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Infection

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Summary

Musculoskeletal infection is a clinical problem with significant direct healthcare costs. The prevalence of infection after closed, elective surgery is frequently estimated to be less than 2%, but in severe injuries, posttraumatic infection rates have been reported as 10% or greater. Although clinical infections are found outside the realm of medical devices, it is clear that the enormous increase of infections associated with the use of implants presents a major challenge worldwide. This review summarizes recent advances in the under-standing, diagnosis, and treatment of musculoskeletal infections.

Keywords

musculoskeletal infection; biofilm; bacteria; biomaterial

BUGS, BONE, AND BIOMATERIALS

In infections involving dead bone and foreign materials, three interacting variables, bacteria, inert surfaces, and viability of host cells and tissues, determine whether infection arises either acutely or over time. The presence of a foreign body is well known to potentiate an infection, and mechanisms for thwarting the action of antibiotic or an antibody include the elaboration of a glycocalyx to form a biofilm, or slime layer.[1] The recurrence of infections is often the result of microbial biofilm formation on the implant, enabling the persistence of bacteria that cause the majority of musculoskeletal infections (Staphylococcus aureus, Acinetobacter baumannii, Pseudomonas aeruginosa, and Klebsiella pneumoniae). *Staphylococcus* species is by far the most studied pathogen in musculoskeletal infections

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and can produce a multilayered biofilm embedded into a glycocalyx. A biofilm may be defined as a microbe-derived sessile community characterized by cells (attached to a substratum, interface, or each other) embedded in a matrix of extracellular polymeric substance, exhibiting an altered phenotype with respect to growth, gene expression, and protein production.[2] Biofilm thickness can vary between a single cell layer to a thick community of cells embedded within a thick polymeric matrix. Recent structural analyses have demonstrated that biofilms possess a sophisticated architecture in which microcolonies can exist, forming intricate networks that provide access to environmental nutrients.[1]

By adopting this sessile mode of life, biofilm-embedded microbes enjoy a number of advantages over their planktonic counterparts. One advantage is the ability of the polymeric matrix to capture and concentrate a number of environmental nutrients such as carbon, nitrogen, and phosphate.[3] Another advantage to the biofilm mode of growth is it enables resistance to a number of removal strategies such as antimicrobial and antifouling agent removal, shear stress, host phagocytic clearance, and host oxygen radical and protease defenses. This inherent resistance to antimicrobial factors is mediated in part through very low metabolic levels and drastically down-regulated rates of cell division (eg, small colony variants) of the deeply embedded microbes. Although low metabolic rates may explain a great deal of the antimicrobial resistance properties of biofilms, other factors may play a role as well, including the ability of biofilms to act as a diffusion barrier to slow down the penetration of some antimicrobial agents.

The last advantage of the biofilm mode of growth is the potential for dispersion through detachment. These micro-colonies may detach under the direction of mechanical fluid shear or through a genetically programmed response that mediates the detachment process. Under the direction of fluid flow, this microcolony travels to other regions of the host to attach and promote biofilm formation on virgin areas. Therefore, this advantage allows a persistent bacterial source population that is resistant to antimicrobial agents and host immune clearance while at the same time enabling continuous shedding to promote bacterial spread.

The glycocalyx develops on devitalized tissue and bone (such as the involucrum) or medically implanted devices to produce an infection. The presence of implants or devitalized tissue is a predisposing factor in the development of infection because they are coated in host proteins, and this coating provides an excellent site for bacterial attachment. Once attached, the bacteria can form a glycocalyx, which protects the bacteria from normal host defenses and systemic antibiotics. In addition, because the nidus of infection can be relatively small and often missed during regular deep culture, biofilm infections are notoriously hard to diagnose.

Besides biofilms, other *Staphylococcus* treatment challenges include the ability of the bacteria to incorporate within the osteoblast, where it is protected from the action of antibiotics or white cells.[4] Novel strategies such as the use of a nanotechnology-derived technique for altering antibiotics through their attachment to polylactic-co-glycolic acid have been investigated to render effective an otherwise ineffective extracellular antibiotic.[5]

Implant materials and their design features may also be important determinants of their relative propensity for bacterial surface colonization. The interaction of the implant surface with attaching host or bacterial tissue (the “race for the surface”) represents the initial phase in a cascade of interactions.[6] Knowledge of these processes and the ability to direct surface implant interactions to promote host tissue attachment and prevent sepsis represent new areas of scientific inquiry.

Novel Ways to Detect Infection

Swabs, needle aspiration, deep culture, and tissue biopsy are the primary methods of obtaining a sample of the bacteria from a wound that are then grown out on a culture media plate. Although a Gram stain result can be available in less than 1 hour, the most useful information is not available for 2 to 3 days. It can take as long as a week to identify the types of the antibiotics to which each organism is sensitive and resistant. This archaic method both delays identification of the infecting organisms and may be inaccurate, especially in a polymicrobial infection or if the bacteria is within a biofilm. Fortunately, new technologies are now present and emerging for both research and clinical applications.

For research, quantitative cultures have been the gold standard. Unfortunately, the technique is tissue-consumptive, has high variance, does not discern for spatial distribution of the bacteria, and does not provide an accurate assessment of the entire wound. Previously, preclinical studies used wound inoculation with a specific species of bacteria. More recently, experimental studies use genetically modified bacteria that emit photons; a *Photorhabdus luminescens lux* operon is inserted into a bacterial chromosome, the bacteria emits light at 486-nm wavelength during normal bacteria respiration, and the amount of photons emitted is determined by the amount of bacteria present. These bacteria can be quantified with the use of a photon-counting camera and localized by superimposing generated luminescent images onto a gray-scale background image. In contaminated musculoskeletal models, bioluminescent bacteria have a high correlation coefficient to quantitative cultures, are not tissue-consumptive, allow for repeat measurements, and are more representative of the entire wound than quantitative cultures that sample a few locations within the wound.[7] This method also has the added benefits of providing spatial distribution of the bacteria and, because there is less variance, much smaller sample sizes are needed to determine statistical significance. Many different species and strains of these bacteria now are available commercially (Caliper Life Sciences, Hopkinton, MA).

Another exciting new technology available for research studies couples multilocus polymerase chain reaction to electrospray ionization/mass spectrometry to provide rapid, high-throughput and precise analysis of bacteria within 5 hours.[3] Bacterial or fungal DNA is amplified by polymerase chain reaction and introduced into a mass spectroscopy by electrospray ionization. The amplification procedure uses 16 S primers, and the primers can be varied to detect fungi and antibiotic resistance genes (eg, mec A). All bacterial DNA is amplified by the primer, not just suspected organisms, like with cultures or conventional polymerase chain reaction. A mathematical algorithm determines the molecular weight of the nucleotides, which is then compared against a database to identify the organism. Clinical studies evaluating this technology are currently underway.

Although culturing bacteria takes days, amplifying DNA takes hours. Accurate, rapid point-of-care devices would be ideal for clinical use. Recently, a microscale fiber (1/30 the size of human hair) has been developed for making precision nanoscale measurements to concentrate and capture cells in seconds (InsituTec Inc, Charlotte, NC). Developments are underway to selectively bind target bacteria present in the sample to the device and count the bacteria cells as they bind through electronic signal. The goal is to develop one-time use cartridges that are inexpensive and quick enough to be used routinely at the point of contact. This technology would enable the selection of the most effective antibiotic and reduce the need for broad-spectrum coverage.

The Use of Bone Morphogenetic Proteins in the Setting of Infection: Unsafe or Advantageous?

Infected fracture nonunions are one of the most difficult conditions treated by orthopaedic surgeons with the treatment goals of eliminating the infection, obtaining bone union, and restoring function. Although it is much easier to achieve union when the hardware is left in place to stabilize the bone, clinical series of patients with infected fractures demonstrate that maintenance of hardware is possible in only a minority of cases.[8] However, clinical and experimental experience suggests that internal fixation of open fractures reduces the infection rate, which is considered to be the result of the stability afforded to the soft tissues and improved revascularization of the zone of injury. Therefore, any intervention that promotes fracture healing in the presence of infection would dramatically improve the treatment of this difficult problem.

Chen and colleagues initiated a series of studies to determine whether the two commercially available bone morphogenetic proteins (BMPs), rh-BMP-2 (Infuse; Medtronic Sophamor Danek, Memphis, TN) and BMP-7 (osteogenic protein-1; Stryker Biotech, Hopkinton, MA), would retain the ability to promote bone formation in an infected critical defect in the rat femur in vivo. Using imaging (plain radiographs and high-resolution computed tomography), histology, and bio-mechanical testing, the authors concluded that both osteogenic protein-1 (BMP-7) and BMP-2 maintain their ability to promote healing of both acutely and chronically infected critical bone defects.[9–11] In a chronic infection model, the authors also found that this effect was potentiated when systemic antibiotic therapy also was administered.[12] Furthermore, in a study measuring mRNA formation, Brick et al found that the upregulation of bone-forming proteins in response to BMP administration was similar in the infected and uninfected defects.[13] The effects of BMPs in humans may be different and less robust than in rodents, and it remains to be proven whether BMPs maintain their osteoinductive capability in infected human wounds. The authors are aware of only one series describing the use of BMP in an infected site in humans,[14] although readers should recognize that this use is off-label.

BMPs have complex but poorly understood interactions with the immune system and may be involved in both the response to sepsis and malignancy. For example, in neonatal mice, BMP signaling is a normal part of the protective innate immune response against viral infection of the central nervous system.[15] Although abnormal BMP-2 expression has been noted in lung, breast, colon, prostate, and pancreatic cancers, so far BMPs appear to be safe

in clinical use. Golden et al noted that in 2043 patients who were each followed for a mean of 2.2 years, the ratio of the observed rate of later malignancy to the expected rate did not indicate an increased risk of malignancy with the use of BMP.[16]

Chronic Orthopaedic Infections: Are Vaccines the Answer?

S. aureus infections are increasingly difficult to eradicate with antimicrobial regimens because the microbe gains resistance factors, highlighting the need to develop novel therapeutic treatments, including vaccine strategies. Overall, vaccine strategies focus on surface-exposed proteins expressed in most clinical strains and target the planktonic phenotype. Each of these strategies provide partial protection against *S. aureus* in animal models, but *S. aureus* vaccines have failed to transition to late-phase clinical trials. In a departure from conventional vaccine strategies, Brady et al focused on *S. aureus* biofilm-specific antigens that elicit a humoral response to develop a protective vaccine.[17] Salient biofilm features, including planktonic cell disbursement from biofilms and biofilm heterogeneity, have also been evaluated as potential therapeutic targets. Studies demonstrate that protein expression is limited to distinct microcolonies within the biofilm in vitro, indicating that protection against an *S. aureus* biofilm may be elicited using a multicomponent vaccine to generate a humoral response that targets biofilm heterogeneity. Administration of a quadrivalent vaccine (at 20 days and a booster at 10 days before *S. aureus* infection) demonstrated a 99% overall reduction in the bacterial population in the vaccinated animals compared with controls. When the vaccinations were accompanied by a postinfection vancomycin treatment regimen, 90% of the treated rabbits cleared their infection compared with 30% of the vancomycin-treated rabbits. Current efforts are focused on the inclusion of planktonic antigens in a multivalent vaccine to eliminate the need for antibiotic treatment of persisting planktonic organisms.

These studies demonstrate that vaccine strategies can target and protect against orthopaedic infections and potentially other biofilm-mediated infections but require broader antigen selection criteria than those typically observed in conventional vaccine development. Vaccines effective against biofilm-mediated infections must account for: 1) both biofilm and planktonic modes of growth and the variation in protein expression between phenotypes; 2) the heterogeneity of protein expression within the bacterial biofilm directed by microenvironment conditions; and 3) cell surface expression that is not impeded by the biofilm matrix.

The Host Immune Response: How Important Is It?

Methicillin-resistant and -sensitive *S. aureus* colonize hosts as a sessile biofilm population. These infections are initiated by inoculation of staphylococci into deeper tissue layers or the bloodstream. Once deep within the host, the bacteria rapidly divide and attach to host extracellular matrix proteins, causing a chronic biofilm infection.[18] The increasing incidence of *S. aureus* in foreign body-related infections, its ease in rapidly developing resistance to multiple antibiotics, and its ability to evade the host-immune response and change from an acute to a chronic infection have lead this organism to receive significant attention.

In preliminary studies, *S. aureus* was shown to elicit a strong inflammatory response, resulting in the migration of large numbers of neutrophils and macrophages to the site of infection. A majority of *S. aureus* strains have been shown to elicit the production of interleukin-1 alpha, interleukin-6, and interleukin-12 p70 in monocytes in vitro, and this may result in biasing the immune response toward a Th1 type response in vivo.[19] Although studies hint that a Th1-biased adaptive immune response could result from *S. aureus* infection, relatively little is known about Th2, Th17, and Treg responses in an in vivo whole cell infection model of *S. aureus*. Also, it is unknown how the host immune system responds to *S. aureus* as it progresses from an acute to a chronic infection that resists clearance by the host immune system.

Shirtliff et al designed a study to evaluate the host immune response to chronic staphylococcal infection and determine if immunomodulation could promote bacterial clearance in a mouse model of indwelling medical device biofilm infection (Shirtliff, personal communication). After implantation with pins with adherent *S. aureus* cells, viable bacteria could be cultured from the infected pin and surrounding bone as long as 49 days postinfection in mice, even in the presence of vancomycin, thus indicating the development of a chronic biofilm-mediated implant infection. The authors then sought to elucidate the host response to the development of this chronic infection (Shirtliff, personal communication). S. aureus-coated pins implanted into mice led to the activation of a CD4 response much like that seen in clinical cases of indwelling medical device infection, including the early production of IgG2b (the dominant Th1-associated IgG subtype) against the biofilm-upregulated antigen SA0486, the presence of Th1 and Th17 cytokines at the implant site, and the suppression of Tregs. These studies suggest that skewing of the host immune response toward proinflammatory Th1 and Th17 responses is a potential mechanism by which *S. aureus* successfully eludes clearance by the host immune system when progressing from an acute to chronic biofilm infection. The authors hypothesized that this may be due to the ability of *S. aureus* to form biofilms on areas of devitalized tissue and vascular insufficiency, which results from tissue damage caused by proinflammatory cytokines.

To test this hypothesis, Shirtliff et al evaluated the host properties during *S. aureus* biofilm-mediated implant infection using two different strains of mice (a Th1/Th17 inflammatory response biased strain and a Th2 and Treg-biased strain). These studies demonstrated that not only is the inflammatory immune response detrimental to the host in both the clearance and prevention of chronic biofilm infection by S. aureus, but that a functional Th2 response is necessary for resolution of the infection. These studies support the concept that modification of the host immune response can potentially lead to the generation of immunomodulatory regimens against methicillin-resistant *S. aureus* and methicillin-sensitive *S. aureus* infections. Furthermore, because the majority of all infections are biofilm-related, this approach may be useful in the development of vaccines against other biofilm-producing bacteria.

SUMMARY

Device-related biofilm infections increase hospital stays and add over $1 billion per year to US hospitalization costs. Because the use and types of indwelling medical devices commonly used in modern health care are continuously expanding, the incidence of biofilm infections will also continue to rise. The central problem with foreign body biofilm infections is their propensity to resist clearance by the host immune system and antimicrobial agents. Compared with their free-floating, planktonic counterparts, microbes within a biofilm are 50 to 500 times more resistant to antimicrobial agents, making therapeutic but nonlethal dosing regimens for the human host impossible. The end result is a conversion from an acute to a persistent, chronic, and recurrent infection, most often requiring device removal. This review summarizes some of the current concepts in the treatment of infections associated with devitalized tissue and indwelling medical devices, including novel methods of diagnosis, treatment, prevention, and clearance of the infection. It is the hope of the authors that this information will stimulate further discussion and research of this complex problem.

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