidis* (26, 27).

More recently, eDNA, previously considered merely as an inert residue of lysed dead cells, has been included in the list of EPS (22, 28). Interest in eDNA is increasing, with regard both to the mechanisms of its origin and to its structural and functional role in biofilms. The mechanisms of production are suggested to be based either on a fratricide mode, resulting from a process similar to necrosis of eukaryotic cells, as in *Enterococcus faecalis*, or on an altruistic suicide, a programmed cell death similar to apoptosis of eukaryotic cells, as in *S. aureus*. Both mechanisms are considered to have evolved for the survival of the bacterial community. The structural and functional roles of eDNA are described by Montanaro et al in this special issue, *Focus on Implant Infections* (29). The investigation into a relationship between the production of the exopolysaccharide component and that of eDNA has been carried out by screening a collection of *S. epidermidis* clinical isolates by means of new fluorescence-based microtiter-plate assays (30). The results indicate a good correlation between PIA and eDNA production, since no strong exopolysaccharide producer has been found without a concomitant production of the eDNA component. Due to its polyanionic nature, eDNA is thought to interact with PIA, which, being partly deacetylated, bears a polycationic charge and thus reinforces the biofilm structure. The rapid assays for exopolysaccharide and eDNA determination, described in this special issue, *Focus on Implant Infections* (30, 31), allow us to screen large collections of isolates and, thus, to achieve information not restricted to that for only a few reference strains. A complete elucidation of biofilm structure and function in staphylococci, the major agent responsible for implant infections, is fundamental for the development of new targeted strategies of treatment. Besides the proposal of innovative enzymatic treatments promoting biofilm destruction, the use of antibiotics efficacious against biofilm bacteria is also under investigation. In cases of infections of deep-seated implants, which may not be easily removed, researchers are focusing on the individuation of the best antibiotic treatment, also including substances with antibiofilm activity.

In fact, numerous studies have demonstrated interestingly not only that the currently used antibiotics are often resisted by bacteria when they are embedded within biofilms, but also that submininal inhibitory concentrations of certain antibiotics may be able themselves to promote, *in vitro*, bacterial biofilm formation. Kaplan, in this special issue, *Focus on Implant Infections*, reviews the studies on this topic (32). It is still unclear whether antibiotic-induced biofilm formation contributes to the frequent failure of antimicrobial therapy in device infections. The study by Gattringer et al on the time-dependent effects of rifampicin on established biofilms of *S. epidermidis* isolated from patients with cardiac implant infections (33) and the study by Hansen et al on an antibiotic-delivering apparatus to reduce the formation of bacterial biofilm on implanted pacing devices (34) both demonstrated that research on doses and delivery times of antibiotics may be promising toward combating biofilm-centered infections, particularly for when the surgical approach is not feasible.

**Antibiofilm photodynamic therapy**

Owing to the worldwide rise in antibiotic resistance and the ability of biofilms to resist the currently used antibiotics, researchers are also steering toward the development of
novel antimicrobial strategies able to support or potentiate antibiotic therapy.

An innovative approach is based on the combination of the action of a photodynamic treatment with antibiotics at concentrations significantly lower than those acting as inhibitory toward biofilms. Photodynamic treatment (PDT) is a process in which microorganisms are treated with a photosensitizer drug and then with low-intensity visible light of an appropriate wavelength (35).

Usually, photodynamic inactivation of microorganisms is based on the effect of a photosensitizer dye, able to target preferentially bacterial structures and avoid those of the eukaryotic tissue cells, and subsequently to be activated by low doses of visible light, so that free radicals or singlet oxygen bactericidal to microorganisms is generated. Besides the application of PDT for the eradication of bacterial biofilm growing in dental plaques and on oral implants, the treatment has proved efficacious against S. epidermidis and S. aureus biofilms (36, 37). Recently Saino et al have demonstrated the efficacy of PDT in inactivating staphylococcal biofilms in many device-related infections which are accessible to visible light (38).

The effectiveness of PDT against staphylococcal biofilms depends on the appropriate choice of the photosensitizer molecule. A greater photo-efficiency for killing bacteria is obtained when the photosensitizer molecule is able to penetrate deeper into the bacterial membranes, thus reaching a higher endocellular concentration (38, 39). If bacterial antibiotic resistance becomes progressively worst, antimicrobial PDT may be a complementary therapy in clinical practice, depending on the pharmacokinetics of the photosensitizer, on the suitability of the wavelength, and on the time of irradiation (40), in addition to being dependent, obviously, on the accessibility of the implant site.

**Biomaterial coatings for osseointegration and antibacterial action**

Even though innovative treatments against implant infections are emerging, and in spite of an estimated low risk (1%-2%) of periprosthetic infection, the increasing number of implants worldwide, often in patients affected by multiple comorbidities, leads us to believe that the total number of implant infections is destined to increase further (19, 41). Therefore control strategies must rely on implant infection prevention. Many studies are addressed to the development of antiinfective and infection-resistant biomaterials, such as materials doped with effective antimicrobial drugs or resurfaced to repel bacterial adhesion.

In dentistry, dental plaque formation is a complex phenomenon involving microbial adhesion to oral surfaces. The biofilm mode of growth protects bacterial plaque against phagocytosis and antibiotics; detaching the biofilm is the only way to destroy the bacterial environment and to prevent caries development and periodontal disease. Many different restorative materials are routinely incorporated in the mouth. Plaque formation also depends on the properties of these materials, such as surface roughness, electrical properties, hydrophobicity, surface free-energy, and fluoride release. Poggio et al have studied many different restorative materials and canal sealers to evaluate and compare some of the features on which the plaque formation may depend (42-44).

In orthopedics, the integration and long-lasting function of an implant within bone tissue requires the preferential adhesion of cells from the osteoblastic lineage (multipotent mesenchymal stromal cells or osteoblasts) and the prevention of bacterial adhesion. Since bacterial adhesion arises from unspecific passive adsorption of proteins of the extracellular matrix, such as fibronectin and collagen, the concept of preventing protein adsorption by a material surface able also to favor osseointegration has recently emerged, and Groll et al have presented and discussed, within this context, the use of surface coatings able to both modulate eukaryotic cell adhesion and bacterial adhesion (45), encouraging the former and inhibiting the latter. In this special issue, Fiedler et al present evidence that copper and silver ion implantation on Ti6AlV4 alloy surfaces stimulates osteoblast proliferation and prevents bacterial contamination, depending osteoblast proliferation, supposedly, on the Cu ions, while the antibacterial effect is mainly on the Ag ions (46).

Another interesting solution is the utilization of bioglasses doped with gold nanoparticles, involving gold as a well-known antibacterial agent. In the work of Grandi et al (47), Au nanocomposite bioactive glasses exhibit an antibacterial effect depending on the chemical interaction between Au nanoparticles and bacterial lysine groups. The more evident effect is seen against gram-positive bacteria, relying on the more abundant presence of lysine in the cell walls of gram-positive bacteria, in comparison with those of gram-negative bacteria.

A different approach, which better applies to clinical ap-
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Pathogenesis of osteomyelitis and a rational basis for vaccine prophylaxis

In a previous special issue of *Focus on Implant Infections*, the pathogenesis of implant-related osteomyelitis, a severe and deep infection of the peri-implant bone, had been interpreted to be the result of the bone damage and the osteolysis by polymorphonuclear neutrophils (PMNs), when they approach biofilm in an attempt to exert their defensive role (49). The tentative attack of PMNs against biofilms are at times either useless or successful, depending on the species of biofilm-producing bacteria. The attack of PMNs against staphyloccocal biofilms has been studied by time-lapse video microscopy, observing that, in the case of *S. aureus*, PMNs move across the biofilm and appear to be able to phagocytize the bacteria, although phagocytosis proceeds incompletely. In contrast, in *S. epidermidis* biofilm, the PMNs were nearly immobile, and phagocytosis was limited only to the nearest bacteria (50). Therefore, biofilms are not fully protected against the attack by phagocytic cells, even though their sensitivity varies among bacterial strains, depending presumably on the biofilm properties (51).

The possible bone damage to be observed can depend not only by a frustrated phagocytosis (50, 52), but also on a staphylococcal invasion of osteoblasts (8). In an early pathogenetic conception of osteomyelitis, *S. aureus* was regarded as a noninvasive extracellular pathogen able to damage host cells only from the outside. However, many studies are demonstrating that *S. aureus* can invade and persist in nonprofessional phagocytes, in particular in osteoblasts (8). Entry of *S. aureus* into osteoblasts is a complex process, which may follow two pathways: either the formation of a fibronectin bridge between bacterial adhesin FnBP and αβ integrin on the osteoblast surface, or an interaction between *S. aureus* protein A (SpA) and tumor necrosis factor receptor (TNFR-1) on the osteoblast surface. Both entrance modalities appear to be followed by a sequence of events which lead either to osteoblast death by apoptosis or to osteoclast activation. Osteolysis and bone destruction follow. Moreover, a variety of adhesin patterns may condition the ability of the different *S. aureus* clinical strains to enter osteoblasts (8, 53). Considering that internalized staphylococci can not only trigger osteolysis, but also evade immune defenses and antibiotics, vaccines against appropriately selected patterns of bacterial adhesins appear an interesting and potentially efficacious control strategy, since they could prevent not only initial bacterial adhesion to biomaterials, but also bacterial invasion of bone cells (54). Adhesin-targeting vaccines could be also assembled with others, to target diverse virulence factors, such as biofilm antigens or surface proteins other than adhesins, and thus to potentiate and expand their efficacy against implant infections and implant-related osteomyelitis (55, 56).

The future

The culture methods still used in microbiology laboratories were developed by Robert Koch in Berlin back in 1884 (57). This old and at its time genial technology has served us well, and it still serves to study on agar plates the floating cells of planktonic bacteria causative of acute diseases. Nevertheless, culture methods are revealing more and more their unsuitability for biofilms and other chronic diseases, highlighting the need for new more appropriate and complete technologies. The future lies, it is hoped, in an in-depth and full demonstration of the sensitivity and accuracy of the new emerging methods, such as the above-mentioned methods based on mass spectroscopy (Ibis and MALDI-TOF), and in their consequent adoption for the diagnosis of bacterial infections. These improvements in diagnosis, together with the validation and adoption of more specific clinical
control strategies [antiinfective or infection-resistant materials also able to promote osseointegration (45-48, 58, 59), disaggregating biofilm agents (22-25), complementary therapies (28, 32-40, 60), vaccines (54-56, 61) and other preventive measures deriving from progress in our knowledge of pathogenesis and molecular epidemiology (8, 20-22, 29, 49-52, 62-73)], could revolutionize the treatment of implant infections.

SIMPLE SUMMARY

Rapid diagnosis of prosthetic joint infections

- A prompt recognition of the etiological agent is a crucial step and an indispensable requirement for undertaking efficacious therapeutic measures.
- Etiological diagnosis for biofilm infections is limited by the frequent failures in detaching and collecting biofilm cells and in culturing them on agar.
- Identification of the etiological agent may be attained by molecular methods, which, in the case of nucleic acid–based methods, require the extraction of DNA and/or RNA from samples, whereas the presence of biofilm and the need for bacteria digestion are critical steps to be completed with appropriate procedures.
- Automated ribotyping is one of the most reliable molecular techniques, although the high cost of the instrument and consumables, together with the need for a previous bacteriological culture of the pathological sample as are some drawbacks.
- A recently applied technique is matrix-assisted laser desorption ionization coupled with time of flight analysis mass spectrometry (MALDI-TOF/MS).
- The PCR-electrospray ionization (ESI)/MS (Ibis) technique, founded on nucleotide base ratios (not base sequences), appears to be a molecular system promising to fulfill the requirements for routine diagnosis in orthopedic surgery.

Biofilm and antibiofilm agents

- Given the resistance of biofilms to conventional antimicrobial agents and to host defenses, most implant-associated infections have a chronic course.
- New knowledge of biofilm structural features is the basis for efficacious antibiofilm therapy.
- eDNA, previously considered merely as an inert residue of lysed dead cells, is included in the list of extracellular polymeric substances of bacterial biofilm.
- Enzymes such as DNase I and dispersin appear to be the most rational and promising agents able to attack biofilm components.
- Research is also focused on the individuation of the best antibiotic treatments, including substances with antibiofilm activity.
- An innovative approach is based on the combination of the action of a photodynamic treatment with antibiotics at concentrations significantly lower than those acting as inhibitory toward biofilms.
Biomaterial coatings for osseointegration and antibacterial action

- In orthopedics, integration and long-lasting function of an implant within bone tissue require the preferential adhesion of cells from the osteoblastic lineage and the prevention of bacterial adhesion.
- Copper and silver ion implantation on commonly used titanium alloy with an aluminium oxide–blasted surface is now under investigation. Ion implantation is a powerful and versatile technology to modify the properties of material surfaces. Atoms of a desired chemical element are ionized, accelerated in electrical fields, and implanted into the surface of materials. Preliminary results indicate antibacterial effects of silver and promotion of osteoblast proliferation by copper, depending on the dosage of the implanted ions.
- Also the utilization of bioglasses doped with gold nanoparticles appears promising for their antibacterial properties.

Pathogenesis of osteomyelitis and rational basis for vaccine prophylaxis

- In an early pathogenetic conception of osteomyelitis, *Staphylococcus aureus* was regarded as a noninvasive extracellular pathogen able to damage host cells only from the outside. However, many studies have demonstrated that *S. aureus* can invade and persist in nonprofessional phagocytes, in particular in osteoblasts.
- Entry of *S. aureus* into osteoblasts is a complex process, which may involve either the formation of a fibronectin bridge between bacterial adhesin FnBP and $\alpha_5\beta_1$ integrin on the osteoblast surface, or an interaction between *S. aureus* protein A (SpA) and tumor necrosis factor receptor (TNFR-1) on the osteoblast surface.
- Since internalized staphylococci can promote osteolysis and bacterial evasion of immune defenses and antibiotics, vaccines against bacterial adhesins appear to be a potentially efficacious control strategy for the prevention of initial bacterial adhesion to biomaterials and of bacterial invasion of bone cells.

**Conflict of Interest Statement:** The Authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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