Review Article

Effects of Metallic Implant on the Risk of Bacterial Osteomyelitis in Small Animals

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Abstract

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Metallic implants are frequently used for stabilization of fractures in dogs and cats. Postoperative bacterial osteomyelitis is a recognized complication following fracture repair and can be influenced by the presence of metallic implants. Metallic implants influence susceptibility to infection through several mechanisms, including corrosion, adherence of biofilm, isolation from the immune response, and compromise of blood supply. Factors that should be considered when using metallic implants include antibiotic prophylaxis, appropriate implant selection, meticulous surgical technique and proper aseptic technique.

Implants, bone infection fracture treatment

In small animal orthopaedic surgery, metallic fixation devices such as bone plates, screws, intramedullary pins, Kirshner wire, and cerclage wire are commonly used for repair of fractures. Factors, which influence the type of implant used for fracture repair, include the surgeon's preference, configuration of the fracture, viability of regional soft tissues, presence or absence of bacterial contamination, and various clinical factors. Clinical factors that may play a role include the age, health and size of the patient, and expected compliance of pet and owner after surgery. Events, which negatively affect any of these factors, may increase the danger of postoperative bacterial infection. The presence of metallic implants at the fracture site may directly or indirectly influence bone infection.

When metallic implants such as plates and screws are implanted in tissues that have been compromised by injury and surgical trauma, an environment conductive to chronic bacterial proliferation is induced (Harrari 1984). Subclinical osteomyelitis may smolder for years, causing chronic pain and limb dysfunction (Daly 1985). In addition, the chronic inflammatory reaction caused by bacteria may have a role in the development of fracture-associated sarcoma (Harrison et al. 1976; Stevenson et al. 1982), recalcitrance of bacterial cryptic infection and delayed or non-union (Jones 1994). Postoperative osteomyelitis is one of the most serious complications after bone fracture treatment in small animal orthopedic surgery (Harrari 1984), and typically is difficult to eliminate and has an unfavorable prognosis.

Metallic implants would ideally be inert when placed in the body. Unfortunately, no material is inert in the biological environment and the interactions are inevitable. Biocompatibility refers to the ability of the material to perform with an appropriate host response in a specific situation (Hansis 1996). Metallic implants having acceptable biocompatibility should be made of corrosion-resistant metals to decrease the danger of bacterial osteomyelitis. However, all metallic material implants in the body can be prone to corrosion as well as implant-associated inflammation. These factors can increase the

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virulence of the bacteria and inhibit the host defense mechanism. Because metallic implants have an inherent risk of infection, it is appropriate for surgeons to optimize factors associated with implant-associated bacterial osteomyelitis risk.

Orthopaedic infection in veterinary medicine

The majority of orthopaedic and soft tissue infections in animals are classified as posttraumatic infection, that is, occurring after surgery or trauma. They can be divided into wound infection involving soft tissue only and osteomyelitis, or true bone infection. Wound infection may progress to osteomyelitis or remain within soft tissue. Likewise, osteomyelitis can have a soft tissue component or be primarily confined to the bone. Infections associated with the device implant play an important role in fracture treatment because lack of tissue integration and associated infections are leading to premature failure of devices implanted (Suci et al. 1998). Infective organisms can reach bone by either hematogenous or direct routes. Although hematogenous osteomyelitis is fairly common in humans (Mader et al. 2000), it is rarely seen in small animal orthopaedic surgery (Parker 1987), and there are only a few reports in immature animals (Dunn et al. 1992; Emmerson and Pead 1999; Gilson and Schwartz 1989). Direct contamination can occur after open reduction of a closed fracture, as a consequence of an open fracture, or by contiguous spread from surrounding soft tissue infection. If properly treated, the majority of open fractures should heal without developing osteomyelitis (Caywood et al. 1978). Osteomyelitis can be expected where there has been overwhelming bacterial contamination in combination with severe trauma, bite wounds (Baranyiová et al. 2003), surgical intervention, or the presence of dead bone, or where metallic implants are used, especially if bone or implants are unstable. Previous studies have reported gram-positive organisms to be more common in canine osteomyelitis, with the most frequent organism isolated in the dog being *Staphylococcus* spp., followed by *Streptococcus* spp. (Love 1989; Parker 1987). Additional organisms frequently isolated include *Escherichia coli* and *Proteus* spp. In the majority of infections, a single organism is identified whereas two organisms are identified in 33% of cases and three organisms in about 15% of cases (Griffiths and Bellenger 1979). Anaerobic bacteria such as *Clostridium* spp. (*C. villosum, C. perfringens, C. welchii*) (Thomson and Eger 1997; Stead and Lawson 1981), *Bacteroides* spp. (*B. gingivalis*) (Johnson et al. 1984), *Peptostreptococci* (*Peptostreptococcus anaerobicus*) (Walker et al. 1983), *Fusobacterium nucleatum* and *Propionibacterium* spp. (Hodgin et al. 1992) have been shown to play a role in osteomyelitis in humans and their role may be underestimated in cases of orthopaedic infections in other animals (Berg et al. 1979). Furthermore, some uncommon organisms such as *Brucella canis* (Smeak et al. 1987), *Clostridium* spp. (Thomson and Eger 1997), *Blastomyces dermatitidis, Corynebacterium renale* (Almaier et al. 1994), *Scedosporium prolificans* (Swerczek et al. 2001), and *Scedosporium inflatum* (Salkin et al. 1992) were also reported in domestic animals from the osteomyelitic bone. It is possible that the routine long-term use of prophylactic antibiotics may act to select for resistant organisms among those persisting in the wounds, resulting in clinical infection at a later time.

Clinical manifestations of severe orthopaedic infections include pain, erythema, and soft tissue swelling with or without drainage. Pain and lameness can be the only clinical signs seen (Dow and Jones 1986). Consistent radiographic changes include bone destruction and periosteal new bone formation with or without soft tissue swelling (Piermattei and Flo 1997; Smeltzer et al. 1997). Classical radiographic changes of osteomyelitis, including sequestrum and involucrum formation, are not always seen (Eereneberg et al. 1994; Robert 1983). Development of significant radiographic and histologic signs of bone infection can occur as soon as a week after infection and progress steadily 3 weeks after

infection (Smeltzer et al. 1997). Loosened orthopaedic implants may or may not be indicative of infection (Sande 1999; Walker 1975). The diagnosis of infection is suspected by identification of clinical signs and radiographic finding. Radiology alone has been shown to have a sensitivity of 62.5% and specificity of 57.1% (Mader et al. 1996) whereas radionuclide imaging of bone after labelling the patient's leukocytes with Indium111 is a sensitive and specific means in the diagnosis of osteomyelitis, even subclinical osteomyelitis which caused delayed and non-union in bone healing (Esterhai et al. 1987; Mader et al. 1996). Nuclear scintigraphy can be an aid in the diagnosis of osteomyelitis, especially of axial skeletal sites. But these techniques are still expensive and require specialized equipment (Lamb 1987). Furthermore, confirmations, which can be sought by pathology and wound culturing, are still popularly performed even though histopathology has shown to have a sensitivity and specificity of 33.3% and 86.3%, respectively in the diagnosis of osteomyelitis (Dernell 1999).

Traditional therapy for posttraumatic wound infection involves improvement of the wound environment and appropriate antimicrobial therapy. Removal of necrotic debris, purulent material and avascular bone segments through debridement is essential (Muir and Johnson 1996; Rudd 1986). Inflammatory cells isolate necrotic bone from the vascular supply through the formation of granulation tissue and this prevents bone resorption leading to formation of sequestra, and impedes healing (Dernell 1999). Wound debridement needs to be combined with appropriate stabilization of unstable fracture fragments (Muir and Johnson 1996) and, if possible, removal or at least minimization of metallic implants (Nečas 1996; Nečas et al. 1998). Reports of treatment response rates in dogs with osteomyelitis approach 90% with appropriate antimicrobial therapy in combination with surgical debridement, which based on culture and sensitivity results (Rudd 1986), preferably using minimum inhibitory concentration values. Furthermore, as an alternative treatment, the open drainage after saucerization and delayed internal fixation plus autogenous cancellous bone grafting was reported that has a successful outcome both in humans and in small animals (Bardet et al. 1983). Cephalosporins have a good result for treatment in long term (4-6 weeks) (Dow and Jones 1986) and in many reports while clindamycin has shown a good spectrum for anaerobic organisms. Successful treatment of osteomyelitis should follow up clinical and radiographic evaluation as well as repeated culturing of the infected area. The definitive treatment of severe cases of osteomyelitis by systemic antibiotics alone can be hampered by the inability to obtain adequate antimicrobial concentrations in infected bone as well as by bacterial adherence and protection in the presence of metallic implants. Newer treatment methods such as antibiotic-impregnated polymethylmethacrylate (PMMA) bead implantation at the site of infection, fusidic acid (Atkin and Gottlieb 1999) may improve the success rate of these complicated cases.

Factors affecting fracture biology and osteomyelitis

The ultimate aim of biomaterials applied to fracture fixation is to restore the structural integrity of the damaged bone. This is dependent upon a complex interplay of the material properties, device design, and physiologic requirements. Important are considerations such as the site and type of fracture, the possible operative approaches, the progress of bone healing, and the desired or feasible program of postoperative care. The obvious clinical requirements are that the material has suitable mechanical properties to fulfill its function of fixation, maintenance of fracture reduction and minimal postoperative effect.

The manifestation of postoperative metallic implant infection is associated with three primary factors: the overall systemic trauma, the local tissue damage resulting from trauma and orthopaedic surgery, and the bacterial contamination of the wound. Extensive systemic trauma can disrupt the body's ability to heal properly due to its negative effects on protein

synthesis and immune system inhibition. The amount of local host damage caused during initial injury and surgery can affect local vascular supply to bone and adjacent soft tissues. The effect of this can be two-fold. First, bone healing can be delayed due to the need for revascularization of devitalized bone tissue. Delayed healing implies longer reliance on the metallic implants used for fracture stabilization, which increases the odds of implant loosening and subsequent implant-associated osteomyelitis. Second, disruption of vascular supply causes local tissue ischemia, predisposing the tissues to bacterial colonization and osteomyelitis. Minimum invasive surgical approaches have a positive effect on early bone healing and prevention of osteomyelitis due to preservation of remaining neurovascular structures and soft tissue integrity (Horstman and Beale 2002; Hulse et al. 1997; Reems et al. 2001). The factor of intraoperative bacterial inoculum can be modified by adherence to hygiene and sterile technique (Printzen 1996). Proper handling of contaminated soft tissue and bony tissues as soon as possible after injury is indicated to decrease the chance of infection. Tissues should be debrided and lavaged copiously to eliminate a potential media for bacterial proliferation and reduce bacterial cell count.

The bacterial wound flora and the local condition of the orthopaedic wound are interrelated. If either factor exceeds the tolerable threshold, infection will become manifest. The level of this breaking point may depend upon certain systemic host factors, surgical technique, type of implanted device, postoperative care, and antibiotic selected usage in orthopedic surgery (Budsberg and Kirsch 2001). It is found that the greater part of infection is not caused by lack of hygiene but by severe local host damage. Thus reduction of local host damage will lower the infection rate even under less than optimal hygienic condition. Theoretically, the so-called aseptic wounds are contaminated. In the aseptic wounds the bacterial density might only be so low that it cannot be proven with normal technique (Printzen 1996). Asymptomatic contamination in aseptic wound seems to increase the risk of an infection, becoming manifest.

There are three scenarios that emerge with respect to mutual influence of bacteria and biomaterials in this complex trauma-induced inflammation. First, in view of the major impact of the trauma, the biomaterial induced component of inflammation during the acute phase is so insignificant that it has no clinically significant effect on the course of any infection. Secondly, the feature of the biomaterial-induced inflammation, perhaps involving the attraction and activation of large numbers of phagocytic cells, may actually enhance the body's defensive capacity and reduce the risk of infection. Thirdly, either the cellular or humoral components of the implant-specific inflammation may be such that they compromise the ability of these cells to deal with the bacteria or they increase the virulence of the bacteria (Kahn and Pritzker 1973), in either case making infection even more likely (Printzen 1996). The increasing amount of collagen synthesis taking place in the wound area in general and around the implant in particular must have some influence on bacterial activity, just as it does on the transport of degradation products from the implant. It is very likely that the fibrous capsule that tends to form around a fixation device, or at least around parts of it, will alter the capacity of both cellular and humoral mechanisms to deal with any bacteria present. A great deal will depend on the relative time scales of tissue repair, bacterial colonization and material degradation.

Interaction of tissue and metallic material after implantation

The almost immediate event that occurs upon implantation of metals is an adsorption of proteins from blood and tissue fluids at the wound site. Later, proteins from cellular activity accumulate in the periprosthetic region (Arens et al. 1996). On the material surface, protein can resorb or remain to mediate tissue-implant interaction. In addition to protein adsorption on the surface of the implant, significant changes also occur on the surface of the material

(Hansis 1996). Interaction of the metallic foreign body with the tissue involves the redox reaction (an electron exchange) at the interface, the hydrolysis (a proton exchange) of oxide hydrate as products of corrosion and the formation of metal organic complexes in the electrolyte (Steinemann 1996). Denatured tissue in contact with the foreign body is the consequence. Surface analytical studies show that the chemical composition of the oxide film changes by incorporating Ca, P, and S. Continued oxide growth affects the ongoing electrochemical events at the tissue-implant interface (Steinemann 1996). The ability for bacteria to proliferate is enhanced in the denatured tissue contacting the metallic implant. Behaviours of metals are variable. Gold, stainless steel, and most other metals react as described above. Titanium and tantalum react differently. Titanium and Tantalum have a reduced foreign body effect and this reduces susceptibility to infection of tissues. In water and tissue fluid, corrosion occurs as an electrochemical process in which oxidation (electron loss of the metal) is coupled with reduction (electron gain of electrolyte components). Reduction of oxygen typically leads to precipitation of hydroxides, hydrous oxides, and oxides on the metal surface. This reaction can also result in changes of pH and local tissue toxicity, both of which can have a negative effect to tissues. Corrosion by-product can also accumulate locally and systemically, resulting in hypersensitivity reaction. *In vitro* studies have revealed that metal ions, even at sublethal doses interfere with differentiation of osteoblasts and osteoclasts. The effect of bone cells *in vivo* is still unknown. These interactions between host and implant can result in delayed healing and osteomyelitis. The goal of current implant design is to create an environment that permits rapid bone healing and reduces tissue-implant interaction.

Host response

The host response to implants placed in bone involves a series of cell and matrix events, ideally culminating in intimate apposition of bone to biomaterial, such as osseointegration. Gaps between bone and implant must be filled, and bone damaged during preparation of the implant site must be repaired. During this time, unfavourable condition such as premature loading leading to micromotion will disrupt the newly forming tissue, resulting in formation of a fibrous capsule.

Morphological studies have revealed the heterogeneity of the bone implant interface, which feature often reported is an afibrilar interfacial zone, comparable to cement lines and laminae limitans. The interface, which is electron-dense interfacial layer, is rich in non-collagenous protein, such as osteopontin and bone sialoprotein, as well as certain plasma proteins, such as alpha-2 HS glycoprotein. These proteins are believed to play a role in cell adhesion and binding of mineral (Printzen 1996). Osteoblast, osteoid, and mineralized matrix are observed adjacent to the lamina limitans-like layer, suggesting that bone is deposited directly on the surface of the implant, extending outward from the biomaterial. Thus, bone formation in the periprosthetic region occurs in two directions, not only does the healing bone approach to biomaterial, but bone also extends from the implant toward the healing bone (Arens et al. 1996; Hansis 1996). Therefore, all host response to metallic implant will protect some cryptic bacteria, which contaminated from the host defense mechanism.

Microbiology consideration

The presence of bacteria in bone alone is not enough to cause disease. It appears that bacteria, vascular occlusion secondary to septic thrombosis, and the resulting bone necrosis are equally important factors in establishing infection (Caywood 1983; Daly 1985). From clinical experience those cases can be recognized in which the wound itself seems to determine or influence its bacterial flora and the virulence of the microorganisms. The evaluation of the microbiological monitoring in many studies revealed two particular types

of bacterial flora, which are coagulase-negative *staphylococci* and b-haemolytic *streptococci*. Most commonly, bacterial infection always caused by *staphylococci* (50-60%) of bone infections in dogs (Johnson 1994) and historically the organism most commonly reported has been *Staphylococcus aureus* (Aron 1979; Braden et al. 1987; Hirsh and Smith 1978; Walker et al. 1983); however, some reports indicated *Staphylococcus intermedius* to be more common (Caywood et al. 1978). In accordance with human literature, *S. aureus* and *S. intermedius* are commonly found (Ieven et al. 1995). Recently *S. schleiferi*, which was shown to be more virulent than other coagulase-negative staphylococcus species and difficult to identify, is also reported (Calvo et al. 2000). Other common organisms include *streptococci, E. coli, Proteus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa,* and *Pasteurella* (*Pasteurella multocida*) are also reported (Johnson 1994; Smith et al. 1989). Uncommon bacteria such as *Actinomyces viscosus* (McMillan et al. 1982) and *Penicillium verruculisum* (Wigny et al. 1990) are also found in bacterial culture (Johnson et al. 1984).

Anaerobic bacteria, such as *Clostridium* spp. (*C. villosum, C. perfringens, C. welchii*) (Thomson and Eger 1997; Stead and Lawson 1981), *Bacteroides* spp. (*B. gingivalis*) (Johnson et al. 1984), *Peptostreptococci* (*Peptostreptococcus anaerobius*) (Walker et al. 1983), *Fusobacterium nucleatum,* and *Propionibacterium* spp. (Hodgin et al. 1992) are usually only found, except for fresh open wounds immediately after accident, in old (Dow and Jones 1987), dirty and surgically insufficiently treated wounds (Dow and Jones 1986), and bite wounds (Johnson et al. 1984; Muir and Johnson 1992). In a study of dogs and cats with osteomyelitis caused by anaerobic bacteria, the radius, ulna, and mandible were the bones commonly affected (Muir and Johnson 1992). Characteristics of anaerobic infection include fetid odor, sequestration of bone fragment, development of draining tracts, lack of response to treatment with aminoglycosides evidence of bacteria with differing morphology in Gram's stained smears of exudates, and failure to isolate bacteria aerobically (Dow and Jones 1987). Anaerobics in an infected wound are always a sign of inadequate wound treatment and surgical debridement.

When metallic material is implanted for orthopaedic purpose, the surface of implants becomes coated with matrix and serum proteins, fibronectin, ions, cellular debris, and carbohydrate (Johnson 1994). *Staphylococci* and some other gram-positive bacteria have cell membrane receptors that bind with fibronectin molecules on implant surface, thus ensuring their adhesion (Gristina et al. 1992). Anaerobic bacteria and gram-negative aerobes attach less firmly via poli and fimbriae that have specific affinity for cellular proteins, matrix protein, and glycolipid. In chronic infections, bone sequestra are colonized by bacteria that bind to exposed collagen matrix protein and hydroxyapatite crystals of the damaged bone (Johnson 1994). Two important mechanisms ensure persistence of adherent bacteria which are slime production and phenotypic transformation. *Staphylococci* and other gram-positive aerobes produce slime, which consists of extracellular polysaccharide, ions, and nutrients. The combination of bacterial slime and host derived material is called glycocalyx. Biofilm enshrouds bacterial colonies and facilitates bacterial adhesion, protect bacteria from phagocytosis and antibodies, alter bacterial susceptibility to drug and induces some adherent bacteria to transform phenotypically to more virulent strains that are more resistant to antimicrobial drugs (Gristina et al. 1992).

Antibiotic prophylaxis in veterinary orthopaedic surgery

The effectiveness of these antibiotic or antimicrobial drugs has been a major reason for the decrease in postoperative wound infection. The judicious use of antibiotics in the surgical setting can be of great benefit in reducing surgical wound infection. However, it should be recognized, that antibiotics are only a small part of surgeon's defenses. On the

infection rate of clean surgical wound, the basic principles such as aseptic technique, atraumatic tissue handling, and decreased surgical time correlate with a decrease in infection rate (Budsberg and Kirsch 2001). The successful use of antibiotic prophylaxis is not only limited to the prevention of infection or reduction of the surgeon's overall wound infection rate but also involves prevention of the development of resistance organisms, allergic or toxic reactions, and controlling client costs. While wound classification and risk assessment are important factors in deciding whether to use antibiotic prophylaxis (Dow and Jones 1987), the surgeon must critically evaluate the individual patient to make the appropriate decision (Love 1989).

Pathogens isolated from infection differ primarily depending on the type of surgical procedure. The appropriate antibiotic should be selected based on the anticipated organism initially, but the final choice of antibiotic should be determined by culture and sensitivity. *Staphylococcus* species from the patient´s skin or the exogenous environment is the usual causative agent. The best alternatives are agents within the beta-lactam group (Budsberg and Kirsch 2001).

Penicillins, such as potassium penicillin G, ampicillin, and amoxicillin has narrow spectrum and potential destruction by bacterial beta-lactamases has been considered limited. A recent study does challenge this notion by showing effectiveness in preventing infection in elective orthopaedic surgeries (Daude-Lagrave et al. 2001). A more traditional recommendation is the use of anti-staphylococcal semi-synthetic penicillins such as oxacillin, cloxacillin, and dicloxacillin. The costs and restricted commercial availability have limited their popular use. The addition of beta-lactamase inhibitors such as clavulanic acid and sulbactam, usually combined with amoxicillin, furnish the desired antimicrobial spectrum including effectiveness against beta-lactamase producing organisms. The lack of commercial availability, in a parenteral form in some countries, has again limited its use. Due to narrow antimicrobial spectrum and prohibitive costs other classes of penicillins are not warranted for routine orthopaedic prophylaxis (Dow and Jones 1986; Rosin 1990).

Cephalosporins are the most commonly used antibiotics for surgical prophylaxis (Love 1989). They are well suited for this role since they are bactericidal over the needed bacterial spectrum (including most gram-positive and some gram-negative organisms), are rarely toxic and relatively inexpensive. There is a large number of research data in pharmacokinetics, serum tissue and bone concentration of these drugs (Daude-Lagrave et al. 2001). One reported about the pharmacokinetics of this drug in dogs showing that a single dose of cefazolin at the beginning of surgery rapidly equilibrated between serum and surgical wound fluid at levels effective against 100% of staphylococcus and 80% of *E. coli* in vitro. These characteristics, along with low toxicity and low cost, make cefazolin an effective prophylactic antibiotic in procedures such as clean orthopaedic surgeries (Anwar et al. 1992), in which the most likely contaminants are normal skin or gastrointestinal flora. Second-generation cephalosporins have a greater gram-negative spectrum than first-generation cephalosporins, as well as a limited anaerobic spectrum, but are less active against gram-positive organisms. With the possible exception of cefoxitin, due to limited spectrum of activity and prohibitive cost, the use of second and third-generation cephalosporins as surgical prophylactic antibiotics is not merited. Cefoxitin may be considered in open fractures (Budsberg and Kirsch 2001) because, like cefazolin, it achieves effective levels against gram-negative organisms in surgical wound tissues and fluid (Rosin et al. 1993). Its primary advantage seems to be the addition of anaerobic efficacy. With regard to cefazolin specifically, evaluation of the present data suggests that the most common dose of 22 $mg \cdot kg^{-1}$ repeated every two to three hours for the duration of the procedure would provide adequate tissue concentrations. This recommendation is fairly conservative given every two hours at 22 $mg \cdot kg^{-1}$, maintains serum concentration at least 10

times MIC for three to four hours (Daude-Lagrave et al 2001). Furthermore, in a canine research model, re-dosing cefazolin at six-hour intervals maintained effective wound concentrations for more than 12 hours (Budsberg and Kemp 1990).

Other antimicrobials, such as lincomycin, enrofloxacin (Duval and Budsberg 1995), and clindamycin, supposedly did penetrate infected bone in concentrations sufficient to kill bacteria (Fitzgerald et al. 1992). Recent reports found *in vitro* clindamycin and enrofloxacin to be effective in the treatment of experimentally induced posttraumatic *S. aureus* osteomyelitis in dogs (Braden et al. 1988; Duval and Budsberg 1995).

Unfortunately, antibiotic penetration of bone does not infer efficacy in treatment of bone infection. Various studies have shown that antimicrobials penetrate infected bone well, but that pathogenic bacteria posses some unique adaptive mechanisms that ensure their adhesion, persistence, and virulence in chronic bone infection (Anwar and Costerton 1990; Budsberg and Kemp 1990; Gristina et al. 1992).

Interactions between antimicrobial agents and bacterial biofilms

Bacteria are common inhabitants of the body, which are normally kept under control by the immune system. However, once bacteria adhere to a material surface, they may form a biofilm in which cells are, for reasons, which have not been fully elucidated, protected from many antagonistic agents. Biofilm bacteria have been protected from complement-mediated opsonic factors, phagocytic cells and antimicrobial agents (Hoyle 1990; Jones et al. 1992). *In vitro* experiments indicate that bacteria colonizing biomedical materials can sometimes withstand many times the dosage of antimicrobial agent sufficient to completely eradicate planktonic (free floating) bacteria (Anwar et al. 1992; Anwar and Costerton 1990). Hypotheses that have emerged to explain the reduced susceptibility of biofilm bacteria to antimicrobial agents, are summarized in Table 1. They can be grouped into two categories, the first category encompasses origins of recalcitrance related to transport limitation within the biofilm, while the second focuses on physiological or metabolic characteristics which microorganisms assume by virtue of life within biofilm (Deighton and Borland 1993; Suci et al. 1998).

Table 1

Hypotheses suggested to explain the reduced susceptibility of biofilm bacteria to antimicrobial agents

Attribute	Mechanism
Transport-related EPS (extracellular polymeric substance)	EPS moieties (e.g. uronic acid) may bind charged compounds: EPS can effect diffusion parameter.
Cellular surface hydrophobicity pH	Mobilization of hydrophilic proteins at cell surface may affect transport of polar compounds. Change in pH through the biofilm could alter efficacy
Physiology-related Decreased metabolic activity	May impede active transport across walls /membranes: agents, which depend on interference with an enzyme involved in repair or regeneration of cellular components may be less effective; agents, which interfere with translation or transcription may be less effective.
Decreased growth rate	May alter effectiveness of agents, which interfere with enzymes involved in replication (Fluroquinolone).
Specific enzyme activity	Increased production of enzymes, which inactivate antimicrobials.

Transport limitations of antimicrobials through biofilms

Biofilm bacteria (such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *S. epidermidis*) are typically enveloped in an extracellular polymeric substance (EPS) matrix. This polymeric network connects cells with one another and to the substratum (Deighton and Borland 1993). Intuitively, one might assume that the EPS matrix shields

the cells from antimicrobial agents, and this interpretation has been invoked to explain biofilm recalcitrance. Certain classes of antimicrobial agents (especially beta lactams) can be inactivated by bacterial enzymes and its maximum values for rates of hydrolysis of beta-lactams are included as part of a transport model.

Physiological traits of biofilm bacteria, which may bestow recalcitrance

Bacteria can be capable of making appropriate responses to environmental changes. Many responses involve regulation of sets of genes (Brown et al. 1990). From many studies, one can predict that biofilm bacteria will differ from their planktonic counterparts at the level of genetic regulation and thus may differ profoundly in many a physiological change inherent to surface-associated growth did not, either fortuitously or by design, affect their susceptibility to antimicrobial agents (Deretic et al. 1994).

An argument that is commonly expressed to refute the claim that hindered transport through biofilms can be responsible for the observed recalcitrance is the following: even if there is a delay in reaching the minimum bactericidal concentration (MBC) in certain portion of the biofilm, if the bulk concentration is larger than the MBC, given enough time, this concentration will be exceeded. This argument ignores the possibility of physiological adaptation such as the possibility that given a time period of sufficient duration, bacteria will adjust to a sublethal concentration of a given antimicrobial agent, this enabling survival during exposure to concentrations exceeding the MBC. Bacteria can adapt rapidly (10-20 min) to environmental stress by altering expression of various proteins (especially the protein content of bacterial cell envelopes) (Brozel et al. 1993). The true mechanism of bacterial resistance to the antimicrobial agents is still unknown. In biomaterial research development, biodegradable orthopedic fixation device with antibiotic embedding has been developed to avoid the unfavorable effects caused by metallic implant such as corrosion, implanted infection and weakening of bone (Sloten et al. 1998). A method how to promote bone healing after implantation in order to decrease the duration of implantation, is developed by enhancing osteoblast adhesion on biomaterial (Anselme 2000). Whereas the biomaterial implant is being developed, the attempt how to prevent the occurrence of cryptic infection is also investigated (Hench 1998; Sloten et al. 1998).

Conclusion

Some complications after fracture fixation with metallic device are acceptable not only for the surgeon, but also the animal (Doherty and Smith 1995). Minor complications such as slight malalignment, hypertrophic callus, and small amount of cryptic infection at the site of healed fractures in patients without clinical or radiographic signs of osteomyelitis are not a serious clinical problem; whereas, major complications, including delayed union, non union, severe malalignment, osteomyelitis, and implant failure are considered as fatal sequelae (Dvořák et al. 2000). Some cryptic infection and the bone-metallic material interaction can also be acceptable in clinical practice. On the other hand, the presence of bacterial infection at the site of metallic implant may result in an interaction that may develop into chronic osteomyelitis and long-term clinical dysfunction.

Cryptic infections are biofilm-mediated, whereby the infective bacteria are able to produce a polysaccharide mucoid peribacterial film (glycocalyx). Glycocalyx promotes bacterial growth and adherence to a foreign material. Cryptic infections persist despite an effective host immune system and appropriate antimicrobial chemotherapy. Such infections are characteristically focalized, seldom cause bacteremia or clinical signs of toxemia and usually persist until the foreign material is removed. Bacteria causing cryptic infection may be dormant for weeks or years and may unpredictably become less adherent in character (Hench 1998), causing signs of localized osteomyelitis and possibly systemic disease.

Bacteria colonizing the metallic implants in dogs may have been present or introduced at the time of the initial operative procedure or may have been haematogenously delivered after implant application. Large metallic implants such as plates and screws require tissue trauma and devitalization during application, provide a large surface area for bacteria with adherent properties, and provide a large mechanical barrier to the immune system. Altered cellular activity associated with prolonged fracture healing and osteomyelitis has been suggested as possible initiating or contributing factors in the development of fracture-associated sarcoma (Harrison et al. 1976; Stevenson et al. 1982). It would therefore be advisable to remove such implants whenever practical and to closely monitor the healing process in animals with treatment with the metallic internal fixation. Furthermore, the reuse of metallic device, which had a corrosion reaction during implantation, may create not only tissue-bone reaction, but also promote cryptic infection. Although cryptic infection, itself, cannot pose any clinical problem, whenever the host defense mechanisms are decreased by any causes (i.e. systemic diseases, hypersensitivity to metallic implants), the bacteria may be recalcitrant and causes the infection leading to implant failure. Furthermore, the invasive surgical technique, intensive sterile technique, and antimicrobial prophylaxis selection for the orthopedic procedure are playing important roles for prevention of bone infection as well.

Vliv kovových implantátů na riziko bakteriální osteomyelitidy u malých zvířat

Ke stabilizaci fraktur u psů a koček se často používají kovové implantáty. Jednou z komplikací chirurgické léčby zlomenin je osteomyelitida bakteriálního původu, jejíž výskyt může být ovlivněn přítomností kovových implantátů v operační ráně. Tyto implantáty ovlivňují vnímavost kosti vůči infekci, a to prostřednictvím několika mechanizmů, jako je koroze, adherence biofilmu, znesnadnění lokálních imunitních pochodů a omezení krevního zásobení v místě lomu. Při použití kovových implantátů je třeba zvážit profylaktické podání antibiotik, zvolit vhodný typ implantátu a při manipulaci s tkáněmi během operace dodržovat atraumatický přístup a aseptické techniky.

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