MICROBIAL BIOFILMS

Implant infections: adhesion, biofilm formation and immune evasion

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Abstract | Medical device-associated infections account for a large proportion of hospital-acquired infections. A variety of opportunistic pathogens can cause implant infections, depending on the type of the implant and on the anatomical site of implantation. The success of these versatile pathogens depends on rapid adhesion to virtually all biomaterial surfaces and survival in the hostile host environment. Biofilm formation on implant surfaces shelters the bacteria and encourages persistence of infection. Furthermore, implant-infecting bacteria can elude innate and adaptive host defences as well as biocides and antibiotic chemotherapies. In this Review, we explore the fundamental pathogenic mechanisms underlying implant infections, highlighting orthopaedic implants and *Staphylococcus aureus* as a prime example, and discuss innovative targets for preventive and therapeutic strategies.

Health-care-associated infections
An infection contracted by a patient while receiving medical care in a hospital or in another health-care facility (synonymous with nosocomial and hospital infection).

Opportunistic pathogens
Microorganisms that generally live harmlessly as commensals but can cause infection in hosts with lowered resistance to disease.

Granulation tissue
New connective tissue and capillaries that replace the fibrin matrix during wound healing.

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https://doi.org/10.1038/s41579-018-0019-y

Biomedical implants, such as prosthetics, catheters and several other devices (BOX 1), have revolutionized medicine, but they increase the infection risk. Indeed, implant infection is one of the most frequent and severe complications associated with the use of biomaterials[1,2]; for example, device-associated infections account for 25.6% of all health-care-associated infections in the USA[3]. In this Review, we focus on orthopaedic implants, because, as prosthetics remain in the body, their infection is particularly problematic. Infections frequently lead to the failure of the prosthetic device, require implant replacement and often cause chronic and/or relapsing disease[1,2]. Furthermore, the diagnosis of orthopaedic implant infections, including identifying the infectious agent and its antimicrobial sensitivity, can be problematic, and they are often hard to treat owing to antimicrobial resistance, tolerance and/or persistence. Orthopaedic implant infections are often caused by *Staphylococcus aureus*, but many other pathogens can cause such infections[4] (TABLE 1).

Implant infection involves complex interactions between the pathogen, the biomaterial and the host immune response to both. Without a foreign body, tissue contamination by opportunistic pathogens is usually spontaneously cleared by host immune defences. By contrast, in implant-associated infections, the biomaterial triggers a local tissue response, which includes acute and chronic inflammation, a foreign body reaction, formation of granulation tissue and, finally, fibrous encapsulation[5]. This generates a niche of immune depression, a *locus minoris resistentiae*[6], which predisposes the implant to microbial colonization and infection[7,8]. Furthermore, the biomaterial is a substrate for bacterial adhesion and biofilm formation. Bacterial adhesion is the first step of biomaterial-related infections, and it paves the way for colonization of the implant. In the newly acquired sessile status, pathogens form microcolonies and produce protective biofilms, which enables them to persist in the hostile host environment. Adhesion and biofilm formation, therefore, have pivotal roles in the pathogenesis of implant infections[9]. For example, substantially fewer *S. aureus* cells are needed to infect rabbits when a foreign material is present at the surgical site than when surgery is performed without implant materials[10]. Moreover, implants enable not only virulent pathogens such as *S. aureus* but also bacteria such as *Staphylococcus epidermidis*, which was formerly considered a mere saprophyte, to survive and thrive[11].

In this Review, we focus on infections of orthopaedic implants, but some of the discussed principles also apply to other implants. We briefly discuss the different types of orthopaedic implant infections, the main infectious agents and the molecular mechanisms that underlie pathogenesis and, finally, we summarize approaches to prevent and treat these infections.

Implant-infecting bacteria

Prosthetic infections usually originate from microbial contamination during surgery. Traditionally, orthopaedic implant infections that develop within 3 months after surgery are classified as early postoperative infections. Delayed (or subacute) infections develop 3–24 months after surgery and late infections after more than 24 months[2,12]. Late infections can be initially asymptomatic surgical infections that become symptomatic

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Box 1 | **Implant materials, devices and sites**

Based on past FDA[162] and Medtech Europe estimates, more than 500,000 types of medical devices have currently entered the global market. Invasive medical devices, including indwelling and implantable devices, represent just a fraction of these. Nonetheless, the number of devices, such as cerebrospinal shunts and urinary and vascular catheters, used globally each year is massive, in the order of hundreds of millions. More than a million cardiovascular electronic devices are implanted worldwide each year[163], and 10 million dental implant procedures are performed[164]. In the USA alone, knee and hip arthroplasty procedures are exceeding 1 million per year[13]. Five to 10 million women currently have artificial breast implants[165]. These examples showcase the myriad devices used in medicine and the vast number of devices used. Depending on the type of device, its level of invasiveness in the body, the anatomical site of insertion and the duration of application (transient, short-term, long-term or permanent), the risk of infection differs. Invasive devices that are inserted into the human body through body orifices and remain in contact with mucous membranes favour the entrance of bacteria from the external environment. Such invasive devices include urinary catheters, tracheal cannulae and tubes, contact lenses and fixed dental prostheses, among others. Intubation with endotracheal tubes provides a conduit for the migration of microorganisms, resulting in a markedly increased risk of pneumonia (6-fold to 20-fold) in patients who are often debilitated and critically ill[166]. The use of urinary catheters is frequently the cause of catheter-associated bacteriuria, which represents one of the most frequent health-care-associated infections. In the current system of classification, the devices described above are distinguished from surgically invasive devices and implants, which are inserted into the body tissues by breaching the skin or mucous membranes. These devices are used in cardiovascular surgery (for example, cardiovascular catheters, pacemakers, stents and heart valves), neurosurgery (for example, neurological shunts and spinal stents), orthopaedic surgery (for example, prosthetic joints, megaprostheses, external and internal fixation systems, artificial ligaments and bone cements), plastic surgery (for example, sutures, breast implants and tissue augmentation implants), ophthalmology (for example, intra-ocular lenses), urology (for example, penile implants), gynaecology (for example, urogynaecological mesh surgical implants), dentistry (for example, dental filling materials and abutments) and in many other applications. Biomaterials are implanted for medical treatments in nearly all anatomical locations of the body, and they interface with all kinds of human tissues. No matter where the surgically invasive device is placed, it is a foreign body. Even a mild tissue response alters the immune defences at the site of implantation, creating a *locus minoris resistentiae*, which is vulnerable to bacterial attack even by weakly virulent, opportunistic pathogens. Most critical adverse effects in terms of morbidity and mortality are generally observed for those implants that are meant to be long term or permanent, are applied to critically ill patients, are life supporting or that reach vital organs such as the heart and brain. However, all surgically invasive devices, especially those in contact with the bloodstream, can potentially cause sepsis.

later or new infections that reach the implant through haematogenous spread[12–14]. A more recent classification distinguishes early infections, which become manifest within 1 month after surgery, and acute haematogenous infections with a duration of symptoms of up to 3 weeks from chronic infections, which persist for more than 3 weeks and require different treatment[12]. Specifically, debridement and implant retention offer an acceptable rate of cure only for early and acute haematogenous implant infections[12,13].

In orthopaedic prosthetic infections, the most commonly isolated microorganisms are Gram-positive cocci: *S. aureus*, coagulase-negative staphylococci (CNS) and enterococci (TABLE 1). Aerobic Gram-negative bacilli, including *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*, are less frequent causes of infection. Anaerobes, including *Propionibacterium acnes* (also known as *Cutibacteriumacnes*), account for 4% of infections[15]. The causative agents vary depending on the type and the site of the implant as well as the time since surgery[16]. Early infections are generally caused by virulent microorganisms, such as *S. aureus*, and by contamination of the surgical site. Delayed infections often are caused by microorganisms of low virulence, such as CNS and *P. acnes*. Late haematogenous infections originate from bacteria that cause skin, respiratory, dental and urinary tract infections[17].

Generally, staphylococci are the most frequent cause of orthopaedic implant infections. A recent study reported differences between geographic areas: *S. aureus* was the most frequent pathogen associated with total arthroplasty in the USA, whereas *S. epidermidis* was slightly more common in Europe[18] (TABLE 1). This difference might be due to different surgical algorithms and reported outcomes[18]. Other factors might be involved, such as the local pathogen prevalence and antibiotic resistance in combination with the type of aseptic and prophylactic measures applied. However, the reference centres considered in the study might not have been representative of the whole region. An earlier study of other European clinics also found *S. aureus* to dominate[4] (TABLE 1).

Antibiotic resistance is an important issue in orthopaedic implant infections[19]. Implant-infecting *S. aureus* strains have high rates of antibiotic resistance, and there is an alarming increase of antibiotic resistance in other species, such as *S. epidermidis*[20]. In periprosthetic joint infections, methicillin-resistant *S. aureus* (MRSA) predominantly caused early infections, whereas methicillin-sensitive *S. aureus* (MSSA) caused delayed and late infections, and the number of MSSA infections was approximately 2.5-fold greater than that of MRSA infections[21]. Up to 40% of *S. epidermidis*[22] and 32% of *S. aureus*[23] strains isolated from orthopaedic postsurgical and implant-related infections were reported to be resistant to gentamicin.

Adhesion to the implant surface

Bacterial adhesion can be divided into two stages: first, primary unspecific reversible attachment; and second, specific irreversible attachment[24]. Initial adhesion to abiotic surfaces is generally unspecific, whereas adhesion

Coagulase-negative staphylococci
(CNS). A broad group of staphylococci devoid of coagulase activity that includes some of the staphylococcal species that often cause hospital-acquired infections.

Total arthroplasty
A reconstructive surgical procedure consisting of replacement of a joint with an artificial prosthesis.

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Table 1 | **Major implant-infecting bacteria causing orthopaedic infections**

Species	Prevalence in medical device infections (%)	Prevalence in knee arthroplasty infections (%)	Prevalence in hip arthroplasty infections (%)	Prevalence in infections involving external fixation (%)	Prevalence in infections involving internal fixation (%)	Refs
Staphylococcus aureus	31.7	21.1	22.2	54.5	47.8	2
	33.8	26.4	24.4	47.8	42.5	4
	13.0 (EU)[a]–31.0 (US)[a]	12.1 (EU)[a]–29.6 (US)[a]	13.6 (EU)[a]–32.6 (US)[a]	ND	ND	18
Coagulase-negative staphylococci	20.2 (US)[a]–39.3 (EU)[a]	21.7 (US)[a]–37.0 (EU)[a]	18.4 (US)[a]–40.7 (EU)[a]	ND	ND	18
Staphylococcus epidermidis	39.0	52.6	48.1	18.2	26.1	2
	31.5	41.8	43.6	15.2	21.9	4
Coagulase-negative staphylococci other than *Staphylococcus epidermidis*	11.6	ND	ND	ND	ND	2
	12.8	ND	ND	ND	ND	4
Streptococcus spp. and *Enterococcus* spp.	10.3 (US)[a]–14.5 (EU)[a]	10.3 (US)[a]–14.5 (EU)[a]	9.1 (US)[a]–12.1 (EU)[a]	ND	ND	18
Enterococcus faecalis	2.4	2.6	0.0	0.0	6.5	2
	4.4	0.5	3.5	8.7	5.3	4
Gram-negative bacteria	ND	4.5 (EU)[a]–6.4 (US)[a]	4.2 (EU)[a]–6.8 (US)[a]	ND	ND	18
Pseudomonas aeruginosa	6.1	10.5	3.7	18.2	4.3	2
	6.7	4.4	2.9	14.1	8.9	4
Escherichia coli	2.4	5.3	0.0	0.0	0.0	2
	1.6	ND	ND	ND	ND	4

EU, data from a European reference clinical setting; ND, not determined; US, data from a US reference clinical setting. [a]Infections after total arthroplasty.

to living tissues involves specific lectin-based or adhesin-based interactions[25]. Bare material surfaces are rapidly covered by extracellular matrix (ECM) proteins and immune protein components when submerged in physiological fluids[26,27].

Similarly, implants are coated with proteins from blood and interstitial fluids within nanoseconds[26], and this process is determined by surface chemistry and wettability of the implant surface[28]. Therefore, adhesins are also the main tool for bacterial attachment to the implant surface inside the body (FIG. 1). *S. aureus* and *S. epidermidis* have multiple mechanisms for attachment and biofilm formation that contribute to their virulence in chronic implant infections[9,29,30].

Adhesion to uncoated abiotic surfaces. Initial bacterial attachment to abiotic surfaces is mediated by nonspecific forces (Lifshitz–van der Waals, Lewis acid–base and electrostatic forces)[31,32], with bacteria behaving like colloidal microparticles. These conditions can easily be simulated in vitro. Nonetheless, even elaborate models of colloidal adhesion, such as the extended Derjaguin–Landau–Verwey–Overbeek theory (XDLVO theory) do not always accurately predict the behaviour of viable bacteria[33,34], which have variable surface properties depending on species, strain, population heterogeneity and cell cycle phase.

Bacterial filamentous cell appendages, such as nanofibres, bacterial pili and pilus-like adhesive structures, also function as adhesins[35]. Some bacterial nanofibres mediate cell adhesion to abiotic surfaces and are involved in biofilm formation[36]. Others specifically bind to host cell surface molecules and/or ECM components, such as collagen and fibronectin, and are involved in implant infection[37]. Some species-specific proteins, for example, the autolysins AtlE[38] from *S. epidermidis* and AtlA[39] from *S. aureus*, mediate binding to abiotic surfaces. AtlE participates in the attachment of *S. epidermidis* to abiotic surfaces, such as naked polystyrene, and it also binds biomolecules such as vitronectin. AtlA is a bifunctional enzyme that undergoes proteolytic cleavage to yield two catalytically active proteins, an amidase and a glucosaminidase[40]. Only the amidase binds the matrix proteins fibrinogen, fibronectin and vitronectin[41]. Thus, AtlA mainly mediates adhesion of *S. aureus* to implants that are coated by host matrix proteins[40]. In *S. aureus*[40] and *Enterococcus faecalis*, AtlA contributes to biofilm formation through its autolytic activity, and *E. faecalis* strains with an *atlA* deletion have a defect in biofilm formation[42].

Adhesion to biotically coated surfaces. Bacterial adhesion to ECM molecules primarily occurs through specific binding of piliated and non-piliated bacterial adhesins to host proteins, and these interactions have been thoroughly reviewed[43]. *S. aureus* has a rich repertoire of adhesins, including cell wall-anchored microbial surface components recognizing adhesive matrix molecules (MSCRAMMs)[27,44] and secretable expanded repertoire adhesive molecules (SERAMs), which are ionically associated with the bacterial cell wall[45,46]. Adhesins are multifunctional and do not just mediate

Wettability
Ability of a solid surface to reduce the surface tension of a liquid in contact with it so that the liquid spreads over the surface and wets it.

Extended Derjaguin–Landau–Verwey–Overbeek theory
(XDLVO theory). A theory that describes the interactions between material surfaces immersed in a liquid, taking into account the different attractive and repulsive forces (Lifshitz–van der Waals, electrical double layer and Lewis acid–base forces). It can be used to predict the interactions of bacteria and biomaterial surfaces.

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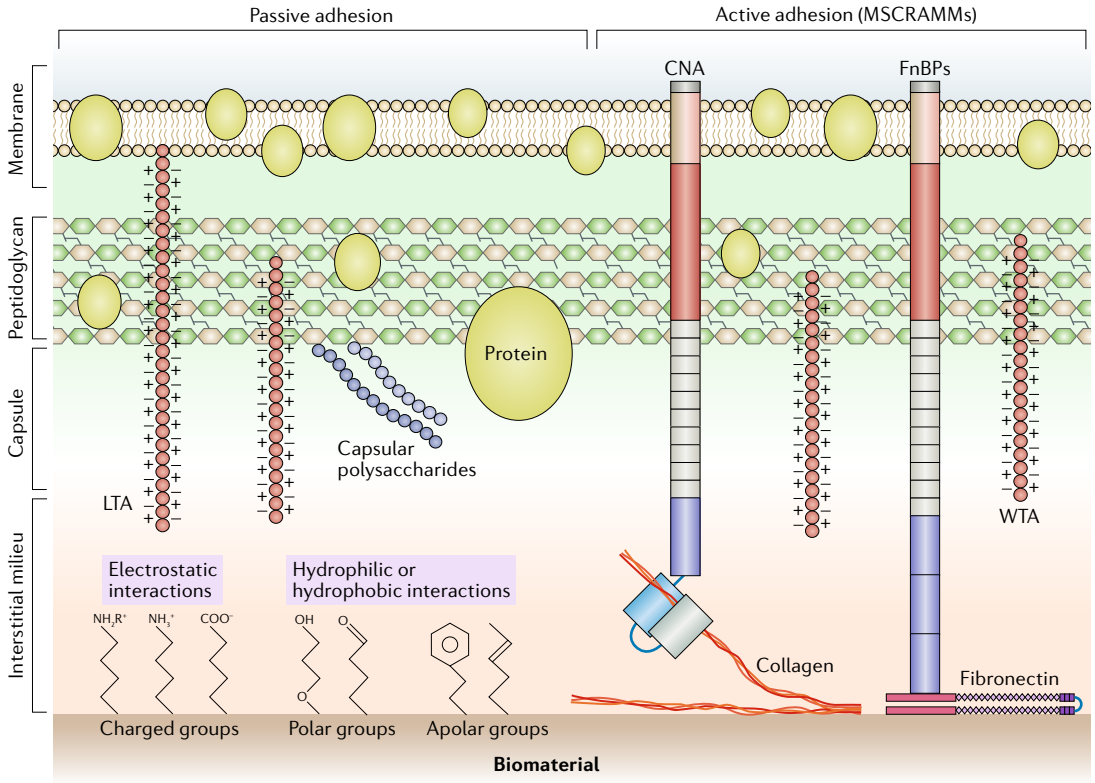

Fig. 1 | **Adhesion of *Staphylococcus aureus* to implant surfaces.** Bacterial adhesion on the biomaterial surface results from a combination of reversible passive mechanisms and irreversible active mechanisms. The latter involves microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), such as the collagen-binding adhesin (CNA) and the fibronectin-binding proteins (FnBPs), which are expressed by most *Staphylococcus aureus* strains and can bind collagen and fibronectin, respectively. LTA, lipoteichoic acid; WTA, wall teichoic acid.

adhesion to ECM proteins[46]. Certain MSCRAMMs modulate the host immune response, and others function as invasins, mediating bacterial internalization into host cells.

The main matrix proteins that are ligands for bacterial adhesins are collagens, fibronectins and fibrinogen. Collagen is a superfamily of over 20 matrix proteins with a structural role in tissues. Type I collagen and bone sialoprotein are the most abundant proteins of the bone matrix. In orthopaedic implant infections, biomaterials interface bone tissues, and, therefore, the ability of *S. aureus* to bind to collagen and to bone sialoprotein is an important virulence trait[47].

Fibronectins are homodimeric glycoproteins that are linked by disulfide bonds at the carboxyl terminus. Hepatocytes secrete soluble fibronectins into the plasma. Fibroblasts secrete fibronectins into the interstitial space, where they associate into high molecular mass insoluble polymers. Fibronectins attach to fibrous collagens and promote adhesion and spreading of cells and regulate the shape of host cells by influencing cytoskeleton assembly[48].

The binding of *S. epidermidis* to fibronectin has been studied at the single molecule level by dynamic force spectroscopy. *S. epidermidis* bound the carboxy-terminal domain of fibronectin, and the interaction was specifically inhibited by heparin[49].

Fibrinogen is synthesized by hepatocytes and is composed of three pairs of non-identical chains. Staphylococcal binding to fibrinogen has a more important role in catheter-related infections compared with orthopaedic implant infections[50].

Race to the surface. Over the years, the concept of 'race to the surface' (REFS[51,52]) has been introduced to describe the competition between host cells and contaminating bacteria to occupy the biomaterial surfaces. The rapid integration of biomaterials into host tissues is key for the success of many implants[53], and there is evidence that prompt integration is also crucial for preventing bacterial adhesion and colonization[54]. In orthopaedics, bone tissue healing around the implant leads to the apposition of bone and integration of the implant into bone tissue, that is, osseointegration. In vitro observations with osteosarcoma cells show that pre-colonizing bacteria drastically change and compromise host cell adhesion to material surfaces[53]. In fact, if bacterial adhesion occurs before tissue repair takes place, host defences cannot prevent surface colonization and biofilm formation[32,54].

Although in vitro models provide mechanistic insights into the interactions of bacteria with host cells and biomaterial surfaces, they often focus on single host cell types and short times of observation. The strengths and limitations of in vitro studies have been reviewed[55],

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Box 2 | **Persister cells**

In implant infections, antibiotic treatment may lead to eradication of most of the susceptible bacterial population, but a small fraction of non-growing persister cells survive and can potentially reconstitute the biofilm when antibiotic therapy is stopped[167]. There is evidence from many in vitro studies and from some in vivo studies that persister cells have a role in clinical infections[168]. Insufficient elimination of persister cells contributes to the recalcitrance and recurrence of biofilm-associated infections. Persister cells that neither grow nor die in the presence of microbicidal antibiotics are important for the tolerance of biofilms to antibiotics. They contribute to the establishment of chronic infections[169,170] and they complicate the treatment of implant infection. Persister cell formation is stimulated under conditions that activate stress signalling, which are all typically present in implant infections, such as growth of biofilms, hostile body environments and sublethal concentrations of antibiotics[160]. The possibilities of provoking 'death in sleep' or awakening dormant persister cells have been explored[171,172]. Attacking the persister and dormant cells in biofilms could help to prevent relapse of implant infection.

but insights into the more complex in vivo events leading to overt symptomatic infections and delayed low-grade infections are needed.

Biofilm formation

Implant-infecting bacteria generally are not sparsely distributed, single, adherent cells but form biofilms, in which bacterial aggregates tightly adhere to the biomaterial surface and are encased in an abundant matrix of extracellular polymeric substances (EPSs)[56].

Biofilms are responsible for the persistence of implant infections (see BOX 2 on persister cells, which contribute to the recalcitrance of implant infection to therapy) and are a source of bacterial dissemination to other body sites. Further, because host immune defences and conventional antimicrobial therapies are often inefficacious against bacteria growing in a biofilm, chronic inflammation arises[57]. The protection offered by life in biofilms applies to different microbial species, including Gram-negative bacteria and fungi[58].

Furthermore, the high cell density in biofilms facilitates high rates of horizontal gene transfer between microorganisms, and conjugation occurs more often between members of biofilm communities than between planktonic bacteria[59].

Stages of biofilm formation. The classic model of biofilm formation, which applies to *S. aureus*[9] and to Gram-negative bacteria such as *P. aeruginosa*[60], involves several stages: adhesion, microcolony formation due to cellular aggregation and EPS production, and macrocolony (presenting as towers) formation due to further remodelling and maturation (FIG. 2). Finally, biofilm dispersal can occur when bacteria return to a planktonic lifestyle. In an alternative model of biofilm formation, an unexpected early dispersal stage occurs, during which a fraction of the bacteria returns to a planktonic state. This stage takes place ~6 hours after *S. aureus* seeding and precedes the formation of towers[61].

Polysaccharide intercellular adhesin. During biofilm formation and maturation, bacteria adhere to each other and produce EPSs to form the biofilm matrix. EPSs include exopolysaccharides, proteins, extracellular DNA (eDNA) and teichoic and lipoteichoic acids[62]. In *S. epidermidis* and *S. aureus*, polysaccharide intercellular adhesin (PIA) is the main polysaccharide of the biofilm matrix. The *icaADBC* locus is responsible for PIA production[63]. Among *S. epidermidis* isolates from orthopaedic implant infections, PIA producers exhibited higher resistance to antibiotics, mainly aminoglycosides[64], than non-producers. Moreover, *icaADBC*-negative strains are susceptible to a broad range of antibiotics[64]. In *S. epidermidis*, environmental stresses, such as high osmolarity, heat and ethanol, substantially increase PIA synthesis and biofilm formation[65]. The induction of PIA synthesis by sodium chloride depends on *rsbU*, which encodes an activator of the first gene of the *sigB* operon[66]. *sigB* is found in members of the Gram-positive genera *Bacillus*, *Listeria* and *Staphylococcus* and is important for rapid adaptation to and survival in stressful environments[67]. However, ethanol stress can induce PIA synthesis and biofilm formation independently of *rsbU*[66]. PIA production is increased during nutrient and iron limitation and when oxygen levels are low. When *S. aureus* experiences stressful conditions in biofilms, the rates of horizontal gene transfer and mutations increase and, thus, *S. aureus* acquires antibiotic resistance faster[68]. A theoretical model suggests that stress-induced genetic variation increases the emergence of antibiotic resistance[69].

Shear stress due to fluid flow is another environmental stressor that influences bacterial behaviour. Shear stress varies with the anatomical location and is high in blood vessels, cerebrospinal fluid shunts and intravascular devices. High shear stress increases the level of expression of PIA in *S. epidermids*[70]. *S. epidermidis* isolates from high-shear environments were more likely to produce PIA-containing biofilms than isolates from low-shear environments. Low-shear isolates have been collected from body fluids, cerebrospinal fluid, eyes, tissues and samples obtained during or immediately following removal of prosthetic devices, whereas high-shear isolates have been obtained from confirmed positive blood cultures from patients with concomitant positive catheter cultures[70]. This suggests that the PIA matrix also protects against shear flow and that matrix composition is adapted to shear stress.

S. epidermidis can switch expression and synthesis of PIA on and off reversibly through the insertion and excision of the insertion sequence IS*256* into and from *icaC*[71]. *S. epidermidis* isolates from biomaterial infections carried *icaADBC* and multiple copies of IS*256*, often in association with the Tn4001 transposon, and were resistant to multiple antibiotics[72].

Stress responses, dissemination of resistance by horizontal gene transfer and on-and-off switching of PIA production by insertions in the *icaADBC* locus or in its regulators all contribute to the adaptation and success of *S. epidermidis* on biomaterial surfaces.

Extracellular DNA. Among the molecular components of the biofilm matrix, eDNA stands out because of its particular versatility. On the basis of in vitro studies, eDNA appears to have four important roles: stabilization and strengthening of the biofilm matrix[73]; gene

Conjugation
A mechanism by which genetic material can transfer between bacterial cells. It involves direct cell-to-cell contact or a bridge-like connection between cells.

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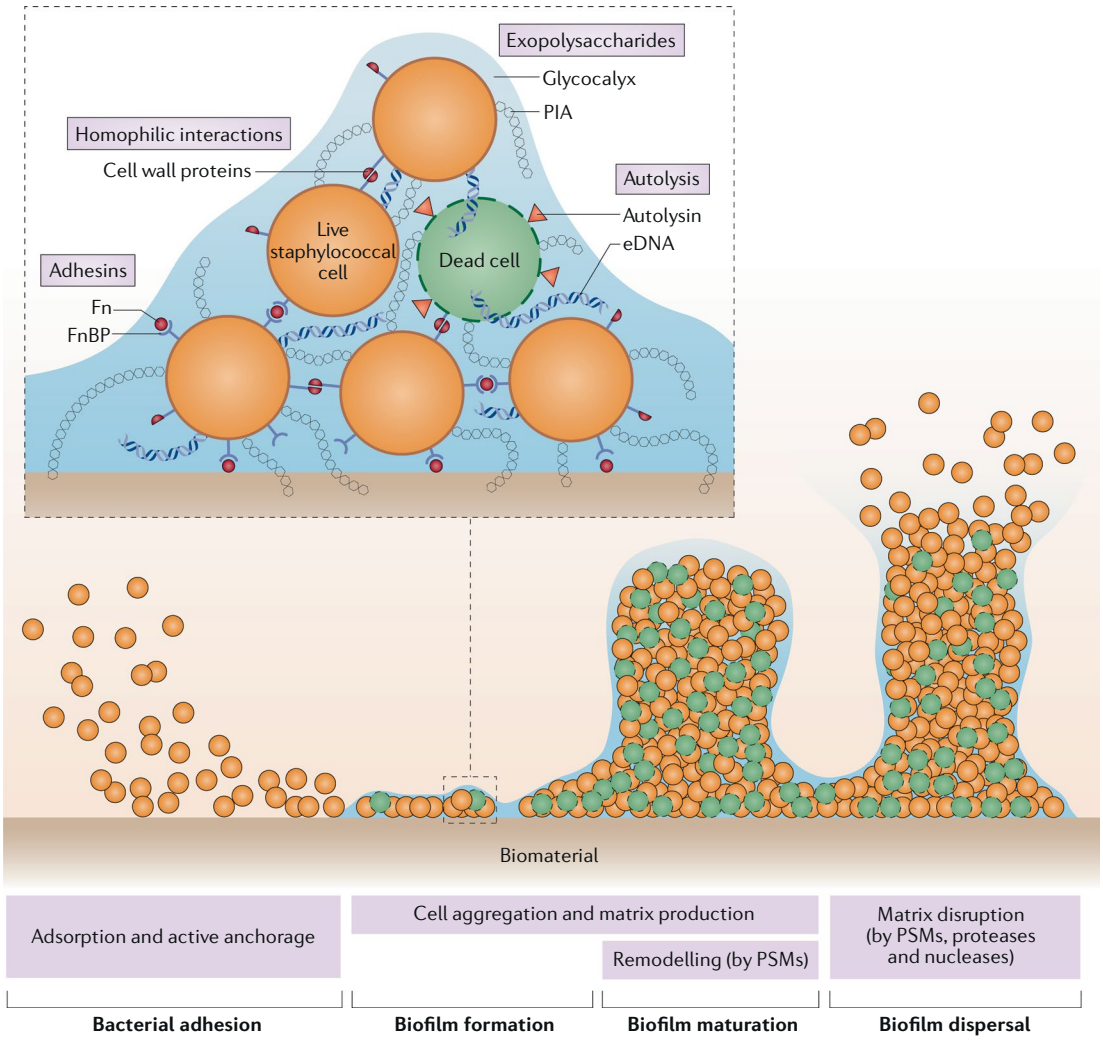

Fig. 2 | **Stages of staphylococcal biofilm formation.** Stable anchorage of bacteria is generally followed by the formation of a biofilm. Intercellular interactions mediated by adhesins and cell wall proteins lead bacteria to cluster together, forming microcolonies. For example, in *Staphylococcus aureus*, fibronectin-binding proteins (FnBPs) bind to fibronectin (Fn) molecules, forming a bridge, and this interaction promotes bacterial aggregation. The production of extracellular polymeric substances is part of the biofilm maturation process, in which the biofilm matrix progressively builds up and larger bacterial aggregates called towers develop. In *S. aureus* and *Staphylococcus epidermidis*, the mechanisms involved in biofilm formation include the expression of the polysaccharide intercellular adhesin (PIA) and the release of extracellular DNA (eDNA) derived from bacterial autolysis and from dead host cells. In *S. epidermidis*, the β-subclass of phenol-soluble modulins (PSMs) contributes to biofilm structuring and leads to the formation of characteristic water channels[95], which are observed in mature biofilms. In *S. aureus* and *S. epidermidis*, PSMs are also involved in biofilm dispersal, together with proteases and nucleases.

transfer between cells[74]; modulation of the innate immune response[75]; and supply of nutrients[76]. Biofilm cells actively release eDNA[77], which contributes to biofilm stability and might be a target for future diagnostics and therapeutics. Interestingly, eDNA production, as observed by confocal laser scanning microscopy, was higher in clinical isolates of *S. aureus* and *S. epidermidis* from infected implants than in controls after 6 hours of biofilm formation[78].

In vitro studies indicate that bacteria produce eDNA either through altruistic suicide or fratricide killing[73,79]. *S. aureus* biofilm cells can be divided into altruists and survivors, the former of which commit suicide by programmed cell death for the sake of the community[80]. By contrast, *E. faecalis* biofilm cells differentiate into attackers and targets. Attackers release killing factors that kill target cells, but they themselves are protected by specific immunity proteins[80].

Teichoic acids. Staphylococci, like most Gram-positive bacteria, contain teichoic acids in their cell envelopes. Teichoic acids are either covalently linked to peptidoglycan as wall teichoic acid or to the cytoplasmic membrane as lipoteichoic acid[81]. Teichoic acids have a strong effect on bacterial adherence to biomaterials and on biofilm formation in *S. aureus* and *E. faecalis*[82,83]. Teichoic acids are an essential part of the *S. epidermidis* cell wall and have an important role in bacterial adhesion. Wall teichoic acids increase the adhesion of *S. epidermidis* to medical devices by binding to adsorbed fibronectin[84].

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Box 3 | **Tissue response to implants**

Permanent implants support the persistence of bacteria[32]. By contrast, resolution of infections can occur when implants are made of degradable materials. Indeed, as observed in a murine model, infections can be cleared once the implants are totally resorbed[173]. The susceptibility of surgically invasive devices to bacterial colonization is due to reduced effectiveness of human immune defences at the implant–tissue interface. For example, microroughness and microporosity on the biomaterial surface provide niches that are too small to be accessible to the large leukocytes but can be easily inhabited by bacteria. In addition to physical exclusion of professional phagocytes, biomaterial surfaces interact with extracellular matrix proteins, such as collagen, fibronectin and elastin; with immune molecules, such as immunoglobulins and components of the complement system; and with proteins of the coagulation cascade and the fibrinolytic systems[174–176].

Protein adsorption on biomaterial surfaces is followed by the Vroman effect (that is, the competitive exchange of adsorbing and desorbing proteins), which changes the composition of the proteinaceous coating of the biomaterial surface[177]. These initial humoral interactions influence the subsequent interaction with blood cells, such as platelets and leukocytes. Neutrophils are the first responders, and they precondition and prime[178] the biomaterial surfaces through the release of chemical factors and neutrophil extracellular traps (NETs), which are web-like structures that consist mainly of decondensed chromatin and antimicrobial factors[179]. Two phenotypes of neutrophil activation have been described to form in response to biomaterials, namely, the N1 phenotype, which is pro-inflammatory and characterized by increased expression of tumour necrosis factor (TNF), and the N2 phenotype, which is anti-inflammatory and produces more vascular endothelial growth factor (VEGF) and matrix metalloproteinase 9 (MMP9), which are important for angiogenesis[178]. Depending on the relative abundance of the two phenotypes, neutrophils can influence the immune response either towards inflammation or resolution. Neutrophils dominate and orchestrate the acute phase of the tissue response after implant surgery, whereas monocytes and macrophages are recruited in the later phase of tissue repair[178]. Over time, leukocytes become exhausted because the implant is a large foreign body that cannot be engulfed.

Regulation of biofilm formation. Biofilm formation depends on the interactions between bacterial cells, both in monospecies and multispecies biofilms, and these interactions are controlled by complex regulatory networks.

Biomaterial colonization leads to changes in gene expression in *S. epidermidis*: *atlE*, *aap*, the gene encoding accumulation associated protein (AAP), *agrBDCA* genes for the Agr quorum sensing system and *icaADBC* genes were upregulated, which indicates that these genes are important for foreign body colonization and potentially for the establishment of implant infections[85,86].

Signalling systems orchestrate gene expression and bacterial behaviour in response to external stimuli, such as stress and cell density. In staphylococci, the Agr quorum sensing system governs the expression of numerous virulence factors and toxins[87], and it is also involved in biofilm dispersal[88]. Another quorum sensing system, involving S-ribosylhomocysteine lyase (LuxS) and autoinducer 2 (AI-2), is also known to regulate biofilm formation, although the effects depend on the staphylococcal species and the environmental conditions[89,90].

Dispersal. Biofilm dispersal can lead to dissemination of the detaching bacteria[88], which can then reach the bloodstream and cause systemic infections[53]. Inhibition of matrix production, enzymatic degradation of EPSs and surfactant molecules all contribute to dispersal[91,92]. In staphylococci, biofilm disruption is characterized by the production of extracellular enzymes and phenol-soluble modulins (PSMs)[93], which are peptide toxins with surfactant-like properties. Several enzymes are secreted, including staphopain cysteine proteases, the V8 glutamyl endopeptidase SspA and staphylococcal nuclease. The relative importance of each enzyme depends on the strain-specific composition of the biofilm matrix[94].

PSMs have an important role in the dispersal phase, especially in implant-associated biofilm infections[95]. The Agr system regulates PSM production in a density-dependent manner. PSMs disrupt the non-covalent forces that strengthen the biofilm matrix, which promotes the formation of channels for the delivery of nutrients to deeper biofilm layers and contributes to the dispersal and dissemination of biofilm clusters to distal sites[95]. In *S. epidermidis*, PSMβ promoted biofilm structuring and detachment in vitro and dissemination from colonized catheters in a mouse model[96].

Several factors, including nitric oxide and cyclic di-GMP, control biofilm dispersal and expression of effector enzymes and surfactant molecules. The role of these different signals varies depending on the bacterial species[91,97].

Immune evasion

The host immune defences not only react to the bacteria that contaminate an implant, they also react to the biomaterial surface of the implant and recognize it as a foreign body. This reaction triggers an inflammatory response that involves the coagulation cascade, complement system, platelets and immune cells, particularly neutrophils[26] (BOX 3). Biomaterial-induced neutrophil activation leads to metabolic exhaustion and depletion of oxidative resources owing to continuous release of reactive oxygen species, which dramatically reduces the capacity of neutrophils to kill bacteria. Decreased bactericidal activity of neutrophils after the exposure to biomaterial surfaces has been documented both in vitro[98,99] and in vivo in guinea pigs[100], and it has been linked to severe biomaterial-related infections[26]. In addition to the exhaustion of immune responses due to the presence of the implant, the bacteria themselves use several strategies to evade host immunity, such as invasion of host cells, toxin production and skewing of the immune response.

Quorum sensing system
A bacterial regulatory system based on signalling molecules (autoinducers) that reflect bacterial cell-population density and regulate gene expression in response to it.

Surfactant molecules
Compounds that lower the surface tension between two liquids or between a liquid and a solid.

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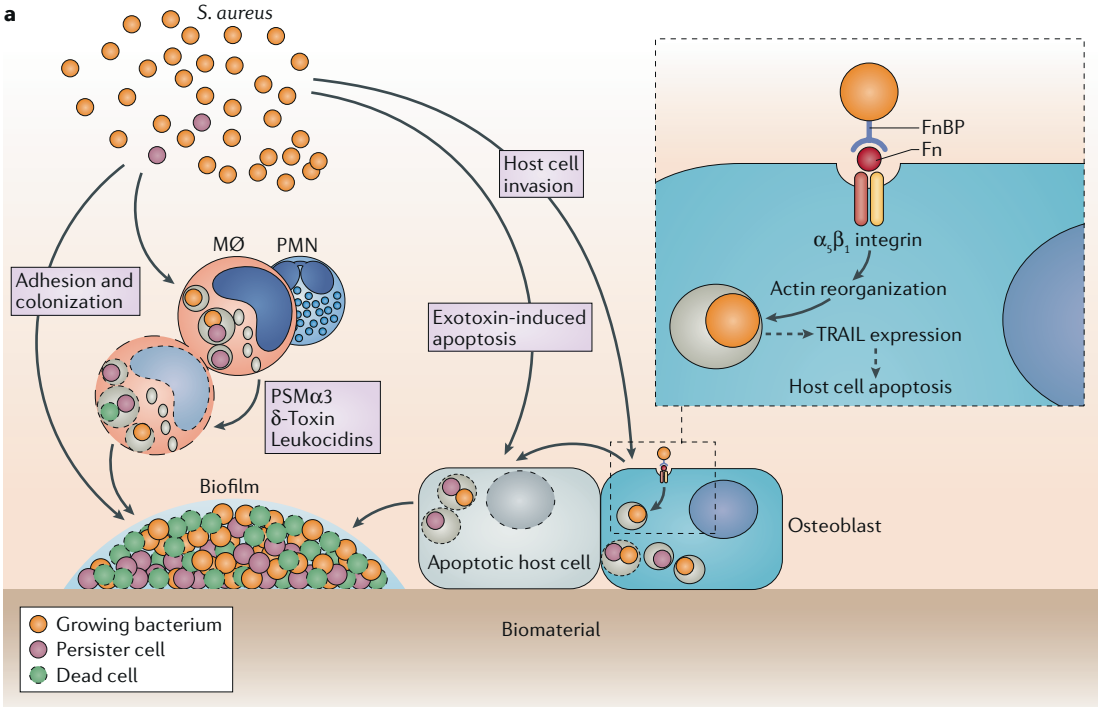
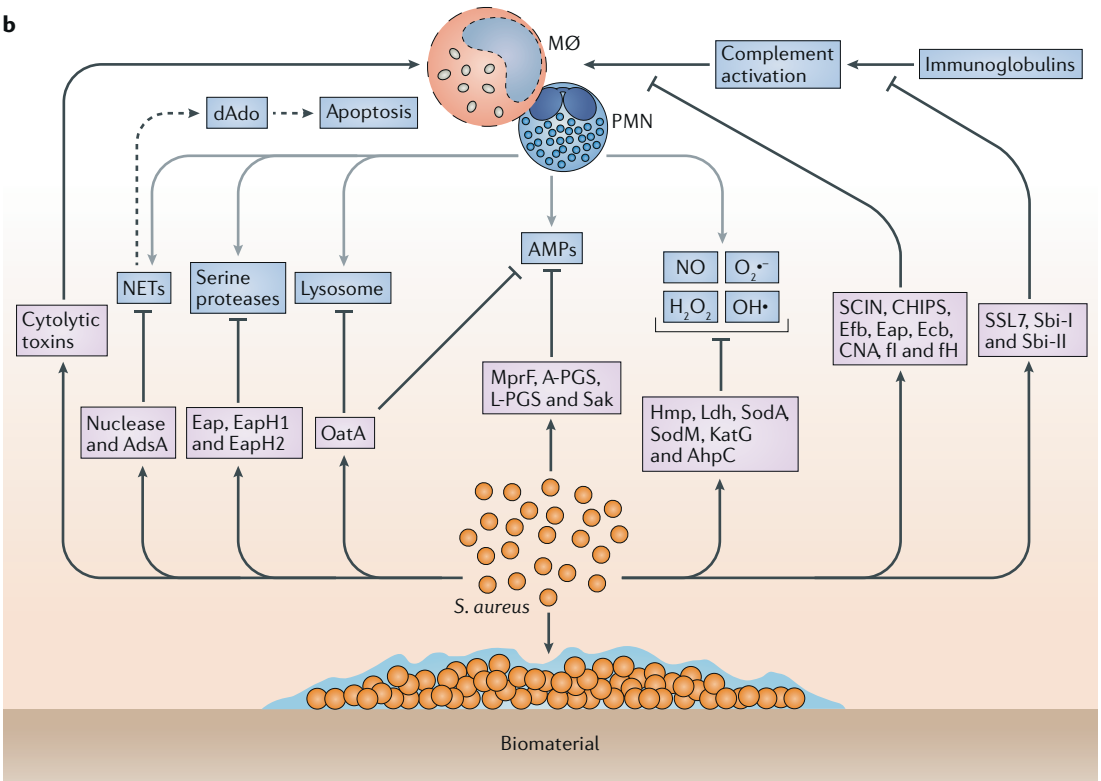
Non-professional phagocytes
A variety of non-immune cells that are capable of phagocytosis, including fibroblasts, osteoblasts, keratinocytes and endothelial cells.

Professional phagocytes
A group of cells that includes phagocytes of the innate immune system, such as neutrophils, monocytes, macrophages, mast cells and dendritic cells.

Invasion of host cells and bone. Bacteria can evade both antibiotics and host defences by hiding in host cells (FIG. 3a). Up to 8% of *S. aureus* cells invade non-professional phagocytes such as osteoblasts within 2 hours of exposure[101]. Inside the cells, *S. aureus* evades antibiotics that are inactive intracellularly and activated professional phagocytes; later, the bacteria can induce apoptosis of host cells and colonize the implant surfaces[102]. Internalization of *S. aureus* into osteoblasts is mediated by fibronectin, which forms a bridge between staphylococcal fibronectin-binding protein (FnBP) and $\alpha_5\beta_1$ integrin on osteoblasts[103]. This interaction upregulates tumour necrosis factor-related apoptosis-inducing ligand (TRAIL, also known as TNFSF10), which induces activation of caspase 8, osteoblast apoptosis and bone destruction[104].





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Small colony variant
Slow-growing variant of bacteria that arises spontaneously and forms small colonies when grown in the laboratory. These variants are implicated in persistent infections.

Canaliculi
Thin, hair-like channels in the bone that link the lacunae with one another and with the Haversian canal.

Lacunae
Small cavities within the bone matrix in which single osteocytes are lodged.

Osteoblast invasion by *S. aureus* also contributes to the pathogenesis of osteomyelitis, as evidenced by internalization of bacteria into skull and thigh bone osteoblasts in embryonic chicks[105]. Both *S. aureus*[101,106] and *Staphylococcus pseudintermedius*[106] invade osteoblasts, whereas clinical isolates of other staphylococcal species, such as *S. epidermidis*, *Staphylococcus lugdunensis*[101,106] and *E. faecalis*[101], show much lower invasion rates than *S. aureus*.

In chronic implant infections, internalized bacteria and a subset of bacteria in biofilm[107] can adopt a small colony variant-like phenotype, which is characterized by slow growth and low levels of cytotoxic factor secretion[107] and which enables the internalized bacteria to survive for long time periods[108,109].

Although *S. aureus* generally is an extracellular pathogen, there is growing evidence that internalization and persistence of *S. aureus* in host cells occur in vivo and have a role in osteomyelitis[110,111]. Indeed, when rat osteoblasts were infected ex vivo with *S. aureus*, the internalized bacteria could initiate infection of open fractures in vivo[112].

In addition to hiding in osteoblasts, *S. aureus* can also enter canaliculi of live cortical bone. In a murine model of osteomyelitis, *S. aureus* reached osteocyte lacunae through the canaliculi and formed biofilms in the lacunae[113]. Hiding in bone tissue contributes to the recalcitrance of orthopaedic implant infections to host defences and antibiotic treatment.

Species-specific strategies to evade host immunity. Different bacterial species and strains use different mechanisms to combat host immune defences. For example, *E. coli* strains isolated from orthopaedic implant infections show a higher resistance to complement than strains from patients who have bacteraemia but non-infected orthopaedic implants[114]. Complement resistance is associated with the synthesis of long-chain lipopolysaccharide[114], and it helps bacteria to survive in the blood and reach the implant, dampens the local immune response and promotes seeding to surrounding tissues.

S. aureus has an exceptional number of mechanisms and virulence factors to evade the host immune response[115,116] (FIG. 3b). *S. aureus* induces leukocyte lysis with toxins, including the γ-haemolysins HlgAB and HlgCB, leukocidin GH and Panton–Valentine leukocidin, and a number of PSMs[93]. Moreover, in a murine model, other toxins, such as α-toxin and leukocidin AB, were involved in the macrophage dysfunction induced by *S. aureus* biofilms[117]. These toxins inhibited macrophage phagocytosis and caused macrophage death.

S. epidermidis has fewer mechanisms to evade immune defences, and its pathogenicity relies mainly on biofilm formation[118]. As a mostly opportunistic pathogen, *S. epidermidis* causes double the number of orthopaedic implant infections compared with infections in the absence of orthopaedic implants[4]. *S. epidermidis* has minimal cytolytic properties, although it encodes similar PSMs as *S. aureus*[93]; however, they are expressed at very low levels[119].

Biofilm formation as an immune evasion mechanism. Bacterial resistance to leukocytes in biofilms was at first explained by a lack of penetration of leukocytes into biofilms and a decreased ability of phagocytes to kill biofilm-encased bacteria. A mature biofilm has a dense polymeric matrix that is difficult to engulf by macrophages. This results in 'frustrated phagocytosis' (REF.[75]), a concept that was first introduced to describe the response of phagocytes to asbestos fibres[120]. However, in vitro studies have produced contrasting results, suggesting that human leukocytes effectively penetrate biofilms[121] and that biofilms are not inherently protected from phagocytic cells[122].

Mouse studies have shown that staphylococcal biofilms can skew the host innate immune response towards an anti-inflammatory, pro-fibrotic response instead of a pro-inflammatory, bactericidal response[75,122]. The biofilms change macrophage polarization from the classic pro-inflammatory towards the anti-inflammatory phenotype[123,124], the latter phenotype being characterized by the production of anti-inflammatory mediators[125,126]. In murine and human implant infections, interleukin-12 (IL-12) promotes the recruitment of myeloid suppressor cells, which contribute to the anti-inflammatory biofilm milieu through their powerful immunosuppressive activity, impairing phagocyte influx and biofilm clearance[127].

Diagnosis, prevention and therapy

The challenge of diagnosing biofilm-associated implant infections. Biofilm infections are not only difficult to treat but also difficult to diagnose owing to the difficult removal of mature biofilms from implant surfaces and the reduced growth of dormant bacteria in biofilms. Sonication of the extracted implant can help. In a recent prospective clinical study, sonication fluid culture

◀ Fig. 3 | **Immune evasion by *Staphylococcus aureus*. a** | During implant infections, bacteria such as *Staphylococcus aureus* can escape the immune response, phagocytosis by professional phagocytes and antibiotic treatment by invading non-professional phagocytes such as osteoblasts. In *S. aureus*, the mechanism of internalization involves binding of fibronectin (Fn) to fibronectin-binding proteins (FnBPs) on the bacterial surface and to $\alpha_5\beta_1$ integrin on the surface of osteoblasts[103]. An FnBP–Fn–$\alpha_5\beta_1$ bridge forms that triggers the active uptake of bacteria by osteoblasts. Within phagosomes, *S. aureus* can cause osteoblast apoptosis by inducing the expression of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) and the consequent activation of caspase 8 (REF.[104]). **b** | The niche created by the implant is an easy place to conquer for pathogens such as *S. aureus*, which is equipped with a broad range of virulence factors and immune evasion strategies. The defence arsenal of this pathogen includes factors that prevent complement activation, opsonization and consequent leukocyte activation and chemotaxis; leukocidal cytolytic toxins; factors that shelter the bacterium from the many bactericidal substances produced by leukocytes, including reactive oxygen species (H_2O_2, $O_2^{\bullet-}$ and $OH^{\bullet}$), enzymes and antimicrobial peptides (AMPs); and factors that disassemble neutrophil extracellular traps (NETs), resulting in the production of pro-apoptotic molecules. AdsA, adenosine synthase A; AhpC, alkyl hydroperoxide reductase; A-PGS, alanyl-phosphatidylglycerol synthase; CHIPS, chemotaxis inhibitory protein of *S. aureus*; CNA, collagen-binding adhesin; dAdo, 2′-deoxyadenosine; Eap, extracellular adherence protein; EapH, extracellular adherence protein homologue; Ecb, extracellular complement-binding protein; Efb, extracellular fibrinogen-binding protein; fH, complement factor H; fI, complement factor I; Hmp, flavohaemoglobin; KatG, catalase; Ldh, L-lactate dehydrogenase; L-PGS, lysyl-phosphatidylglycerol synthase; MØ, macrophage; MprF, multiple peptide resistance factor; OatA, *O*-acetyl transferase; PMN, polymorphonuclear neutrophil; PSMα3, phenol-soluble modulin α3; Sak, staphylokinase; Sbi, second immunoglobulin-binding protein; SCIN, staphylococcal complement inhibitor; Sod, superoxide dismutase; SSL7, staphylococcal superantigen-like protein 7.

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was the most sensitive individual diagnostic method of delayed and late infections compared to peri-implant tissue culture, synovial culture and histology[128].

Traditional culture methods depend on the ability of bacteria to grow in a defined culture medium. Molecular techniques are changing our view from the classical one pathogen, isolated during acute infection and grown in the laboratory, to a more nuanced view that includes non-culturable organisms and polymicrobial and chronic infections[129]. A series of molecular methods has been developed to provide rapid, culture-independent diagnostics, including the Ibis PLEX-ID technology[130], MALDI-TOF mass spectroscopy[131], next-generation sequencing[132] and other technologies[133].

These technologies have already become indispensable tools for research on bacterial phylogenetics and taxonomy and for epidemiological surveillance. Despite the many advantages, the routine use of these techniques in the management of patients faces critical obstacles, such as high cost and insufficient validation[134,135], and, thus, culture-independent methods are not generally adopted in the clinic. In clinical diagnosis, conventional swab and broth cultures with their known limitations are still used. In addition, measuring α-defensin in synovial fluid can help in the clinical diagnosis of prosthetic joint infections[136].

Preventive strategies. Risk factors for surgical site infections and the regimen for their control have been scrutinized, especially with regard to MRSA infections[137]. For example, preoperative anaemia increases the incidence of prosthetic joint infections to 4.3% from the 2.1% observed in patients without anaemia[138].

Surgical site infections in major cardiac and orthopaedic surgeries can be effectively reduced with preoperative patient 'care bundle' approach[139]. Care bundles are a group of best evidence-based interventions that, when administered together, give maximum outcome benefit. An example of a simple care bundle is by decolonization of nasal and extranasal sites in nasal carriers of *S. aureus*[140]. Prophylaxis with amoxicillin and clavulanic acid decreases the proportion of people developing MRSA infections[141,142]. Anti-infective biomaterials currently represent a main preventive strategy[143,144].

Biomaterial-based preventive strategies. A way to interfere with microbial adhesion is modifying the microtopology and nanotopology of the implant surface. Coating the implant with surfactants or hydrophilic polymer brush systems can generate antifouling, adhesion-resistant or even bacteria-repellent surfaces[14,60]. In orthopaedics, in addition to resisting bacterial adhesion, ideal biomaterials should promote rapid tissue integration and the fast adhesion of host cells[145,146]. Surface nanotopographies, which are inspired by nature, can confer superhydrophobicity, antifouling and bacteria-repelling properties and bactericidal activity[147,148] (see REF.[143] for an in-depth discussion of anti-infective biomaterials). Some anti-infective biomaterials are at an advanced stage of development or have entered clinical use (for example, biomaterials endowed with antimicrobial and antibiotic substances, bactericidal and adhesion-resistant coatings, and intrinsically antimicrobial biomaterials), whereas others are in preclinical development (for example, nanomaterials, systems targeting biofilm physiology and immune-modulatory systems).

Conventional therapeutic approaches. Conservative strategies based on debridement and implant retention can be considered only for prosthetic joint infections that are diagnosed during the first month after implantation. In late prosthetic joint infections, the options of treatment are two-stage implant exchange, one-stage implant exchange, permanent resection arthroplasty (for example, when a previous two-stage procedure failed or when risk of relapse is unacceptable) and amputation[13]. Revision surgery of chronic infections can be made in one or two stages. Two-stage exchange consists of debridement of all non-viable tissues, resection of the infected implant with or without placement of a temporary antibiotic-impregnated cement spacer and delayed reimplantation of a new prosthesis in a separate surgery, after infection has been eradicated. In one-stage exchanges, prosthetic reimplantation occurs in the same surgical procedure[149].

Rifampicin is an important antibiotic drug for the treatment of staphylococcal prosthetic joint infections, as it is effective against staphylococcal biofilms. Rifampicin diffuses well within bone tissue, bacterial biofilms and host cells and, as it inhibits DNA-dependent RNA synthesis independently of bacterial metabolic activity and growth, it is efficacious even against difficult-to-treat dormant bacteria[150]. Owing to the high risk of emergence of resistance[151], rifampicin should be used in combination with other antimicrobials[12,152].

Innovative antimicrobials. Antimicrobial peptides (AMPs) are derived from defence peptides with anti-infective and/or immunomodulatory activity[153]. Fusaricidin (also known as LI-F), a class of cyclic lipopeptide antibiotics, is active against ESKAPE pathogens[154], inhibits biofilm formation in vitro and in vivo, and eradicates mature biofilms of *P. aeruginosa* and *S. aureus*[155].

Antisense oligomers for antimicrobial therapy are designed to silence specific genes. An interesting class of antisense molecules are peptide nucleic acids (PNAs)[156]. In vitro, hybrid-conjugated AMP–PNA molecules efficiently delivered an antibacterial PNA targeting the essential *acpP* gene, which encodes the acyl carrier protein, into *E. coli*[157]. PNA-peptide conjugates targeting the *rpoD* gene were active against Gram-negative bacteria even in vivo[158].

Conclusions

Implant infections are a serious clinical problem. As mentioned above, the implanted material compromises host defences and provides a foothold for bacteria, which have an armamentarium of mechanisms to be successful in this niche, including biofilm formation, persister cell formation, immune evasion, osteoblast invasion and antimicrobial resistance. Therefore, prevention of implant infections is crucial, and it starts with the knowledge of the multiple risk factors that favour their onset and spans the preoperative, intraoperative or postoperative periods[159].

ESKAPE pathogens
Acronym for a group of pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species) that are some of the main species that cause antimicrobial-resistant infections.

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Because antibiotics at sublethal concentrations stimulate the formation of persister cells[160] (BOX 2), care should be taken to give antibiotics at adequate concentrations. Furthermore, invasion of host cells contributes to the failure of antimicrobial therapy, as conventional extracellular antibiotics often do not reach the intracellular bacteria at sufficient concentrations. The near impermeability of host cells to common antibiotics causes inadequate bacterial clearance, resulting in unresolved infections. Novel strategies that will improve drug penetration into host cells and that will be active in dormant bacteria are under study. Cell-penetrating cationic polymers with antibacterial activity against intracellular *S. aureus* appear promising[161]. The search for innovative therapeutic strategies and for anti-infective implant biomaterials may inspire well-founded hopes to overcome the severe infective complications associated with implant surgery.

Published online 2 May 2018

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Acknowledgements
The authors gratefully acknowledge M. P. Landini for the inspiring atmosphere at the Rizzoli Orthopaedic Institute. This work was supported by the POR-FESR 2014–2020 grant (Project No. 725827) and by the "5 per mille" grants to the Rizzoli Orthopaedic Institute.

Author contributions
All authors researched data for the article, contributed substantially to discussion of the content and wrote, reviewed and edited the manuscript before submission.

Competing interests
The authors declare no competing interests.

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