**Microbial Biofilms and Surgical Site Infection: A Review of Selective Prevalence, Mechanistic Aetiology, Management and Treatment**

Charles E. Edmiston PhD, CIC, FISDA, FSHEA, FAPIC Emeritus Professor of Surgery, Department of Surgery, Medical College of Wisconsin, Milwaukee, USA

Andrew J. McBain BSc PhD Professor of Microbiology, Manchester Pharmacy School The University of Manchester, UK

Martin Kiernan MPH MClinRes RN Visiting Clinical Fellow
Richard Wells Research Centre
University of West London, UK

 David J. Leaper DSc, MD, ChM, FRCS, FACS, FACS Emeritus Professor of Surgery University of Newcastle upon Tyne, UK

Correspondence to: profdavidleaper@doctors.org.uk

Running head: Biofilm-associated surgical site infections

Key words: biofilms, surgical site infection, device-related infection, antibiotic recalcitrance, mechanistic aetiology

**Background**

The global impact of surgical site infections (SSIs) on healthcare systems is considerable, 80% of SSIs are related to formation of a microbial biofilm. Biofilms play a significant role in the pathogenesis of implantable device-related infection and are also important in persistent postoperative skin and soft tissue wound infections.

**Method**

PubMed and OVID databased were search for relevant articles regarding biofilm-associated infection in surgery, including epidemiology, diagnosis, treatment and management.

**Results**

Biofilm-associatedinfections increase utilization of healthcare resources, prolonged length of stay, increased cost of antibiotic therapy, result in additional surgical revisions and extended rehabilitation postdischarge. *S. aureus* and *S. epidermidis* are the most common isolates recovered from device-related infections. Early infection occurs within two weeks of implantation and associated with intraoperative wound contamination; late-onset infections are often occult clinical findings, prolonging disease recognition by weeks, months and in some cases, years. Biofilm is a physical barrier against penetration of antibiotics, antibodies and granulocytic cell populations. The ideal strategy for preventing biofilm-associated SSI is to prevent intraoperative contamination through rational use of effective surgical care bundles, including appropriate antibiotic prophylaxis and adequate skin antisepsis prior to surgery.

**Conclusion**

Management of postoperative biofilm-associated infections involves; surgical debridement followed by irrigation with biocidal agent; removal of infected device followed by insertion of antimicrobial adjuncts such as antimicrobial spacers, beads or sutures together with selective therapeutic agents that penetrate the mature biofilm. Finally, institution should have policies and procedures in-place for management, sterilization compliance and handling of biomedical devices prior to implantation.

**Introduction**

The Centers for Disease Control and Prevention of the United States (CDC) has reported that 51.4 million in-patient surgical procedures were performed in the United States and approximately 400,000 of these procedures were complicated by a surgical site infection (SSI) with an associated mortality as high as 25%.1-4 Data available from Europe in 2009 indicate that in excess of 61 million surgical procedures were undertaken for inpatient surgery alone and it is likely that infection rates are similar to elsewhere in the world, imposing a significant burden on healthcare resources, much of which in Europe is publicly funded.5

This global impact of SSIs on healthcare systems is considerable and, according to some reports, as many as 80% of these SSIs may be related to the formation of a microbial biofilm.6-10 Many of the microbial populations associated with SSIs and other chronic wounds, exist within the biofilm matrix as a heterogeneous) community.11, 12 The presence of a microbial biofilm, within host tissue or on the surface of a biomedical device, poses a significant clinical dilemma when attempting to eradicate subsequent infections. Biofilm-mediated infections exhibit resistance to host defenses, often contributing to an excessive or inappropriate inflammatory response, leading to complement activation and formation of immune complexes, which in turn lead to tissue injury.13,14 In addition, biofilms are notoriously recalcitrant to antimicrobial therapy, often resulting in therapeutic failure following traditional parenteral antibiotic therapy.9,12

While biofilms play a significant role in the pathogenesis of implantable device-related infection, they also play a significant role in persistent skin and soft tissue wound infections in post-surgical military personnel who have deployment-related injuries.15 The presence of a polymicrobial biofilm is likely to be the major risk factor for relapsing infection after these skin and soft tissue injuries because of the increased biofilm microbial bioburden, severity of disease, recalcitrance and enhanced inflammatory response within the affected-tissues. *Acinetobacter baumannii* and *Pseudomonas aeruginosa* have been the predominant isolates recovered in both mono-microbial and polymicrobial infections in this group of surgical (military) patients.15,16 It has only been within the past 30 years that clinicians have realized the important role that microbial biofilms play in both tissue-based and device-related infections, although they have been recognized for over 50 years as being ubiquitous within the natural environment and in the oral cavity, as dental plaque**.**17

Biofilm-associatedimplant infections are almost always accompanied by increased utilization of healthcare resources including: a prolonged length of stay; increased cost of antibiotic therapy; increased risk of the development of antimicrobial resistance, additional surgical revisions and extended rehabilitation following hospital discharge.18 *S. aureus* and *S. epidermidis* are the two most common isolates recovered from documented device-related infections. According to widely accepted criteria, early infections occur within two weeks of implantation and are associated with intraoperative infection or wound contamination in the immediate postoperative period. Infections which present years postoperatively were traditionally thought to have been initiated via the delivery of microorganisms to the primary site following an unrelated bacteraemia. However, late-onset, implant-related infections are often associated with occult (subclinical) clinical findings which may prolong disease recognition by weeks, months and in some cases, years, with resolution often requiring reoperation and removal of the infected device. 19, 20 In addition, infections associated with implants are often difficult to diagnose because the organisms responsible may lay deep within localised biofilms, evading sampling or requiring sonication to release detectable organisms. Because of the difficulty in treating these serious, life-threatening, infections after major prosthetic surgery, particularly hip and knee replacement, current emphasis focuses on prevention through fastidious technique and appropriate skin-antisepsis which includes innovative wound irrigation techniques using effective biocidal agents.21

**Clinical implications of biofilm-associated biomedical device infections**

More than 900,000 abdominal wall hernia repairs are performed yearly in the United States.22 In England this surgery is the most common procedure undertaken annually, with over 60,000 procedures carried out in 2011/2012.23 Contamination of implanted mesh usually occurs at the time of implantation or later, exogenously, in the early postoperative period. Several clinical studies have suggested that the infection rate is highly variable and dependent upon the type of mesh used for abdominal repair ranging from 2.5% to > 6% with polypropylene and polyester, or 1% to > 9% with expanded polytetrafluoroethylene (ePTFE). The level of microbial-contamination (bioburden) that develops upon the surface of a synthetic mesh reportedly depends upon the type of material and the structural surface characteristics of the device.24 For example, in a clinical study, meshes made of multifilament Dacron supported a well-developed and dense biofilm. Meshes which exhibit a hydrophobic surface such as ePTFE have been viewed as being able to resist microbial adherence.25 However, following “conditioning” of the device surface with blood and tissue proteins and after prolonged exposure to bacterial contamination, both Gram-positive and Gram-negative organisms are able to form a dense biofilm. It would appear that the benefit of surface hydrophobicity, acting as a deterrent against bacterial adherence to a device, has only a short-term benefit *in vivo*. Biofilm-associated mesh infections delay the wound healing process by interfering with the in-growth of host tissues through the mesh pores. *S. aureus* colonization of the mesh surface for example, induces fibroblast death (apoptosis), thereby inhibiting the proliferation of these cells during the maturation period of wound healing. Biofilm formation on the surface of a synthetic mesh may result in persistent, chronic infection with draining sinuses, mesh extrusion and enteric fistula formation. Most of these chronic biofilm infections are associated with staphylococci but mesh infections following abdominal surgery may also involve Gram negative bacteria. It has been suggested that relapsing infection, of the type observed with chronic mesh infections, is almost always associated with biofilm-forming microorganisms and many of these isolates in addition to being recalcitrant to therapy may also express multi-drug resistance. 26 Resolution of these infections requires complete mesh removal, along with other foreign bodies such as residual suture material, followed by simultaneous reconstruction. Patients require a prolonged period of follow-up to monitor the possibility of occult infection which may occur years after mesh removal.

The pathophysiology of vascular graft infection is more clearly understood when it is characterized as a biofilm-mediated infection. The development of a groin sinus tract after insertion of an aorto-femoral prosthetic bypass graft is an example of a biofilm-mediated, vascular graft infection.27 These infections are often characterized as late-onset, occurring weeks to months post-implantation, and the presentation may be occult with no systemic signs of infection. Traditional culture methodology often fails to recover any isolates which is often due to ineffective sampling and the localization of the infection. However, when a retrieved graft segment is sonicated, *S. epidermidis* is often recovered in numbers exceeding 6-logs.27 The occurrence of a late-onset biofilm-mediated vascular graft infection often follows a series of sequential events.19, 27 First, the device is contaminated at the time of insertion by a biofilm-forming organism; a process facilitated again by surface conditioning with blood and tissue fluid protein (Figure 1). Once the organism is adherent to the surface of the graft, microcolony aggregation begins to form following the secretion of an extracellular matrix which eventually leads to development of a mature biofilm. The organisation and maturation of biofilm is a dynamic process which paradoxically requires few contaminating organisms to initiate the infectious process. Localised reduced metabolic activity, due to limited substrate availability within the microbial biofilm aggregate, and production of extracellular matrix, contributes to the physiological conditions that foster recalcitrance to both host immune defenses and antimicrobial therapy. Typically, the biofilm spreads slowly over the exterior surface of the graft, eventually involving the graft-to-artery anastomosis, reducing anastomotic tensile strength, leading to development of a pseudo-aneurysm and eventual graft failure which is often heralded by catastrophic haemorrhage.19, 27 During this process there is little or no spread of organisms to the peri-graft tissues, nor is the development of sepsis likely; which is clinically very different to early-onset vascular graft infections involving *S. aureus* or Gram-negative pathogens (usually *E. coli*) which presents as a graft-tissue based infection. The incidence of SSI following vascular surgery is reported to be in the range of 5% to 15%; higher in diabetic patients, patients colonized with MRSA, and after procedures requiring a groin incision.19,27 Early-onset infections are characterized by wound dehiscence and purulent drainage often within days of surgery. Signs of SSI may include failure of graft healing (incorporation) within the surrounding tissues, sinus tract formation, or late erosion into adjacent bowel mediated by a chronic inflammatory process (with attendant, often catastrophic, enteric haemorrhage) due to the presence of a mature bacterial biofilm (Figure 2) on the surface of the graft.

Within the past 20 years the number of total hip replacements in the United States has increased 2-fold to > 250,000 while the number of total knee replacements has increased almost 5-fold to > 500,000.28 The risk of infection following total joint replacement is 0.5% to approximately 2% for hips and knees and 2% to 9% following ankle replacement. Data from the United Kingdom (UK) mandatory surveillance program, in which all hospitals undertaking joint replacement surgery are required to submit data, report an infection rate of 0.6% for primary hip replacement and 0.5% for primary knee in 2014/15. These data are however not fully validated, being based on in-patient and readmission figures, and therefore not all outcomes are included.29 Biofilm-mediated infection of orthopaedic implants can occur either as an exogenous process, with contamination of the device occurring during surgery (or early in the postoperative period), or through a bacteraemia at any time after surgery. The vast majority of exogenous infections occur in the early postoperative phase, particularly in patients who are at risk of poor wound healing, early superficial dehiscence or multiple co-morbid risk factors (Figure 3). Peri-prosthetic joint infections (PJI) can be classified as “early”, “delayed” or “late.” Early infections occur within the first two months; delayed infections occur between the third month and up to two years postoperatively and late infections are diagnosed more than two years postoperatively. The most common organisms associated with PJI are the coagulase-negative staphylococci (30%-43%) and *S. aureus* (12%-23%). These are followed by streptococci (9%-10%), enterococci (3%-7%), Gram-negative bacilli (3%-65) and miscellaneous anaerobes, such as *Propionibacterium* and *Peptostreptococcus* spp. (2%-4%) which have been underestimated as pathogens involved in implant infections.30 Polymicrobial infection is observed in approximately 10% of cases but 10%-30% of clinical cases present as culture-negative. As with other biofilm-mediated infections, traditional antibiotic therapy has limited value in the treatment of PJI. Therapeutic efficacy dictates that a sufficient concentration of antibiotic, much greater than the MIC90 for most likely pathogens, must diffuse from the bloodstream into the tissuespace and then into the biofilm which is essentially a “third compartment”. It has been documented that the MIC required to inhibit or kill most microorganism within a mature biofilm is often 100 to 1000 times the accepted MIC90 for device-related microbial pathogens.31, 32 The use of antibiotics in the treatment of PJI is adjunctive to surgical management, which may involve a single or two-stage (first debridement and creation of an articulated spacer) revision.

Prosthetic joint infections are catastrophic for both patient and practitioners, management may involve years of additional medical, surgical and rehabilitation care with no guarantee of successful resolution. The device-adherent (biofilm) organisms may express recalcitrant phenotypes and therefore require a much higher minimal bactericidal concentration (MBC) to resolve infections which is the most likely cause of therapeutic failure. The optimal strategy is prevention which requires full compliance with an appropriate interventional perioperative care bundle that minimises the risk of wound/device contamination and therefore postoperative infection.33

**Mechanistic aspects of biofilm formation in host tissues and implantable devices**

A microbial biofilm is an organized community of bacteria, enveloped within a self-produced matrix, which can attach, for example, to a wound surface (Figure 4).34  The formation of a differentiated, multicellular community gives a biofilm defense against UV light, bacteriophages, biocides, antibiotics, immune system responses, and many environmental stresses.35 The biofilm, on an organizational level, allows bacteria to survive intrinsic and extrinsic defenses which could easily kill the single cell, planktonic form of bacteria. The first step in establishment of a biofilm involves adherence of the organism to a conditioned surface such as a host cell, wound surface or implantable device (figure 5).36 In the presence of plasma, *S. aureus* forms a biofilm which has a unique composition, composed of sheaths of fibrin and glycocalyx.37 These substances serve to anchor the matrix to the infected cell or inert biomedical device surface. In the process of biofilm formation the cells attach irreversibly to surfaces and begin to divide, forming microcolonies, and produce the extracellular polymers that define structural components of the biofilm.38These extracellular polymeric substances (EPS) consist primarily of polysaccharides which can be detected microscopically and by chemical analysis. EPS provides the matrix or structure for microbial biofilms which are highly hydrated (98%) not a mere homogeneous monolayer of “slime” but is heterogeneous with “water channels” that allow transport of essential nutrients and oxygen to the cells.39 Biofilms also have a propensity to act as filters to entrap particles of various kinds, including minerals and host components such as fibrin, RBCs and platelets.

Phenotypic heterogeneity is an important biofilm characteristic and biofilm-associated organisms can grow more slowly than planktonic organisms occupying the same niche because of localized nutrient and/or oxygen depletion.40  Bacterial cells may detach from the biofilm as a result of physical disturbance, cell growth and division or the spontaneous release or biofilm cell aggregates.41 These detached (planktonic) cells can potentially metastasize to local and distant sites in the host causing a systemic infection. Planktonic bacteria are dispersed using appendages as fimbria, cilia, and flagella, which support their motility but also give the bacteria a sense of “touch”. After the bacterium attaches irreversibly to the surface, change in the phenotype of the bacterium occurs and rapidly with a substantial number of new proteins being expressed within the first hour during which the bacterium attaches. Once irreversible attachment has occurred, the bacterium changes to a biofilm phenotype and may rapidly begin to grow depending on nutrient availability and differentiate, eventually, recalcitrant biofilm.

**Why are biofilms difficult to eradicate using antimicrobials?**

Surgical site infections (SSIs) associated with implants are difficult to eradicate using antibiotic regimens which would typically exhibit effectiveness against the same bacteria growing under planktonic conditions (Figure 6).42  In addition, clinical laboratory results based upon *in vitro* susceptibility of planktonic cells provide little clinical therapeutic guidance. The biofilm functions as a physical barrier against penetration of many antibiotics, antibodies and granulocytic cell populations. Multiple mechanisms are responsible for antimicrobial recalcitrance, most of which relate to phenotypic changes and multi-cellularity, rather than the genetic adaptation responsible for antibiotic resistance exemplified by MRSA. This is evidenced by the fact that cells dispersed from a biofilm, if tested before significant cell division occurs, can exhibit susceptibility comparable to planktonic cells.43 Growth-rate is a key mediator of bacterial susceptibility to many antimicrobial agents, even in planktonic cells.44, 45

Bacteria which are not actively dividing may be refractory to some antimicrobials through a process that has been termed “drug-indifference”.46 This is where the role of the biofilm matrix is particularly important since immobilization can confer marked phenotypic variation in cellular growth-rate within the biofilm due to localized depletion of nutrients and oxygen (for aerobic cells). However, growth-rate alone does not fully account for the extent of biofilm recalcitrance.47 The “persister cell” hypothesis, which was originally proposed in 1944, answers some of the questions posed on the process of biofilm recalcitrance. It was originally noted that staphylococcus cultures could not be sterilized with penicillin; an antibiotic to which the test bacterium was highly susceptible. Surviving cells, which were called “persister cells”, occurred at a frequency of approximately one per million of the bacterial cell present in the original culture.48 Surviving persister cells are apparently phenotypic variants which did not result from stable genetic alteration and are essentially indifferent to antimicrobials. Therefore, persister cells represent an important facet in the current explanation of biofilm recalcitrance, with survival during antimicrobial treatment and later proliferation, having been protected from phagocytosis by the biofilm matrix.49

**Biofilms, infection and wound healing in chronic surgical wounds**

The early stages of biofilm formation may occur rapidly, even in acute wounds such as burns and sutured surgical wounds healing by primary intention, and may result in delayed healing, such as superficial wound dehiscence, or a greater risk of developing overt infection.50 Biofilms are may be present in up to 70% of open wounds, healing by secondary intention, and this figure probably increases in chronic wounds, particularly in the presence of multiple, underlying and unmet co-morbidities. 51,52 It is commonly observed that the presence of bacteria (or the bioburden) in chronic, open wounds (such as venous and diabetic foot ulcers and pressure injuries) presents as a continuum from contamination through colonization to local and systemic infection. This may be worsened by underlying pathological processes such as venous or arterial insufficiency, diabetes, pressure damage or an occult malignant process.53 The difficulty of assessing infection, particularly in these chronic wounds, is not made easier by conventional processing and analysis of microbiological swabs. The presence of bacterial biofilms on a wound surface can encourage and excite an underlying, inappropriate and excessive host inflammatory response (through stimulation of neutrophils and macrophages) to cause a prolonged release of nitric oxide, inflammatory cytokines and free radicals and delayed healing.51,52 This concept, helps to explain why chronic wounds fail to heal and why some surgical wounds fail to heal, often with superficial skin dehiscence, without classical, clinical signs of acute infection (cellulitis, pus formation and pain) and a failure to identify/harvest micro-organisms from the dehisced wound. Although there is likely to be biofilm-related structures in or on most wounds these cannot be recognized without sophisticated laboratory testing.

The ideal way to manage a biofilm-mediated surgical wound would be to prevent it occurring during a surgical procedure through the rational use of a care bundle which includes the use of antibiotic prophylaxis in addition to adequate skin antisepsis prior to surgery, the use of antimicrobial-coated sutures and maintenance of normothermia and optimal tissue perfusion**.**54Prevention of biofilm reformation in open wounds involves adequate wound irrigation or cleansing using antiseptics, the use of negative pressure wound therapy (NPWT) or the use of antimicrobial dressings**.**55-57 Once there is wound dehiscence following bacterial attachment to a wound bed with biofilm formation it is probable that only maintenance debridement can control it (as in chronic open wounds). The diversity of biofilm phenotypes directly relates to successful attachment and infection in the wound, together with resistance to host response and antimicrobial therapy. The role that biofilms may play in delaying healing in sutured surgical or traumatic wounds is less clear, although it may account for early dehiscence of wounds after sutures or staples have been removed. Separation of the sutured skin is often accompanied with little evidence of acute inflammation or pus formation and cultures obtained from the margins of the wound are often negative, failing to yield responsible organism. Several unanswered questions remain, particularly for acute surgical wounds: does biofilm formation turn an acute wound into a chronic one (this may be relevant in early diabetic foot ulcers); does biofilm formation precede donor or recipient site infection, or burns colonization prior to infection, particularly with the pseudomonads? Microbial attachment and biofilm formation may also occur within deep wounds and these biofilms would be clinically relevant in prosthetic, orthopaedic and vascular SSIs.

**Diagnosis of acute and late onset biofilm infection in surgical patients**

Several investigators have suggested a diagnostic guideline which may help to recognise the presence of a biofilm-based infection within host tissues and selective clinical presentationswhich can be summarised asfollows:7, 58

1. Microbiological evidence of a localized or foreign body-associated infection post-surgery.
2. Microscopic (light or electron optic) evidence of the accumulation of microbes in aggregates.
3. Medical history, documenting a biofilm predisposing condition such as an implanted biomedical device, infective endocarditis, previous device-related infection.
4. Recurrent infection (site-specific) with organisms which are clonally identical.
5. Documented history of antimicrobial failure or therapeutic recalcitrance (persistent infection) despite selection of appropriate antimicrobial agent based on laboratory sensitivity testing and site of infection (both dose and duration).
6. Presence of local or systemic signs and symptoms of infection that resolved primarily with appropriate antimicrobial therapy, only to recur following termination of therapy.

In some clinical situations it may be assumed that a biofilm-based infection is present. However, in the case of a chronic inflammatory process involving fascia (deep) or organ-space involvement, the true nature of the infection may not be evident until the wound is actually visualized at surgical revision. The recalcitrant nature of these infections requires that all infected tissue be excised because residual biofilm may not respond to traditional antimicrobial therapeutic measures, even though planktonic (free-floating) forms of the responsible organisms may be susceptible to traditional antimicrobial agents.32

**Treatment and management of biofilm-mediated infections**

At present there are no evidence-based studies focusing on the therapeutic efficacy of selective strategies for managing biofilm-associated SSIs. However, in general the current therapeutic options can be characterized as follows:

1. Tissue-based infection: Surgical debridement, with negative pressure therapy when indicated, to remove devitalized tissue, followed by irrigation preferably with a biocidal agent followed by parenteral antibiotics.21, 59, 60
2. Device-related infection: Removal of an infected device followed by insertion of antimicrobial adjuncts such as antimicrobial spacers, beads or sutures together with parenteral antibiotics.28, 61-63
3. Antimicrobial agents: Selection of a therapeutic agent that can penetrate the mature microbial biofilm, such as linezolid, daptomycin, rifampin or possibly ceftaroline.26, 64, 65

**Biofilm-associated infections: the future**

Unfortunately, biofilm-associated infections are likely to increase in number due in-part to the continued growth of implanted medical devices. While investigators are currently focusing on biofilm-resistant polymers or surface coatings which discourage microbial attachment, these efforts are in progress and unlikely to radically alter the risk of infection within the immediate future. Prevention of intraoperative contamination offers the greatest benefit for any patient receiving an implantable medical device. Therefore, meticulous surgical technique, use of perioperative care bundles and awareness of the various possible avenues of intraoperative contamination that can occur at the time of implantation is reflective of a rational strategy for improving surgical patient outcome. Finally, every institution should have specific policies and procedures in-place for the management, sterilization compliance and handling of biomedical devices prior to surgical implantation.

**Figure Legends**

Figure 1. Surface contamination (following condition of graft surface with whole blood and 10% serum albumin) of a double velour Dacron graft 8 hours post contamination (*Staphylococcus epidermidis*).

Figure 2. Documentation of confluent *Staphylococcus epidermidis* biofilm on surface of double velour Dacron at 72 hours post contamination.

Figure 3. Presentation of a 78 year old male with a prosthetic graft infection after total knee arthroplasty. The patient had multiple co-morbid risk factors: 20 year history of rheumatoid arthritis, 30-year history of type II diabetes, morbidly obesity (BMI 49), smoker, psoriasis, remote site infection (diabetic foot infection of right foot) and and ASA >3.

Figure 4**.** The cellular and extracellular components of a biofilm. The wound biofilm is visualized using florescence microscopy in conjunction with FISH (red), to indicate eubacteria; ConA (green), to indicate biofilm matrix and other ConA-reactive material; and with Hoechst 33252 (blue) for the detection of nucleic acids.66

Figure 5. Simplified model illustrating sequential biofilm development based on knowledge of biofilms in aquatic, oral and clinical environments. In this example, a mixture of bacterial species in planktonic form adhere to the implant surface (1) and develop into a biofilm (2) with the associated deposition of enveloping matrix material. Dispersion (3) if and when it occurs, can enable cells to colonize elsewhere. The diagram also shows aggregation (where cell clusters form), co-aggregation, where taxonomically distinct bacteria are believed to form aggregates through specific surface receptor interactions and co-adhesion, and where co-aggregated cells attach to an extant biofilm.

Figure 6. Main mechanisms believed to be responsible biofilm recalcitrance which include (i) gradients of nutrient and gases which may be established, resulting in local variation in bacterial phenotype, including areas of dormancy; (ii) reduced penetration of antimicrobials through interaction with the biofilm matrix and by enzymes within the matrix including β-lactamases; (iii) phenotypically specialized recalcitrant variants including persister cells which can be protected from immune cells by the matrix and survive sub-inhibitory antimicrobial exposure allowing regrowth following treatment; and (iv) clusters of taxonomically distinct bacteria.

**References**

1. Reed D, Kemmerly SA. Infection control and prevention: a review of hospital-acquired infections and the economic implications. The Oscher J 2009; 9: 27-31.
2. Shepard J, Ward W, Milstone A, et al. Financial impact of surgical site infections on hospital: the hospital management perspective. JAMA Surg 2013; 148: 907-914.

3. De Lissovoy G, Fraeman K, Hutchins V, et al. Surgical site infection: incidence and impact on hospital utilization and treatment costs. Am J Infect Control 2009; 37: 387-397.

4. Herwaldt LA, Cullen JJ, Scholz D, et al. A prospective study of outcome, healthcare resource utilization, and cost associated with postoperative nosocomial infections. Infect Control Hosp Epidemiol 2006; 27: 1291-1298.

5. World Health Organization (WHO), European Health Information Gateway.Total number of inpatient surgical procedures per year. http://portal.euro.who.int/en/indicators/hfa-indicators/hfa\_539-6031-total-number-of-inpatient-surgical-procedures-per-year/.

6. Edward R, Harding KG. Bacteria and wound healing. Current Opinion in Infect Dis 2004; 17: 91-96.

7. Hall-Stoodley L, Stoodley P, Kathju S, et al. Towards diagnostic guidelines for biofilm-associated infections. FEMS Immmunol- Med Microbiol 2012; 65: 127-145.

8. Percival SL. Biofilms and their potential role in wound healing. Wound 2004; 16: 234-240.

9. Romling U, Balsalobre C. Biofilm infections, their resilience to therapy and innovative treatment strategies. J Intern Med 2012; 272: 541-561.

10. National Institute of Health Research on microbial biofilms: PA Number: 2002, PA-03-047 [http://grants.nih.gov/grants/guide/pa-files/PA-03-047.html].

11. Dowd SE, Wolcott RD, Sun Y, et al. Polymicrobial nature of chronic diabetic foot ulcer infections determined using bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP). PloS One 2008; 3: e3326.

12. Edmiston CE, Krepel CJ, Marks RM, et al. Microbiology of Explanted Sutures Segments from Infected and Non-Infected Surgical Cases. J Clin Microbiol 2013;51:417-421.

13. Hoiby N, Ciofu O, Johansen HK, et al. The clinical impact of bacterial biofilms. Int J Oral Sci 2011;3:55-65.

14. Jenson PO, Givskov M, Bjarnsholt T, et al. The immune system versus. *Pseudomonas aeruginosa* biofilms. FEMS Immunol Med Microbiol 2011;59:292-305.

15. Akers KS, Mende K, Cheatle K, et al. Biofilms and perisistent wound infections in United States military trauma patients a case-control analysis. BMC Infect Dis 2014; 14: 190 [http://www.biomedcentral.com/1471-2334/14/190].

16. Buchan B, Ledeboer N, Edmiston CE. Acinetobacter Infections: Epidemiology and Pathogenesis of a Significant Healthcare-Associated Pathogen. Healthcare Infection 2011;16:6-17.

17. Costerton JW, Cheng KJ, Geesey GG, et al. Bacterial biofilms in nature and disease. Annu Rev Microbiol. 1987;41:435-464.

18. Del Pozo JL, Patel R. The challenge of treating biofilm-associated bacterial infections. Clin Pharmacol Ther. 2007 Aug;82(2):204-209.

19. Hasanadka R, Seabrook GR, Edmiston CE. Vascular Graft Infections. In: Rello J, Vanes J, Kollef M, eds. 2nd ed. Critical Care Infectious Diseases. Boston: Kluwer Academic Publishers. 2007, pp. 555-566.

20. Hasanadka R, Seabrook GR, Edmiston CE. Vascular Graft Infections. In: Rello J, Vanes J, Kollef M, eds. 2nd ed. Critical Care Infectious Diseases. Boston: Kluwer Academic Publishers. 2007 pp. 531-542.

21. Barnes S, Spencer M, Graham D, et al. Surgical wound irrigation: a call for evidence-based standardization of practice. Am J Infect Control 2014; 42: 525-529.

22. Engelsman AF, van der Mei HC, Ploeg RJ, et al. The phenomenon of infection with abdominal wall reconstruction. Biomaterials 2007; 28: 2314-2327.

23. British Hernia Society. Association of Surgeons of Great Britain and Ireland (2013) Commissioning guide: Groin Hernia. 2013. https://www.rcseng.ac.uk/healthcare-bodies/docs/published-guides/hernia

24. Engelsman AF, van der Mei HC, Busscher HJ, et al. Morphological aspects of surgical mesh as a risk factor for bacterial colonization. Br J Surg 2008; 95: 1051-1059.

25 Sanchez CJ, Mende K, Beckius ML, et al. Biofilm formation by clinical isolates and the implication in chronic infections, BMC Infect Dis. 2013 Jan 29;13:47. doi: 10.1186/1471-2334-13-47.

26. Edmiston EC, Goheen MP, Seabrook GR, Johnson CP, Lewis BD, Brown KR, Towne JB. Impact of selective antimicrobial agents on staphyococcal adherence to biomedical devices. Am J Surg 192:344-354.

27. Bandyk DF, Black MR. Infection in prosthetic vascular grafts. In Rutherford RB, Johnson KW, eds. Vascular Surgery. 6th ed. Phialdelphia, PA: Elsevier Saunders 2005:875-6894.

28. Del Pozo JL, Patel R. Clinical Practice. Infection associated with prosthetic joints. N Eng J Med 2009; 361: 787-794.

29. Public Health England. Surgical site infections (SSI) surveillance: NHS hospitals in England (2014/2015).<https://www.gov.uk/government/uploads/system/uploads/> attachment\_data/file/484874/Surveillance\_of\_Surgical\_Site\_Infections\_in\_NHS\_Hospitals\_in\_England\_report\_2014-15.pdf.

30. Portillo, ME, Corvec S, Borens O, et al. "Propionibacterium acnes: an underestimated pathogen in implant-associated infections." Biomed Res Int 2013: 804391.

31. Costerton JW, Lewandowski Z, Caldwell DE, et al. Microbial biofilms. Ann Rev Microbiol 1995; 49: 711-745.

32. Edmiston CE. Prosthetic device infections in surgery. In Nichols RL, Nyhus LM, eds. Update Surgical Sepsis. Philadelphia, PA; JB Lippincott. 1993, pp. 444-468.

33. Kim DH, Spencer M, Davidson SM, et al. Institutional prescreening for detection and eradication of methicillin-resistant *Staphylococcus aureus* in patients undergoing elective orthopaedic surgery. J Bone Joint Surg 2010; 92: 1820-1826.

34. Nemoto K, Hirota K, Ono T, et al. Effect of varidase (streptokinase) on biofilm formed by *Staphylococcus aureus*. Chemother 2000; 46: 111-115.

35. James GA, Ge Zhao A, Usui M, et al. Microsensor and transcriptomic signatures of oxygen depletion in biofilms associated with chronic wounds. Wound Repair Regen 2016;24:373-383.

36. Gilbert P, Maira-Litran T, McBain AJ, et al.The physiology and collective recalcitrance of microbial biofilm communities. Adv Microb Physiol. 2002;46:202-256.

37. Arnold WV, Shirtliff ME, Stoodley P. (2013) Bacterial biofilms and periprosthetic infections. J Bone Joint Surg Am 2013;;95:2223-229.

38. Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010;8:623-633.

39. Stoodley L, Sidhu S, Nistco L. et al. Kinetics and morphology of polymicrobial biofilm formation on polypropylene mesh. FEMS Immunol Med Microbiol. 2012:283-290;

40. Gilbert P, Maira-Litran T, McBain AJ, et al.The physiology and collective recalcitrance of microbial biofilm communities. Adv Microb Physiol 2002;46:202-256.

41. Davies DG, Marques CN. A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. J Bacteriol. 2009;191:1393-403.

42. Gilbert P, Maira-Litran T, McBain AJ, et al. The physiology and collective recalcitrance of microbial biofilm communities. Adv Microb Physiol 2002;46:202-256.

43. Stewart PS. 2015 Antimicrobial Tolerance in Biofilms. Microbiol Spectr. 2015; Jun;3 doi: 10.1128/microbiolspec.MB-0010-2014

44. Hunt SM, Werner EM, Huang B, t al.Hypothesis for the role of nutrient starvation in biofilm detachment. Appl Environ Microbiol 2004;70: 7418-7425.

45. Werner E, Roe F, Bugnicourt A, et al. Stratified growth in Pseudomonas aeruginosa biofilms. Appl Environ Microbiol 2004;70:6188-6196.

46. Jayaraman R. Bacterial persistence: some new insights into an old phenomenon. J Biosci 2008;33:795-805.

47. Gilbert P, Maira-Litran T, McBain AJ, et al. The physiology and collective recalcitrance of microbial biofilm communities. Adv Microb Physiol 2002;46:202-256.

48. Bigger JW. Treatment of staphylococcal infection with penicillin. Lancet

1944;2:497-500.

49. Conlon BP, Rowe SE, Lewis K. 2015. Persister cells in biofilm associated infections. Adv Exp Med Biol 2015;831:1-9.

50. Percival SL, Hill KE, Williams DW, et al. A review of the scientific evidence for biofilms in wounds. Wnd Repair Regeneration 2012;20:647-657.

51. Wolcott RD, Rhoads D, Dowd SE. Biofilms and chronic wound inflammation. J Wnd Care 2008; 7: 333-34.

52. Wolcott RD, Rhoads DD, Bennett ME, et al. Chronic wounds and the medical biofilm paradigm. J Wnd Care 2010;19:45-53.

53. Wolcott RD, Rhoads DD. A study of biofilm-based wound management in subjects with critical limb ischemia. J Wnd Care 2008.17:145–155.

54. Leaper D, Ousey K. Evidence update on prevention of surgical site infection. Current opinion in infectious diseases 2015; 28:158-163.

55. Wolcott RD, Rumbaugh KP, Stewart PS, Dowd SE. Biofilm maturity studies indicate sharp debridement opens a time-dependent therapeutic window. J Wnd Care 2010;19:320–328.

56. Wolcott RD, Kennedy JP, Dowd SE. Regular debridement is the main tool for maintaining a healthy wound bed in most chronic wounds. J Wnd Care 2009; 18:54-56.

57. Wolcott RD, Cox S. More effective cell-based therapy through biofilm suppression. J Wnd Care 2013; 22:26-31.

58. Hall MR, McGillicuddy E, Kaplan LJ. Biofilm: basic principles, pathophysiology, and implications for clinicians. Surg Infect 2014; 15: 1-7.

59. Cardinal M, Eisenbud DE, Armstrong DG, et al. Serial surgical debridement: a retrospective study on clinical outcomes in chronic lower extremity wounds. Wnd Repair and Regeneration 2010;17:306-311.

60. Leaper DJ, Meaume S, Apelqvist J, et al. Debridement methods of non-viable tissue in wounds. In: Advanced Wound Repair Therapies. Farrar D, ed. Woodhead Publishers. Cambridge, UK, 2011.

61. Edmiston CE, Daoud F, Leaper DJ. Is there an evidence-based argument for embracing an antimicrobial (triclosan)-coated suture technology to reduce the risk for surgical-site infections?: a meta-analysis. Surg 2013;154:89-100.

62. Edmiston CE, Bruden, Rucinski M, et al. Reducing the risk of surgical site infections: does chlorhexidine gluconate provide a risk reduction benefit? Am j Infect Control 2013;41:S49-S55.

63. Griffin JW, Guillot SJ, Redick JA, et al. Removed antibiotic-impregnated cement spacers in two-stage revision joint arthroplasty do not show biofilm formation *in vivo*. J Arthroplasty 2012;27:1796-1799.

64. Barber KE, Werth BJ, McRoberts JP, et al. A novel approach utilizing biofilm time-kill curves to assess the bactericidal activity of ceftaroline combinations against biofilm-producing methicillin-resistant *Staphylococcus aureus*. Antimicrob Agent Chemother 2014;58:2989-2992.

65. Seaton RA, Malizos KN, Viale P, et al. Daptomycin use in patients with osteomyelitis: a preliminary report from the EU-COREsm database. J Antimicrob Chemother 2013;68:1642-1649.

66. Oates A, Bowling FL, Boulton AJ, et al. The visualization of biofilms in chronic diabetic foot wounds using routine diagnostic microscopy methods. J Diabetes Res 2014 doi: 10.1155/2014/153586.