Bingyun Li · Thomas Fintan Moriarty
Thomas Webster · Malcolm Xing *Editors*

Racing for the Surface

Pathogenesis of Implant Infection and Advanced Antimicrobial Strategies

Springer

Racing for the Surface

Bingyun Li • Thomas Fintan Moriarty
Thomas Webster • Malcolm Xing
Editors

Racing for the Surface

Pathogenesis of Implant Infection and Advanced Antimicrobial Strategies

Springer

Editors
Bingyun Li
Department of Orthopaedics
West Virginia University
Morgantown, WV, USA

Thomas Webster
Department of Chemical Engineering
Northeastern University
Boston, MA, USA

Thomas Fintan Moriarty
Microbiology Lab
AO Research Institute Davos
Davos Platz, GR, Switzerland

Malcolm Xing
Engineering and Medicine
University of Manitoba
Winnipeg, MB, Canada

ISBN 978-3-030-34474-0 ISBN 978-3-030-34475-7 (eBook)
https://doi.org/10.1007/978-3-030-34475-7

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Foreword

With the implantation of new devices and novel technologies to mitigate pain and improve function, unforeseen, sometimes serious complications are currently being observed that may limit the lifetime of the implant. In orthopedic surgery, chief amongst these adverse events is implant infection. Whether the implant is a permanent prosthesis (such as in joint replacement or a spinal device), or a biodegradable polymeric device (e.g., for fracture fixation or reconstruction of deficient ligaments), acute and chronic infection can occur. Infection of an implant induces local inflammation, subsequent pain and poor function, and may necessitate removal of the implant, aggressive debridement of the local tissues, and prolonged antibiotic treatment. Infections of orthopedic implants are a substantial burden to society and the healthcare system and a devastating financial and psychosocial event in the lives of patients and their families.

Are orthopedic implant infections common? In joint replacement surgery of the lower extremity, periprosthetic infection is the leading cause of failure of total knee replacements and is amongst the top three reasons for failure of total hip replacements. Given the fact that joint replacement of the hip and knee total more than one million cases per year in the USA alone, and the infection rate varies from about 0.5 to 10% or more depending on the complexity of the case, implant infection is a potential scenario that cannot be ignored. Substantial clinical and basic research has been performed to identify the causative organisms and provide antiseptics and antibiotics to accompany radical surgical debridement, and if necessary, implant removal. However, these secondary measures do not address the major challenge in this area, namely how to prevent the occurrence of infection primarily and in the longer term. These issues have great relevance to clinical practice. The population is aging and individuals want to remain active at all ages including in their later years of life. At the same time, the immune system ages, making the elderly more susceptible to infection, compared to younger individuals. Furthermore, advances in the treatment of chronic diseases such as diabetes, cancer, and others are enabling individuals to enjoy an extended life span despite a compromised and aging immune system. The possibility of late infection of an implant looms over these individuals because of their suboptimal immune status.

Thus, there are major challenges that present themselves to orthopedic clinicians, researchers, device manufacturers, government regulators, insurance companies, and other related parties. These challenges pertain to the selection and optimization of patients who will be receiving orthopedic implants, the appropriate use of peri-operative antibiotic prophylaxis, implant choice and surgical technique, and postoperative care to improve patient outcomes. Surgical devices need to have an established track record of safety, efficacy, and cost-effectiveness, and be relatively straightforward for the surgeon to implant. Recent studies have demonstrated that the properties of the device including its composition, shape, topography, and surface properties, as well as the addition of specialized treatments and coatings may have a major impact on the development of implant infection. In this regard, the compendium of chapters in this book on current developments in the field of orthopedic implant infection is both timely and indispensable to the broadening of our knowledge base on a subject that continues to plague orthopedic surgeons and patients alike.

June 2, 2019

Stuart Goodman
Mary Ellenburg Professor of Surgery
Professor of Orthopaedic Surgery
Stanford University
Stanford, California

Preface

Medical Device Infections: Is Anyone Paying Attention?

Infection is a problem that has always (and consistently) been an issue for medical devices, and understandably so. Whenever a medical device is surgically inserted into the body, an open wound to the environment creates an ideal opportunity for numerous and diverse microorganisms to penetrate and grow uncontrollably on exposed tissue and on an implant. While your tissues have the ability to at least partially fight such bacteria presence, today's orthopedic implants do not. As if this was not cause for alarm alone, consider that no matter how sterile and clean a surgical room is, the *patient* carries a significant number of bacteria with them to surgery—it is on our *own* skin.

From the start, it appears to be a losing battle to keep bacteria from infecting surgical wounds and colonizing implants. But at one time, there *was* hope: antibiotics. Please make no mistake about it. Antibiotics have significantly helped patients and reduced or eliminated infection in millions of patients worldwide. But times have changed. As is now well documented and agreed upon in the medical community, bacteria can quickly mutate around antibiotics to render them useless. Antibiotics are not the solution anymore, and this has led to startling statistics.

As just one example, the U.S. Centers for Disease Control has predicted more deaths from antibiotic-resistant bacteria than all cancers combined by 2050. Think about this. If this doesn't get your attention, I am not sure anything will. More deaths from bacteria than *all* cancers combined. Bacteria infecting medical devices that we have no antibiotics to kill. Antibiotic-resistant bacteria seemingly transferring from livestock to humans to infect them with no treatment. And, the problems go on. The problems with antibiotic-resistant bacteria are enormous and, yes, *we* created it.

But who is paying attention? Certainly, when examining resources being used to fight cancer versus fighting infection, cancer wins out. And trust me, it should not be a competition between which diseases to fight, but it is clear that developing solutions to reverse the growing problem of antibiotic-resistant bacteria is not

receiving the resources needed. Where are our federal agencies with the funding needed to reverse this alarming problem? Where is big Pharma, who it seems has all but given up by closing their antibiotic divisions? FDA approval of new antibiotics has plummeted with no clear change in strategy apparent. Are medical device statistics even accurate, considering healthcare reimbursement strategies and the negative connotations that come with an orthopedic clinical practice with device infections? How do we develop strategies to reverse medical device infections if we do not even have accurate infection statistics? Where are the solutions, and even before that, where is the conversation?

It is for these reasons (and more) that we introduce this book centered on novel solutions to fight orthopedic medical device infections. We chose orthopedic medical devices to emphasize since it is the largest medical device market with significant patient complications due to infection. We commend the authors who wrote chapters for this book as they are the ones offering novel solutions to kill or inhibit bacteria growth on implants at a time that is increasingly difficult to get funding, receive regulatory approval, and ultimately commercialize such strategies. In this book, you will see chapters on nanotechnology, carbon nanotubes, novel antibacterial metals, chiral stereochemical strategies, obscure elements on the period table like tellurium, new polymers, bioinspired surfaces, new chemical compounds, polysaccharides, new coatings, hydrogels, smart surfaces, and so much more to combat medical device infection. This book is not short on ideas which we are sure will motivate you to even develop your own.

So who is paying attention? I can tell you at least some people who are: we are, the researchers in each of these chapters. And while all of these ideas may not be the ultimate solution for reversing the growing health crisis around antibiotic-resistant bacteria, they are *ideas* that are needed *now*! These researchers are starting the conversation and keeping momentum going. They are paying attention and so should you.

Morgantown, WV, USA — Bingyun Li
Davos, GR, Switzerland — Thomas Fintan Moriarty
Boston, MA, USA — Thomas Webster
Winnipeg, MB, Canada — Malcolm Xing

Contents

Part I
Clinical Significance of Infection

When the Race Is Lost: The Clinical Impact of Prosthetic Joint Infections

Justin Vaida and Matthew J. Dietz

Abstract Joint arthroplasty, a procedure that can relieve patients of life-altering and debilitating pain, has proven to be so successful that over 1,000,000 procedures are performed annually in the USA alone. However, a prosthetic joint infection represents a devastating complication for patients which can lead to not only revision surgery but possible permanent loss of function, amputation, and even death. Infection can present not only in the immediate postoperative period but at any point for the duration of the implant's life.

The challenges confronting providers are numerous. Diagnostic testing has varying sensitivities and specificities depending on duration of infection meaning there is no true "gold standard" for testing. Additionally, once the diagnosis of infection is made, treatment options are limited and have high rates of morbidity and mortality.

Given the impending rise in total joint arthroplasty case volume and subsequent revision case volume due to PJI, an urgent need exists for continued work in the development of preventive, diagnostic, and therapeutic tools.

Keywords Joint arthroplasty · Infection · Biofilm · PJI · TJA · Diagnosis · Treatment

Epidemiology/Incidence

The goal of total joint arthroplasty (TJA) is to relieve a patient of debilitating joint pain by replacing the biological joint surface with prosthetic implants. This surgery is most often performed in the setting of severe osteoarthritis although rheumatoid arthritis and osteonecrosis of the hip are other common indications. A patient becomes an appropriate surgical candidate only after conservative measures such as

J. Vaida (✉) · M. J. Dietz
Department of Orthopaedics, West Virginia University, Morgantown, WV, USA
e-mail: Justin.vaida@hsc.wvu.edu

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_1

anti-inflammatory medications, physical therapy, and various injections have been exhausted and failed. TJA has proved to be one of the most successful surgeries performed, with consistently high patient satisfaction scores [1, 2]. The success of the procedure has led to more than 370,000 primary total hip arthroplasties (THA) and 680,000 primary total knee arthroplasties (TKA) performed in 2014 in the USA alone [3].

Periprosthetic joint infection (PJI) is one of the most feared and devastating complications of TJA due to clinical challenges in diagnosis and treatment and the extreme financial, physical, and emotional costs to the patient [4]. Despite evolving preoperative and intraoperative regimens to reduce infection risk, estimates of PJI incidence rates for both primary hip and knee arthroplasties range from approximately 0.5–2.0%, with a slightly higher rate of PJI in TKA compared to THA [5–8]. More troubling is that failure rates after the first line of treatment for PJI often exceed 25% with an increasing failure rate with repeated subsequent revision procedures. With an average cost of $116,383 for an infected total hip arthroplasty and $88,623 for an infected total knee arthroplasty, a substantial burden is placed on the healthcare system [9, 10]. Additional costs are assumed by the patient in extended rehabilitation, prolonged hospital stays, and emotional costs [11, 12]. Ultimately, PJI can lead to not only revision surgery but also possible is permanent loss of function, amputation (Fig. 1), and even death, with the 1-year mortality of PJI in THA at 7% between stages of a two-stage revision and 33% within 5 years of completion of revision [13, 14].

Among the many challenges facing clinicians are shifting definitions of PJI, myriad diagnostic tests that, while helpful in aggregate, lack 100% accuracy, treatment regimens that have unacceptably high failure rates, and a spectrum of disease representing a moving and evolving target. With a projected 1.9 million combined primary total hip and knee arthroplasties expected to be performed annually by 2030 in the USA alone [3], there is an expected proportional increase in the infection burden. Moreover, some research has indicated this projection may prove too conservative and a more dramatically exponential increase in the TJA caseload may be borne out [15]. Therefore, it is imperative that clinicians and basic scientists continue research into preventing, diagnosing, and treating this devastating complication.

Overview of Challenges

The diagnosis and treatment of PJI depends largely on two factors: the time since index surgery and the duration of a patient's signs and symptoms. While in reality the infectious process occurs on a continuum and does not adhere to discrete intervals, the sensitivity and specificity of various diagnostic tests as well as treatment algorithms have historically been categorized by these two crucial factors. Because successful eradication of infection depends in part on the identification of where a patient falls on this spectrum, several categorizations have been proposed [16–19].

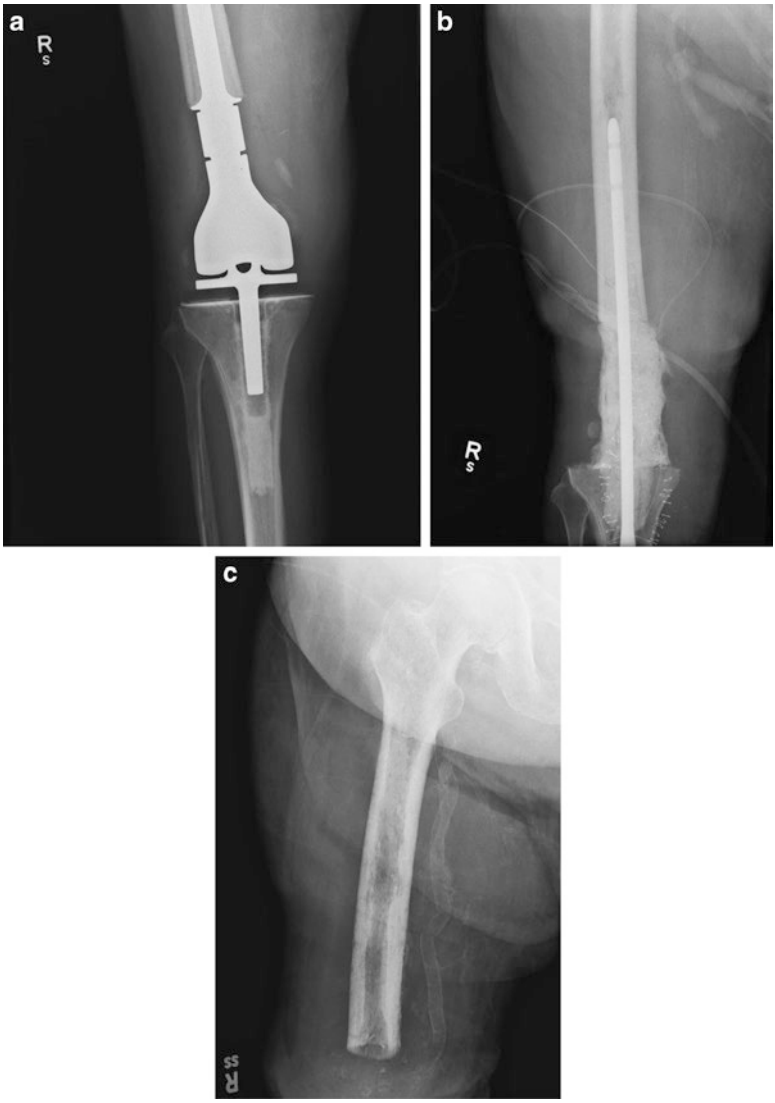


Fig. 1 (**a**–**c**) Progression in a single patient from infected revision total knee arthroplasty to knee fusion and ultimately to amputation due to recurrent infections. This patient eventually suffered multiorgan system failure and succumbed to his infection

For diagnostic and therapeutic purposes, PJI has been delineated into three broad categories [20]:

- Early postoperative infection: within 4 weeks of index surgery.
- Acute hematogenous infection (AHI): less than 3 weeks of symptoms. These infections typically cause an abrupt onset of symptoms that progress rapidly in severity.

- Chronic (late) hematogenous infection (CHI): seeded from a remote source and can be present at any point in the life of the patient after TJA, from months to years [21, 22]. Symptoms develop more gradually.

Part of the rationale for temporal categorization is directly related to one of the unique challenges inherent in PJIs: the ability of infective bacteria to form a "biofilm"—a metabolically cooperative colony surrounded by an extracellular glycocalyx (Fig. 2). Unfortunately, the same synthetic joint surface of the prosthesis which so successfully relieves pain in a patient makes the formation of this biofilm easier. Bacteria can adhere to prosthetic surfaces and resist mechanical disturbance making biofilm eradication especially challenging and often necessitates complete implant removal. Further, the glycocalyx protects bacteria not only from antibiotics and host antibodies but also detection from diagnostic testing. Biofilms have been found in samples from confirmed PJI cases in which preoperative cultures were negative [23]. It has been postulated that PJIs exhibit alternating periods of quiescent growth and acute exacerbations caused by the release of bacteria [24]. In the quiescent phases, few if any symptoms may be present, while during acute phases, symptoms may be limited locally to the affected joint or systemically manifesting as a fever, malaise, or frank septicemia. It is imperative that when a clinician suspects PJI, the patient is treated as soon as possible as a mature biofilm may be less likely to develop into acute PJI and implants may possibly be retained.

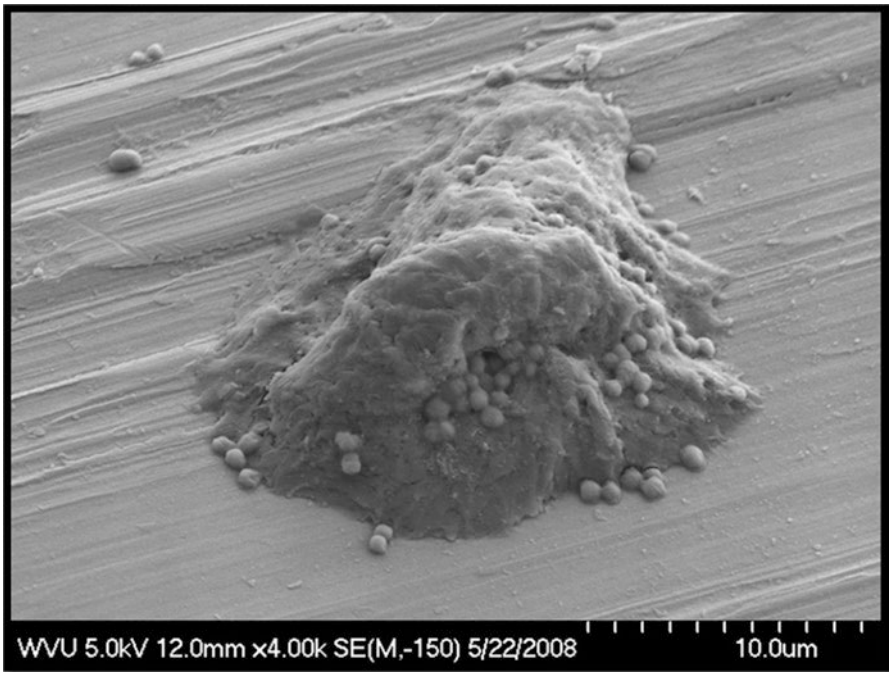


Fig. 2 Scanning electron microscopy image of *Staphylococcus aureus* biofilm adherent to a stainless steel disc

It should be noted that this categorization is more to help in the development of treatment algorithms and research purposes than based on a demonstrated bacterial threshold as studies have shown biofilm formation within mere hours of joint inoculation [25]. Therefore, the consideration of infection as a spectrum rather than a distinct acute/chronic dichotomy is likely more accurate.

Host Risk Factors

Prevention remains the best step in getting a head start in the race to the surface and several risk factors have been identified which aid clinicians in identifying patients predisposed towards PJI (Table 1). These include characteristics considered both modifiable, such as elevated body mass index, poorly controlled diabetes, tobacco

Table 1 Host risk factors for PJI/SSI in TJA [122]

Host risk factors for PJI/SSI in TJA	
Modifiable	Nonmodifiable
Active infection	Age
Alcoholism	ASA score > 2
Cardiovascular disease	Bariatric surgery
• Congestive heart failure	
• Cardiac arrhythmia	
Chronic kidney disease	Chronic anticoagulation
Chronic obstructive pulmonary disease	Hemiplegia/paraplegia
Clotting disorders	HBV
Depression	Osteonecrosis
Diabetes mellitus	Previous joint infection
• HbA1c	
• Serum glucose	
Drug abuse	Previous joint surgery
Frailty	Previous infection
HIV/AIDS	Sex
Immunosuppression	Transplant
Intra-articular steroid/viscosupplement injection	
Malnutrition	
MRSA colonization	
Obesity	
Peripheral vascular disease	
Psychosis	
Renal disease	
Rheumatoid arthritis	

Outlines the modifiable and nonmodifiable risk factors known to impact the risk for prosthetic joint infection as described by Cizmic et al.
HbA1c hemoglobin A1c, *HIV* human immunodeficiency virus, *AIDS* acquired immunodeficiency syndrome, *MRSA* Methicillin-resistant *Staphylococcus aureus*, *ASA* American Society of Anesthesiologists, *HBV* hepatitis B

use, alcohol consumption, and immunosuppression, as well as nonmodifiable, such as previous joint surgery and previous PJI [26, 27].

Body Mass Index (BMI)

BMI is recognized as a major risk factor with postoperative infection rates 6.7 times higher after TKA and 4.2 times higher after THA in patients with BMI $\geq$ 35 kg/m^2 compared to nonobese patients [28]. This risk elevates incrementally with each point increase in BMI > 25 kg/m^2 (hazard ratio of 1.09 per unit) [29, 30].

Diabetes

For diabetic patients, a hemoglobin A1c level of 7.5 has been shown to almost triple the risk of PJI in TKA when compared to those below this threshold [31]. Studies have demonstrated PJI rates in both TKA and THA rising from 0.8 to 5.4% with an A1c of 7.7 or higher compared to an A1c $\leq$ 7.6 [32].

Lifestyle Factors

Several other lifestyle factors have demonstrated an elevated risk of PJI. For instance, current tobacco users have more than double the risk of PJI than nonsmokers (OR 2.16 [1.57–2.97]) and this risk persists even after smoking cessation (OR 1.52 [1.16–1.99]) [33]. Alcohol consumption in the perioperative period has been shown to increase the risk of PJI and alcohol cessation is recommended at least 4 weeks prior to surgery to reduce postoperative morbidity [34–36]. Several of these lifestyle factors are related to effects on wound healing and coagulation.

Modifiable Medical Risk Factors

While some TJA patients suffer from chronic conditions such as rheumatoid arthritis (RA) which elevate the risk of PJI, these patients should be medically optimized prior to surgery [37]. For instance, those on biologic disease-modifying antirheumatic drugs (DMARDs) should discontinue these medications in the perioperative period (approximately 2 weeks before and after surgery, based on drug half-life) as numerous studies have shown an increased risk of surgical site infections (SSIs)/PJIs with perioperative use of these medications [38–40]. This elevated risk is borne out in patients using glucocorticoids within 90 days of surgery as well due to immu-

nosuppressive effects [37]. However, those on nonbiologic DMARDs (e.g., methotrexate, leflunomide) can continue these medications throughout the perioperative period. Finally, anemia has consistently been found to be a factor in both the incidence and failure of treatment of PJI and is another example of a modifiable medical risk factor [34, 35, 41, 42].

Nonmodifiable Risk Factors

Several significant nonmodifiable risk factors such as prior PJI and prior joint surgery have been identified. A history of prior joint infection increases the risk of PJI 5.0–21.0 times [43–45]. Patients with a history of previous joint surgery have almost tripled the risk of PJI [46]. While risk factors such as these cannot be modified, an awareness of these patient factors and heightened surveillance enables clinicians to detect PJI in its early stages thereby increasing the likelihood of treatment success. Further, it should be noted that, while many host factors exist, they should all be taken in the context of the patient to determine the overall risk of PJI with no single factor providing a definitive risk assessment.

To synthesize several of these risk factors, calculators are available online enabling clinicians to compute the overall risk of PJI. Risk factors utilized include BMI, sex, race, insurance status, smoking, drug abuse, prior surgeries, and various comorbidities [47]. While these calculators do not yield an absolute risk assessment, they can be a useful aid in guiding diagnostic testing.

Diagnosis

The diagnosis of PJI remains challenging due to the lack of a single test with 100% accuracy. Instead, a clinician must rely on a combination of clinical history, physical examination, imaging, laboratory testing including serological and synovial markers, microbiological culture, and intraoperative findings.

Clinical Presentation

Most often, PJI is suspected initially due to patient symptomatology rather than incidental findings on imaging or laboratory testing in an asymptomatic patient. Because a patient can become infected even years after surgery, any patient with a history of a TJA presenting with a painful joint should raise a clinician's suspicion. Unlike an acute infection typically associated with an abrupt onset of rapidly progressing symptoms, chronic PJI represents an indolent infection with gradual progression of less severe symptoms. In the settings of both acute and chronic PJIs, patients most

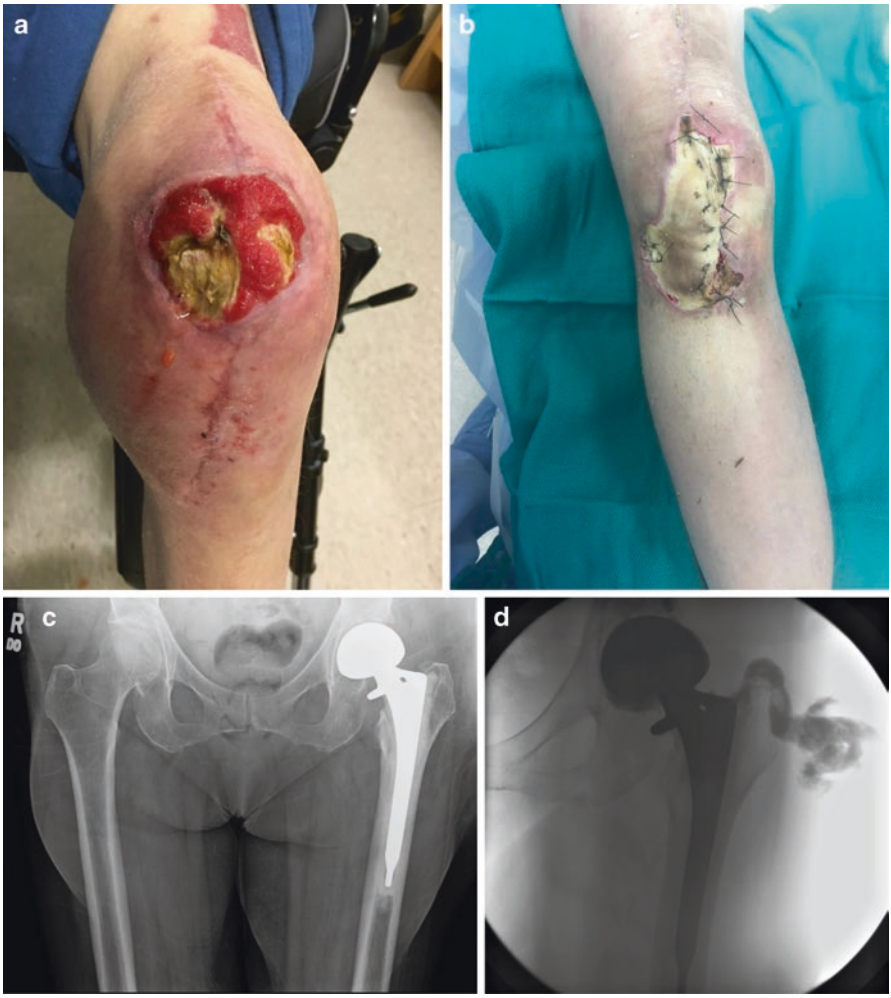


Fig. 3 (**a**, **b**) Clinical pictures of a suspected sinus tract and wound complication in the setting of a presumed PJI in a TKA. (**c**) Radiograph of a suspected infected THA. (**d**) Joint arthrogram confirming a sinus tract communicating from the joint directly to the skin surface. The presence of a sinus tract is diagnostic of a PJI

often present with a progressively painful joint [48]. Fever, while specific for PJI, is inconsistently present and has been found in 32.5% of early postoperative infections, 75.5% of AHI, and 14.0% of CHI [48]. Fever is also frequently present in the immediate postoperative period as a normal physiological response to surgery [49]. Local signs of inflammation such as warmth or diffuse swelling are more easily visible at the knee due to the more superficial nature of the joint. The presence of warmth in TKA and THA was found in 50% and 14% cases of PJI, respectively, while effusions were found in 75% and 29%, respectively [50]. Local warmth or hyperemia can be

Table 2 International Consensus Meeting (ICM) on Musculoskeletal Infection criteria [70]

Major criteria (at least one of the following)				Decision
Two positive growths of the same organism using standard culture methods				Infected
Sinus tract with evidence of communication to the joint or visualization of the prosthesis				
Minor criteria	**Threshold**		**Score**	**Decision**
	Acute	Chronic		
Serum CRP (mg/L)	100	10	2	Combined preoperative and postoperative score:
or				≥6 infected
D-Dimer (μg/L)	Unknown	860		3–5 inconclusive[a]
Elevated serum ESR (mm/h)	No role	30	1	<3 not infected
Elevated synovial WBC (cells/μL)	10,000	3000	3	
or				
Leukocyte esterase	++	++		
or				
Positive alpha-defensin (signal/cutoff)	1.0	1.0		
Elevated synovial PMN (%)	90	70	2	
Single positive culture			2	
Positive histology intraoperative (whites/HPF)			3	
Positive intraoperative purulence			3	

International Consensus Meeting criteria for diagnosing a PJI. Acute infections defined as occurring less than 3 months from index arthroplasty, and acute hematogenous PJI, defined as symptoms occurring for less than 6 weeks but more than 3 months from index surgery

CRP C-reactive protein; *ESR* erythrocyte sedimentation rate; *WBC* white blood cell; *PMN* polymorphonuclear; *HPF* high-power field

Reprinted from The Journal of Arthroplasty, 34(2s), Shohat et al., Hip and Knee Section, What is the Definition of a Periprosthetic Joint Infection (PJI) of the Knee and the Hip? Can the Same Criteria be Used for Both Joints?: Proceedings of International Consensus on Orthopedic Infections, S325-s327, Copyright (2019), with permission from Elsevier

[a]Consider further molecular diagnosis

confusing in the acute postoperative period as this can often be related to increased blood flow in the area due to the normal healing response after surgery.

These signs may be accompanied by skin changes such as erythema, puckering, or obvious drainage of fluid. Drainage must be examined with particular scrutiny as it may be the result of a sinus tract or fistula communicating directly with the joint (Fig. 3a–d). The presence of a sinus tract or abscess represents deep involvement and, due to its high sensitivity and specificity, represents one of the major criteria for diagnosis of PJI (Table 2).

On physical examination, a number of signs may be present with manipulation of the joint such as crepitus or bogginess. The patient may feel pain with either palpation or range of motion especially at extremes of motion. The reported presence of stiffness or restricted range of motion varies widely but has been reported as high as 74% in TKA and 85% in THA [50]. Conversely, laxity may be appreciated

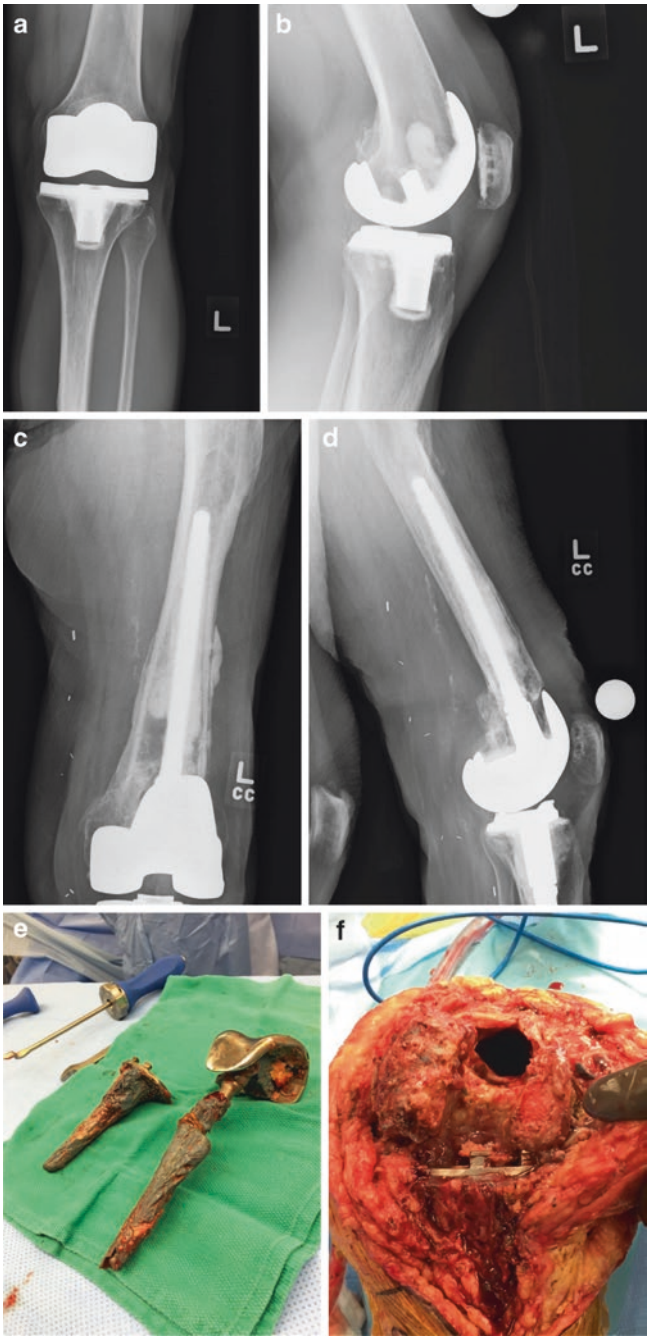


Fig. 4 (**a**, **b**) Normal postoperative radiographs showing a well-fixed prosthesis in a TKA with normal cement fixation. (**c**, **d**) Radiographs of a prior revision total knee arthroplasty in the setting of a PJI; classic signs of infection are visible including osteolysis, bone remodeling, loss of cement fixation, heterotopic bone formation, and migration of the prosthesis within the intramedullary canal. (**e**, **f**) Intraoperative findings demonstrating infection and loose femoral and tibial components

as a function of component subsidence or loosening. Unfortunately, all of these findings are fairly nonspecific.

Imaging

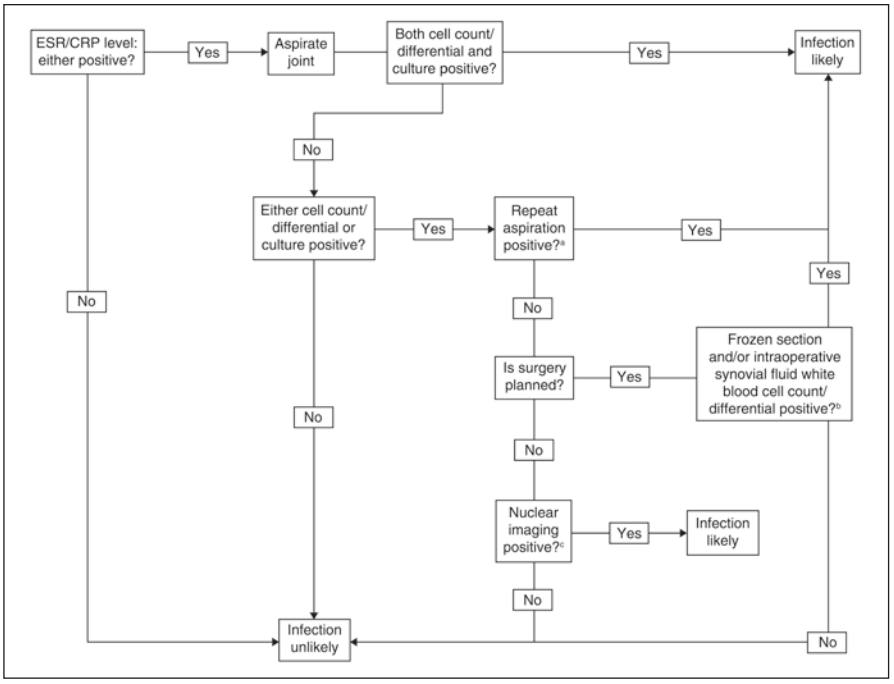
Initial imaging consists of anterior–posterior (AP) and lateral radiographs (X-rays) and should be compared to previous X-rays if available (Fig. 4a, b). Additional specialized views are not routinely acquired. Several signs are possible on X-ray imaging to support a diagnosis of PJI. Osteolysis, as represented by radiolucency, may be visible and may be indirectly seen as manifested by component loosening or subsidence, as the foundation upon which it rests has been compromised. Sinus tracts may be inferred by the appearance of a distinct interruption of the cortex. Finally, a generalized periosteal reaction may be seen (Fig. 4c–f). Unfortunately, X-rays are fraught with limitations due to their low sensitivity as a significant amount of cortical disruption must be present in order to be visible on an X-ray, diminishing their utility especially in early infections. Further, distinguishing loosening as a result of PJI vs. aseptic etiology is challenging [51]. Despite these limitations, the low cost and ease of acquisition make radiographs an ideal component of initial workup of a painful joint.

Advanced imaging modalities include bone scintigraphy, or "bone scan," which may be utilized when infection is strongly suspected but serological markers or synovial fluid analysis are equivocal. Leukocyte, antigranulocyte, and combined leukocyte and bone marrow scintigraphy are also available. All scintigraphy modalities are fairly sensitive (80–99%); however, the specificities among the tests vary widely due to false positives from conditions such as a fracture or bone remodeling. This trend is especially true in the first 12 months following surgery as periprosthetic bone remodeling continues. Therefore, these studies are typically reserved for the setting of chronic or low-grade infection and can be a useful adjunct in diagnosis. Positron emission tomography can be employed and has a sensitivity of 70% and specificity of 84%. However, due to its high cost, it is rarely used [52].

Computerized tomography (CT), magnetic resonance (MR), and ultrasound imaging are not routinely employed in the diagnosis of PJI due to their significant limitations. CT and MR both suffer from beam hardening and projection data noise resulting in image artifacts, making it difficult to distinguish bony architecture surrounding the prosthesis. Ultrasound is most useful in evaluating small areas of soft tissue architecture or the presence of fluid collections or sinus tracts [53], but does not provide useful information about bone morphology or implant position.

Criteria

Once a thorough history and clinical examination raise suspicion for a PJI, the algorithm endorsed by the American Academy of Orthopaedic Surgeons (AAOS) begins with the noninvasive serum biomarkers C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) [54] (Fig. 5). Due to their high sensitivities, they have been used as reliable predictors of the absence of infection [55]. Recent meta-analysis, however, suggests sensitivities of 88% and 75% and specificities of 74% and 70%, respectively and debate continues as to their utility [56]. It should be noted that CRP increases postoperatively from a baseline, peaking on postoperative day 2–3 and normalizing by day 21 [57]. Therefore, the thresholds used are >100 mg/L in the acute phase and >10 mg/L in the chronic phase. Similarly, ESR



Algorithm for patients with a higher probability of hip or knee periprosthetic joint infection.
[a] Perform repeat aspiration when a discrepancy exists between the probability of infection and the result of the initial aspiration culture
[b] Perform frozen section when the diagnosis has not been established at the time of surgery; synovial fluid white blood cell count and differential may also be obtained intraoperatively
[c] Nuclear imaging modalities: labeled-leukocyte imaging combined with bone or bone marrow imaging, F-18 fluorodeoxyglucose–positron emission tomography, gallium imaging, or label-leukocyte imaging
CRP = C-reactive protein, ESR = erythrocyte sedimentation rate

Fig. 5 An algorithm endorsed by the American Academy of Orthopaedic Surgeons used in the diagnosis of a PJI. (Reprinted with permission from Della Valle et al., Diagnosis of Periprosthetic Joint Infections of the Hip and Knee, Journal of the American Academy of Orthopaedic Surgeons, 18(12), 762, https://journals.lww.com/jaaos/Fulltext/2010/12000/Diagnosis_of_Periprosthetic_Joint_Infections_of.6.aspx#pdf-link)

increases immediately postoperatively, though it demonstrates a slow and irregular decline for several months after surgery and thus has more diagnostic utility in a chronic PJI with a threshold of >30 m/h [57, 58]. Ultimately CRP and ESR, as with other serum and synovial markers, must be interpreted with respect to time from index surgery.

Research is underway examining the utility of D-Dimer, a fibrinolytic by-product. One study found that using a threshold of 850 ng/mL resulted in a sensitivity of 89% and a specificity of 93% compared to a combined CRP/ESR sensitivity and specificity of 84% and 47%, respectively [59]. Further studies are underway to validate its utility but, due to its success, it is included in the current International Consensus Meeting on Musculoskeletal Infection (ICM) criteria (Table 2).

Joint Aspiration

If either ESR or CRP is elevated, the clinician should consider aspiration of the concerned joint for synovial fluid analysis which provides the most direct non operative assessment. If no serological markers are elevated, PJI is unlikely though continued clinical suspicion warrants aspiration. Analysis of synovial fluid yields a variety of diagnostic tests including cell count, culture, and inflammatory biomarkers. Synovial fluid cell count with differential and leukocyte esterase is most commonly obtained. Leukocyte count (WBC), leukocyte esterase, and polymorphonuclear cell percentage (PMN%) have demonstrated sensitivities of 89%, 77%, and 89%, respectively, and specificities of 86%, 95%, and 86%, respectively. Alpha-defensin is a marker which has grown in popularity due to its reported 97% sensitivity and 96% specificity, though recently its sensitivity of point of care testing and sensitivity after prior treatment for PJI have both been called into question [60–62]. Regardless, initial promising studies have warranted its inclusion in the current ICM criteria. As with serological markers, synovial markers are affected by proximity to surgery. For example, the threshold for WBC in the acute period is 10,000 cell/μL and 3000 cells/μL in the chronic period. Several other synovial markers, such as interleukin-6 (IL-6), IL-8, and CRP, demonstrate high sensitivities and specificities but are not routinely available at all institutions and therefore are not included in the standard workup for PJI.

Culture

Joint aspiration also allows for bacterial and fungal culture of synovial fluid which has been found to be 94% specific and subsequently is part of the criteria for PJI, though two positive cultures are required as part of the major criteria. However, while culture may seem a likely candidate for a "gold standard" diagnostic test, it has also been found to have only 62% sensitivity [62]. Further, a review of the most

recent literature indicates a culture-negative PJI rate from 7.0% to as high as 42.1% [63, 64]. The most common risk factors identified for culture-negative results were antecedent antibiotic use and presence of postoperative wound drainage. A culture-negative sample may be due to the presence of a biofilm in a quiescent state with a relative lack of planktonic bacteria to sample. Though the outcome of culture-negative PJIs is similar to that caused by known organisms, a negative culture hinders diagnosis and presents challenges in postoperative treatment as antibiotics cannot be tailored to specific sensitivities [65].

Various bacteria have been implicated in PJIs with a preponderance for gram-positive organisms with *Staphylococcus aureus* (*S. aureus*) the most prevalent followed by coagulase-negative *Staphylococcus* [64]. Finally, in rare cases, PJI may result from fungal or mycobacterium species. In the case of fungal PJIs, which account for <1% of all PJIs, cultures may require up to 4 weeks of incubation [66, 67].

Next-generation (next-gen) sequencing, capable of sequencing all DNA present in a sample concurrently, allows for a more complete assessment of the microbes present. Studies have shown its ability to outperform traditional microbial culture with an 89.3% sensitivity vs. 60.7%. Moreover, next-gen sequencing was able to detect bacteria in 81.8% of culture-negative samples from PJIs and 25.0% in presumed aseptic culture-negative revisions [68]. Finally, next-gen sequencing has demonstrated an ability in some cases to reveal a polymicrobial infection, which on culture initially grew only a single organism [69]. This finding may explain failure of some prior therapies and aid antibiotic selection in future cases. Further studies are underway but, due to the relatively high cost, next-gen sequencing is not a first-line test but may prove to be a useful adjunct.

Ultimately, these tests should be interpreted in the context of a patient's clinical history and presentation. To consolidate information and offer guidelines for clinicians, recommendations made by the Musculoskeletal Infection Society in 2011 and further amended at the International Consensus Meeting (ICM) in 2013, set forth guidelines to aid in the diagnosis of PJI. These guidelines were further amended and externally validated for chronic infections in 2018 and found to have a sensitivity of 97.7% and specificity of 99.5% [70] (Table 2). A robust debate continues within the orthopedic community as to the importance of each of the individual components; however, the establishment and revisiting of the Musculoskeletal Infection Society (MSIS) criteria provides a discussion and common roadmap for clinicians for the diagnosis of PJI.

Treatment

Once the diagnosis of PJI has been made, treatment is beset by its own challenges. There are a myriad of treatment regimens available with numerous surgical techniques, implant options, and antimicrobial therapies from which to choose. However, despite advancements and research over the last several decades, there remains a high recurrence rate. For instance, one study of 1.5 million infected TKA knees

found a 26% recurrence rate of infection after first-line treatment [71]. The selection of treatment and success of that regimen require a keen clinical acumen and continued surveillance.

Suppressive Antibiotic Therapy (SAT)

For some patients, medical treatment alone via long-term antibiotic suppression may be the only choice. The goal of therapy is to reduce or at least keep in check the bioburden and thus the incidence of systemic effects cause by the bacterial infection. The scope of patients for whom this treatment would be considered is narrow, as it is not a curative option, but typically includes those medically unsuitable to undergo surgery, those who refuse surgery, and those for whom surgery would not improve functional outcomes. Antibiotic therapy should be continued for the remainder of the patient's life; however, adverse reactions may limit the patient's ability to tolerate such therapy. Several small studies indicate moderate success with 68.5–86.2% of patients maintaining a functioning prosthesis [72–74]. However, complete resolution of a PJI requires surgical intervention; therefore, SAT should only be considered a palliative option.

Surgery

Because of the presence of biofilm which prevents antibiotics from fully permeating and completely eradicating an infection, the only definitive procedure for the elimination of a PJI is open surgery.

Debridement and Irrigation with Implant Retention (DAIR)

DAIR involves an open exposure of the joint to visualize and access the prosthetic implants. Most surgeons employ a surgical/mechanical debridement along with a chemical debridement. All surfaces are scrubbed with an antiseptic solution and irrigated with 6–9 L of sterile saline via low-pressure lavage while the easily exchanged components are removed and replaced, both to improve access to the joint and decrease the bioburden present. The indications for the procedure are narrow: early postoperative infections and acute hematogenous infections. Further, patients must be appropriate surgical candidates, have a microbial isolate of low virulence, and no sinus tract or wound complication present [75]. Several factors have been associated with treatment failure including presence of bacteremia and infection caused by *S. aureus* or *Enterococci* [76].

The advantage is a much faster, less expensive procedure with less morbidity and quicker recovery time. The operative time and blood loss are greatly increased when

components are removed which is avoided in this procedure. However, numerous studies have shown failure rates of 56–76% across multiple PJI chronicities caused by multiple organisms [77–79]. Outcomes appear to be improving, however, with time from surgery (<7 days) as a major factor in that success [80], and subsequent risk of failure rising with each additional day from the onset of symptoms [81]. For the right patient population, DAIR is a viable option that can be considered.

Exchange Arthroplasty

The current definitive treatment for PJI is an open procedure in which all components are removed and new components are implanted. There is an ongoing debate in the orthopedic community as to whether component exchange should be done in a single procedure or in two stages.

Single-Stage Exchange

In a single-stage exchange procedure, infected components and bone cement are removed, tissue is aggressively debrided, and definitive implants are placed in the same surgical setting. Local antibiotics are delivered during the procedure while postoperatively patients receive 4–6 weeks of intravenous antibiotics tailored to culture growth and sensitivities obtained from samples obtained during surgery. Indications include absence of bacteremia, positive isolation of causative organism with sensitivities, and minimal bone and soft tissue loss [82, 83]. Relative contraindications include bacteremia, culture-negative PJI, poor bone stock for fixation of new components, presence of a sinus tract, and soft tissue deficiencies which would preclude adequate closure of the wound [84–88].

The advantages of one-stage exchange compared to two-stage exchange are clear: decreased morbidity and mortality, reduced cost, and earlier functional return as only one surgery is required. However, research is conflicting with some data suggesting equivalence or superiority to two-stage exchange [89–91] while others suggest an elevated risk of reinfection [92]. However, research comparing the two treatment options suffer from heterogeneity of patient populations and are predominantly retrospective in nature. Therefore, several multicenter, prospective, randomized, controlled studies are underway to answer this important clinical question.

Two-Stage Exchange

In a two-stage exchange, the removal of infected components and reimplantation of new components occurs in two surgeries separated in time by the retention of an intra-articular polymethyl methacrylate (PMMA) spacer implanted during the first

procedure. The PMMA spacer, which may be retained for several months or even years, is impregnated with antibiotics which passively elute over time. The patient additionally receives 4–6 weeks of intravenous and/or oral antibiotics. While there is no definitive threshold as to when it is safe to reimplant, most surgeons will monitor serological and/or synovial markers. When they feel it is clinically appropriate, the spacer is removed and new components are implanted in a second procedure.

The PMMA spacer utilized during a two-stage exchange may be static, preventing motion through the implantation of a joint-spanning rod, or dynamic which allows partial to full range of motion (Fig. 6a, b). Due to the exothermic reaction (82–86 °C in femurs and 115 °C in tibias) that occurs during the curing of PMMA, the scope of antibiotics able to be incorporated is limited to those with heat stability, such as vancomycin or tobramycin [93]. Recent investigations suggest antibiotics once considered "heat-sensitive," such as ceftazadime, may be more resilient than previously thought but until more data are available, the antibiotic arsenal able to be incorporated into PMMA spacers remains limited [94, 95]. Further, the antibiotics in a PMMA spacer elute in a burst fashion, peaking in concentration on postoperative days two and three and quickly decreasing over several weeks. Finally, the spacer cannot be redosed with antibiotics once implanted. Research has demonstrated low-dose intraosseous or intra-articular vancomycin administration results in equal or better tissue and synovial fluid concentrations when compared to systemic administration and minimizes side effects [96, 97]. Methods to improve drug delivery options may provide improved treatment success allowing for minimization of both dosing concentrations and time needed for treatment.

Though the two-stage exchange procedure is associated with high rates of infection control for those patients who complete both stages (83–89.8%), it has also been shown to have substantial mortality [13, 92]. Prior reports in the 90–95% success range likely did not account for the attrition due to death between stages [89, 98]. After just the first stage for TKA, the 30-day readmission rate is 11.1% and 90-day mortality rate is 2.6% [99]. The 1-year and 5-year mortalities for completed TKA two-stage revision have been reported as 4.33% and 21.64%, respectively [100]. While considered the "gold standard" in the USA, two-stage revision leaves room for improvement of outcomes and places a substantial burden on the patient.

Resection Arthroplasty

When the race is truly lost in the face of recalcitrant infections, several procedures are available as salvage options to maximize the function and health of the patient. The decision to employ one of these options is a result of host factors, namely, poor soft tissue envelope or bone stock, a patient's physical and emotional exhaustion, and a reluctance or inability to continue with therapy.

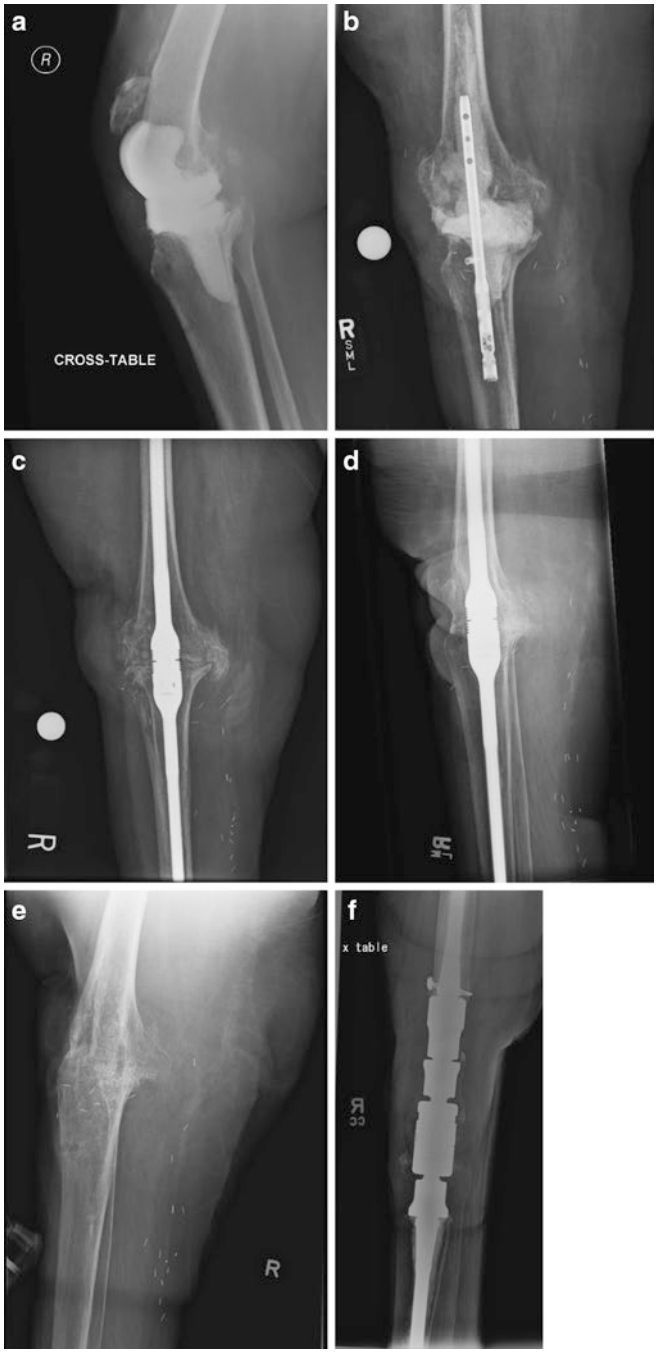


Fig. 6 (**a**) Lateral radiograph of an infected articulating spacer with an extensor mechanism disruption as demonstrated by the high-riding patella. (**b**) The same patient status post placement of a static spacer, in this case a humeral nail with high dose antibiotic cement. (**c**) Following explant of the static spacer, an intramedullary nail was placed for the knee fusion. (**d**) Due to the patient's poorly controlled diabetes and renal dysfunction requiring dialysis, 2 years later she presented with a reinfected implant though healed fusion. (**e**) The infected nail was removed and after debridement, the healed fusion was able to be retained. (**f**) If bony fusion is unobtainable, intercalary fusion presents another treatment option

Knee Arthrodesis and Above the Knee Amputation

In the setting of an infected TKA, knee arthrodesis (KA) may be employed to provide a stable, painless knee via intramedullary nail or compression plating which may be augmented temporarily by external fixation once all infected TKA components have been removed (Fig. 6c–f). The intramedullary nail may be coated with PMMA impregnated with antibiotics for further infection control. The knee is fused in full extension and therefore requires a rehabilitative period for gait training. Once fused, the patient can ambulate on their native leg and KA requires a lower energy expenditure (0.16 mL O_2/kg/min vs. 0.20 mL O_2/kg/min) when compared to an above the knee amputation (AKA) [101, 102]. The retention of the native knee, however, does retain a possible nidus for latent infection and reinfection rates of 5.4–10.6% have been reported [103]. Further, a significantly higher rate of postoperative complications has been associated with KA when compared to AKA [104]. Conversely, KA has been associated with improved functional outcomes and lower mortality when compared to AKA [105, 106]. Further, AKA has been associated with more systemic complications, longer hospital stays, and higher readmission rates [107]. Barring poor host factors such as severe comorbidities and poor soft tissue envelope or bone stock, KA is the preferred salvage procedure compared to its lower incidence of complications, lower mortality, and superior functional outcomes [104, 108].

Girdlestone and Hip Disarticulation

In the setting of recurrent THA infection, the Girdlestone procedure provides the surgeon with a salvage procedure in which prosthetic components are removed and infection is controlled at the expense of joint functionality (Fig. 7a–d). After implant removal, an osteotomy is performed just above the greater trochanter resecting the femoral neck and head. The hip capsule remains and fibrous tissue fills in creating a pseudarthrosis. No bony fusion occurs, allowing for a limited range of motion [109]. Patient satisfaction varies widely with the procedure (13–83%) with resolution of infection occurring in 80–100% of cases [110]. Conversely, a hip disarticulation, in which the entire lower extremity is amputated at the level of the hip joint, is considered a morbid procedure and reserved typically for life-threatening scenarios such as systemic sepsis or extreme soft tissue compromise [111]. In addition to limitations with weight bearing, special postoperative considerations exist such as wheelchair use requiring a special balance of weights to compensate for loss of the entire limb.

Adjunctive Antibiotic Therapy

Postoperative antibiotics are required regardless of procedure selected by the surgeon. Ideally, a causative organism with sensitivities is identified prior to surgery. Multiple samples are taken at the time of implant removal, and patients are started

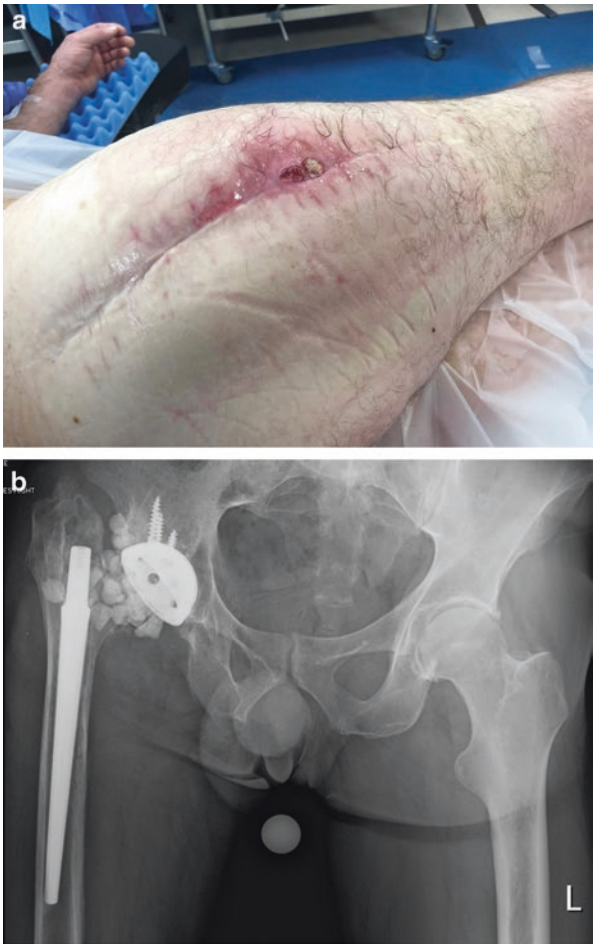


Fig. 7 (**a**) Clinical picture of a draining wound in a male with a history of multiple hip surgeries and eventual partial hip exchange for MRSA infection. Given his medical comorbidities, it was decided to proceed with a Girdlestone procedure, as any attempt for reimplantation would likely result in repeat infection. (**b**) Radiograph of the patient's prior partial hip resection. The femoral stem and acetabular cup were retained with placement of nonabsorbable antibiotic-impregnated PMMA beads. (**c**) Postoperative radiograph after debridement and removal of all components with placement of absorbable calcium sulfate high-dose vancomycin beads. (**d**) Radiograph 5 months after a Girdlestone procedure demonstrating the greater trochanter articulating with the pelvis. The patient now ambulates with a shoe lift and crutches

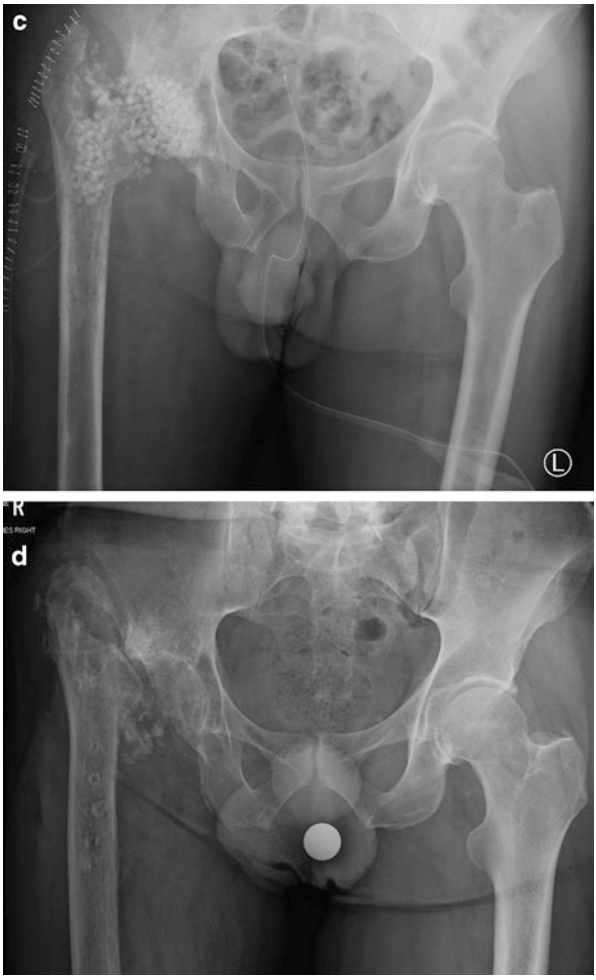


Fig. 7 (continued)

immediately postoperatively on empiric intravenous antimicrobials. Once sensitivities are obtained, drugs are then tailored to the specific organism(s). Multiple studies have demonstrated a success rate of 90–100% with the use of 6 weeks or less of intravenous antibiotics [112, 113] with one study directly examining a 1-week course vs. 6-week and finding no superiority [114]. Bernard et al. examined the outcomes of 144 patients treated with DAIR, one- and two-stage exchange, Girdlestone, or KA and found no advantage to antibiotic therapy longer than 6 weeks [115]. To date, no studies published have directly compared exclusively

oral vs. exclusively intravenous antibiotics. However, studies have demonstrated equivalent efficacy of beginning antibiotic therapy parenterally and then transitioning to oral [116–118]. The Infectious Disease Society of America currently endorses a 4–6-week course of intravenous or highly bioavailable oral antibiotics with a six-week course endorsed for more virulent organisms.

Negative Pressure Wound Therapy (NPWT)

NPWT or "wound vac" is often applied as an adjunct for wound closure and to contend with postoperative drainage in both primary and revision cases. Studies have demonstrated reduced wound exudate, fewer dressing changes required postoperatively, and a decreased rate of superficial wound infections [119, 120]. Given that the rate of infection increases 29% for TKA and 42% for THA for each additional day of postoperative drainage, many surgeons apply NPWT prophylactically [121].

Call to Action

Despite surgical, technological, and medical advances, the infection rate in total joint arthroplasty has remained largely unchanged for the last 30 years. Some have speculated infection can never truly be prevented and subsequently healthcare providers must remain ever vigilant in the face of this devastating complication. The race for the surface may not be a winning vs. losing proposition but rather is likely a continuous spectrum. The treatment for each patient should be as individualized as the clinical scenario and depends on host factors, microbial isolates, and treatment regimens available.

As bacteria adapt to our arsenal, so too must we continue our efforts against them. There is a critical knowledge gap and growing need for continued treatment evolution in the way of improved osteoinductive/antimicrobial materials at time of a reimplant, in addition to prevention technology. Given the impending rise in total joint arthroplasty case volume and subsequent revision case volume due to PJI, an urgent need exists for continued work in the development of preventive, diagnostic, and therapeutic tools.

Acknowledgements The authors would like to thank Suzanne Danley for her assistance and critical review of the manuscript.

References

1. Scott CE, Bugler KE, Clement ND, MacDonald D, Howie CR, Biant LC (2012) Patient expectations of arthroplasty of the hip and knee. J Bone Joint Surg Br 94(7):974–981
2. Palazzo C, Jourdan C, Descamps S, Nizard R, Hamadouche M, Anract P, Boisgard S, Galvin M, Ravaud P, Poiraudeau S (2014) Determinants of satisfaction 1 year after total hip arthroplasty: the role of expectations fulfilment. BMC Musculoskelet Disord 15:53
3. Sloan M, Premkumar A, Sheth NP (2018) Projected volume of primary total joint arthroplasty in the U.S., 2014 to 2030. J Bone Joint Surg Am 100(17):1455–1460
4. Bozic KJ, Ries MD (2005) The impact of infection after total hip arthroplasty on hospital and surgeon resource utilization. J Bone Joint Surg Am 87(8):1746–1751
5. Namba RS, Inacio MC, Paxton EW (2013) Risk factors associated with deep surgical site infections after primary total knee arthroplasty: an analysis of 56,216 knees. J Bone Joint Surg Am 95(9):775–782
6. Edwards JR, Peterson KD, Mu Y, Banerjee S, Allen-Bridson K, Morrell G, Dudeck MA, Pollock DA, Horan TC (2009) National Healthcare Safety Network (NHSN) report: data summary for 2006 through 2008, issued December 2009. Am J Infect Control 37(10):783–805
7. Huotari K, Peltola M, Jamsen E (2015) The incidence of late prosthetic joint infections: a registry-based study of 112,708 primary hip and knee replacements. Acta Orthop 86(3):321–325
8. Ong KL, Kurtz SM, Lau E, Bozic KJ, Berry DJ, Parvizi J (2009) Prosthetic joint infection risk after total hip arthroplasty in the Medicare population. J Arthroplast 24(6 Suppl):105–109
9. Kapadia BH, McElroy MJ, Issa K, Johnson AJ, Bozic KJ, Mont MA (2014) The economic impact of periprosthetic infections following total knee arthroplasty at a specialized tertiary-care center. J Arthroplast 29(5):929–932
10. Kapadia BH, Banerjee S, Cherian JJ, Bozic KJ, Mont MA (2016) The economic impact of periprosthetic infections after total hip arthroplasty at a Specialized Tertiary-Care Center. J Arthroplast 31(7):1422–1426
11. Whitehouse JD, Friedman ND, Kirkland KB, Richardson WJ, Sexton DJ (2002) The impact of surgical-site infections following orthopedic surgery at a community hospital and a university hospital: adverse quality of life, excess length of stay, and extra cost. Infect Control Hosp Epidemiol 23(4):183–189
12. de Lissovoy G, Fraeman K, Hutchins V, Murphy D, Song D, Vaughn BB (2009) Surgical site infection: incidence and impact on hospital utilization and treatment costs. Am J Infect Control 37(5):387–397
13. Berend KR, Lombardi AV Jr, Morris MJ, Bergeson AG, Adams JB, Sneller MA (2013) Two-stage treatment of hip periprosthetic joint infection is associated with a high rate of infection control but high mortality. Clin Orthop Relat Res 471(2):510–518
14. Choi HR, Beecher B, Bedair H (2013) Mortality after septic versus aseptic revision total hip arthroplasty: a matched-cohort study. J Arthroplast 28(8 Suppl):56–58
15. Kurtz S, Ong K, Lau E, Mowat F, Halpern M (2007) Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg Am 89(4):780–785
16. Tsukayama DT, Goldberg VM, Kyle R (2003) Diagnosis and management of infection after total knee arthroplasty. J Bone Joint Surg Am 85-A(Suppl 1):S75–S80
17. Tsukayama DT, Estrada R, Gustilo RB (1996) Infection after total hip arthroplasty. A study of the treatment of one hundred and six infections. J Bone Joint Surg Am 78(4):512–523
18. Parvizi J, Gehrke T (2014) Definition of periprosthetic joint infection. J Arthroplast 29(7):1331
19. McPherson EJ, Tontz W Jr, Patzakis M, Woodsome C, Holtom P, Norris L, Shufelt C (1999) Outcome of infected total knee utilizing a staging system for prosthetic joint infection. Am J Orthop (Belle Mead NJ) 28(3):161–165

20. Li C, Renz N, Trampuz A (2018) Management of Periprosthetic Joint Infection. Hip Pelvis 30(3):138–146
21. Luthringer TA, Fillingham YA, Okroj K, Ward EJ, Della Valle C (2016) Periprosthetic joint infection after hip and knee arthroplasty: a review for emergency care providers. Ann Emerg Med 68(3):324–334
22. Cook JL, Scott RD, Long WJ (2007) Late hematogenous infections after total knee arthroplasty: experience with 3013 consecutive total knees. J Knee Surg 20(1):27–33
23. Costerton W, Veeh R, Shirtliff M, Pasmore M, Post C, Ehrlich G (2003) The application of biofilm science to the study and control of chronic bacterial infections. J Clin Invest 112(10):1466–1477
24. Costerton JW (2005) Biofilm theory can guide the treatment of device-related orthopaedic infections. Clin Orthop Relat Res (437):7–11
25. Barth E, Myrvik QM, Wagner W, Gristina AG (1989) In vitro and in vivo comparative colonization of Staphylococcus aureus and Staphylococcus epidermidis on orthopaedic implant materials. Biomaterials 10(5):325–328
26. Alamanda VK, Springer BD (2019) The prevention of infection: 12 modifiable risk factors. Bone Joint J 101-b(1_Supple_A):3–9
27. Kee JR, Mears SC, Edwards PK, Barnes CL (2017) Modifiable risk factors are common in early revision hip and knee arthroplasty. J Arthroplast 32(12):3689–3692
28. Namba RS, Paxton L, Fithian DC, Stone ML (2005) Obesity and perioperative morbidity in total hip and total knee arthroplasty patients. J Arthroplast 20(7 Suppl 3):46–50
29. Smith JO, Frampton CMA, Hooper GJ, Young SW (2018) The impact of patient and surgical factors on the rate of postoperative infection after total hip arthroplasty—a New Zealand Joint Registry Study. J Arthroplast 33(6):1884–1890
30. Wagner ER, Kamath AF, Fruth KM, Harmsen WS, Berry DJ (2016) Effect of body mass index on complications and reoperations after total hip arthroplasty. J Bone Joint Surg Am 98(3):169–179
31. Cancienne JM, Werner BC, Browne JA (2017) Is there a threshold value of hemoglobin A1c that predicts risk of infection following primary total hip arthroplasty? J Arthroplast 32(9s):S236–s240
32. Tarabichi M, Shohat N, Kheir MM, Adelani M, Brigati D, Kearns SM, Patel P, Clohisy JC, Higuera CA, Levine BR, Schwarzkopf R, Parvizi J, Jiranek WA (2017) Determining the threshold for HbA1c as a predictor for adverse outcomes after total joint arthroplasty: a multicenter, retrospective study. J Arthroplasty 32(9s):S263–S267.e1
33. Bedard NA, DeMik DE, Owens JM, Glass NA, DeBerg J, Callaghan JJ (2019) Tobacco use and risk of wound complications and periprosthetic joint infection: a systematic review and meta-analysis of primary total joint arthroplasty procedures. J Arthroplast 34(2):385–396.e4
34. Bozic KJ, Lau E, Kurtz S, Ong K, Rubash H, Vail TP, Berry DJ (2012) Patient-related risk factors for periprosthetic joint infection and postoperative mortality following total hip arthroplasty in Medicare patients. J Bone Joint Surg Am 94(9):794–800
35. Bozic KJ, Lau E, Kurtz S, Ong K, Berry DJ (2012) Patient-related risk factors for postoperative mortality and periprosthetic joint infection in medicare patients undergoing TKA. Clin Orthop Relat Res 470(1):130–137
36. Poultsides LA, Ma Y, Della Valle AG, Chiu YL, Sculco TP, Memtsoudis SG (2013) In-hospital surgical site infections after primary hip and knee arthroplasty—incidence and risk factors. J Arthroplast 28(3):385–389
37. Cordtz RL, Zobbe K, Hojgaard P, Kristensen LE, Overgaard S, Odgaard A, Lindegaard H, Dreyer L (2018) Predictors of revision, prosthetic joint infection and mortality following total hip or total knee arthroplasty in patients with rheumatoid arthritis: a nationwide cohort study using Danish healthcare registers. Ann Rheum Dis 77(2):281–288
38. Goodman SM, Springer B, Guyatt G, Abdel MP, Dasa V, George M, Gewurz-Singer O, Giles JT, Johnson B, Lee S, Mandl LA, Mont MA, Sculco P, Sporer S, Stryker L, Turgunbaev M, Brause B, Chen AF, Gililland J, Goodman M, Hurley-Rosenblatt A, Kirou K, Losina E, MacKenzie R, Michaud K, Mikuls T, Russell L, Sah A, Miller AS, Singh JA, Yates A

(2017) 2017 American College of Rheumatology/American Association of Hip and Knee Surgeons guideline for the perioperative management of antirheumatic medication in patients with rheumatic diseases undergoing elective total hip or total knee arthroplasty. Arthritis Rheumatol 69(8):1538–1551
39. Momohara S, Kawakami K, Iwamoto T, Yano K, Sakuma Y, Hiroshima R, Imamura H, Masuda I, Tokita A, Ikari K (2011) Prosthetic joint infection after total hip or knee arthroplasty in rheumatoid arthritis patients treated with nonbiologic and biologic disease-modifying antirheumatic drugs. Mod Rheumatol 21(5):469–475
40. Suzuki M, Nishida K, Soen S, Oda H, Inoue H, Kaneko A, Takagishi K, Tanaka T, Matsubara T, Mitsugi N, Mochida Y, Momohara S, Mori T, Suguro T (2011) Risk of postoperative complications in rheumatoid arthritis relevant to treatment with biologic agents: a report from the Committee on Arthritis of the Japanese Orthopaedic Association. J Orthop Sci 16(6):778–784
41. Pruzansky JS, Bronson MJ, Grelsamer RP, Strauss E, Moucha CS (2014) Prevalence of modifiable surgical site infection risk factors in hip and knee joint arthroplasty patients at an urban academic hospital. J Arthroplast 29(2):272–276
42. Swenson RD, Butterfield JA, Irwin TJ, Zurlo JJ, Davis CM 3rd (2018) Preoperative anemia is associated with failure of open debridement polyethylene exchange in acute and acute hematogenous prosthetic joint infection. J Arthroplast 33(6):1855–1860
43. Pugely AJ, Martin CT, Gao Y, Schweizer ML, Callaghan JJ (2015) The incidence of and risk factors for 30-day surgical site infections following primary and revision total joint arthroplasty. J Arthroplast 30(9 Suppl):47–50
44. Bongartz T, Halligan CS, Osmon DR, Reinalda MS, Bamlet WR, Crowson CS, Hanssen AD, Matteson EL (2008) Incidence and risk factors of prosthetic joint infection after total hip or knee replacement in patients with rheumatoid arthritis. Arthritis Rheum 59(12):1713–1720
45. Bedair H, Goyal N, Dietz MJ, Urish K, Hansen V, Manrique J, Hamilton W, Deirmengian G (2015) A history of treated Periprosthetic joint infection increases the risk of subsequent different site infection. Clin Orthop Relat Res 473(7):2300–2304
46. Kunutsor SK, Whitehouse MR, Blom AW, Beswick AD (2016) Patient-related risk factors for periprosthetic joint infection after total joint arthroplasty: a systematic review and meta-analysis. PLoS One 11(3):e0150866
47. Prosthetic Joint Infection risk calculator. https://icmphilly.com/ortho-applications/prosthetic-joint-infection-pji-risk-calculator/
48. Amanatullah D, Dennis D, Oltra EG, Marcelino Gomes LS, Goodman SB, Hamlin B, Hansen E, Hashemi-Nejad A, Holst DC, Komnos G, Koutalos A, Malizos K, Martinez Pastor JC, McPherson E, Meermans G, Mooney JA, Mortazavi J, Parsa A, Pecora JR, Pereira GA, Martos MS, Shohat N, Shope AJ, Zullo SS (2019) Hip and knee section, diagnosis, definitions: proceedings of international consensus on orthopedic infections. J Arthroplasty 34(2s):S329–s337
49. Ghosh S, Charity RM, Haidar SG, Singh BK (2006) Pyrexia following total knee replacement. Knee 13(4):324–327
50. Zajonz D, Wuthe L, Tiepolt S, Brandmeier P, Prietzel T, von Salis-Soglio GF, Roth A, Josten C, Heyde CE, Ghanem M (2015) Diagnostic work-up strategy for periprosthetic joint infections after total hip and knee arthroplasty: a 12-year experience on 320 consecutive cases. Patient Saf Surg 9:20
51. Tigges S, Stiles RG, Roberson JR (1994) Appearance of septic hip prostheses on plain radiographs. AJR Am J Roentgenol 163(2):377–380
52. Verberne SJ, Sonnega RJ, Temmerman OP, Raijmakers PG (2017) What is the accuracy of nuclear imaging in the assessment of periprosthetic knee infection? A meta-analysis. Clin Orthop Relat Res 475(5):1395–1410
53. Sofka CM (2007) Current applications of advanced cross-sectional imaging techniques in evaluating the painful arthroplasty. Skelet Radiol 36(3):183–193
54. Della Valle C, Parvizi J, Bauer TW, Dicesare PE, Evans RP, Segreti J, Spangehl M, Watters WC 3rd, Keith M, Turkelson CM, Wies JL, Sluka P, Hitchcock K (2010) Diagnosis of periprosthetic joint infections of the hip and knee. J Am Acad Orthop Surg 18(12):760–770

55. Spangehl MJ, Masri BA, O'Connell JX, Duncan CP (1999) Prospective analysis of preoperative and intraoperative investigations for the diagnosis of infection at the sites of two hundred and two revision total hip arthroplasties. J Bone Joint Surg Am 81(5):672–683
56. Berbari E, Mabry T, Tsaras G, Spangehl M, Erwin PJ, Murad MH, Steckelberg J, Osmon D (2010) Inflammatory blood laboratory levels as markers of prosthetic joint infection: a systematic review and meta-analysis. J Bone Joint Surg Am 92(11):2102–2109
57. Larsson S, Thelander U, Friberg S (1992) C-reactive protein (CRP) levels after elective orthopedic surgery. Clin Orthop Relat Res (275):237-42
58. Nazem K, Motififard M, Yousefian M (2016) Variations in ESR and CRP in total knee arthroplasty and total hip arthroplasty in Iranian patients from 2009 to 2011. Adv Biomed Res 5:148
59. Shahi A, Kheir MM, Tarabichi M, Hosseinzadeh HRS, Tan TL, Parvizi J (2017) Serum D-Dimer test is promising for the diagnosis of periprosthetic joint infection and timing of reimplantation. J Bone Joint Surg Am 99(17):1419–1427
60. Stone WZ, Gray CF, Parvataneni HK, Prieto HA (2019) Clinical evaluation of alpha defensin test following staged treatment of prosthetic joint infections. J Arthroplast 34:1446
61. Carli AV, Abdelbary H, Ahmadzai N, Cheng W, Shea B, Hutton B, Sniderman J, Philip Sanders BS, Esmaeilisaraji L, Skidmore B, Gauthier-Kwan OY, Bunting AC, Gauthier P, Crnic A, Logishetty K, Moher D, Fergusson D, Beaule PE (2019) Diagnostic accuracy of serum, synovial, and tissue testing for chronic periprosthetic joint infection after hip and knee replacements: a systematic review. J Bone Joint Surg Am 101(7):635–649
62. Lee YS, Koo KH, Kim HJ, Tian S, Kim TY, Maltenfort MG, Chen AF (2017) Synovial fluid biomarkers for the diagnosis of periprosthetic joint infection: a systematic review and meta-analysis. J Bone Joint Surg Am 99(24):2077–2084
63. Yoon HK, Cho SH, Lee DY, Kang BH, Lee SH, Moon DG, Kim DH, Nam DC, Hwang SC (2017) A review of the literature on culture-negative periprosthetic joint infection: epidemiology, diagnosis and treatment. Knee Surg Relat Res 29(3):155–164
64. Tande AJ, Patel R (2014) Prosthetic joint infection. Clin Microbiol Rev 27(2):302–345
65. Berbari EF, Marculescu C, Sia I, Lahr BD, Hanssen AD, Steckelberg JM, Gullerud R, Osmon DR (2007) Culture-negative prosthetic joint infection. Clin Infect Dis 45(9):1113–1119
66. Phelan DM, Osmon DR, Keating MR, Hanssen AD (2002) Delayed reimplantation arthroplasty for candidal prosthetic joint infection: a report of 4 cases and review of the literature. Clin Infect Dis 34(7):930–938
67. Bosshard PP (2011) Incubation of fungal cultures: how long is long enough? Mycoses 54(5):e539–e545
68. Tarabichi M, Shohat N, Goswami K, Alvand A, Silibovsky R, Belden K, Parvizi J (2018) Diagnosis of periprosthetic joint infection: the potential of next-generation sequencing. J Bone Joint Surg Am 100(2):147–154
69. Ivy MI, Thoendel MJ, Jeraldo PR, Greenwood-Quaintance KE, Hanssen AD, Abdel MP, Chia N, Yao JZ, Tande AJ, Mandrekar JN, Patel R (2018) Direct detection and identification of prosthetic joint infection pathogens in synovial fluid by metagenomic shotgun sequencing. J Clin Microbiol 56(9)
70. Shohat N, Bauer T, Buttaro M, Budhiparama N, Cashman J, Della Valle CJ, Drago L, Gehrke T, Marcelino Gomes LS, Goswami K, Hailer NP, Han SB, Higuera CA, Inaba Y, Jenny JY, Kjaersgaard-Andersen P, Lee M, Llinas A, Malizos K, Mont MA, Jones RM, Parvizi J, Peel T, Rivero-Boschert S, Segreti J, Soriano A, Sousa R, Spangehl M, Tan TL, Tikhilov R, Tuncay I, Winkler H, Witso E, Wouthuyzen-Bakker M, Young S, Zhang X, Zhou Y, Zimmerli W (2019) Hip and knee section, what is the definition of a periprosthetic joint infection (PJI) of the knee and the hip? Can the same criteria be used for both joints? Proceedings of International Consensus on Orthopedic Infections. J Arthroplast 34(2s):S325–s327
71. Cochran AR, Ong KL, Lau E, Mont MA, Malkani AL (2016) Risk of reinfection after treatment of infected total knee arthroplasty. J Arthroplast 31(9 Suppl):156–161
72. Goulet JA, Pellicci PM, Brause BD, Salvati EM (1988) Prolonged suppression of infection in total hip arthroplasty. J Arthroplast 3(2):109–116

73. Rao N, Crossett LS, Sinha RK, Le Frock JL (2003) Long-term suppression of infection in total joint arthroplasty. Clin Orthop Relat Res 414:55–60
74. Siqueira MB, Saleh A, Klika AK, O'Rourke C, Schmitt S, Higuera CA, Barsoum WK (2015) Chronic suppression of periprosthetic joint infections with oral antibiotics increases infection-free survivorship. J Bone Joint Surg Am 97(15):1220–1232
75. Haasper C, Buttaro M, Hozack W, Aboltins CA, Borens O, Callaghan JJ, de Carvalho PI, Chang Y, Corona P, Da Rin F, Esposito S, Fehring TK, Sanchez XF, Lee GC, Martinez-Pastor JC, Mortazavi SM, Noiseux NO, Peng KT, Schutte HD, Schweitzer D, Trebse R, Tsiridis E, Whiteside L (2014) Irrigation and debridement. J Arthroplast 29(2 Suppl):100–103
76. Wouthuyzen-Bakker M, Sebillotte M, Lomas J, Taylor A, Palomares EB, Murillo O, Parvizi J, Shohat N, Reinoso JC, Sanchez RE, Fernandez-Sampedro M, Senneville E, Huotari K, Barbero JM, Garcia-Canete J, Lora-Tamayo J, Ferrari MC, Vaznaisiene D, Yusuf E, Aboltins C, Trebse R, Salles MJ, Benito N, Vila A, Toro MDD, Kramer TS, Petersdorf S, Diaz-Brito V, Tufan ZK, Sanchez M, Arvieux C, Soriano A (2019) Clinical outcome and risk factors for failure in late acute prosthetic joint infections treated with debridement and implant retention. J Infect 78(1):40–47
77. Koyonos L, Zmistowski B, Della Valle CJ, Parvizi J (2011) Infection control rate of irrigation and debridement for periprosthetic joint infection. Clin Orthop Relat Res 469(11):3043–3048
78. Fehring TK, Odum SM, Berend KR, Jiranek WA, Parvizi J, Bozic KJ, Della Valle CJ, Gioe TJ (2013) Failure of irrigation and debridement for early postoperative periprosthetic infection. Clin Orthop Relat Res 471(1):250–257
79. Odum SM, Fehring TK, Lombardi AV, Zmistowski BM, Brown NM, Luna JT, Fehring KA, Hansen EN (2011) Irrigation and debridement for periprosthetic infections: does the organism matter? J Arthroplast 26(6 Suppl):114–118
80. Tsang SJ, Ting J, Simpson A, Gaston P (2017) Outcomes following debridement, antibiotics and implant retention in the management of periprosthetic infections of the hip: a review of cohort studies. Bone Joint J 99-b(11):1458–1466
81. Volpin A, Sukeik M, Alazzawi S, Haddad FS (2016) Aggressive early debridement in treatment of acute periprosthetic joint infections after hip and knee replacements. Open Orthop J 10:669–678
82. Buchholz HW, Elson RA, Engelbrecht E, Lodenkamper H, Rottger J, Siegel A (1981) Management of deep infection of total hip replacement. J Bone Joint Surg Br 63-b(3):342–353
83. Jackson WO, Schmalzried TP (2000) Limited role of direct exchange arthroplasty in the treatment of infected total hip replacements. Clin Orthop Relat Res 381:101–105
84. Lange J, Troelsen A, Solgaard S, Otte KS, Jensen NK, Soballe K (2018) Cementless one-stage revision in chronic periprosthetic hip joint infection. Ninety-one percent infection free survival in 56 patients at minimum 2-year follow-up. J Arthroplast 33(4):1160–1165.e1
85. Bori G, Navarro G, Morata L, Fernandez-Valencia JA, Soriano A, Gallart X (2018) Preliminary results after changing from two-stage to one-stage revision arthroplasty protocol using cementless arthroplasty for chronic infected hip replacements. J Arthroplast 33(2):527–532
86. Wolf M, Clar H, Friesenbichler J, Schwantzer G, Bernhardt G, Gruber G, Glehr M, Leithner A, Sadoghi P (2014) Prosthetic joint infection following total hip replacement: results of one-stage versus two-stage exchange. Int Orthop 38(7):1363–1368
87. Jenny JY, Lengert R, Diesinger Y, Gaudias J, Boeri C, Kempf JF (2014) Routine one-stage exchange for chronic infection after total hip replacement. Int Orthop 38(12):2477–2481
88. Raut VV, Siney PD, Wroblewski BM (1994) One-stage revision of infected total hip replacements with discharging sinuses. J Bone Joint Surg Br 76(5):721–724
89. Leonard HA, Liddle AD, Burke O, Murray DW, Pandit H (2014) Single- or two-stage revision for infected total hip arthroplasty? A systematic review of the literature. Clin Orthop Relat Res 472(3):1036–1042
90. George DA, Logoluso N, Castellini G, Gianola S, Scarponi S, Haddad FS, Drago L, Romano CL (2016) Does cemented or cementless single-stage exchange arthroplasty of chronic periprosthetic hip infections provide similar infection rates to a two-stage? A systematic review. BMC Infect Dis 16(1):553

91. Haddad FS, Sukeik M, Alazzawi S (2015) Is single-stage revision according to a strict protocol effective in treatment of chronic knee arthroplasty infections? Clin Orthop Relat Res 473(1):8–14
92. Romano CL, Gala L, Logoluso N, Romano D, Drago L (2012) Two-stage revision of septic knee prosthesis with articulating knee spacers yields better infection eradication rate than one-stage or two-stage revision with static spacers. Knee Surg Sports Traumatol Arthrosc 20(12):2445–2453
93. Vaishya R, Chauhan M, Vaish A (2013) Bone cement. J Clin Orthop Trauma 4(4):157–163
94. Carli AV, Sethuraman AS, Bhimani SJ, Ross FP, Bostrom MPG (2018) Selected heat-sensitive antibiotics are not inactivated during polymethylmethacrylate curing and can be used in cement spacers for periprosthetic joint infection. J Arthroplast 33(6):1930–1935
95. Samara E, Moriarty TF, Decosterd LA, Richards RG, Gautier E, Wahl P (2017) Antibiotic stability over six weeks in aqueous solution at body temperature with and without heat treatment that mimics the curing of bone cement. Bone Joint Res 6(5):296–306
96. Young SW, Zhang M, Freeman JT, Mutu-Grigg J, Pavlou P, Moore GA (2014) The Mark Coventry Award: higher tissue concentrations of vancomycin with low-dose intraosseous regional versus systemic prophylaxis in TKA: a randomized trial. Clin Orthop Relat Res 472(1):57–65
97. Roy ME, Peppers MP, Whiteside LA, Lazear RM (2014) Vancomycin concentration in synovial fluid: direct injection into the knee vs. intravenous infusion. J Arthroplast 29(3):564–568
98. Toulson C, Walcott-Sapp S, Hur J, Salvati E, Bostrom M, Brause B, Westrich GH (2009) Treatment of infected total hip arthroplasty with a 2-stage reimplantation protocol: update on "our institution's" experience from 1989 to 2003. J Arthroplasty 24(7):1051–1060
99. Browne JA, Cancienne JM, Novicoff WM, Werner BC (2017) Removal of an infected hip arthroplasty is a high-risk surgery: putting morbidity into context with other major nonorthopedic operations. J Arthroplast 32(9):2834–2841
100. Lum ZC, Natsuhara KM, Shelton TJ, Giordani M, Pereira GC, Meehan JP (2018) Mortality during total knee periprosthetic joint infection. J Arthroplast 33(12):3783–3788
101. Conway JD, Mont MA, Bezwada HP (2004) Arthrodesis of the knee. J Bone Joint Surg Am 86-a(4):835–848
102. Waters RL, Perry J, Antonelli D, Hislop H (1976) Energy cost of walking of amputees: the influence of level of amputation. J Bone Joint Surg Am 58(1):42–46
103. Balato G, Rizzo M, Ascione T, Smeraglia F, Mariconda M (2018) Re-infection rates and clinical outcomes following arthrodesis with intramedullary nail and external fixator for infected knee prosthesis: a systematic review and meta-analysis. BMC Musculoskelet Disord 19(1):361
104. Chen AF, Kinback NC, Heyl AE, McClain EJ, Klatt BA (2012) Better function for fusions versus above-the-knee amputations for recurrent periprosthetic knee infection. Clin Orthop Relat Res 470(10):2737–2745
105. Wu CH, Gray CF, Lee GC (2014) Arthrodesis should be strongly considered after failed two-stage reimplantation TKA. Clin Orthop Relat Res 472(11):3295–3304
106. Son MS, Lau E, Parvizi J, Mont MA, Bozic KJ, Kurtz S (2017) What are the frequency, associated factors, and mortality of amputation and arthrodesis after a failed infected TKA? Clin Orthop Relat Res 475(12):2905–2913
107. Carr JB 2nd, Werner BC, Browne JA (2016) Trends and outcomes in the treatment of failed septic total knee arthroplasty: comparing arthrodesis and above-knee amputation. J Arthroplast 31(7):1574–1577
108. Khanna V, Tushinski DM, Soever LJ, Vincent AD, Backstein DJ (2015) Above knee amputation following total knee arthroplasty: when enough is enough. J Arthroplast 30(4):658–662
109. Castellanos J, Flores X, Llusa M, Chiriboga C, Navarro A (1998) The Girdlestone pseudarthrosis in the treatment of infected hip replacements. Int Orthop 22(3):178–181
110. Cordero-Ampuero J (2012) Girdlestone procedure: when and why. Hip Int 22(Suppl 8):S36–S39

111. Zalavras CG, Rigopoulos N, Ahlmann E, Patzakis MJ (2009) Hip disarticulation for severe lower extremity infections. Clin Orthop Relat Res 467(7):1721–1726
112. Mittal Y, Fehring TK, Hanssen A, Marculescu C, Odum SM, Osmon D (2007) Two-stage reimplantation for periprosthetic knee infection involving resistant organisms. J Bone Joint Surg Am 89(6):1227–1231
113. McKenna PB, O'Shea K, Masterson EL (2009) Two-stage revision of infected hip arthroplasty using a shortened post-operative course of antibiotics. Arch Orthop Trauma Surg 129(4):489–494
114. Hsieh PH, Huang KC, Lee PC, Lee MS (2009) Two-stage revision of infected hip arthroplasty using an antibiotic-loaded spacer: retrospective comparison between short-term and prolonged antibiotic therapy. J Antimicrob Chemother 64(2):392–397
115. Bernard L, Legout L, Zurcher-Pfund L, Stern R, Rohner P, Peter R, Assal M, Lew D, Hoffmeyer P, Uckay I (2010) Six weeks of antibiotic treatment is sufficient following surgery for septic arthroplasty. J Infect 61(2):125–132
116. Bassetti M, Cadeo B, Villa G, Sartor A, Cainero V, Causero A (2014) Current antibiotic management of prosthetic joint infections in Italy: the 'Udine strategy'. J Antimicrob Chemother 69(Suppl 1):i41–i45
117. Darley ES, Bannister GC, Blom AW, Macgowan AP, Jacobson SK, Alfouzan W (2011) Role of early intravenous to oral antibiotic switch therapy in the management of prosthetic hip infection treated with one- or two-stage replacement. J Antimicrob Chemother 66(10):2405–2408
118. Farhad R, Roger PM, Albert C, Pelligri C, Touati C, Dellamonica P, Trojani C, Boileau P (2010) Six weeks antibiotic therapy for all bone infections: results of a cohort study. Eur J Clin Microbiol Infect Dis 29(2):217–222
119. Redfern RE, Cameron-Ruetz C, O'Drobinak SK, Chen JT, Beer KJ (2017) Closed incision negative pressure therapy effects on postoperative infection and surgical site complication after total hip and knee arthroplasty. J Arthroplast 32(11):3333–3339
120. Karlakki SL, Hamad AK, Whittall C, Graham NM, Banerjee RD, Kuiper JH (2016) Incisional negative pressure wound therapy dressings (iNPWTd) in routine primary hip and knee arthroplasties: a randomised controlled trial. Bone Joint Res 5(8):328–337
121. Patel VP, Walsh M, Sehgal B, Preston C, DeWal H, Di Cesare PE (2007) Factors associated with prolonged wound drainage after primary total hip and knee arthroplasty. J Bone Joint Surg Am 89(1):33–38
122. Cizmic Z, Feng JE, Huang R, Iorio R, Komnos G, Kunutsor SK, Metwaly RG, Saleh UH, Sheth N, Sloan M (2019) Hip and knee section, prevention, host related: proceedings of international consensus on orthopedic infections. J Arthroplasty 34(2s):S255–s270

Complications in Orthopedic Trauma Surgery: Fracture-Related Infection

Marc Antoine Burch, T. Fintan Moriarty, Richard Kuehl, Andrew Foster, and Mario Morgenstern

Abstract Fracture-related infection (FRI), in particular when associated with internal fixation hardware, is one of the most dreaded complications in orthopedic trauma surgery. Often hard to diagnose, these infections require input from both surgical and microbiological specialists. As such, these infections extend beyond the sole control of basic orthopedic surgery and demand the input of a multidisciplinary team of specialists in order to be adequately and comprehensively treated. FRI can lead to compromised new bone formation, bone necrosis, and failure for the fracture to heal. It can also result in considerable bone defects created when the infected necrotic bone is surgically removed. The reconstruction of these large bone defects can become a significant, secondary challenge, even for experienced surgeons. The burden of FRI is demonstrated not only in terms of the costs of repeated operative revision and prolonged hospital stay, but also in morbidity and loss of function for the affected patient. In this chapter we summarize the current clinical practices in the diagnosis and management of FRI. In addition, we list several domains in which scientific and technical developments are most needed, such as antimicrobial delivery and diagnostics, and which have the greatest potential to impact clinical practice.

Keywords Fracture-related infection · Orthopedic device-related infection · Fracture · Osteomyelitis · Bacterial infection · Biofilm

M. A. Burch
AO Research Institute Davos, Davos, Switzerland

Clinic for Orthopedics and Trauma Surgery, University Hospital of Basel, Basel, Switzerland

T. F. Moriarty · A. Foster
AO Research Institute Davos, Davos, Switzerland

R. Kuehl · M. Morgenstern (✉)
Clinic for Orthopedics and Trauma Surgery, University Hospital of Basel, Basel, Switzerland
e-mail: mario.morgenstern@usb.ch

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_2

Fracture-Related Infection

A fracture-related infection (FRI) is a bacterial or fungal infection present at a fracture site. In addition to the usual inconvenience encountered with musculoskeletal infection (e.g., pain and loss of function), FRI can additionally lead to osteolysis and consequently compromised stability of the implant, which might ultimately lead to failure of the fracture to heal. This biomechanical component increases the complexity of FRI over and above other musculoskeletal infections. This becomes even more challenging when devitalized, necrotic, infected bone must be excised, leading to large bone defects. Finally, the development of an antibiotic-tolerant bacterial biofilm at the surface of the implanted device often means the implant must be removed. Of course, large bone defects coupled with implant removal can make maintaining biomechanical stability required for later fracture healing a significant challenge. Although many people may be aware of peri-prosthetic joint infection (PJI), it is worthwhile to highlight the features that distinguish FRI from PJI. The main differences between FRI and PJI is the presence of a fracture, which needs eventually to heal, and the possibility to remove the foreign material once this healing is completed (not possible for a joint prosthesis). FRI may also be associated with greater soft-tissue damage, and a wider range of potential pathogens.

The aim of this chapter is to focus on the clinical problem of FRI, with the aim that future scientific endeavors may fully understand the clinical problem of FRI, and make tailor-made solutions building from the current, and real, clinical problems.

Definition

The clinical and scientific literature has provided data on postoperative infection rates of fracture fixation since the advent of internal fracture fixation. However, a clear definition and the establishment of the term *"Fracture-Related Infection"* is relatively new and follows a consensus process initiated in 2016 by an international expert panel [1, 2]. Importantly, in the recent definition, FRI is not subdivided according to the time of presentation to the clinic (e.g., acute vs. chronic, as described by Willeneger in 1986 [3]) because of a lack of scientific evidence supporting such a classification, particularly when it comes to treatment strategies. Also, no reference of the anatomic location or the depth of infection (e.g., superficial, deep, organ/space infection, as in the surgical site infection definition) is made in the new definition, which is also a break from similar past initiatives. The definition includes two levels of certainty for the diagnostic criteria: An infection is present if at least one "confirmatory criteria" can be diagnosed (Table 1 and Fig. 1). The presence of suggestive criteria requires further investigation in order to look for confirmatory criteria [2].

Table 1 Definition criteria for the diagnostic of fracture-related infection [1]

Confirmatory criteria	
Clinical	• Fistula (abnormal communication between two spaces, here most often connecting the fracture site and the skin surface) • Wound breakdown (with communication to the bone or the implant) • Purulent drainage from the wound or presence of pus during surgery
Laboratory	• Presence of microorganisms in deep tissue specimens confirmed by histopathological examination • Phenotypically indistinguishable pathogens identified by culture from at least two separate deep tissues/implants
Suggestive criteria	
Clinical	• Pain, redness, swelling, warmth, loss of function (dolor, rubor, tumor, calor, function laesa), fever • Persistent, increasing, or new-onset wound discharge • New onset of joint effusion
Radiological	• Osteolysis • Implant loosening • Sequester (necrotic bone fragment, often within the cancellous part of the bone) • Failure of progression of bone healing (i.e., nonunion) • Presence of periosteal bone formation (at localizations other than the fracture site or in case of a consolidated fracture)
Laboratory	• Pathogenic organism identified by culture from a single deep tissue/implant specimen • Elevated serum inflammatory markers (white blood cells count, C-Reactive Protein)

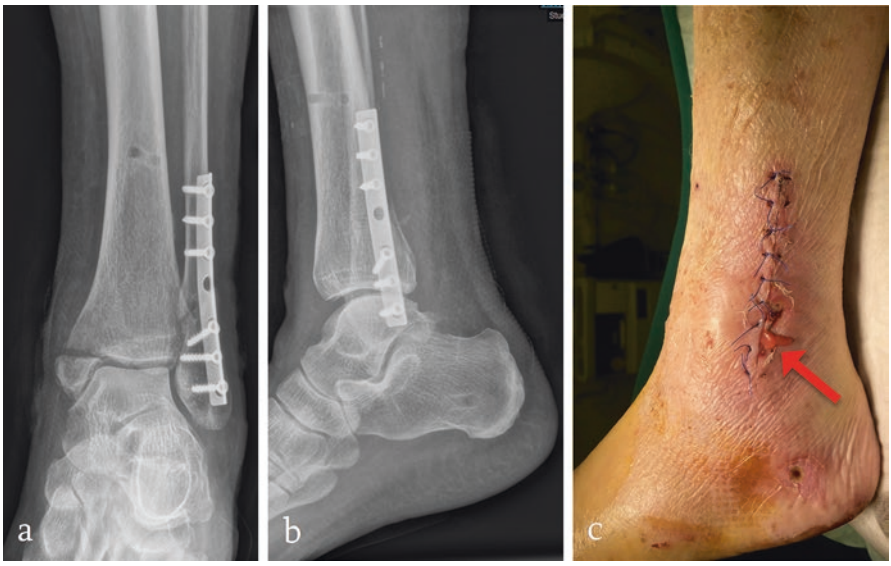


Fig. 1 FRI 4 days after osteosynthesis of a fracture of the lateral malleolus. (**a**, **b**) Conventional radiographic pictures, wherein no evidence of infection is seen at this early stage. (**c**) Clinical presentation, showing that the distal wound is swollen and red and a purulent drainage confirms the diagnosis (red arrow)

Etiology

There are two routes for bacterial colonization of a bone fracture and/or the osteosynthesis device used to fix it. First and most common, *exogenous* contamination takes place either at initial trauma in the case of an open fracture, during surgery, or postoperatively (e.g., in the case of poor wound care). Secondly, and far less commonly, *hematogenous* contamination occurs due to infection at a distant site causing bacteremia/fungemia, which then reaches the implant.

Staphylococcus aureus (*S. aureus*), being either methicillin resistant (MRSA) or sensitive (MSSA), is isolated in approximately 30% of all cases. This Gram-positive bacterium is endowed with virulence factors such as a coagulase, which is believed to help the bacteria escape the host immune system by forming a coagulum around itself [4]. *S. aureus* also possesses microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), which are adhesion proteins with the function of attachment to host structures including bone and collagen [5]. Typically, FRIs caused by *S. aureus* are florid infections with acute manifestations both locally and systemically. Infection with *S. aureus* also provokes osteolysis around implants, which leads to loosening of the fixation within a few weeks.

For other Staphylococcal species, symptoms may be much less dramatic. The coagulase-negative staphylococci (CoNS) are an important group of pathogens in FRI. It includes *Staphylococcus epidermidis* (*S. epidermidis*), an ubiquitous skin commensal [6] which typically develops a biofilm, but does not possess many of the toxins possessed by *S. aureus*. Infection with *S. epidermidis* mostly progresses in a subacute, silent manner, often without clear clinical symptoms [7]. Such low-grade infections may remain overlooked for months and further complicates definitive fracture healing. Another CoNS, *Staphylococcus lugdunensis*, may rather mimic the acute manifestation of *S. aureus* [8, 9]. Other pathogens in the CoNS group include *Staphylococcus saprophyticus*, *Staphylococcus haemolyticus*, and *Staphylococcus simulans*, though these are relatively rarely involved in FRI.

Further Gram-positive cocci known to provoke FRI are enterococci (*Enterococcus faecalis*, *Enterococcus faecium*), human gut-commensals [10, 11] and streptococci (*Streptococcus viridans*, *Streptococcus agalagtiae*, *Streptococcus pyogenes*), which are also often found in human feces or skin samples [12, 13]. Bacilli such as *Bacillus subtilis* or *Bacillus cereus* may also be opportunistic pathogens for open fractures [14].

Gram-negative bacteria account for 15–30% of FRI pathogens [15]. The most common species are Enterobacteriaceae (*Escherichia coli (E. coli)*, *Klebsiella* species, *Serratia* species) which again are normally found in the human gut flora [16], and *Pseudomonas aeruginosa (P. aeruginosa)*, a potent biofilm producer which was shown to be associated with poor healing in FRI [17].

Anaerobic bacteria such as *Cutibacterium* (formerly *Propionibacterium) acnes* and *Clostridium* species are occasionally found to be involved in FRI. *Cutibacterium acnes* is more often present in wounds of the upper extremity [18], probably due to its location in the pilosebaceous gland of the dermis, which are numerous in the shoulder region [19]. *Clostridium perfringens* is more likely to infect open fractures

that have been contaminated with soil [20] and is one of the best-known causative organisms of gas gangrene, a lethal condition if not rapidly treated. Those anaerobic bacteria may be missed in clinical practice in case of improper intraoperative sample harvesting due to their intolerance to oxygen.

Fungal FRI are rarely cited and most commonly affect immunocompromised patients. Usual organisms comprise *Candida* species and *Aspergillus* [21].

Biofilm Formation

As bacteria proliferate on the surface of implants or avascular, necrotic tissue fragments, they initiate the elaboration of the so-called biofilm. The composition of this structure varies according to the bacteria responsible for its development, but mainly consists of a polymeric matrix offering shelter for the microorganisms against cells of the immune system and antibiotic activity. The current concept of the dynamic life cycle of the biofilm consists of a continuum of three phases: attachment, accumulation and maturation, and dispersal [22].

In the first, attachment, bacteria encode adhesion molecules, such as MSCRAMMs, which permits the settlement of individual microbes on the surface of an implant or the margins of the wound. The second phase, accumulation, is where the bacteria divide to increase their numbers. Simultaneously, a shift in the molecular production of the bacteria in order to increase the production of extracellular polymeric substance (EPS) is observed [5], which ultimately leads to the maturation of the biofilm [23]. Clinical studies investigating the treatment of FRIs exhibit a trend toward increased success rates if antibiotic therapy is initiated within 3 weeks after primary fracture fixation, with decreasing success rates if the therapy is initiated thereafter [24]. This finding could be explained through the progressive maturation of the biofilm.

Finally, the third phase of a biofilm's life cycle is dispersal. Perhaps following a lack of local resources, some microorganisms escape the biofilm, which can lead to expansion of the biofilm or creation of a distant new settlement of the microorganism. In theory, this can result in seeding of distant implants, such as a prosthesis.

Incidence and Prevention

Preventing the development of FRI is a matter of optimized patient care at all levels, starting with prehospital management of the injury, intraoperative surgical and asepsis technique, postoperative wound care and follow-up [25].

In closed fractures (i.e., a fracture where the skin envelope is not disrupted), the risk of developing an FRI is around 1%; however, in comminuted high energy open fractures where bony fragments are displaced and skin coverage is breached, the infection rates rise to up to 15–20% [26, 27]. The extent of the trauma, especially the degree of soft-tissue damage, is predictors for a subsequent infection. In order to

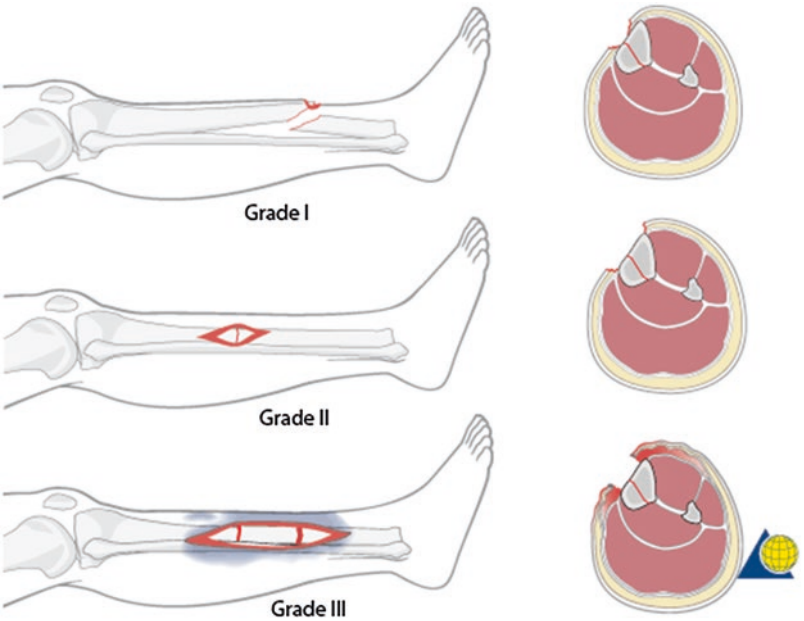


Fig. 2 Schematic overview of the Gustilo–Anderson classification. Due to soft-tissue destruction, a contamination of the wound in grade III is often unavoidable. This situation is correlated with a risk of developing FRI of 15–20% (from: AO surgery reference, principles of management of open fractures, classification of open fracture)

Table 2 A simplified Gustilo and Anderson classification of open fractures [34, 35]

Gustilo type	I	II	III
Wound size	<1 cm	1–10 cm	>10 cm
Soft-tissue damage	Minimal	Moderate	Extensive
Contamination	Minimal	Moderate	Extensive
Infection rate	1–2%	8%	15–20%

stratify the risk of subsequent infection, open fractures can be graded using the Gustilo and Anderson classification, whereby infection risk increases with wound severity (Fig. 2 and Table 2) [28, 29].

Prevention of infection is one of the most important goals in the treatment of open fractures. Systemic antibiotic prophylaxis as well as surgical debridement and irrigation are proven to significantly reduce the risk of infection [25, 30]. Systemic antibiotics should be given as soon as possible, and the specifics (agent and duration) are dependent on the abovementioned degree of soft-tissue injury. The antibiotic prophylaxis should target Gram-positive organisms, whereas additional coverage for Gram-negative organisms should be considered for patients with high-energy open fractures. Several studies showed that a prolonged course of prophylaxis over 72 h did not further decrease the risk of developing FRI [31]. Some authors even found that a shorter course of antibiotic prophylaxis of only

1 day in type III open fractures resulted in no increased infection risk [32]. It should be mentioned that any open wound contaminated with soil might harbor Clostridium. Penicillin or Amoxicillin/Clavulanate should therefore be added to the normal prophylaxis [33].

The Role of Local Antibiotic Administration in the Prevention of FRI

The term local antibiotic administration refers to the application of antibiotics directly to the fracture site, respectively the operative situs, either via an antibiotic-loaded carrier or as a free substance. A systematic review by Morgenstern et al. in 2018 showed a statistically significant reduction of FRI after open fractures when antibiotics were administrated directly to the wound during the primary operation (in addition to the systemically administered antibiotic) versus systemic administration alone [36]. The carrier most often used in the different cohort was tobramycin-loaded poly-(methyl methacrylate) (PMMA, i.e., bone cement).

Despite the fact that loading antibiotics into PMMA bone cement is an established method of local antibiotic delivery, particularly in infections associated with prosthetic joints, its use in the context of prophylaxis for FRI is limited due to its nonbiodegradable nature necessitating the need for further surgery to remove it. Thus, biodegradable carriers are desirable alternatives; however, evidence proving a beneficial effect in infection prophylaxis is scarce [36]. Current commercially available options include gentamicin-loaded collagen fleeces and antibiotic-loaded calcium sulfate paste or pellets (Fig. 3).

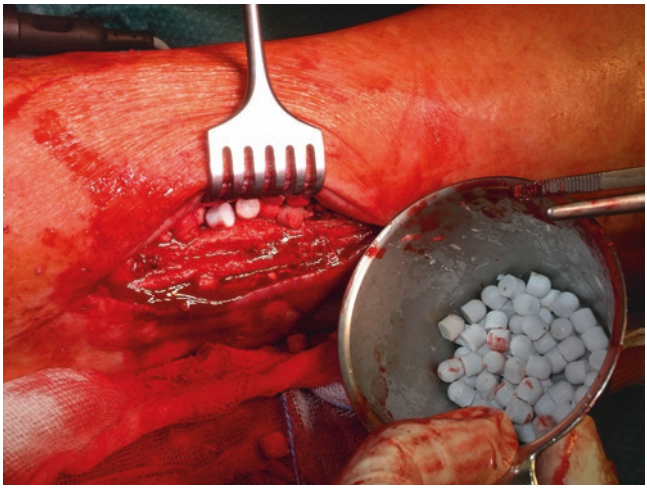


Fig. 3 Insertion of calcium sulfate pellets loaded with vancomycin. These pellets are biodegradables and allow for primary closure of the wound without subsequent removal, which makes these ideal for the prevention of infection in high-risk situations. (With the kind permission of Prof. V. Alt, Universitätsklinikum Regensburg, Germany)

The application of antibiotics without any carrier has also been proposed and rather the antibiotic solution or powder is applied. The advantages of this method are low cost and availability. This approach is already popular in elective spinal surgery and arthroplasty [37] where it reportedly helped decrease the postoperative infection up to fourfold [38, 39]. However, its value in infection prophylaxis for open fractures remains unclear since the evidence remains scarce and somewhat contradictory [40, 41]. The local injection of aqueous gentamicin and tobramycin in the operative wound in the case of open fractures has been studied by Lawing et al. With an odds-ratio of 0.43, the group found a positive effect of locally applied aqueous gentamicin [41]. Similarly, Lovallo et al. [42] showed in shoulder arthroplasty that an additional local injection of antibiotics was an independent predictor of lower infection rates. The application of rifampicin powder was studied only in an animal bone defect model and showed a reduction in infection rate [43]. One note of caution with the use of such "naked" antibiotics in situ, in which delivery is uncontrolled, is that there could be a risk for extremely high local concentrations with toxicity concerns, but also the development of bacterial resistance.

Diagnosis

The assessment and diagnosis of FRI is based on clinical, radiological, and laboratory findings. Those might be subtle signs or, on the contrary, obvious abnormalities, depending on the causative pathogen, the timing and dynamic of the infection, the immune status of the host or the experience of the physician. The definitive diagnosis should be made in the presence of one of the confirmatory criteria (see Table 1), most often after obtaining the microbiological results from samples taken intraoperatively. Therefore, the cornerstone of diagnosis is proper sampling, which is done during surgical debridement.

Clinical Signs

The classical inflammatory signs such as pain, redness, swelling, and warmth might be present and should alert the physician, especially when localized in the vicinity of the operated zone. However, "low-grade infections," which are mainly caused by less virulent pathogens might lack such typical infection symptoms. The presence of a sinus, a fistula, or a wound breakdown with direct communication with the involved bone or osteosynthesis material are considered as a confirmatory criterion. A subtle sign is the onset of joint effusion in case of FRI with direct synovial connection, for instance after operative reduction of an intraarticular fracture [1].

Radiological Signs

The different radiological modalities are all of value for different aspects of FRI; however, none can offer any certainty about a definitive diagnosis.

Plain radiography is the investigation of first choice. This modality is inexpensive as well as widely available and allows for judgment of implant positioning, fracture reduction, bone union, and progress of osseous healing. However, computed tomography (CT Scan) allows for a more detailed visualization of those aspects, though is not always necessary (Fig. 4). Repeated follow-up X-rays allow judgment of infection evolution and are standard practice over time.

A CT scan permits a three-dimensional assessment of the bony structure and fracture consolidation and has the best sensitivity for detecting sequestrate, i.e., necrotic bone fragments. Intramedullary gas can occasionally be seen, most often as a sign of infection [44]. CT has limited sensitivity for soft-tissue changes and the visualization of the zone of interest may be troubled by metal artifacts provoked by the implants in the immediate vicinity.

There is limited value for ultrasonography in the context of FRI. It may be used to assess surrounding soft tissues but does not allow a high-resolution visualization of bone or implant. It permits however guidance of probes used for diagnostic punctures, confirms a joint effusion, or defines a collection in the overlying soft tissues.

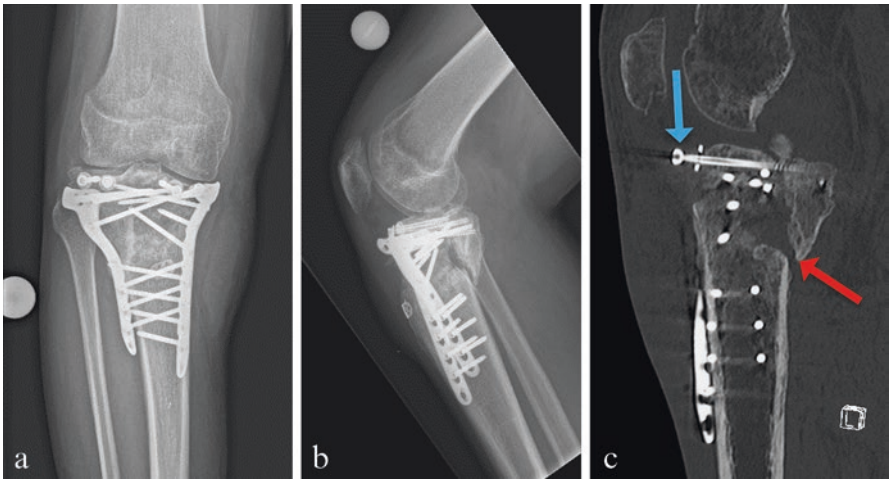


Fig. 4 A case of FRI 12 weeks after osteosynthesis of a fracture of the proximal tibia demonstrating the value of a CT Scan over conventional radiography. (**a** and **b**): conventional radiographs (**a**: frontal view, **b**: lateral view), (**c**): CT Scan, sagittal view. Loosening of a screw (blue arrow) and failure to heal of the posteromedial fragment of the tibia plateau (red arrow) are evident on a CT scan but are difficult to see in conventional radiography. Both features are suggestive criteria for FRI

The best modality in detecting soft-tissue changes and bone marrow edema is magnetic resonance imaging (MRI) [44]. MRI studies can also be valuable in detecting osseous changes such as peri-cortical abscesses or cortical defects [45]. Drawbacks are the limited availability, the time of image acquisition, and some contraindications such as claustrophobia, or presence of metallic implants in the patients' body (e.g., cardiac pacemakers, intracranial aneurysm coils). Orthopedic/trauma implants may cause artifact formation, which may be reduced with new protocols. A further limitation of MRI in postoperative and post-traumatic setting is the presence of scarring tissue, which may exhibit analogous signal intensity as does infected tissues [44, 46].

Nuclear imaging, a method in which a radioisotope is injected in a patient's body before acquiring its emission with a gamma-camera might become more common in clinical practice. Modern uses include triple-phase, gallium, leukocyte-labeled, and ^{18}F-fluorodeoxyglucose scans. In triple phase, the radioisotope used is technetium-99m-labeled MDP (Tc^{99m}-MDP), followed by the image acquisition in three phases: angiographic, soft tissue, and bone-Phase. Tc^{99m}-MDP has the tendency to concentrate in zones of increased osteoblastic activity and shows high uptake in all three phases in case of osteomyelitis [47]. Unfortunately, since triple-phase scans shows a decreased specificity in profaned bone, its place in diagnosing FRI is marginal [48].

Gallium-67-scintigraphy (Ga-67) has a higher specificity than triple-phase scans in bone infections and is especially used to diagnosis spine infections. The radioisotope binds various plasmatic proteins, particularly Transferrin, and accumulates in infected areas. The major drawback is the time needed to complete the study, the scan having to be made 48–72 h after the injection. This modality is also the one requiring the most radiation in comparison to the other nuclear-imaging modalities we present [49].

Indium-111 or Technetium-99-hexamethylpropyleneamine oxime (Tc-99m-HMPAO) are isotopes used to mark leukocytes, which accumulate in zones of increased bone turnover in case of infection. However, since the cells also accumulate in bone marrow, this method is often used in combination with a bone marrow scan that uses a Tc99m-labeled colloid. The discrepancy between both studies reveals an infectious focus. This could make leukocyte-labeled scans the method of choice to diagnose FRI in unclear cases [50, 51]. When the images are compared to an additional CT scan, its specificity and sensitivity can reach up to 97% and 100%, respectively. Major drawbacks are a poor global availability, a long acquisition time, high costs, and center-dependent protocols [47, 52].

Laboratory Findings

White blood cell counts (WBC) and C-reactive proteins (CRP) are unspecific markers of inflammation which are of little value in diagnosing FRI. They can nevertheless provide information about the evolution of an infection [53]. Serum immune biomarkers such as interleukin 6 (IL-6), human-α-defensin 1-3, or neutrophil-elastase 2 have been found to have some specificity in the setting of PJI, but have not been studied for FRI to date [44, 53, 54].

Microbiological Findings

Deep tissue samples that were harvested during surgical debridement at the site of perceived infection are the gold standard for diagnosing FRI. Peripheral blood cultures are mostly culture-negative since the majority of bacteria remain in the biofilm at the fracture site [44]. Tissue samples must be transported to the microbiology lab in a sterile tube. In case of pus collection, it is advisable to collect it in blood culture bottles, which have been shown to achieve high sensitivity [55]. The use of swabs for sampling has been shown to lead to unacceptable false-negative and false-positive results and is therefore not recommended [56].

Removed implants should be sent for sonication in a sterile container. The ultrasonic bath disrupts the biofilm on the implant and allows better detection of the bacteria [15, 57]. In order to be considered as a true causative organism, rather than a contaminant, the identical bacteria must be isolated in at least two out of the five samples [44]. Some bacteria exhibit a slower growth pattern or need special conditions in order to be detected in the microbiology laboratory. Examples for the former are so-called small colony variants (SCV), i.e., metabolically altered variants of common bacteria such as *S. aureus*, *S. epidermidis*, *E. coli*, or *P. aeruginosa*. Those variants can harbor genetic alterations leading not only to reduced growth, but also atypical colony morphology, and unusual biochemical characteristics, which complicates the microbiological identification [58]. Interestingly, SCV can survive in mammalian cells such as fibroblasts and osteoblasts, making their identification and eradication even more difficult. Such an entity is rarely described in FRI [59], much more often in PJI [60]. Other slow-growing organisms include aerobic Gram-positive bacilli and Peptostreptococcus species [61]. These organisms typically involve anaerobic bacteria such as *Cutibacterium acnes* and *Clostridium spp.* which might not be recovered in cultures unless appropriate conditions are maintained. It is therefore recommended to rapidly transfer tissue samples to an anaerobic environment and to extend the incubation time of both aerobic and anaerobic culture media for 14 days in case such an infection is suspected [62, 63].

Polymerase chain reaction (PCR) could become an efficient, rapid, and precise method to detect pathogens in FRI. It has been shown in one study to be non-inferior to traditional bacterial culture, delivering results within 5 h [64]. The powerful amplification of this method may however provoke false-positive results by expanding contaminants [65]. In any case, traditional bacterial culture is still needed in order to test the antibiotic susceptibility of the microorganisms, as genotypic tests are not yet 100% indicative of phenotypic resistance.

Treatment

The objective of treatment in the case of FRI is fracture healing, eradication or suppression of the infection, restoration of functionality, and prevention of chronic osteomyelitis [15]. Infection eradication is not always the sole or primary goal in treatment, since the osteosynthesis device may be definitively removed (and the biofilm upon it) once the fracture is healed.

These goals can be achieved by one of two surgical principles: debridement and implant retention (sometimes termed DAIR in the literature, for Debridement, Antibiotics, Implant Retention) or debridement and implant removal and replacement. The latter can be achieved either with direct (single stage) exchange, or with implant replacement after an implant-free interval.

The cornerstone in FRI treatment is the operative debridement. In order to allow adequate reduction of the bacterial load, all necrotic materials should be judiciously excised. Copious rinsing of the wound should occur, either with saline or with the addition of an antiseptic such as polyhexanide [66]. This operation also allows collection of biopsies for culture. The stability of the fracture fixation must be assessed, and any loose material, including implants and bone, removed. Good quality tissue sampling should ensure optimal identification of the responsible pathogens. The condition of the soft tissue (i.e., degree of swelling, presence of necrosis, see Fig. 5) guides the decision between primary (i.e., immediate), or delayed closure. Living soft-tissue envelope contributes to infection eradication and bone healing by providing perfusion. If closure of the wound with the remaining tissues is not possible, plastic surgery is required.

There has been some debate about the preoperative antibiotic prophylaxis for these revision procedures, some seeing it as a risk for a successful tissue culture, while others prioritize the prevention of secondary surgical site infection. A recent review of PJI concluded that the risk of the latter exceeds the small amount of growth-negative culture due to preoperative prophylaxis and therefore recommends preoperative antibiotic prophylaxis [67]. Such data are unavailable in the setting of FRI at the present time.

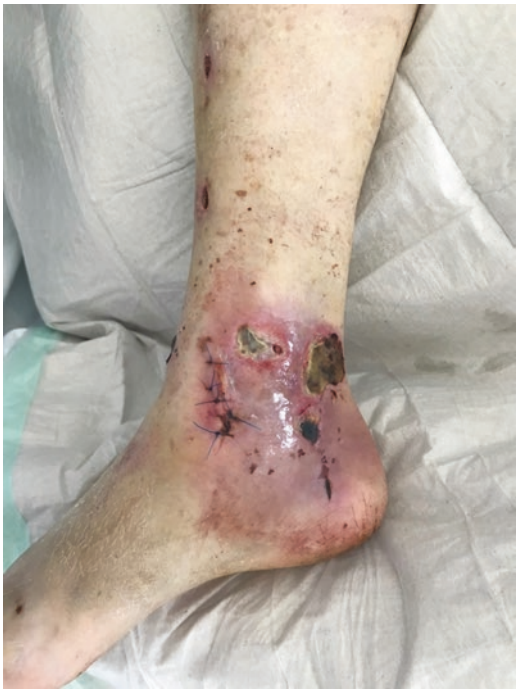


Fig. 5 FRI early after osteosynthesis of a fracture of the medial malleolus. An extended soft-tissue necrosis is seen, which has to be excised. Reconstructive surgical techniques (e.g., skin graft, local flap) probably have to be used

Stability is one of the most important factors in order to achieve fracture healing: it was shown in animal models that a fracture could heal even in the presence of an infection if the implant remained stable [68]. In such a case, the retention of the implanted material (i.e., DAIR) can be tempting. Besides stability, preconditions for DAIR are vital soft tissue envelope, absence of necrotic bone, ability to perform a proper debridement, and importantly, the time interval between fracture fixation and FRI manifestation. DAIR is believed to exhibit better results when the infection is recognized, and the treatment initiated within 3 weeks after primary fixation [24, 69, 70]. After this time, treatment success rates may drop, and this is believed to be due to the development of a mature biofilm on the surface of the device.

If the above-stated conditions are not fulfilled, the removal of all foreign material is advised to achieve fracture healing. Depending on the situation, the non-healed fracture can be stabilized temporarily with an external fixator or with a new internal fixation device.

In case of infection with a so-called "difficult to treat" organism (i.e., an organism against which no biofilm-active antibiotic is available due to antibiotic resistance of the pathogen, drug intolerance of the patient, or incompatible drug interactions), only a "suppressive" antibiotic strategy is an option. It is continued until the fracture is consolidated and the removal of the internal osteosynthesis construct. Finally, in case of consolidated fracture at the time of FRI diagnosis, all fixation material should be removed and an osteomyelitis antibiotic treatment with a duration of 6 weeks should be initiated.

The adequate choice of systemic antibiotic therapy is of upmost importance whether for retention or exchange/removal strategy. Decision should be made in an interdisciplinary setting, taking in account the resistance profile of the pathogen as well as the host physiology (existence of allergy against antibiotics, immunosuppression, kidney and liver function, conditions such as diabetes mellitus). The course of the treatment is often initiated intravenously and continued in a *per os*, targeted fashion once culture results are available. In Table 3, an antibiotic schema proposed by Zimmerli in 2015 is listed [71]. It is important to note that the rate of penetration of antibiotics in the biofilm may vary and that many antibiotics seem to be unable or poorly able to fully penetrate the biofilm [23, 72]. Of special interest, rifampin (in case of staphylococcal biofilms) and ciprofloxacin (in case of Gram-negative biofilms) have displayed bactericidal activity against biofilms [73].

Finally, as a consequence of the initial trauma as well as of the treatment, the soft-tissue envelope of the affected limb is in many cases compromised. Early discussion with plastic surgeons helps define the best curative strategy to ensure a vital soft-tissue envelope overlaying the bone. The scope of soft-tissue reconstruction is broad, from simple skin graft to free flaps, where plastic and vascular surgery expertise is required (Fig. 6).

Dead Space Management

As stated above, adequate debridement is an essential step in the treatment of FRI. However, this procedure can lead to extensive void formation, a poorly vascularized "dead space" which is filled with hematoma, providing a weak point for

Table 3 Proposed antibiotic treatments according to pathogen (Adapted from Zimmerli [71] and Metsemakers [74])

Pathogen	Antibiotic
Staphylococcus spp.	
Methicillin sensible	Flucloxacillin + Rifampin, followed by Rifampin + Ciprofloxacin or Rifampin + Cotrimoxazole
Methicillin resistant	Vancomycin/Daptomycin + Rifampin, followed by Rifampin + Ciprofloxacin or Rifampin + Cotrimoxazole
Streptococcus spp.	Penicillin or Ceftriaxon, followed by Amoxicillin or Clindamycin
Enterococcus spp.	
Penicillin sensible	Amoxicillin or Penicillin
Penicillin resistant	Vancomycin or Daptomycin or Linezolid
Enterobacteriaceae	β-lactam according to sensibility
Nonfermenters	third- or fourth-generation Cephalosporin with nonfermenter activity, followed by Ciprofloxacin
Cutibacterium spp.	Penicillin or Ceftriaxon, followed by Amoxicillin or Clindamycin
Gram-negative anaerobes	Metronidazole
Fungus	antifungal agent according to sensibility
Culture-negative	β-lactam, followed by Rifampin + Levofloxacin

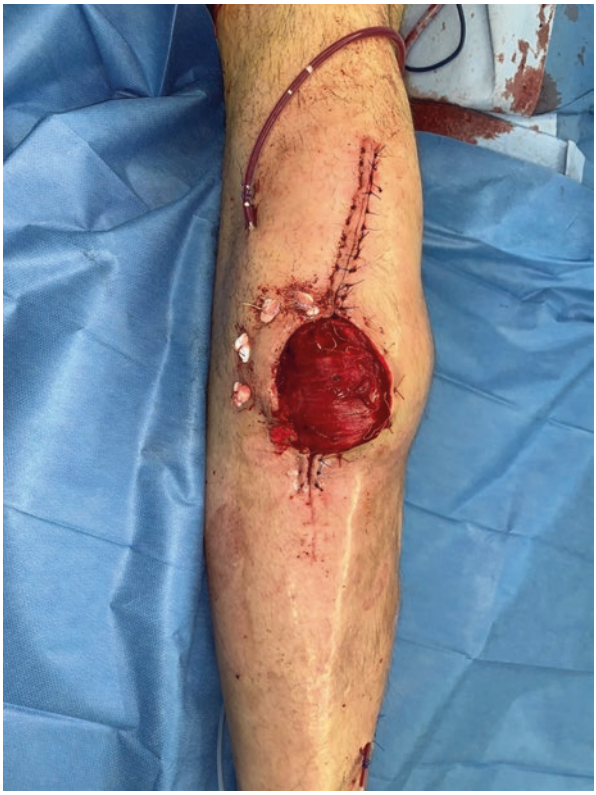


Fig. 6 An example of regional flap surgery: The medial part of the musculus Gastrocnemius has been used in order to cover the anterior part of the knee in a case of FRI after osteosynthesis of a fracture of the proximal tibia

bacterial growth and biofilm formation. Because of the poor vascularization, adequate antibiotic concentrations might not be obtained in the void. Therefore, the local application of antibiotics was shown to be beneficial in this situation [75, 76]. Depending on the extension of the dead space (cavitary, segmental, intramedullary), different antibiotic-loaded bone void fillers may be used.

The oldest and best-known carrier is PMMA. It can be used in the form of a cement spacer, mainly in the case of a bone defect, or as cement beads. The most common antibiotics used with this method are gentamicin or tobramycin, bactericidal aminoglycosides active against many Gram-positive and Gram-negative strains [77]. Another commonly used antibiotic in PMMA is vancomycin, a bactericidal tricyclic glycopeptide inhibiting bacterial cell wall synthesis. It is the drug of choice for treating MRSA infections [74]. Vancomycin is a large molecule unable to penetrate the outer membrane of Gram-negative bacteria, showing therefore no action against such microbes [78].

Notably, rifampin, the most effective drug acting against a staphylococcal biofilm, seems to interfere with the ability of PMMA to harden [79] and is therefore not recommended. However, Likine et al. [80] recently described a method allowing for the incorporation of rifampin and tobramycin in PMMA. The authors described a delayed but successful hardening of the cement.

The drawback of PMMA is that only 10% of the incorporated antibiotics are released and that most of them are released in the first days (so called "burst-release"). Therefore, subtherapeutic antibiotic levels are released, which increase the risk of developing bacterial resistance and bacterial growth on the spacer [74, 81]. Furthermore, another issue is the burdensome reoperation to extract PMMA, which bears again the risk of a perioperative exogenous infection (Fig. 7).

Fig. 7 Application of PMMA beads loaded with gentamicin around a plate on the lateral aspect of the femur. Those beads have to be surgically removed in a further operative session. (With the kind permission of Prof. V. Alt, Universitätsklinikum Regensburg, Germany)

PMMA may be used to fill bone defects (e.g., created after extensive debridement) and replaced for instance with cancellous bone graft once the infection is healed (Fig. 8).

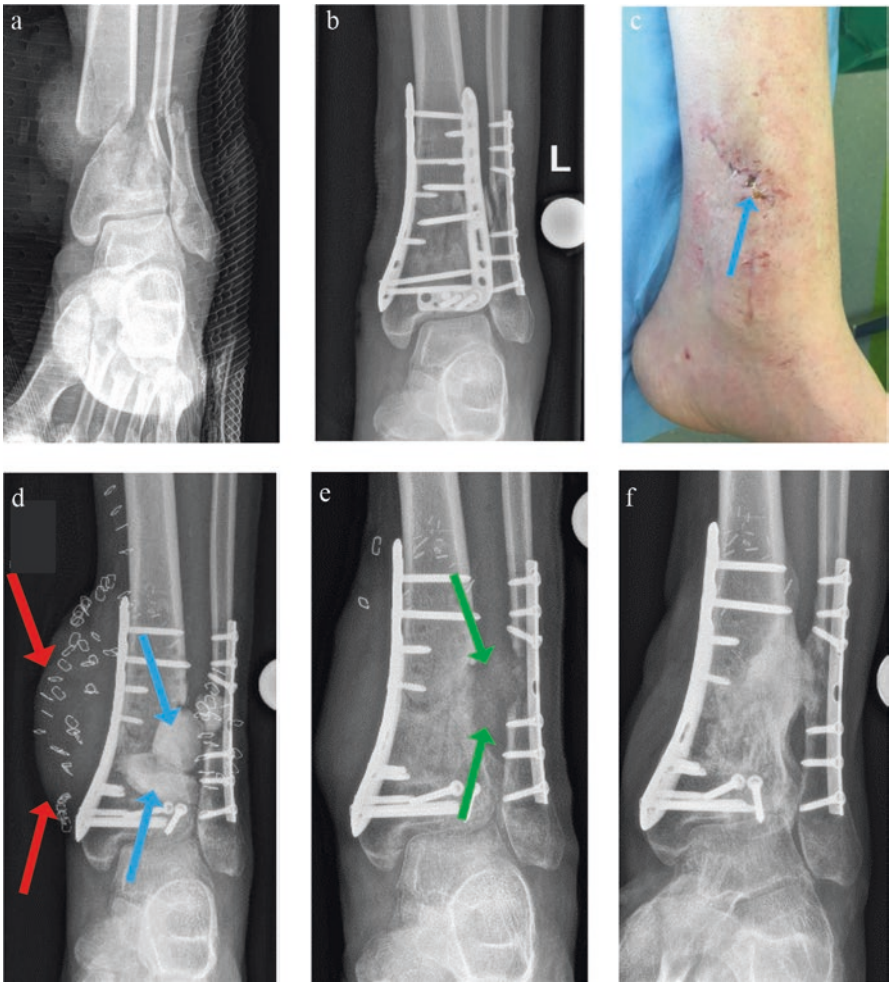


Fig. 8 Open fracture of the distal tibia and fibula. (**a**): first radiograph at arrival in the emergency room. (**b**): fracture reduction and plate osteosynthesis. (**c**): clinical presentation several months after initial treatment. The presence of a discrete fistula with purulent discharge (blue arrow) is the only confirmatory criteria for FRI. (**d**): radiography after operative revision: the posterior plate (central on picture **b**) has been removed. A large defect was created during debridement and was filled with antibiotic-loaded PMMA (blue arrows). The soft tissue had also to be extensively debrided, which required plastic coverage (free muscle flap, red arrows). (**e**): Situation after removal of the PMMA. The infection is now controlled, and the defect is filled with a cancellous bone graft (pale "cloud" defined by the green arrows). (**f**): consolidated fracture, the cancellous bone graft has matured, and healing is completed

Commercially available degradable carriers include gentamicin-loaded collagen fleeces and ceramics such as calcium sulfate, calcium phosphate loaded with tobramycin or gentamicin. Gentamicin-loaded collagen fleeces release the majority of the incorporated antibiotics within the first hours and days after application; however, degradation may take up to 8 weeks [82]. These fleeces have shown good clinical results; however, the evidence is limited due to the small scale of the clinical trials [83, 84]. In recent years, various publications have also shown promising results for biodegradable ceramics [85]. The success rates were comparable with PMMA, however without the need of a consecutive surgical removal of the latter [86]. Some concern remains regarding the dissolution of calcium sulfate pellets, which can produce an acidic environment as well as seroma, both of which are deleterious for wound healing. Those side effects are less common with calcium phosphate, probably due to its delayed dissolution.

Emerging Strategies Against FRI

Prevention

Since the application of local antibiotics as an adjunct to systemic prophylaxis reduces the incidence of FRI in open fracture cases [36, 87], its application may be considered clinically useful. In terms of future innovations, the optimal carrier is one important issue. The ideal material should be easy to use, biocompatible or bioresorbable (i.e., not needing secondary removal surgery), mechanically strong, osteoinductive, and with a predictable antibiotic-eluting profile [88]. Such an ideal material does not exist at the present time, although more interventions enter the clinic every few years.

Some success was attained with a combination of vaccination in adjunction to a standard antibiotic regimen, in the form of a quadrivalent vaccine aimed at glucosaminidase, an ABC transporter lipoprotein, a conserved hypothetical protein, and a conserved lipoprotein, which was tested in vitro and in vivo and displayed a better clearance of *S. aureus* biofilm in addition to antibiotics than antibiotics alone in a rabbit model [89]. A solution against biofilm formation on a foreign material may reside in a hardware coating, with numerous publications in the scientific literature emerging on a regular basis. Silver, for instance, which has been used as an antimicrobial agent for centuries, has been recently reported to show extended anti-biofilm activity in the form of silver oxynitrate (Ag_7NO_{11}) [90]. Antimicrobial peptides (AMPs), antimicrobial molecules found in the innate immunity of plants and animals [91], have also shown activity against biofilm formation [92, 93]. Monolaurin, a fatty acid which seems to exhibit antibacterial as well as antifungal and antiviral properties has also been studied [94]. Chitosan, a polysaccharide able to "carry" antibiotics, exhibits good biocompatibility in vitro [95], and so does a direct coating with antibiotics, e.g., Vancomycin, which inhibited biofilm formation in an in vitro study [96]. Notably, gentamicin-loaded coated implants (e.g., intramedullary nails) already exist and are commercially available.

Diagnosis

High quality clinical studies are needed in order to refine the definition of FRI and identify subcategories in which adaptation of treatment could optimize outcomes, e.g., acute vs. chronic infection.

Further clinical studies assessing nuclear imaging could evaluate the cost-effectiveness of those expensive techniques. New imaging modalities using quantum dot-labeled anti-biofilm antibodies in combination with bioluminescence and fluorescence in vivo are being use in laboratories and could be an interesting area for translation to clinical practice [97].

Whereas several interesting biomarkers have been isolated in PJI, none have been found to have enough specificity to help diagnosing FRI. The determination of an immune mediator (e.g., cytokine, acute phase protein, AMP) could have the potential to greatly enhance diagnostic accuracy [44].

Studies specifically using PCR as an addition to traditional culture in the context of FRI should be conducted, not only in order to accelerate pathogen determination in a clinical setting, but also to assess if this method permits a reduction in the rate of FRI without pathogen identification [97].

Treatment

FRI with antibiotic-resistant bacteria are challenging to treat. Of particular concern is the development of resistance to biofilm-active antibiotics (Rifampin and Ciprofloxacin) [91], and an effort to expand the range of antibiotic substances is needed.

In addition to the development of an anti-biofilm vaccine, progression in the field of biofilm-disaggregating agents such as DNase I, an eDNA-digesting enzyme, or Dispersin B, a hydrolase able to degrade poly-N-acetylglucosamine produced in *Staphylococcus* spp. biofilms [97] is of special interest. Another target for biofilm disruption is quorum sensing (QS), the interbacterial communication with small signal molecules [98]. Hentzer et al. conducted experiments showing that disruption of this means of communication reduced the expression of virulence factors in vitro and helped mice to get rid of a *P. aeruginosa* pulmonary infection in vivo [99].

The emergence of antibiotic resistance led researchers to seek alternative antimicrobial therapy. Bacteriophages are viruses discovered in the early twentieth century by Twort [100, 101]. Although having been commonly used in the former Soviet Union in the last 60 years [102], this treatment option has only been marginally valued in western society. Its resurgence in recent years, with laboratory as well as clinical studies offers new perspective in the treatment of recalcitrant infections. Of special interest is the possible ability of bacteriophages to attack bacterial biofilms, which have been demonstrated against MRSA biofilms in vitro [103, 104]. There is no literature about its use in FRI to date.

Conclusion

FRI is a challenging clinical problem, distinct from PJI and warrants customized innovations in prevention, diagnosis, and treatment. Currently, FRI rates remain high in open fractures in particular, despite best medical practices, and therefore, new innovations are required. Increasing antibiotic resistance in common pathogens only exacerbates this situation. Advances in biomaterial design for the prevention, diagnosis, and treatment for FRI have, therefore, not only great potential for significant clinical impact in socioeconomic costs, but also patient welfare.

References

1. Metsemakers WJ, Morgenstern M, McNally MA et al (2018) Fracture-related infection: a consensus on definition from an international expert group. Injury 49:505–510
2. Metsemakers WJ, Kortram K, Morgenstern M et al (2018) Definition of infection after fracture fixation: a systematic review of randomized controlled trials to evaluate current practice. Injury 49:497–504
3. Willenegger H, Roth B (1986) Treatment tactics and late results in early infection following osteosynthesis. Unfallchirurgie 12:241–246
4. Thomer L, Schneewind O, Missiakas D (2016) Pathogenesis of Staphylococcus aureus bloodstream infections. Annu Rev Pathol 11:343–364
5. Nishitani K, de Mesy Bentley KL, Daiss JL (2016) Implant-associated biofilm. Principles of orthopedic infection management. AO Foundation, Davos
6. Nguyen TH, Park MD, Otto M (2017) Host response to Staphylococcus epidermidis colonization and infections. Front Cell Infect Microbiol 7:90
7. Sabate Bresco M, Harris LG, Thompson K et al (2017) Pathogenic mechanisms and host interactions in Staphylococcus epidermidis device-related infection. Front Microbiol 8:1401
8. Gahukamble AD, McDowell A, Post V et al (2014) Propionibacterium acnes and Staphylococcus lugdunensis cause pyogenic osteomyelitis in an intramedullary nail model in rabbits. J Clin Microbiol 52:1595–1606
9. Lourtet-Hascoet J, Bicart-See A, Felice MP, Giordano G, Bonnet E (2016) Staphylococcus lugdunensis, a serious pathogen in periprosthetic joint infections: comparison to Staphylococcus aureus and Staphylococcus epidermidis. Int J Infect Dis 51:56–61
10. Lee K, Lee KM, Kim D, Yoon SS (2017) Molecular determinants of the thickened matrix in a dual-species Pseudomonas aeruginosa and Enterococcus faecalis biofilm. Appl Environ Microbiol 83
11. Frank KL, Guiton PS, Barnes AM et al (2013) AhrC and Eep are biofilm infection-associated virulence factors in Enterococcus faecalis. Infect Immun 81:1696–1708
12. Castillo Pedraza MC, Novais TF, Faustoferri RC et al (2017) Extracellular DNA and lipoteichoic acids interact with exopolysaccharides in the extracellular matrix of Streptococcus mutans biofilms. Biofouling 33:722–740
13. Chao Y, Marks LR, Pettigrew MM, Hakansson AP (2014) Streptococcus pneumoniae biofilm formation and dispersion during colonization and disease. Front Cell Infect Microbiol 4:194
14. Nguyen L, Minville V, Bensafi H et al (2007) Open leg fracture with Bacillus cereus infection. Ann Franc D'anesth Reanim 26:780–783
15. Trampuz A, Zimmerli W (2006) Diagnosis and treatment of infections associated with fracture-fixation devices. Injury 37(Suppl 2):S59–S66

16. Osterblad M, Hakanen A, Manninen R et al (2000) A between-species comparison of antimicrobial resistance in enterobacteria in fecal flora. Antimicrob Agents Chemother 44:1479–1484
17. Berkes M, Obremskey WT, Scannell B et al (2010) Maintenance of hardware after early postoperative infection following fracture internal fixation. J Bone Joint Surg Am 92:823–828
18. Zaid M, Chavez MR, Carrasco AE et al (2019) Cutibacterium (formerly Propionibacterium) acnes clavicular infection. J Bone Joint Infect 4:40–49
19. Patel A, Calfee RP, Plante M, Fischer SA, Green A (2009) Propionibacterium acnes colonization of the human shoulder. J Shoulder Elbow Surg 18:897–902
20. Ibnoulkhatib A, Lacroix J, Moine A et al (2012) Post-traumatic bone and/or joint limb infections due to Clostridium spp. Orthop Traumatol Surg Res 98:696–705
21. Azzam K, Parvizi J, Jungkind D et al (2009) Microbiological, clinical, and surgical features of fungal prosthetic joint infections: a multi-institutional experience. J Bone Joint Surg Am 91(Suppl 6):142–149
22. Joo HS, Otto M (2012) Molecular basis of in vivo biofilm formation by bacterial pathogens. Chem Biol 19:1503–1513
23. Stewart PS (2015) Antimicrobial tolerance in biofilms. Microbiol Spectr 3
24. Kuehl R, Tschudin-Sutter S, Morgenstern M et al (2019) Time-dependent differences in management and microbiology of orthopaedic internal fixation-associated infections: an observational prospective study with 229 patients. Clin Microbiol Infect 25:76–81
25. Metsemakers WJ, Onsea J, Neutjens E et al (2017) Prevention of fracture-related infection: a multidisciplinary care package. Int Orthop 41:2457–2469
26. Patzakis MJ, Wilkins J (1989) Factors influencing infection rate in open fracture wounds. Clin Orthop Relat Res 243:36–40
27. Kortram K, Bezstarosti H, Metsemakers WJ, Raschke MJ, Van Lieshout EMM, Verhofstad MHJ (2017) Risk factors for infectious complications after open fractures; a systematic review and meta-analysis. Int Orthop 41:1965–1982
28. Yim GH, Hardwicke JT (2018) The evolution and interpretation of the Gustilo and Anderson classification. J Bone Joint Surg Am 100:e152
29. Gustilo RB, Anderson JT (1976) Prevention of infection in the treatment of one thousand and twenty-five open fractures of long bones: retrospective and prospective analyses. J Bone Joint Surg Am 58:453–458
30. Chang Y, Kennedy SA, Bhandari M et al (2015) Effects of antibiotic prophylaxis in patients with open fracture of the extremities: a systematic review of randomized controlled trials. JBJS Rev 3
31. Luchette FA, Borzotta AP, Croce MA et al (2000) Practice management guidelines for prophylactic antibiotic use in penetrating abdominal trauma: the EAST Practice Management Guidelines Work Group. J Trauma 48:508–518
32. Dunkel N, Pittet D, Tovmirzaeva L et al (2013) Short duration of antibiotic prophylaxis in open fractures does not enhance risk of subsequent infection. Bone Joint J 95-B:831–837
33. Anderson A, Miller AD, Brandon BP (2011) Antimicrobial prophylaxis in open lower extremity fractures. Open Access Emerg Med 3:7–11
34. Thakore RV, Francois EL, Nwosu SK et al (2017) The Gustilo-Anderson classification system as predictor of nonunion and infection in open tibia fractures. Eur J Trauma Emerg Surg 43:651–656
35. Guerra MTE, Gregio FM, Bernardi A, Castro CC (2017) Infection rate in adult patients with open fractures treated at the emergency hospital and at the ULBRA university hospital in Canoas, Rio Grande do Sul, Brazil. Rev Brasil Ortop 52:544–548
36. Morgenstern M, Vallejo A, McNally MA et al (2018) The effect of local antibiotic prophylaxis when treating open limb fractures: a systematic review and meta-analysis. Bone Joint Res 7:447–456
37. Otte JE, Politi JR, Chambers B, Smith CA (2017) Intrawound vancomycin powder reduces early prosthetic joint infections in revision hip and knee arthroplasty. Surg Technol Int 30:284–289

38. Sweet FA, Roh M, Sliva C (2011) Intrawound application of vancomycin for prophylaxis in instrumented thoracolumbar fusions: efficacy, drug levels, and patient outcomes. Spine 36:2084–2088
39. Bakhsheshian J, Dahdaleh NS, Lam SK, Savage JW, Smith ZA (2015) The use of vancomycin powder in modern spine surgery: systematic review and meta-analysis of the clinical evidence. World Neurosurg 83:816–823
40. Singh K, Bauer JM, LaChaud GY, Bible JE, Mir HR (2015) Surgical site infection in high-energy peri-articular tibia fractures with intra-wound vancomycin powder: a retrospective pilot study. J Orthop Traumatol 16:287–291
41. Lawing CR, Lin FC, Dahners LE (2015) Local injection of aminoglycosides for prophylaxis against infection in open fractures. J Bone Joint Surg Am 97:1844–1851
42. Lovallo J, Helming J, Jafari SM et al (2014) Intraoperative intra-articular injection of gentamicin: will it decrease the risk of infection in total shoulder arthroplasty? J Shoulder Elbow Surg 23:1272–1276
43. Shiels SM, Tennent DJ, Wenke JC (2018) Topical rifampin powder for orthopedic trauma part I: Rifampin powder reduces recalcitrant infection in a delayed treatment musculoskeletal trauma model. J Orthop Res 36:3136–3141
44. Morgenstern M, Kuhl R, Eckardt H et al (2018) Diagnostic challenges and future perspectives in fracture-related infection. Injury 49(Suppl 1):S83–S90
45. Lee YJ, Sadigh S, Mankad K, Kapse N, Rajeswaran G (2016) The imaging of osteomyelitis. Quant Imaging Med Surg 6:184–198
46. Rajashanker B, Whitehouse RW (2015) Chapter 53. Bone, joint and spinal infection. In: Grainger & Allison's diagnostic radiology, 6th edn. Churchill Livingstone, New York, pp 1241–1242
47. Mettler F, Guiberteau M (2012) Chapter 8. Skeletal system. In: Essentials of nuclear medicine imaging, 6th edn. Saunders Elsevier, Philadelphia, pp 296–300
48. Christian S, Kraas J, Conway WF (2007) Musculoskeletal infections. Semin Roentgenol 42:92–101
49. Palestro CJ, Torres MA (1997) Radionuclide imaging in orthopedic infections. Semin Nucl Med 27:334–345
50. Al-Sheikh W, Sfakianakis GN, Mnaymneh W et al (1985) Subacute and chronic bone infections: diagnosis using In-111, Ga-67 and Tc-99m MDP bone scintigraphy, and radiography. Radiology 155:501–506
51. Govaert GA, FF IJ, McNally M, McNally E, Reininga IH, Glaudemans AW (2017) Accuracy of diagnostic imaging modalities for peripheral post-traumatic osteomyelitis—a systematic review of the recent literature. Eur J Nucl Med Mol Imaging 44:1393–1407
52. Glaudemans AW, de Vries EF, Vermeulen LE, Slart RH, Dierckx RA, Signore A (2013) A large retrospective single-centre study to define the best image acquisition protocols and interpretation criteria for white blood cell scintigraphy with (9)(9)mTc-HMPAO-labelled leucocytes in musculoskeletal infections. Eur J Nucl Med Mol Imaging 40:1760–1769
53. van den Kieboom J, Bosch P, Plate JDJ et al (2018) Diagnostic accuracy of serum inflammatory markers in late fracture-related infection: a systematic review and meta-analysis. Bone Joint J 100-B:1542–1550
54. Xie K, Dai K, Qu X, Serum YM (2017) Synovial fluid interleukin-6 for the diagnosis of periprosthetic joint infection. Sci Rep 7:1496
55. Portillo ME, Salvadó M, Trampuz A et al (2015) Improved diagnosis of orthopedic implant-associated infection by inoculation of sonication fluid into blood culture bottles. J Clin Microbiol 53(5):1622–1627
56. Aggarwal VK, Higuera C, Deirmengian G, Parvizi J, Austin MS (2013) Swab cultures are not as effective as tissue cultures for diagnosis of periprosthetic joint infection. Clin Orthop Relat Res 471:3196–3203
57. Yano MH, Klautau GB, da Silva CB et al (2014) Improved diagnosis of infection associated with osteosynthesis by use of sonication of fracture fixation implants. J Clin Microbiol 52:4176–4182

58. Vaudaux P, Kelley WL, Lew DP (2006) Staphylococcus aureus small colony variants: difficult to diagnose and difficult to treat. Clin Infect Dis 43:968–970
59. Ronde-Oustau C, Lustig S, Dupieux C, Ferry T, Lyon BJ (2017) Implant-associated ESBL-Klebsiella pneumonia producing small colony variant bone and joint infection in a healthy 40-year-old man. BMJ Case Rep 2017
60. Tande AJ, Osmon DR, Greenwood-Quaintance KE, Mabry TM, Hanssen AD, Patel R (2014) Clinical characteristics and outcomes of prosthetic joint infection caused by small colony variant staphylococci. MBio 5:e01910–e01914
61. Proctor RA, von Eiff C, Kahl BC et al (2006) Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol 4:295–305
62. Butler-Wu SM, Burns EM, Pottinger PS et al (2011) Optimization of periprosthetic culture for diagnosis of Propionibacterium acnes prosthetic joint infection. J Clin Microbiol 49:2490–2495
63. Post V, Richards R, Moriarty TF (2016) Microbiology. In: Principles of orthopedic infection management. Georg Thieme Verlag, Stuttgart, pp 29–44
64. Renz N, Cabric S, Morgenstern C, Schuetz MA, Trampuz A (2018) Value of PCR in sonication fluid for the diagnosis of orthopedic hardware-associated infections: has the molecular era arrived? Injury 49:806–811
65. Panousis K, Grigoris P, Butcher I, Rana B, Reilly JH, Hamblen DL (2005) Poor predictive value of broad-range PCR for the detection of arthroplasty infection in 92 cases. Acta Orthop 76:341–346
66. Kramer AAO, Below H et al (2013) Wound antiseptics today—an overview. Antiseptics in surgery—update 2013. Lindqvist Book Publishing, Berlin, pp 85–111
67. Wouthyzen-Bakker M, Benito N, Soriano A (2017) The effect of preoperative antimicrobial prophylaxis on intraoperative culture results in patients with a suspected or confirmed prosthetic joint infection: a systematic review. J Clin Microbiol 55(9):2765–2774
68. Rittmann WW, Perren S (1974) Cortical bone healing after internal fixation and infection. Springer Verlag, Berlin
69. Zimmerli W, Widmer AF, Blatter M, Frei R, Ochsner PE (1998) Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. Foreign-Body Infection (FBI) Study Group. JAMA 279:1537–1541
70. Tschudin-Sutter S, Frei R, Dangel M et al (2016) Validation of a treatment algorithm for orthopaedic implant-related infections with device-retention-results from a prospective observational cohort study. Clin Microbiol Infect 22:457 e1–457 e9
71. Zimmerli W (2015) Orthopaedic implant-associated infections: update of antimicrobial therapy. Der Orthopade 44:961–966
72. Jefferson KK, Goldmann DA, Pier GB (2005) Use of confocal microscopy to analyze the rate of vancomycin penetration through Staphylococcus aureus biofilms. Antimicrob Agents Chemother 49:2467–2473
73. Zimmerli W, Sendi P (2019) Role of Rifampin against Staphylococcal biofilm infections in vitro, in animal models, and in orthopedic-device-related infections. Antimicrob Agents Chemother 63:e01746
74. Metsemakers WJ, Kuehl R, Moriarty TF et al (2018) Infection after fracture fixation: current surgical and microbiological concepts. Injury 49:511–522
75. Calhoun JH, Henry SL, Anger DM, Cobos JA, Mader JT (1993) The treatment of infected nonunions with gentamicin-polymethylmethacrylate antibiotic beads. Clin Orthop Relat Res:23–27
76. Rand BC, Penn-Barwell JG, Wenke JC (2015) Combined local and systemic antibiotic delivery improves eradication of wound contamination: an animal experimental model of contaminated fracture. Bone Joint J 97-B:1423–1427
77. Webb JC, Gbejuade H, Lovering A, Spencer R (2013) Characterisation of in vivo release of gentamicin from polymethyl methacrylate cement using a novel method. Int Orthop 37:2031–2036

78. Quintiliani R, Courvalin P (1995) Mechanisms of resistance to antimicrobial agents. In: Manual of clinical microbiology, 6th edn. ASM, Washington, DC, p 1319
79. Anguita-Alonso P, Rouse MS, Piper KE, Jacofsky DJ, Osmon DR, Patel R (2006) Comparative study of antimicrobial release kinetics from polymethylmethacrylate. Clin Orthop Relat Res 445:239–244
80. Likine EF, Seligson D (2019) Rifampin and tobramycin combination with PMMA antibiotic cement. Eur J Orthopaed Surg Traumatol Orthoped Traumatol 29:499–500
81. Inzana JA, Schwarz EM, Kates SL, Awad HA (2016) Biomaterials approaches to treating implant-associated osteomyelitis. Biomaterials 81:58–71
82. Zilberman M, Elsner JJ (2008) Antibiotic-eluting medical devices for various applications. J Control Release 130:202–215
83. Bennett-Guerrero E, Ferguson TB Jr, Lin M et al (2010) Effect of an implantable gentamicin-collagen sponge on sternal wound infections following cardiac surgery: a randomized trial. JAMA 304:755–762
84. Chaudhary S, Sen RK, Saini UC, Soni A, Gahlot N, Singh D (2011) Use of gentamicin-loaded collagen sponge in internal fixation of open fractures. Chin J Traumatol 14:209–214
85. Ferguson J, Diefenbeck M, McNally M (2017) Ceramic biocomposites as biodegradable antibiotic carriers in the treatment of bone infections. J Bone Joint Infect 2:38–51
86. McKee MD, Li-Bland EA, Wild LM, Schemitsch EH (2010) A prospective, randomized clinical trial comparing an antibiotic-impregnated bioabsorbable bone substitute with standard antibiotic-impregnated cement beads in the treatment of chronic osteomyelitis and infected nonunion. J Orthop Trauma 24:483–490
87. Craig J, Fuchs T, Jenks M et al (2014) Systematic review and meta-analysis of the additional benefit of local prophylactic antibiotic therapy for infection rates in open tibia fractures treated with intramedullary nailing. Int Orthop 38:1025–1030
88. El-Husseiny M, Patel S, MacFarlane RJ, Haddad FS (2011) Biodegradable antibiotic delivery systems. J Bone Joint Surg 93:151–157
89. Brady RA, O'May GA, Leid JG, Prior ML, Costerton JW, Shirtliff ME (2011) Resolution of Staphylococcus aureus biofilm infection using vaccination and antibiotic treatment. Infect Immun 79:1797–1803
90. Lemire JA, Kalan L, Bradu A, Turner RJ (2015) Silver oxynitrate, an unexplored silver compound with antimicrobial and antibiofilm activity. Antimicrob Agents Chemother 59:4031–4039
91. Moriarty TF, Kuehl R, Coenye T et al (2016) Orthopaedic device-related infection: current and future interventions for improved prevention and treatment. EFORT Open Rev 1:89–99
92. Mansour SC, de la Fuente-Nunez C, Hancock RE (2015) Peptide IDR-1018: modulating the immune system and targeting bacterial biofilms to treat antibiotic-resistant bacterial infections. J Pept Sci 21:323–329
93. De Brucker K, Delattin N, Robijns S et al (2014) Derivatives of the mouse cathelicidin-related antimicrobial peptide (CRAMP) inhibit fungal and bacterial biofilm formation. Antimicrob Agents Chemother 58:5395–5404
94. Gil D, Shuvaev S, Frank-Kamenetskii A, Reukov V, Gross C, Vertegel A (2017) Novel antibacterial coating on orthopedic wires to eliminate pin tract infections. Antimicrob Agents Chemother 61:e00442
95. Greene AH, Bumgardner JD, Yang Y, Moseley J, Haggard WO (2008) Chitosan-coated stainless steel screws for fixation in contaminated fractures. Clin Orthop Relat Res 466:1699–1704
96. Jennings JA, Carpenter DP, Troxel KS et al (2015) Novel antibiotic-loaded point-of-care implant coating inhibits biofilm. Clin Orthop Relat Res 473:2270–2282
97. McConoughey SJ, Howlin R, Granger JF et al (2014) Biofilms in periprosthetic orthopedic infections. Future Microbiol 9:987–1007
98. Whiteley M, Diggle SP, Greenberg EP (2017) Progress in and promise of bacterial quorum sensing research. Nature 551:313–320

99. Hentzer M, Wu H, Andersen JB et al (2003) Attenuation of Pseudomonas aeruginosa virulence by quorum sensing inhibitors. EMBO J 22:3803–3815
100. Abedon ST, Kuhl SJ, Blasdel BG, Kutter EM (2011) Phage treatment of human infections. Bacteriophage 1:66–85
101. Deresinski S (2009) Bacteriophage therapy: exploiting smaller fleas. Clin Infect Dis 48:1096–1101
102. Sulakvelidze A, Kutter E (2005) Bacteriophage therapy in humans. In: Bacteriophages: biology and application. CRC, Boca Raton, FL, pp 381–436
103. Tkhilaishvili T, Lombardi L, Klatt AB, Trampuz A, Di Luca M (2018) Bacteriophage Sb-1 enhances antibiotic activity against biofilm, degrades exopolysaccharide matrix and targets persisters of Staphylococcus aureus. Int J Antimicrob Agents 52:842–853
104. Henriksen K, Rorbo N, Rybtke ML et al (2019) P. aeruginosa flow-cell biofilms are enhanced by repeated phage treatments but can be eradicated by phage-ciprofloxacin combination. Pathog Dis 77:ftz011

Periprosthetic Joint Infection

Aron Keshishian, Andrew Foster, Georg Matziolis, T. Fintan Moriarty, and Henk Eijer

Abstract Total joint arthroplasty (TJA), the implantation of an artificial joint replacement, has become a relatively commonly practiced operation in many parts of the world. The most common reasons for performing a TJA are to restore mobility and alleviate symptoms of osteoarthritis, and reconstruction after fracture. The procedure is generally well tolerated and successful; however, periprosthetic joint infection (PJI) is the most dreaded complication. In this chapter, we will provide an overview of PJI from the perspective of an orthopedic surgeon, with the aim of providing an insight into the challenges and unresolved issues that may in the future be addressed by basic science and innovations in biomedical engineering. We describe the pathophysiology of acute and chronic PJI, the difficulties in diagnosing PJI, and the current treatment concepts, including both surgery and antibiotic therapy. Finally, we will provide an overview of the innovations in the field that may soon be translated to the clinic.

Keywords Periprosthetic joint infection · Total joint arthroplasty · Staphylococcus aureus · MRSA · Surgical side infection · Antibiotic therapy · One-stage revision · Two-stage revision · DAIR · Total hip arthroplasty · Total knee arthroplasty

A. Keshishian
AO Research Institute Davos, Davos, Switzerland

Regionalspital Emmental AG, Burgdorf, Switzerland

A. Foster · T. F. Moriarty
AO Research Institute Davos, Davos, Switzerland

G. Matziolis
Waldkrankenhaus Rudolf Elle Eisenberg, Universitätsklinik Jena, Eisenberg, Germany

H. Eijer (✉)
Regionalspital Emmental AG, Burgdorf, Switzerland
e-mail: henk.eijer@spital-emmental.ch

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_3

Introduction

TJA is a commonly practiced operation, with 1,186,955 such procedures performed in the USA in 2018 [1]. The main reason for receiving a TJA is pain and/or lack of mobility due to osteoarthritis; however, other reasons for TJA include fracture, osteonecrosis, and rheumatoid arthritis. In general, TJA alleviates pain and may improve the range of motion of the operated joint. TJA is most commonly performed on the hip, knee, and shoulder, although it is also possible in the elbow, ankle, and the first carpometacarpal joint. One of the first distinctions to be made between implants is the use of cement. The prosthetic components are often fixed within the bone with bone cement, although the use of bone cement is reducing due to the availability of cementless implants with improved material design and surface coatings. Cement is, however, still commonly used in total knee and total shoulder arthroplasty [2]. The decision on whether or not to use bone cement depends on the joint involved, the type of prosthesis and the underlying reason for the TJA. Although generally a very successful procedure, the most common complications of TJA are periprosthetic fracture, dislocation, periprosthetic joint infection (PJI), and thrombosis. The number of TJAs has been rising in industrialized nations over recent decades, and the amount of PJI has unfortunately risen in parallel [3]. PJI occurs in 0.5–1.1% of cases after hip TJA and can be up to 2% for knee TJA [4–6].

Against a background of increasing total number of PJI, and the significant burden on the affected patients and the healthcare system, there has been an increase in both clinical and basic science research into PJI in recent years. The present chapter gives an overview of the problem of PJI from an orthopedic surgeon's point of view. The goal is to provide the basic research scientist with an overview of the clinical problem, so that they may develop interventions targeting the areas of most pressing clinical need.

Pathophysiology of PJI

The entry of the contaminating pathogen into the wound is the first step in developing a PJI. Infections that occur within the first year after surgery are caused by microorganisms that are introduced during or shortly after surgery [6]. If PJI develops later than a year after initial TJA, and when no other local cause of infection is obvious, the bacteria arrive at the prosthesis via a hematogenous route.

The beginning of infection is the direct or indirect (e.g., from colonized surrounding soft tissue) adherence of bacteria to the surface of the prosthesis [7]. *Staphylococcus aureus (S. aureus)* is known to be particularly capable of sticking to the surface of the prosthesis due to the presence of surface proteins with specific affinity for human targets such as collagen, fibronectin, and bone [6]. In fact, the risk of seeding on the prosthetic surface in a *S. aureus* bacteremia has been reported to be between 30% and 40% [8]. An interesting question is whether the material

characteristics of the implanted device also influence the risk of bacterial adhesion [7, 9]. In most cases, this has been studied under laboratory conditions, and extrapolation from preclinical data to clinical practice is a challenge [10]. In one of the few clinical studies on this topic, the bacterial load was measured on polyethylene components and compared with that on titanium or cobalt–chromium surfaces [11]. The authors found that polyethylene components had a significantly higher bacterial burden. These findings help inform the clinician about whether an exchange of the polyethylene component is sufficient in the context of surgical debridement and antibiotics or whether removal of all components is necessary for a particular case of infection.

After the bacteria adhere to the surface of the implant, the next step is to produce a biofilm, whereby they multiply within a self-formed extracellular matrix consisting of polysaccharides, proteins, lipids, and extracellular DNA [7] (Fig. 1). The biofilm matrix also comprises host-derived fibrin, neutrophils, erythrocytes, fibroblasts and other host cells [6, 7, 12]. When growing within a biofilm, the bacteria enter into an antibiotic tolerant phenotype, and are protected by the surrounding matrix from host defense mechanisms [13].

From a clinical perspective, the formation of an antibiotic tolerant biofilm on the implant is crucially important as often the implant must be removed to cure the infection, which is a significant burden on the patient. Biofilm-growing bacteria may also be difficult to culture in the clinical microbiology lab. These biofilm infections may therefore be missed, leading to a delay of diagnosis, and the progression of the infection [14]. The detection of bacteria within a biofilm may be improved by the use of sonication, whereby the biofilm is removed and cultured. This of course, also necessitates removal of the device from the patient [14].

Although PJI may develop in any patient, there are several known risk factors for PJI. From the patient side, this includes obesity, diabetes mellitus, rheumatoid arthritis, immunosuppressive medication, high ASA score (American Society of

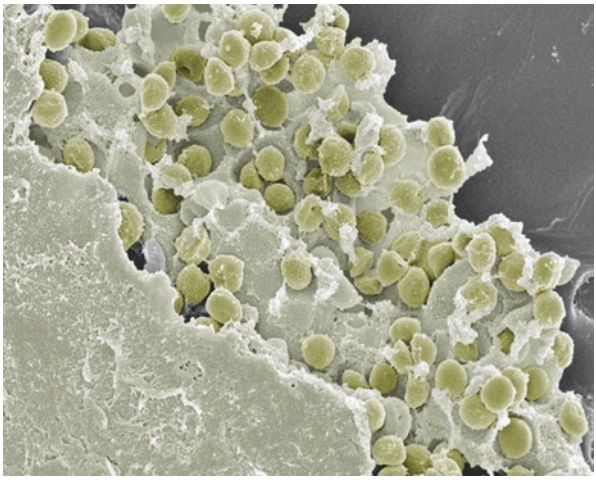


Fig. 1 Biofilm of *S. aureus* on the surface of a titanium orthopedic device. Note the surface of the material, visible on the upper right-hand side, is colonized by coccoid bacteria covered in an extracellular matrix. The extracellular matrix is composed of bacteria-secreted components, as well as host materials. (Unpublished image from the authors)

Anesthesiologists score, describes the patient's general health condition), smoking and malignancy [6]. Risk factors that are not influenced by the patient are, e.g., increased surgical duration and significant blood loss [15].

Classification and Clinical Presentation of Infection

PJI is often classified into one of three categories: (1) acute postoperative PJI, (2) chronic PJI, and (3) an acute hematogenous PJI [16]. An acute postoperative PJI occurs directly after the joint replacement. The cause is an infection with bacteria that have entered the wound during or shortly after the operation. Although studies have shown that wound colonization during the operation is common (up to 25%) [17], infection occurs as aforementioned in 0.5–2% of the cases [4–6]. Whether the wound becomes infected or not is due to the combination of the virulence of the pathogen, the patient's immune response, the affected joint, and the condition of the surrounding soft tissue.

According to the Centers for Disease Control (CDC) in the USA, an acute PJI is one which occurs within 4 weeks postoperatively, or if symptoms are present for up to 4 weeks [18]. Acute postoperative infections are typically caused by bacteria with relatively greater virulence and pathogenicity [6]. Classical symptoms include local pain, redness, swelling, drainage from the wound, and fever with an acute onset (Fig. 2). An acute PJI will eventually lead to osteomyelitis of the affected bone and possibly sepsis if left untreated. It is important that treatment commences early, as PJI may be life or limb threatening. In addition, the biofilm on the device is believed

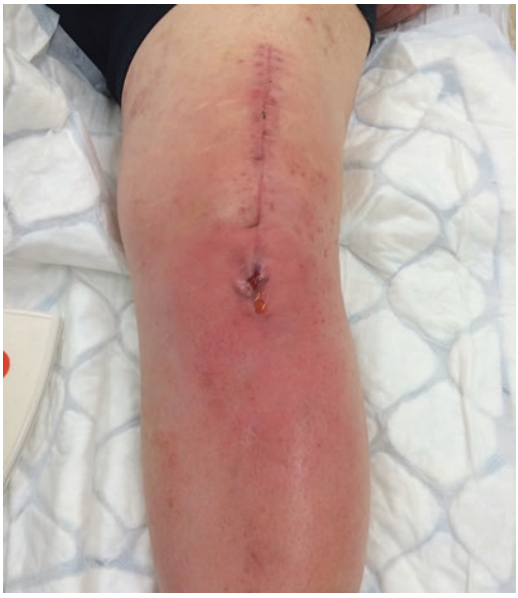


Fig. 2 Acute PJI after implantation of a total knee arthroplasty with redness, swelling, and drainage from the distal part of the wound

to mature with time: in the early weeks after infection onset the biofilm is immature and believed to be more easily treated by antibiotics [14].

In cases where the infection appears after more than 3 months after the operative procedure, the infection is considered a chronic PJI. These chronic infections are often associated with subclinical (or low grade) symptoms of infection and are usually caused by less virulent microorganisms such as coagulase-negative staphylococci (CoNS). The clinical presentation may include a loosening of the prosthesis. Fever, erythema, pain and/or the presence of a sinus tract are still possible, but less frequent than in cases of acute infection [6]. The subacute nature of the infection may also mean a delay in presentation of the patient and make diagnosis a greater challenge. In certain cases, a patient may experience chronic pain due to an unrecognized infection and, at a certain point, develop symptoms of an acute infection. In such cases, the infection is defined as a chronic PJI.

In hematogenous seeded infection, the bacteria seed the prosthesis via the blood, due to an infection at a distant site such as a dental or chronic wound abscess [19]. These infections can occur at any time after surgery, and are a lifelong risk for the patient, which is approximately 0.069% per prosthesis per year [20]. These infections are often times caused by pathogens with a higher pathogenicity as are seen in acute postoperative infection, and symptoms and treatment protocols are, therefore, also similar to acute postoperative infection [6].

Etiology of Periprosthetic Joint Infection

The most common microorganisms causing PJI are CoNS and *S. aureus.* In one study, approximately 54% of hip and knee PJI were caused by these pathogens, both causing 27% of infections [6]. Infections with more than one bacteria (i.e., polymicrobial infections) are seen in 15% of hip and knee PJI [6]. In shoulder arthroplasties, *Cutibacterium acnes* (*C. acnes*, formerly *Propionibacterium acnes)* is the second most prevalent microorganism due to the proximity to the axilla where *C. acnes* is typically resident. *C. acnes* is also a common pathogen after other surgeries of the shoulder than TJA, such as rotator cuff repair or osteosynthesis [21–23]. Up to 14% of infections are so-called culture-negative infections, whereby biopsies do not yield bacterial growth, but an infection is diagnosed through other symptoms such as elevated blood markers, radiographs or wound appearance [6]. These cases of culture-negative infections are still treated as infections; however, antibiotic treatment can be a challenge in the absence of an antibiotic resistance profile of the infecting pathogen.

In recent decades there has been a general trend of increasing prevalence of antibiotic resistant pathogens in the hospital setting. The impact of antibiotic resistance in PJI on treatment success rates has been studied [24]. A number of studies have shown poorer clinical outcome in PJI caused by Methicillin-resistant *S. aureus* (MRSA) than those caused by sensitive strains [25, 26]. This has not been a universal finding however, as comparable outcomes for MRSA and Methicillin-sensitive

S. aureus (MSSA) treatment in PJI have been found [27, 28]. International guidelines offer alternative antibiotic regimens for MRSA, which may provide adequate treatment coverage when properly followed.

Rifampin is a critical antibiotic in the treatment of PJI as it is the antibiotic with activity against biofilm-producing staphylococci [29, 30]. Resistance to rifampin can be observed more frequently after rifampin monotherapy and inadequate surgical debridement [31] and so it is recommended that rifampin is only given as a combination therapy [29]. In cases where rifampicin resistance develops during treatment, options become severely restricted and the patient may be faced with an untreatable infection that can only be managed by lifelong suppressive antibiotic therapy, or severe resection of an infected joint.

Diagnosis of Periprosthetic Joint Infection

Diagnosing an infection after a joint replacement can be difficult, especially in the case of chronic and low-grade infections without the classical symptoms of infection [6]. There are many ways in which patients present with an infection following arthroplasty and therefore there are many parameters to consider when definitively diagnosing PJI. The preoperative evaluation should include the medical history, a physical examination, measurement of blood indicators such as C-reactive protein and erythrocyte sedimentation rate and blood cultures. Radiographs of the affected joint are also recommended, which may reveal osteolysis and implant loosening. Further imaging modalities such as computed therapy, magnetic resonance imaging, and positron emission tomography-computed tomography are possible but not recommended in the routine diagnostics of PJI [32]. Furthermore, a diagnostic aspiration of joint fluid including total cell count, differential leukocyte count, and bacterial culture of synovial fluid must be performed. Elevated leucocytes ($>1.7 \times 10^3$) in the synovial fluid have a sensitivity of 94% and a specificity of 88% when PJI is suspected [33].

When an operative procedure is required, common practice dictates that 5–6 intraoperative tissue samples should be taken to increase the chance of detecting a microorganism and achieving a correct diagnosis [34]. Culturing of the bacteria can be followed by advanced molecular detection methods such as polymerase chain reaction (PCR) or fluorescence in situ hybridization (FISH), when infection is difficult to diagnose [35, 36]. These techniques have to date not been adopted in routine clinical practice. An increase in recovery of bacteria from the biofilm can be achieved through the use of sonication. In this case, the implant must be taken out and submitted to the lab for sonication and culture. The energy applied to the prosthesis can dislodge the biofilm and release the bacteria lining the prosthesis [37–39]. This technique is becoming more prevalent in clinical microbiology labs, although is not yet widespread except in specialist centers.

Recently, a new definition of PJI was published, which should serve to increase consistency in patient care, and clinical research studies. In this definition, a PJI

may be diagnosed based on a scoring system with major criteria (positive intraoperative cultures or the presence of a sinus tract) and minor criteria (e.g., elevated CRP in serum or positive preoperative alpha-defensin testing). This new definition was found to have a higher sensitivity compared to the older criteria published in 2011 (97.7 vs. 79.3%) [40].

Prevention of Periprosthetic Joint Infection

Prophylactic antibiotics are routinely given within 1 h prior to surgery to ensure antibiotics are in the bloodstream during the operative procedure. Antibiotics may be continued for 24 h after the operation as it may offer greater postoperative protection of the healing incisions [41, 42]. If the operation time is longer than 4 h, a second dose of antibiotics are usually given, again to protect the patient until wound closure (more specifically, re-dosing is recommended after two half-lives [43]). A large meta-analysis on antibiotic prophylaxis recommended that it should be commenced 120–0 min prior to operation [44]. Administering antibiotic prophylaxis too late (after the first incision) almost doubles the risk of surgical site infection (SSI) while giving it too early (more than 120 min before incision), the risk of infection is five times higher [44]. However, there are retrospective studies that showed no difference in the rate of PJI if there is a single dose of antibiotics compared with multiple doses postoperatively [42]. To date there is no prospective study that compares single versus multiple antibiotic doses. In cases of increased risk of PJI, it is shown that a prolonged antibiotic therapy (7 days) decreases the risk of PJI [45].

There are a number of considerations, other than the use of prophylactic antibiotics, that have been considered to reduce the risk of infection. The routine addition of antibiotics to bone cement in primary TJA is only recommended for patients with a high risk of PJI [15]. The use of laminar airflow in the operating room to reduce airborne bacteria, has been investigated in several studies, but did not show a significant reduction in PJI and SSI [18, 43]. Body exhaust suits showed no benefit in preventing PJI: in one study, the reoperation rate due to infection after total hip arthroplasty and total knee arthroplasty was even higher with the use of negative pressure helmets and are, therefore, not recommended [15, 43]. Similarly, there is no clear recommendation if immunosuppressive therapy should be stopped, or adjusted in general prior to surgery [43]. What is recommended with supportive evidence, is that there should be strict glycemic control regardless of whether or not diabetes is diagnosed preoperatively [43]. Also, preoperative weight reduction is recommended in obese patients [18, 43]. Other risk factors for PJI are also the chronic use of alcohol, smoking, higher age, male gender, and a certain medical comorbidities such as diabetes mellitus [18].

Revision surgery, such as replacement of an old to a new prosthesis or osteosynthesis in periprosthetic fractures, is also connected to higher infection rates [6]. Revision surgeries have increased infection rate due to the prolonged operating time and the possibility that there was an undiagnosed infection of the joint at the time of

revision (i.e., operating a presumed aseptic loosening case, which was in fact a subacute, chronic infection of the prosthesis). Prior surgery on the index joint also increases the risk of PJI on primary arthroplasty [6]. Wound healing complications also lead to higher deep infection rates [46]. Postoperative risk factors are hematoma, wound drainage, and wound dehiscence [47].

General Concepts in the Treatment of Periprosthetic Joint Infection

Once diagnosed, PJI is a serious complication for the patient and is a challenging scenario for the surgeon. In general, patients are treated with one of two options: (1) treatment with prosthesis retention or (2) with the exchange of the prosthesis. Acute PJI may be treated with antibiotics and debridement, without removing the implant (prosthesis retention) if there is no sign of loosening of the prosthesis and the time since onset of symptoms is shorter than 3–4 weeks or the time since total joint arthroplasty is shorter than 30 days [16, 34]. Retaining the implant offers many benefits to the patient including less operation time, no bone loss, shorter stay in hospital, shorter rehabilitation and is, therefore, an attractive strategy whenever possible. Antibiotic therapy and surgical debridement must be combined in every case of PJI. Guidelines are available for antibiotic selection, but the choice may be complicated by case-specific factors. As a general rule, antibiotics are given intravenously for at least 2 weeks and then changed to oral therapy, often for 3 months. However, this can vary depending on the type of bacteria, on the patient, and local prescribing preferences. The cooperation of the treating surgeon with an infectious disease physician facilitates the choice of treatment and is also common practice. Beside antibiotic therapy, debridement of the infected and non-vital tissue is one of the most important parts of every operation on PJI, regardless of whether the implant is retained or not. Beyond these key concepts that are true for all types of PJI, subsequent therapy can be influenced by the specific case, and by the classification of PJI. In the following section, we will describe the key features of acute and chronic PJI, and how this distinction can influence the treatment.

Acute Infection

For acute infections, when the implant is stable (i.e., osteolysis is not observed), the implant may be retained. This treatment is abbreviated to: debridement, antibiotics, irrigation, and retention (DAIR) (Fig. 3). This approach has been shown to result in 68–85% eradication of the infection, when applied to eligible patients [48]. It has also been shown that all easily removed, modular parts of the prosthesis (like the head in hip replacement and the inlay in knee replacement) are exchanged, the

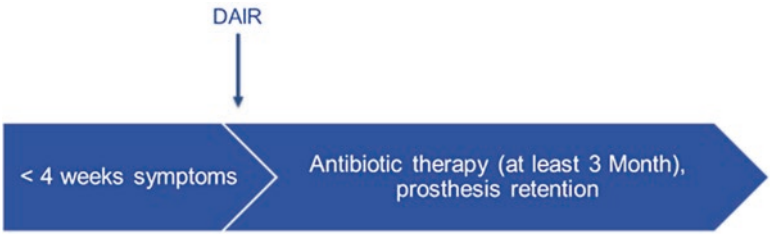


Fig. 3 Treatment of acute PJI with DAIR and implant retention

clearance rate of the infection can be further improved (up to 84% after 3 years) [16, 49, 50]. It is not clear if multiple debridement and irrigation procedures are superior to a single procedure [49] and the decision for any subsequent revisions (so-called "second look" operation) is generally based on the condition of the patient.

Chronic Infection

In cases of chronic infection, DAIR is not usually an option, unless the goal of treatment is not to eradicate the infection (i.e., suppression or palliative therapy is the goal). In patients with chronic infection, the prosthesis is always removed, the joint debrided, and a new prosthesis implanted. The new implant can be placed during the initial surgery, (a so-called one-stage exchange) or in a subsequent surgery after an interim stage where a spacer may be implanted (two-stage exchange). The choice of a one- or two-stage exchange depends on the type of infection, but also on the infecting pathogen, the severity of the infection and, of course, the preference of the operating surgeon (Fig. 4). The two-stage exchange is preferred and still more frequently performed method in the USA [49], whereas the one-stage exchange has gained in popularity in Europe [51]. In either case, debridement must be as thorough as possible to remove all of the infected, necrotic tissue. The benefits of the one-stage operation include a reduced hospitalization time, shorter operation time, faster mobilization, lower costs, while having comparable outcomes in selected patient populations [49, 52]. Several studies postulate that patients only qualify for one-stage exchange when there is minimal loss of bone due to osteolysis and a microbiological diagnosis has been obtained [52]. Poorer outcomes can be expected when a one-stage exchange is performed for a polymicrobial infection or when Gram-negative bacteria such as *Pseudomonas aeruginosa*, or MRSA are the cause of the infection [53]. If these conditions are recognized and avoided, one-stage exchange shows comparable outcomes to two-stage exchange [49, 52, 53].

In a two-stage exchange, the duration of the prosthesis-free interval is dependent on the patient, the infecting pathogen, and the surgeon. The interval can vary from 2 to 6 weeks or more. Placing a new prosthesis after the interval can be difficult due to shortening of the limb and scarring of the tissue, and so a spacer may be used in

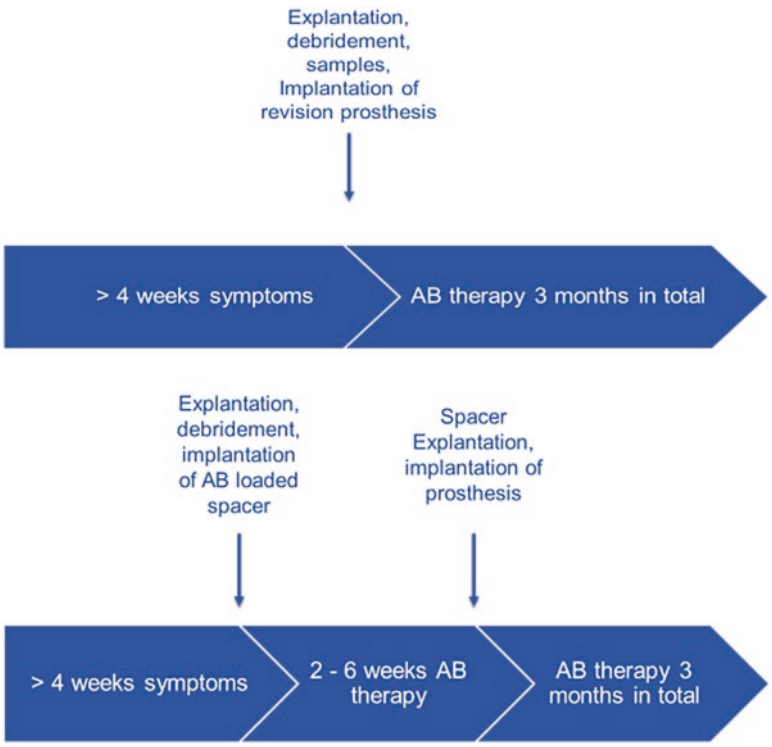


Fig. 4 Timetable of one-stage exchange and two-stage exchange in chronic PJI. *AB* antibiotic

the intervening period (Fig. 5) [49, 54]. Sometimes, after the antibiotic therapy in the prosthesis-free interval, there is an antibiotic holiday for 2 weeks. After that, another aspiration is performed to see if the bacteria are completely eradicated and the new implant can be inserted.

The spacers used in the two-stage exchange procedure are composed of polymethylmethacrylate (PMMA) bone cement and may be handmade in the operating room, or, prepurchased preformed spacers are also available [49] (Fig. 5). Since the patient receiving the spacer is being treated for infection, antibiotics can be added to the cement as an adjunct to therapy. It is also possible to use antibiotic-loaded beads to deliver antibiotics locally [55], which may have an equivalent effect to antibiotic-loaded cement spacers [56]. Antibiotic-loaded PMMA can also be commercially bought or mixed with antibiotics in the operating room, selected on the antibiotic susceptibility of the infecting pathogen. Several studies have shown improved treatment success of an antibiotic-loaded cement spacer over a "normal" spacer [57]. A range of antibiotics are available commercially, at least in Europe; however, a wider range of antibiotics have been incorporated in an off-label manner to better match the antibiotic susceptibility profile of the infecting pathogen. Any such antibiotic loaded into bone cement should be thermally stable (cement cures in

Fig. 5 On the left side a commercially available cement spacer for the hip and on the right a custom-made cement spacer after explantation are shown. The bone cement is colored so that it can be more easily distinguished from the bone in revision surgery

an exothermic reaction), water soluble, and be available as a powder to be suitable for use in bone cement [58]. Antibiotics which lose their antimicrobial activity when added to bone cement include tetracyclines and rifampin [58]. Tetracycline has a decreased activity due to thermal instability and rifampin has impeded cement curing [59].

Every operation has the chance of acquiring a "new" infection, so whenever a new implant is being put in, prophylactic antibiotics should also be given preoperatively (this is also true for a one-stage exchange) [49, 60]. When one follows these rules (i.e., using one or two stage as recommended by the guideline), successful eradication of infection is 85–100% (Fig. 6) [6].

For a certain percentage of patients, the abovementioned treatment regimens are not successful, and alternative measures need to be considered. Treating options in these situations are limited to removal of the prosthesis (e.g., Girdlestone situation in total hip arthroplasty), lifelong antibiotic suppression, arthrodesis (fusion of the bones which are involved forming the original joint), or amputation. The final selection will be based upon the patient's preference, age, and health condition.

Antibiotic suppression therapy is another last resort treatment option, that aims to control the symptoms and progression of osteolysis but does not target eradication of infection. Suppressive antibiotic therapy should follow similar principles in the choice of antibiotic agent as regular antibiotic therapy, which is based on the

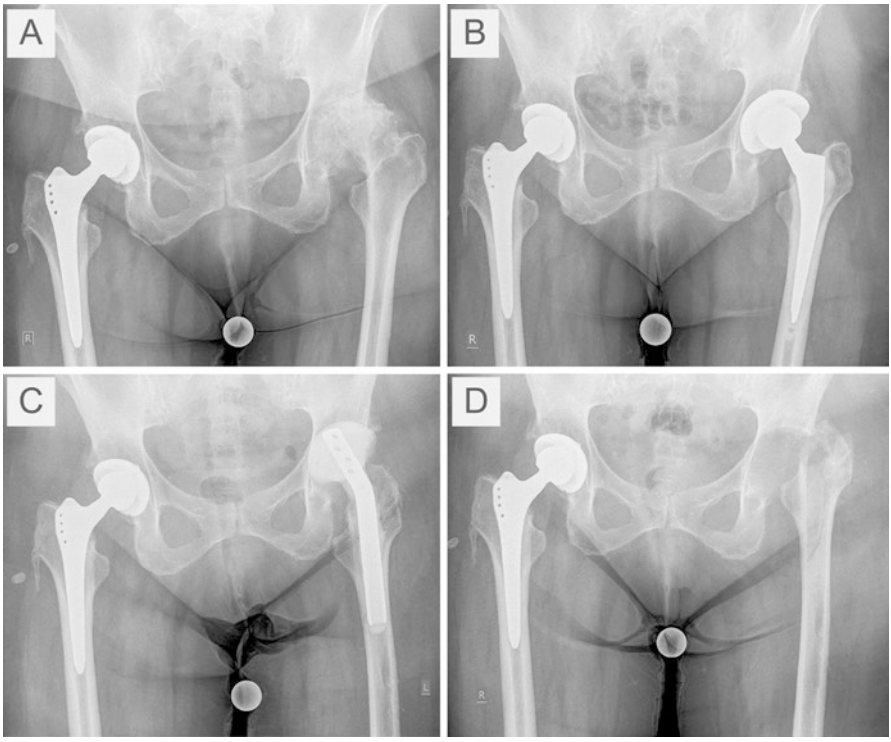


Fig. 6 (**a**) A 67-year-old female patient presented with osteoarthritis of the left hip. (**b**) The patient underwent TJA and a chronic infection developed, the patient underwent a two-stage exchange procedure. In (**c**), the prosthesis and the bone cement is removed and a custom-made antibiotic-loaded cement spacer is put in. In (**d**) the cement spacer is also removed due to clinically persistent infection signs (Girdlestone situation). If infection is controlled, the patient qualifies for implantation of a revision prosthesis

resistance profile of the microorganisms, host allergies (if present) and is usually orally administered. This can also be the treatment of choice for relapsing infections or whenever the patient's condition does not allow for surgery [61]. Amputation and arthrodesis are the final alternative option, but are rarely performed in hip and knee replacement in modern times (about 0.1% of all patients undergo amputation after total knee arthroplasty) [62].

Considering that more and more patients are likely to become infected with highly resistant pathogens, new interventions will be required to enable better treatment options for these patients. Some emerging strategies in the field will be described in the following section.

Emerging Strategies to Prevent and Treat PJI

There is a wide range of antimicrobial therapies in the preclinical literature; however, comparatively few of these have advanced to clinical trials or clinical practice. The following are examples of new technologies that are beginning to be translated to the clinic.

Recently, the use of antibiotic-loaded hydrogel in primary and revision arthroplasty has been described [63–65] and can reduce SSI in TJA significantly [63]. In a study of 380 patients, the antibiotic added to the hydrogel was selected by the surgeon, and included vancomycin, gentamicin, or a combination of vancomycin and meropenem. The hydrogel reduced the SSI rate from 6% in the control group to 0.6% in the treatment group [63]. Furthermore, another study compared two-stage revision surgery against one-stage revision surgery with adjunctive antibiotic-loaded hydrogel. The one-stage revision surgery with hydrogel application showed a significant reduction in length of hospital stay and length of antibiotic treatment, with an equivalent infection recurrence rate. The use of antibiotic-loaded hydrogel showed no impairment of osseointegration [64, 65]. However, the mean follow-up of both studies is relatively short compared to the lifetime of a prosthesis and prolonged follow-up studies on the performance of this hydrogel are expected in the coming years.

Silver-coated implants have shown preclinical efficacy against the formation of biofilms [66] and bacterial resistance to silver is not a clinical concern at the present time. Silver-coated implants are only used in megaprosthesis and on demand in special cases of revision TJA at the present time. In a case-control study, it was shown that the use of silver coating in megaprosthesis showed a significant reduction of PJI and also a higher curing rate if DAIR was performed after recurrent PJI if silver-coated implants were used before [67].

Another innovation adopted from spinal surgery has been the application of vancomycin powder to the wound after total knee arthroplasty [68]. A number of studies have investigated this practice in spinal surgery; however, this practice did not decrease PJI, but showed a significantly higher rate in postoperative wound healing issues [69]. The differences may need to be further investigated in future prospective trials, before widespread use can be recommended.

A recent preclinical study on monoclonal antibodies which target *S. aureus* surface protein A showed promising results in reducing mortality in *S. aureus* (MSSA and MRSA) bacteremia in a mouse model and in in vitro studies [70]. Due to the high rate of *S. aureus* PJI, passive immunization is a promising target for further research in PJI [70]. Historically, passive and active vaccines against *S. aureus* have a poor record in clinical trials [71]. This is believed to be due to the multifactorial virulence of this pathogen and blocking a few virulence factors will not inactivate all and infections still persist. A polyvalent vaccine is currently under investigation for PJI, though results are at present not available.

An innovation that has only recently emerging in the clinical literature is the use of bacteriophages in PJI. Bacteriophages are selective, self-replicating viruses that

infect specific bacteria and lyse them regardless of an antibiotic resistance mechanism they may possess. These bacteriophages have shown promising results in preclinical and clinical studies [72–75], especially when combined with antibiotics [76]. The enzymes used by bacteriophages to break open the bacteria are known as endolysins and are also under investigation in the scientific literature [77]. However, at the present time, these have not yet been introduced to clinical studies. Finally, in an animal model it was shown that when using porous coated prosthesis such as calcium phosphate, that these coatings may be loaded with gentamicin solution immediately prior to implantation, and can reduce orthopedic device-related infection, at least in laboratory animal studies [78].

Conclusion

PJI is a challenging complication that is expected to become even more prevalent in the future due to the increasing number of TJA performed and increasing antibiotic resistance. The most important subject for future research has to be the prevention of infection according to the theory that "prevention is better than cure." Innovations in antimicrobial strategies to overcome antibiotic resistance will be increasingly important. Similarly, allowing bone regeneration postinfection should be a target for future research, as lack of bone stock can significantly reduce treatment options. Should such innovations come to the clinic in the future, they will have the potential to significantly impact upon clinical practice in the near term.

References

1. American Joint Replacement Registry (2018) Fifth AJRR annual report on hip and knee arthroplasty data 2018. Am Joint Replace Regist:319
2. SIRIS (2012) Swiss National Joint Registry. Siris:63. http://www.swissorthopaedics.ch/images/content/SIRIS/170516_SIRISAnnualReport2015_Finalcopie.pdf
3. Wengler A, Nimptsch U, Mansky T (2014) Hüft- und Kniegelenkersatz in Deutschland und den USA. Dtsch Arztebl. https://doi.org/10.3238/arztebl.2014.0407
4. Chitre AR, Sadiq S (2007) The incidence of deep prosthetic infections in a specialist orthopaedic hospital: a 15-year prospective study. J Bone Joint Surg Br 89-B(2):281–281. https://doi.org/10.1302/0301-620x.89b2.19159
5. Gundtoft PH, Overgaard S, Schonheyder HC, Moller JK, Kjærsgaard-Andersen P, Pedersen AB (2015) The "true" incidence of surgically treated deep prosthetic joint infection after 32,896 primary total hip arthroplasties. Acta Orthop 86(3):326–334. https://doi.org/10.3109/17453674.2015.1011983
6. Tande AJ, Patel R (2014) Prosthetic joint infection. Clin Microbiol Rev 27(2):302 LP–302345. https://doi.org/10.1128/CMR.00111-13
7. Gallo J, Kolár M, Novotný R, Riháková P, Tichá V (2003) Pathogenesis of prosthesis-related infection. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 147(1):27–35. https://doi.org/10.5507/bp.2003.004

8. Sendi P, Banderet F, Graber P, Zimmerli W (2011) Periprosthetic joint infection following Staphylococcus aureus bacteremia. J Infect 63(1):17–22. https://doi.org/10.1016/j.jinf.2011.05.005
9. Sorrentino R, Cochis A, Azzimonti B et al (2017) Reduced bacterial adhesion on ceramics used for arthroplasty applications. J Eur Ceram Soc 38:963–970. https://doi.org/10.1016/j.jeurceramsoc.2017.10.008
10. Gómez-Barrena E, Esteban J, Medel F et al (2012) Bacterial adherence to separated modular components in joint prosthesis: a clinical study. J Orthop Res 30(10):1634–1639. https://doi.org/10.1002/jor.22114
11. Karbysheva S, Grigoricheva L, Golnik V, Popov S, Renz N, Trampuz A (2019) Influence of retrieved hip- and knee-prosthesis biomaterials on microbial detection by sonication. Eur Cell Mater 37:16–22. https://doi.org/10.22203/eCM.v037a02
12. Gbejuade HO, Lovering AM, Webb JC (2015) The role of microbial biofilms in prosthetic joint infections. Acta Orthop 86(2):147–158. https://doi.org/10.3109/17453674.2014.966290
13. Mozina SS, Klancknik A, Raspor P (2013) Mechanisms of microbial resistance in biofilms. Biofilms Bioeng (January):311–332
14. McConoughey SJ, Stoodley P, Kathju S et al (2014) Biofilms in periprosthetic orthopedic infections. Future Microbiol 9(8):987–1007. https://doi.org/10.2217/fmb.14.64
15. Fillingham Y, Parsa A, Oshkukov S, Greenward AS (2018) International consensus meeting on musculoskeletal infection. Part II. Hip Knee 4:303–304. https://icmphilly.com/wp-content/uploads/2018/11/Hip-and-Knee.pdf
16. Aggarwal VK, Rasouli MR, Parvizi J (2013) Periprosthetic joint infection: current concept. Indian J Orthop 47(1):10–17. https://doi.org/10.4103/0019-5413.106884
17. Fitzgerald RH, Peterson LFA, Washington JA, Van Scoy RE, Coventry MB (1973) Bacterial colonization of wounds and sepsis in total hip arthroplasty. J Bone Joint Surg A 55(6):1242–1250. https://doi.org/10.2106/00004623-197355060-00011
18. Related H, Factors L, Related H, et al (2018) Part I: General assembly. International Consensus meeting on Prosthetic Joint Infection. Hip and Knee. https://doi.org/10.1016/j.diagmi
19. Toms AD, Davidson D, Masri BA, Duncan CP (2006) The management of peri-prosthetic infection in total joint arthroplasty. J Bone Joint Surg Br 88(2):149–155. https://doi.org/10.1302/0301-620x.88b2.17058
20. Huotari K, Peltola M, Jämsen E (2015) The incidence of late prosthetic joint infections. Acta Orthop 86:321–325. https://doi.org/10.3109/17453674.2015.1035173
21. Saper D, Capiro N, Ma R, Li X (2015) Management of Propionibacterium acnes infection after shoulder surgery. Curr Rev Musculoskelet Med 8(1):67–74. https://doi.org/10.1007/s12178-014-9256-5
22. Kadler BK, Mehta SS, Funk L (2015) Propionibacterium acnes infection after shoulder surgery. Int J Shoulder Surg 9(4):139–144. https://doi.org/10.4103/0973-6042.167957
23. Both A, Klatte TO, Lübke A et al (2018) Growth of Cutibacterium acnes is common on osteosynthesis material of the shoulder in patients without signs of infection. Acta Orthop 89(5):580–584. https://doi.org/10.1080/17453674.2018.1489095
24. Li B, Webster TJ (2018) Bacteria antibiotic resistance: new challenges and opportunities for implant-associated orthopedic infections. J Orthop Res 36:22–32. https://doi.org/10.1002/jor.23656
25. Ryu DJ, Kang JS, Moon KH, Kim MK, Kwon DG (2014) Clinical characteristics of methicillin-resistant Staphylococcus aureus infection for chronic periprosthetic hip and knee infection. Hip Pelvis 26(4):235–242. https://doi.org/10.5371/hp.2014.26.4.235
26. Salgado CD, Dash S, Cantey JR, Marculescu CE (2007) Higher risk of failure of methicillin-resistant Staphylococcus aureus prosthetic joint infections. Clin Orthop Relat Res 461:48–53. https://doi.org/10.1097/BLO.0b013e3181123d4e
27. Lora-Tamayo J, Murillo O, Iribarren JA et al (2013) A large multicenter study of methicillin-susceptible and methicillin-resistant staphylococcus aureus prosthetic joint infections managed with implant retention. Clin Infect Dis 56:182–194. https://doi.org/10.1093/cid/cis746

28. Sousa R, Abreu MA (2018) Treatment of prosthetic joint infection with debridement, antibiotics and irrigation with implant retention—a narrative review. J Bone Joint Infect 3(3):108–117. https://doi.org/10.7150/jbji.24285
29. Zimmerli W, Trampuz A, Ochsner P (2004) Prosthetic-joint infections. N Engl J Med 351(16):1645–1654. https://doi.org/10.1056/NEJMra040181
30. Zimmerli W, Sendi P (2019) Role of rifampin against staphylococcal biofilm infections in vitro, in animal models, and in orthopedic-device-related infections. Antimicrob Agents Chemother 63(2):e01746–e01718. https://doi.org/10.1128/AAC.01746-18
31. Achermann Y, Eigenmann K, Ledergerber B et al (2013) Factors associated with rifampin resistance in staphylococcal periprosthetic joint infections (PJI): A matched case-control study. Infection 41(2):431–437. https://doi.org/10.1007/s15010-012-0325-7
32. Love C, Palestro CJ (2016) Nuclear medicine imaging of bone infections. Clin Radiol 71(7):632–646. https://doi.org/10.1016/j.crad.2016.01.003
33. Trampuz A, Hanssen AD, Osmon DR, Mandrekar J, Steckelberg JM, Patel R (2004) Synovial fluid leukocyte count and differential for the diagnosis of prosthetic knee infection. Am J Med 117(8):556–562. https://doi.org/10.1016/j.amjmed.2004.06.022
34. Osmon DR, Berbari EF, Berendt AR et al (2013) Executive summary: diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 56(1):1–10. https://doi.org/10.1093/cid/cis966
35. Qu X, Zhai Z, Li H et al (2013) PCR-based diagnosis of prosthetic joint infection. J Clin Microbiol 51(8):2742–2746. https://doi.org/10.1128/JCM.00657-13
36. Lippmann T, Braubach P, Ettinger M, Kuehnel M, Laenger F, Jonigk D (2019) Fluorescence in situ hybridization (FISH) for the Diagnosis of periprosthetic joint infection in formalin-fixed paraffin-embedded surgical tissues. J Bone Joint Surg Am 101(2):e5. https://doi.org/10.2106/JBJS.18.00243
37. Evangelopoulos DS, Stathopoulos IP, Morassi GP et al (2013) Sonication: a valuable technique for diagnosis and treatment of periprosthetic joint infections. Sci World J 2013:375140. https://doi.org/10.1155/2013/375140
38. Trampuz A, Piper KE, Jacobson MJ et al (2007) Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med 357(7):654–663. https://doi.org/10.1056/nejmoa061588
39. Zhai Z, Li H, Qin A et al (2014) Meta-analysis of sonication fluid samples from prosthetic components for diagnosis of infection after total joint arthroplasty. J Clin Microbiol 52(5):1730–1736. https://doi.org/10.1128/JCM.03138-13
40. Parvizi J, Tan TL, Goswami K et al (2018) The 2018 definition of periprosthetic hip and knee infection: an evidence-based and validated criteria. J Arthroplasty 33(5):1309–1314.e2
41. Yates AJ Jr, Committee AA of H and KSE-BM (2018) Postoperative prophylactic antibiotics in total joint arthroplasty. Arthroplast Today 4(1):130–131. https://doi.org/10.1016/j.artd.2018.01.003
42. Tan T, Shohat N, Rondon A et al (2019) Perioperative antibiotic prophylaxis in total joint arthroplasty: a single dose is as effective as multiple doses. J Bone Joint Surg Am 101:429–437. https://doi.org/10.2106/JBJS.18.00336
43. Parvizi J, Shohat N, Gehrke T (2017) Prevention of periprosthetic joint infection. Bone Joint J 99-B(4 Supple B):3–10. https://doi.org/10.1302/0301-620X.99B4.BJJ-2016-1212.R1
44. De Jonge SW, Gans SL, Atema JJ, Solomkin JS, Dellinger PE, Boermeester MA (2017) Timing of preoperative antibiotic prophylaxis in 54,552 patients and the risk of surgical site infection. Medicine 96(29):e6903. https://doi.org/10.1097/MD.0000000000006903
45. Inabathula A, Dilley JE, Ziemba-Davis M et al (2018) Extended oral antibiotic prophylaxis in high-risk patients substantially reduces primary total hip and knee arthroplasty 90-day infection rate. J Bone Joint Surg Am 100(24):2103–2109. https://doi.org/10.2106/JBJS.17.01485
46. Wagenaar FCBM, Löwik CAM, Zahar A, Jutte PC, Gehrke T, Parvizi J (2019) Persistent wound drainage after total joint arthroplasty: a narrative review. J Arthroplasty 34(1):175–182. https://doi.org/10.1016/j.arth.2018.08.034

47. Jämsen E, Huhtala H, Puolakka T, Moilanen T (2009) Risk factors for infection after knee arthroplasty a register-based analysis of 43,149 cases. J Bone Joint Surg Am 91(1):38–47. https://doi.org/10.2106/JBJS.G.01686
48. Grammatopoulos G, Kendrick B, McNally M et al (2017) Outcome following debridement, antibiotics, and implant retention in hip periprosthetic joint infection—an 18-year experience. J Arthroplasty 32(7):2248–2255. https://doi.org/10.1016/j.arth.2017.02.066
49. Kuiper JW, Willink RT, Moojen DJF, van den Bekerom MP, Colen S (2014) Treatment of acute periprosthetic infections with prosthesis retention: review of current concepts. World J Orthop 5(5):667–676. https://doi.org/10.5312/wjo.v5.i5.667
50. Hirsiger S, Betz M, Stafylakis D, Götschi T, Lew D, Uçkay I (2019) The benefice of mobile parts' exchange in the management of infected total joint arthroplasties with prosthesis retention (DAIR procedure). J Clin Med 8(2):226. https://doi.org/10.3390/jcm8020226
51. Wongworawat MD (2013) Clinical faceoff: one-versus two-stage exchange arthroplasty for prosthetic joint. Clin Orthop Relat Res 471(6):1750–1753. https://doi.org/10.1007/s11999-013-2882-1
52. Klouche S, Leonard P, Zeller V et al (2012) Infected total hip arthroplasty revision: one- or two-stage procedure? Orthop Traumatol Surg Res 98(2):144–150. https://doi.org/10.1016/j.otsr.2011.08.018
53. Jackson WO, Schmalzried TP (2000) Limited role of direct exchange arthroplasty in the treatment of infected total hip replacements. Clin Orthop Relat Res:101–105
54. Charette RS, Melnic CM (2018) Two-stage revision arthroplasty for the treatment of prosthetic joint infection. Curr Rev Musculoskelet Med 11(3):332–340. https://doi.org/10.1007/s12178-018-9495-y
55. Kuiper JWP, Vos SJ, Saouti R et al (2013) Prosthetic joint-associated infections treated with DAIR (debridement, antibiotics, irrigation, and retention). Acta Orthop 84(4):380–386. https://doi.org/10.3109/17453674.2013.823589
56. Hsieh PH, Shih CH, Chang YH, Lee MS, Shih HN, Yang WE (2004) Two-stage revision hip arthroplasty for infection: comparison between the interim use of antibiotic-loaded cement beads and a spacer prosthesis. J Bone Joint Surg Am 86(9):1989–1997. https://doi.org/10.2106/00004623-200409000-00018
57. Staats K, Sevelda F, Kaider A et al (2017) The influence of antibiotic-loaded cement spacers on the risk of reinfection after septic two-stage hip revision surgery. Infection 45(6):885–891. https://doi.org/10.1007/s15010-017-1081-5
58. Soares D, Leite P, Barreira P, Aido R, Sousa R (2015) Antibiotic-loaded bone cement in total joint arthroplasty. Acta Orthop Belg 81(2):184–190
59. Joseph TN, Chen AL, Di Cesare PE (2003) Use of antibiotic-impregnated cement in total joint arthroplasty. J Am Acad Orthop Surg 11(1):38–47. https://doi.org/10.5435/00124635-200301000-00006
60. Mortazavi SMJ, Vegari D, Ho A, Zmistowski B, Parvizi J (2011) Two-stage exchange arthroplasty for infected total knee arthroplasty: predictors of failure. Clin Orthop Relat Res 469(11):3049–3054. https://doi.org/10.1007/s11999-011-2030-8
61. Rodriguez-Merchan EC (2015) Knee fusion or above-the-knee amputation after failed two-stage reimplantation total knee arthroplasty. Arch Bone Joint Surg 3(4):241–243. https://www.ncbi.nlm.nih.gov/pubmed/26550586
62. Hungerer S, Kiechle M, von Rüden C, Militz M, Beitzel K, Morgenstern M (2017) Knee arthrodesis versus above-the-knee amputation after septic failure of revision total knee arthroplasty: comparison of functional outcome and complication rates. BMC Musculoskelet Disord 18(1):443. https://doi.org/10.1186/s12891-017-1806-8
63. Romanò CL, Malizos K, Capuano N et al (2016) Does an antibiotic-loaded hydrogel coating reduce early post-surgical infection after joint arthroplasty? J Bone Joint Infect 1:34–41. https://doi.org/10.7150/jbji.15986
64. Boot W, Gawlitta D, Nikkels PGJ et al (2017) Hyaluronic acid-based hydrogel coating does not affect bone apposition at the implant surface in a rabbit model. Clin Orthop Relat Res 475(7):1911–1919. https://doi.org/10.1007/s11999-017-5310-0

65. Capuano N, Logoluso N, Gallazzi E, Drago L, Romanò CL (2018) One-stage exchange with antibacterial hydrogel coated implants provides similar results to two-stage revision, without the coating, for the treatment of peri-prosthetic infection. Knee Surg Sport Traumatol Arthrosc 26(11):3362–3367. https://doi.org/10.1007/s00167-018-4896-4
66. Gallo J, Panacek A, Prucek R et al (2016) Silver nanocoating technology in the prevention of prosthetic joint infection. Materials (Basel) 9(5):E337. https://doi.org/10.3390/ma9050337
67. Wafa H, Grimer RJ, Reddy K et al (2015) Retrospective evaluation of the incidence of early periprosthetic infection with silver-treated endoprostheses in high-risk patients: case-control study. Bone Joint J 97-B(2):252–257. https://doi.org/10.1302/0301-620X.97B2.34554
68. Tailaiti A, Shang J, Shan S, Muheremu A (2018) Effect of intrawound vancomycin application in spinal surgery on the incidence of surgical site infection: a meta-analysis. Ther Clin Risk Manag 14:2149–2159. https://doi.org/10.2147/TCRM.S185296
69. Hanada M, Nishikino S, Hotta K, Furuhashi H, Hoshino H, Matsuyama Y (2019) Intrawound vancomycin powder increases post-operative wound complications and does not decrease periprosthetic joint infection in primary total and unicompartmental knee arthroplasties. Knee Surg Sports Traumatol Arthrosc 27(7):2322–2327. https://doi.org/10.1007/s00167-019-05498-z
70. Taha M, Abdelbary H, Ross FP, Carli AV (2018) New innovations in the treatment of PJI and biofilms—clinical and preclinical topics. Curr Rev Musculoskelet Med 11(3):380–388. https://doi.org/10.1007/s12178-018-9500-5
71. Proctor RA (2015) Recent developments for Staphylococcus aureus vaccines: clinical and basic science challenges. Eur Cell Mater 30(734):315–326. https://doi.org/10.22203/eCM.v030a22
72. Kaur S, Harjai K, Chhibber S (2016) In vivo assessment of phage and linezolid based implant coatings for treatment of methicillin resistant S. aureus (MRSA) mediated orthopaedic device related infections. PLoS One 11(6):e0157626. https://doi.org/10.1371/journal.pone.0157626
73. Yilmaz C, Colak M, Yilmaz BC, Ersoz G, Kutateladze M, Gozlugol M (2013) Bacteriophage therapy in implant-related infections: an experimental study. J Bone Joint Surg Am 95(2):117–125. https://doi.org/10.2106/JBJS.K.01135
74. Wright A, Hawkins CH, Änggård EE, Harper DR (2009) A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant Pseudomonas aeruginosa; a preliminary report of efficacy. Clin Otolaryngol 34(4):349–357. https://doi.org/10.1111/j.1749-4486.2009.01973.x
75. Rose T, Verbeken G, De Vos D (2014) Experimental phage therapy of burn wound infection: difficult first steps. Int J Burns Trauma 4(2):66–73
76. Akanda ZZ, Taha M, Abdelbary H (2018) Current review—the rise of bacteriophage as a unique therapeutic platform in treating peri-prosthetic joint infections. J Orthop Res 36(4):1051–1060. https://doi.org/10.1002/jor.23755
77. Fischetti VA (2018) Development of phage lysins as novel therapeutics: a historical perspective. Viruses 10(6):E310. https://doi.org/10.3390/v10060310
78. Thompson K, Petkov S, Zeiter S et al (2019) Intraoperative loading of calcium phosphate-coated implants with gentamicin prevents experimental Staphylococcus aureus infection in vivo. PLoS One 14(2):e0210402. https://doi.org/10.1371/journal.pone.0210402

Perspectives on Biomaterial-Associated Infection: Pathogenesis and Current Clinical Demands

Dan Bai, Jingjie Chen, Peng Li, and Wei Huang

Abstract In this chapter, an overview of current medical implant devices and infection problems associated with implantation is provided, bridging the gap between material engineering and clinical practice. The pathogenesis, common pathogens, and infection sites are listed, alongside the details of up-to-date strategies and guidelines for diagnosis and treatment of biomaterials-associated infections. Through the combined understanding of microbial pathogenicity, drug resistance, patients' immune response processes, and current clinical practices, we can tackle the problem of biomaterials-associated infection via multidisciplinary approaches. To meet the clinical demands and challenges in future, strategic design of intelligent biomaterials is in need to reduce implantation device-caused infections, improving the patient's quality of life.

Keywords Biomaterial-associated infection · Implant-related infection · Nosocomial infection · Drug resistance · Intelligent biomaterials

Introduction

Biomaterial-associated infection is one of the major complications in the clinical use of implanted materials, occurring in both permanent implants and temporary devices. Since the first permanent pacemaker was successfully implanted into the human body 60 years ago, the number of surgical cases using implants has increased significantly in the past decades, such as arthroplasty in joint surgery, intervertebral disc implants in spinal surgery, fracture internal fixation in traumatology, prosthetic valves in cardiac surgery, pacemakers, and various implants and filling materials in

D. Bai · J. Chen · P. Li (✉) · W. Huang
Xi'an Institute of Flexible Electronics (IFE) and Xi'an Institute of Biomedical Materials and Engineering (IBME), Northwestern Polytechnical University (NPU), Xi'an, Shaanxi, People's Republic of China
e-mail: iampli@nwpu.edu.cn

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_4

orthopedics, improving the quality of life of many patients. In recent 30 years of biomaterial evolution, biomaterials have been used in many implantation occasions such as fiber membranes for dialysis, artificial lung, auxiliary heart (segmented polyurethane), intraocular lens, dental adhesive, artificial bone, guide wire, and drug delivery system (e.g., microcapsule). In China, the output capacity of biomaterials such as bio-polyamide (bio-PA) and bio-polytrimethylene terephthalate (bio-PTT) has been put into large-scale industrial production which reached about 678,710 tonnes in total and 170,960 tonnes in the year of 2015 alone [1–4]. The compound annual growth rate (CAGR) of biomaterials was predicted to be over 10–20% till the year of 2020 [1, 4]; the Asia pacific orthopedic biomaterial market is predicted to grow with a CAGR of 12.6% during 2017–2023 [3] (Fig. 1).

While the industry of biomaterials has been thriving recently, the annual overall incidence of implantation device-caused infection is about 2–3% [2], and relatively few biomaterials have been designed with effective infection prevention property. Apart from the surgical operation and perioperative preventive measures, development of intelligent biomaterials is the key factor for implant design. Microbial proliferation can cause physical damage to the implant, such as loosening, dislocation, and structural instability, apart from causing systemic infection symptoms such as fever or embolism. Alongside bioactivity and biocompatibility, the chemical composition and physical properties are crucial for biomaterial design.

Infection around prosthesis implantation is a serious complication. Infections around implant and/or implant device often greatly reduce the patient's quality of life, by subjecting them to chronic pain and inconvenience. According to recent studies, biomaterial-associated infections are the most common cause of revision in the first 5 years after the initial replacement of the implantation [5]. In many cases,

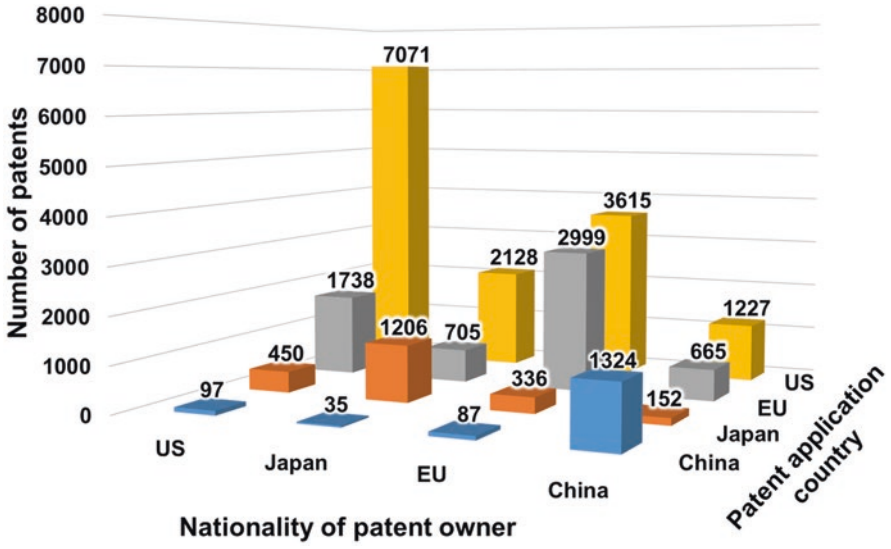
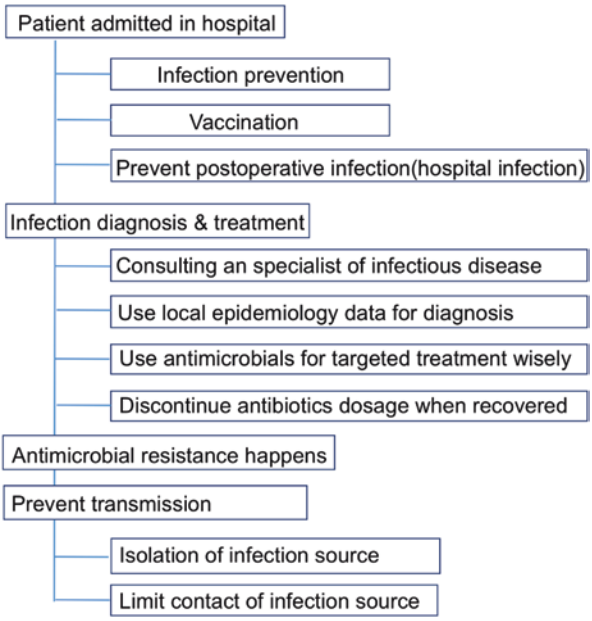


Fig. 1 Statistics of medical biomaterial-related intellectual property worldwide [1, 4]

infection around the prosthesis also means prolonged hospital stays, from weeks to months. For implantation-related nosocomial infections, long-term hospitalization, multiple surgeries, and anesthesia will increase the risk of patients' exposure to multidrug-resistant pathogens, resulting in secondary complications (pulmonary embolism, intubation-related sepsis, antibiotic-associated diarrhea, hemorrhoids, etc.), even the risk of death. Replacement surgery often requires more than one additional surgery to treat these infections with treatment of peripheral bone, muscle, and soft tissues. Consequences of biomaterial-associated infection have become a socioeconomic problem for the medical resource distribution and public health care system. Although progress has been made in preoperative, intraoperative, and postoperative management alongside the greatly improved surgical techniques, the infection rate has not decreased significantly over the past two decades. In the case of implantation infection, the only solution is systemic management of infection prevention before its occurrence. Treatment involving complete removal of all infected soft tissue and bone around the prosthesis has devastating consequences for patients. Therefore, no effort should be spared in reducing the risk of biomaterial-associated infections and effectively diagnosing and treating existing infections (Scheme 1).

For implantation such as artificial joint devices, infection after long-term implantation is a severe problem. The presence of foreign biomaterials in the human body for a long time may cause the patient's innate immune function to decline. When the



Scheme 1 Guidelines for prevention of implantation infection and antibiotic resistance. (US Center for Disease Control and Prevention [6])

surface of implant biomaterials becomes colonized with an infectious flora, the risk of developing infectious diseases cannot be avoided. The difficulty in controlling biomaterial-associated infectious diseases is that it is necessary not only to evaluate the antimicrobial properties at the time of manufacture but also to confirm the effectiveness after long-term placement [7]. However, methods for evaluating the long-term usage of biomaterials in human body environments and their associated material properties still have not been fully investigated. Although evaluation methods for cultured cells and tissues can be studied in various ways, there are fewer studies to investigate changes that occur within human physiological conditions [7]. To confirm whether a newly developed implant meets the required criteria, it is essential to evaluate the long-term characteristics of the biomaterial. Meanwhile, in order to facilitate biomaterial development, it is important to set long-term performance evaluation methods. In the case of medical surgery biomaterials, it is necessary to evaluate what may eventually occur 10 or 20 years after implantation in the human body. Now it is difficult to carry out long-term monitoring even in animal model experiments, which is challenging for biomaterial characterization and evaluation.

Strategies for the prevention, diagnosis, and treatment of biomaterial-associated infections have evolved over the past few years. Most hospitals comply with strategies agreed on by major professional societies. Since infections around the prosthesis have been recognized as the most serious complication of artificial implantation, more attention has been paid to the development of intelligent biomaterials with infection resilience [8–10]. In order to effectively prevent and treat infections in the future while maintaining the function of implants, multidisciplinary collaboration between medical specialists, material science researchers, and the industry needs to be established. This chapter focuses on the pathogenesis of biomaterial-associated infections, current clinical demands of infection-reducing biomaterials, and recent research of infection-reducing strategies, intended to further facilitate research in this area.

Pathogenesis of Biomaterial-Associated Infection

As the phrase “the race for the surface” suggests [11, 12], the fate of biomaterial implants is influenced by a competition between host tissue cell integration and bacterial colonization at their surfaces. Microorganisms may enter the patient’s body during the surgery. Recent studies also suggested that biomaterial-associated infections might be lifestyle related. Physical conditions including past surgical history, diabetes (blood glucose >200 mg/L or HbA1C >7%), nutrition deficiency, obesity (BMI > 40 kg/m^2), chronic liver disease or kidney diseases, excessive smoking (>1 pack/day), excessive drinking, and drug abuse would put the patient at higher risk of biomaterial-associated infections [13].

In due course of implantation, if biomaterials cause damage to the epithelium and the mucosal barrier, the implant or implant device may weaken the host’s

defense system and provide a growing niche for microorganisms, allowing pathogens to access blood circulation and deep tissues. Meanwhile, biomaterials may release soluble components and form high-density fibrous tissue membranes around the implant or implant device, which would act as a mechanical barrier preventing immune responsive macrophages from migrating to the interface and allowing pathogens to survive near the implant. Implanted biomaterials may also interfere with the physiological process of anti-infection through surface–media interactions; tissues around the implant site may be prone to infection diffusion [14]. The choice of implantation biomaterials is crucial because the physical and chemical properties of the biomaterial you choose determine their capacity for preventing or inducing adsorption, infection, and inflammation under healthy physiological conditions when it interacts with different microorganisms.

Mechanistic studies of bacterial and fungal biofilm formation on implantation biomaterials has not received sufficient attention yet. Microorganisms can form biofilms that protect microbes against antibiotics and from the body's own immune system. Biofilm formation helps pathogens adapt to chemical and physical conditions of microenvironment, the biochemical interactions of the host defense, and also antibiotic regimes, assisting in intercellular communication and nutrition for pathogen proliferation [15–19].

As shown in Fig. 2, once attached to the surfaces, bacteria or fungi adhere firmly. The pathogens rapidly grow into microcolonies and secrete extracellular polymeric substances (EPS) to form a three-dimensional matrix cell structure termed biofilm. EPS consists of polysaccharides, proteins, and sometimes extracellular DNA (eDNA). Polysaccharide intercellular adhesin (PIA) process also happens which involves staphylococcal surface protein (60 kDa) [20, 21]. After maturation, the biofilm can disperse causing the bacteria to diffuse and spread [22], seeding acute infections [23]. It is difficult to eradicate biofilms due to their characteristics; the host cells around the biofilm are in a dormant state. The only effective solution is to prevent the formation of bacterial biofilms via strategic design of biomaterials.

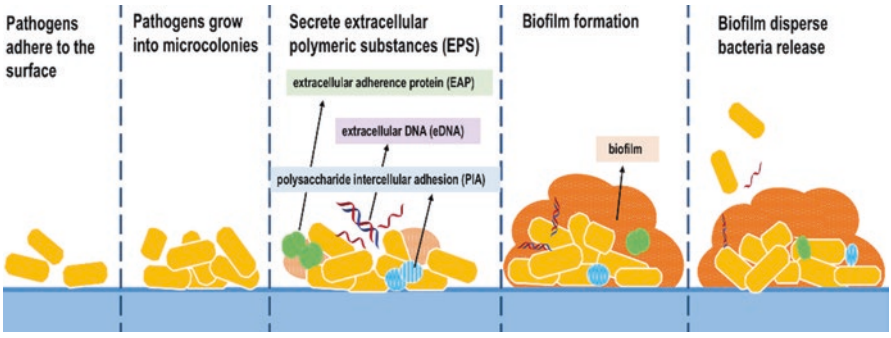


Fig. 2 Mechanism of biofilm formation on a biomaterial surface

Diagnosis and Treatment of Biomaterial-Associated Infections

Early diagnosis of biomaterial-associated infections and the severity of the infections are still quite challenging. At present, there is a lack of consensus of treatment procedure among the specialists, the clinical features of infection around the prosthesis are still not clear, especially how to distinguish biomaterial-associated infections from the failure of the implant to remain sterile; the diagnostic criteria are still controversial, and the choice of suitable antibiotics or surgical methods for treatment is still inconclusive. There is an unmet need of a global-scale survey-based statistics to develop guidelines for handling biomaterial-associated infections and general clinically supported guidelines for the use of various treatments. Patients with persistent or recurrent infections often require multiple surgeries, which can lead to anatomical damage (muscle contractures, bone defects, loss of soft tissue coverage, etc.), which may require additional operation for joint fixation, Girdlestone procedure, or even amputation. Patients with persistent infections are often under great stress due to chronic pain (Fig. 3).

There are many classifications of infections around prosthesis based on different stages, each with its own criteria. As commonly agreed by many specialists, the simplest classification is to divide biomaterial-associated infections into early infections and late infections. Early infection refers to an infection that occurs within 3–4 weeks after the implantation of the prosthesis or the onset of symptoms [24]. Early acute

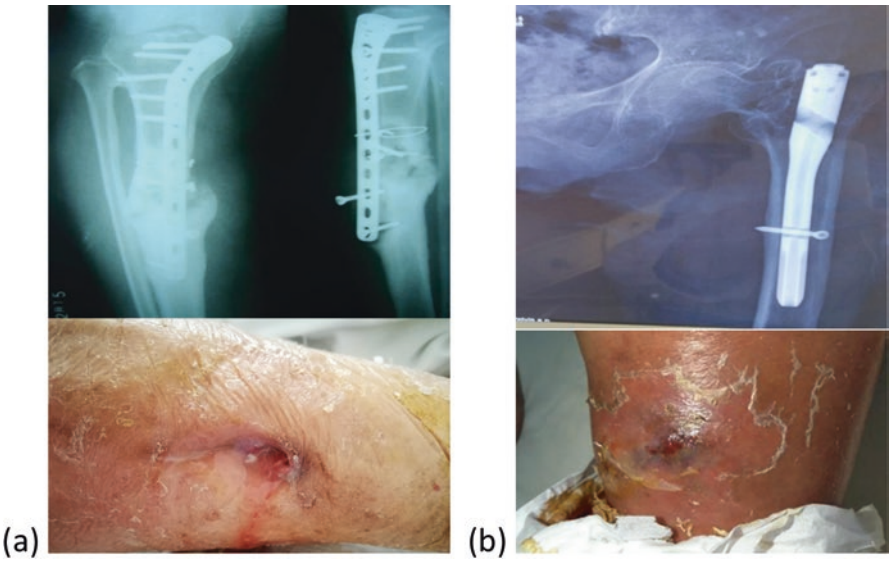


Fig. 3 (**a**) Infection after knee arthroplasty with visible sinus; (**b**) tissue infection around the prosthesis after total hip arthroplasty with visible osteonecrosis after removal of the prosthesis. (Photographs courtesy of Prof. Fanpu Ji at Department of Infectious Diseases, 2nd Hospital of Xi'an Jiaotong University, with consent of patients)

infection symptoms are usually caused by intraoperative misconducts; biofilm production is less or immature in this stage [25]. Infections that occur after 3–4 weeks are classified as late infections, meaning that these infections are caused by blood sources, even several years or decades after surgery. Infections that occur after more than 4 weeks after surgery often accompanied with persistent pain at infection site, and low-virulence pathogens such as coagulase-negative staphylococci, enterococci, or Propionibacterium acnes (P. acnes) are more common source. Acute homogenous infection may happen more than 2 years after surgery, due to bloodborne dissemination; the clinical symptoms are typical redness, heat, and pain, the pathogen source often includes streptococcus and gram-negative bacilli with culture-positive rate <50% [24]. Infection stage classification has certain significance for the treatment plan, but it must be emphasized that infection is a continuous and coherent process. Follow-up treatment must not be based solely on the stage classification; other factors such as the stability of the prosthesis, the presence of sinus, pathogenic virulence, and patients' relevant medical history should also be taken into consideration.

For early infection cases, it may be reasonable to retain the prosthesis. For advanced infections in late stages, the prosthesis, all foreign bodies, and infected bone and soft tissue should be removed [26]. If the prosthesis is implanted close to the surface of the skin, infections are usually discovered in early stages with redness, swelling, heat, and pain around the implant device or implant. Pain is the most important clinical symbol of infection; if pain suddenly occurs after an asymptomatic period, then clinical examination must be performed. Formation of fistula and/or exudation around the implantation part of the body is also considered as a sign of local infection; serum examination of biomarkers should be carried out [25, 27]. Systemic immune and neural symptoms such as fever and muscle dysfunction may occur later as implants and devices gradually become impaired. Infected artificial joints such as hip or knee implants can cause walking pain and walking instability. Infected prosthetic heart valve may cause fatigue as the patient has less cardiac output, eventually leading to severe heart failure. An effective surgery with antibiotic treatment plan is needed to alleviate the infection and pain, and restore function, yet there are still no clear treatment guidelines to ensure more than 90% success rate of long-term treatment.

Alongside echocardiography and scintigraphic imaging (X-ray, CT, fMRI) methods, laboratory-based biochemistry and immunoassay play an important role in the development in the diagnosis of biomaterial-associated infections. In serum testing, elevated levels of indicators such as procalcitonin (PCT), erythrocyte sedimentation rate (ESR), sedimentation rate (sed rate), C-reactive protein (CRP) may be associated with infections. In urine testing, a positive leukocyte esterase test indicates infection. If noninvasive tests fail to diagnose infections, puncturing to collect cerebrospinal fluid and/or synovial fluid from the implantation area that is suspected of being infected must be performed in the operating room with strict aseptic procedures. Patients should cease their antibiotic doses 10–14 days prior to puncture. Specimens obtained by puncture should be sent to the nearest qualified laboratory as soon as possible for further tests and must be cultured for at least 14 days to ensure that slow-growing pathogens can be detected. Increased white blood cell (WBC)

count or increased percentage of neutrophils (PMN%) should be considered a red flag. If clinical manifestations and serological tests are highly suspected of infection around the prosthesis, but bacterial culture test is negative, an open surgical biopsy should be performed. Biopsy specimens collected from around the prosthesis area are more accurate for examination of bacterial culture or histological analysis [5]. If at least two tissue culture tests around the prosthesis have found the same pathogen, an infection could be concluded. Special attention is due if the patient is seriously suspected to have a periprosthetic infection, even if the above diagnostic criteria are not met; infection should be considered with the help of further examination and treatment. Formation of biofilm may significantly reduce the sensitivity of traditional microbial culture techniques, making pathogenic examinations difficult. Currently, there is limited consensus in the diagnosis gold standards and treatment methods; thus, different guidelines should be considered to understand the limitations of each type of detection method. The application and analysis combined with the examination of actual patients' condition need multidisciplinary cooperation. In addition, the sensitivity of qualitative and quantitative examination via biochemical and histological analyses could be further improved with techniques such as sonication, real-time quantitative PCR, metagenomic next-generation sequencing (mNGS), and Ibis T5000 universal biosensor system.

Causative Pathogens of Biomaterial-Associated Infections

Upon usage, biomaterials directly or indirectly contact or interact with the human body components (e.g., organs, tissues, cells, and proteins). Most prostheses such as vascular and blood vessel stains are embodied under the skin within the body and do not have an opening surface for infection. However, implants used in dental treatments usually have extended structure from within the tissue to outside the tissue implant contact point. Biomaterials placed in such a fashion with exposed parts which create the niche of polysaccharide and hemidesmosome secretion are susceptible to infection. Although adhesion, repair, and immune function are retained in the surrounding tissues of implants, the binding part between implanted biomaterials and the tissue mucosa has a much weaker protective mechanism. If inflammation reaches the bones along the tissue surface, especially if an implant has uneven structure, it is difficult to remove the infected surrounding tissue, since at present there is no effective early diagnostic techniques against peri-implant inflammation.

Biomaterial-Associated Infection-Related Drug Resistance

Drug resistance of pathogens is the main enemy we face in the first line of designing anti-infection biomaterials. Just as penicillin-resistant bacteria have already existed before the appearance of penicillin, most of the drug-resistant pathogens have existed in nature long before drug discovery. If antibiotic drugs are continually

applied, the susceptible strains of pathogens may be destroyed, and the resistant strains may survive and eventually proliferate through mutation and evolution. The use of antibiotics means the selection of more resistant pathogens. The prevalence of drug-resistant pathogens may increase when pathogenic microorganisms are frequently exposed to antibiotics. As shown in Table 1, the pathogens in biomaterial-associated infections often include gram-negative bacteria, aerobic gram-positive bacteria, fungi, and even mixed strain of pathogens. The American College of Orthopaedic Surgeons (AAOS) clinical guidelines for the diagnosis of prosthetic infections strongly recommend against the use of antibiotics prior to infection diagnosis. If antibiotics are applied before sample collection for diagnostic tests, the influence of biofilm formation often leads to negative culture results of pathogen culture tests. At present, about 15–20% of implantation infection patients have negative clinical bacterial culture, and the negative results may make diagnosis by doctors perplexing.

As shown in Table 2, multidrug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA) and *Staphylococcus epidermidis* are common sources of infection. Small colony variants (SCVs) including *S. aureus*, *Pseudomonas aeruginosa* (*P. aeruginosa*), and several other bacteria can even grow within the temporary spacer containing gentamicin [46]. Life-threatening pathogens such as enterobacteria, non-fermenting bacteria (e.g., *Acinetobacter* spp., *Pseudomonas* spp.) are resistant to penicillin, cephalosporin, quinolone, and carbapenem, which are classified as 3MRGN (multidrug-resistant gram-negative) or 4MRGN according to Kommission für Krankenhaushygiene und Infektionsprävention (KRINKO) [47]. These multidrug-resistant pathogens are resistant to all known Class 3 or Class 4 antibiotic drugs. When nosocomial outbreak occurs or when there are infections caused by drug-resistant pathogens, there is little to do clinically.

With the drug resistance problem in mind, two aspects must be considered while deciding on the treatment of biomaterial-associated infections: the annihilation of the pathogen by effective drug dosing regimens and the suppression of emergence of resistant pathogen strains. In order to suppress the emergence of resistant pathogen strains, firstly, antibiotics should not be prescribed when the patient is only a carrier without symptoms or when test results of infections are inconclusive; secondly, the antibiotic regimens with sufficient dosage should be stopped immediately after the infection symptoms cease to exist; thirdly, the use of a single antibiotic drug should be avoided in order to decrease the selective pressure of drug resistance; last but not least, nosocomial infections should be prevented with strict regulations, and human-to-human transmission routes must also be prohibited effectively.

Clinical Demands: Desirable Properties of Infection-Reducing Biomaterials

The US Public Law 105-230: Biomaterials Access Assurance Act of 1998 and the FDA guidance of the International Standard ISO 10993-1 [48] insist that biomedical evaluations of implantation biomaterials be required carried out before implantation.

Table 1 Cases of causative pathogens in biomaterial-associated infections and antimicrobial drugs used [28–32]

Causative pathogens	Drugs	Product name	Side effect	Remarks
MRSA (methicillin-resistant *Staphylococcus aureus*)	Lincosamide	Clindamycin	Neuromuscular blocking effect	Can be used in combination with gentamicin
	Lipopeptide	Saptomycin	Myopathy	
	Ceftaroline	Teflaro	Diarrhea, nausea, and rash	
	Tetracycline	Tetracycline	Can cause tooth staining, enamel dysplasia, and liver damage	
	Sulfonamide	Trimethoprim/sulfamethoxazole	Crystalluria	The resistance of bacteria to these drugs is widespread
MSSA (methicillin-sensitive *Staphylococcus aureus*)	Cephamycin	Cefoxitin; cefmetazole; Cefminol	Contraindicated for penicillin allergy	Cefoxitin is not recommended for infants under 3 months
	Cephalosporin	Cefuroxime	Contraindicated for penicillin allergy; adjusting dose for hepatic dysfunction	Can be used in combination with anaerobic drugs (such as metronidazole) to treat abdominal and pelvic infections; alcohol and alcoholic beverages are prohibited
	β-lactam/β-lactamase inhibitor	Amoxicillin/clavulanic acid	Contraindicated for penicillin allergy	
Coagulase-negative staphylococci	β-lactam/β-lactamase inhibitor	Amoxicillin/clavulanic acid; Cefoperazone/sulbactam	Contraindicated for penicillin allergy	
	Tetracycline	Tetracycline	Can cause tooth staining, enamel dysplasia, and liver damage	
	Lipopeptide	Daptomycin	Myopathy	

Gram-positive organisms (e.g., enterococci)	Cephalosporin	Cefazolin	Adjusting dose for renal dysfunction; contraindicated for penicillin allergy	Can work synergistically with aminoglycosides
	Fosfomycin	Fosfomycin calcium capsules; fosfomycin sodium	Dizziness, headache, nausea, weakness, dyspepsia	Not recommended non–urinary tract infections are
	Cephalosporin	Cefuroxime	Contraindicated for penicillin allergy; adjusting dose for hepatic dysfunction	Can be used in combination with anaerobic drugs (such as metronidazole) to treat abdominal and pelvic infections; alcohol and alcoholic beverages are prohibited
Pseudomonas aeruginosa	Cephalosporin	Cefoperazone	Contraindicated for penicillin allergy; adjusting dose for hepatic dysfunction	Can be used in combination with anaerobic drugs (such as metronidazole) to treat abdominal and pelvic infections; alcohol and alcoholic beverages are prohibited
	Carbapenem	Imipenem; meropenem; panipenem	Nausea, vomiting, diarrhea, and so on	Combination with valproic acid or divalproic acid is not recommended
	Monocyclic β-lactams	Aztreonam		Can be used in combination with anaerobic drugs (such as metronidazole) to treat abdominal and pelvic infections
Fungi	Polyene	Amphotericin B deoxycholate	Fever, chills, hypotension, nausea, or tachycardia	
	Flucytosine	Flucytosine	Nausea, diarrhea, rash, hallucinations, headaches, dizziness, and so on	Can be combined with amphotericin B
	Pyrrole	Clotrimazole, ketoconazole	Liver and kidney toxicity	
	Triazole	Posaconazole	Bilirubinemia, elevated aminotransferase, hepatocyte damage, nausea, and vomiting	Can be combined with amphotericin B

(continued)

Table 1 (continued)

Causative pathogens	Drugs	Product name	Side effect	Remarks
Streptococci	Penicillin	Penicillin G	Dosage should be reduced in case of lactating women and patients with renal dysfunction, to avoid allergic reactions	Can work synergistically with aminoglycosides
	Ceftolozane	Tazobactam	Contraindicated for penicillin allergy	Not recommended for complex abdominal infection (cIAI) or complex urinary tract infection (cUTI)
	Cephamycin	Cefoxitin; cefmetazole; cefminol	Contraindicated for penicillin allergy	Cefoxitin is not recommended for infants under 3 months
	Oxycephalosporins	Latamoxef	Can lead to thrombin deficiency, thrombocytopenia, and dysfunction leading to bleeding	
Nonfermenting gram-negative bacilli	Cephalosporin	Cefotaxime; ceftriaxone; cefoperazone; ceftazidime	Contraindicated for penicillin allergy; adjusting dose for hepatic dysfunction	Can be used in combination with anaerobic drugs (such as metronidazole) to treat abdominal and pelvic infections; alcohol and alcoholic beverages are prohibited
	Monocyclic β-lactam	Aztreonam		Can be used in combination with anaerobic drugs (such as metronidazole) to treat abdominal and pelvic infections

Enterobacteriaceae	Cephalosporin	Cefepime	Contraindicated for penicillin allergy; adjusting dose for hepatic dysfunction	Can be used in combination with anaerobic drugs (such as metronidazole) to treat abdominal and pelvic infections; alcohol and alcoholic beverages are prohibited
	Sulfonamide	Trimethoprim/ sulfamethoxazole	Crystalluria	The resistance of bacteria to these drugs is widespread
	Cephamycin	Cefoxitin; cefmetazole; cefminol	Contraindicated for penicillin allergy	Cefoxitin is not recommended for infants under 3 months
	β-lactam/β-lactamase inhibitor	Amoxicillin/clavulanic acid	Contraindicated for penicillin allergy	
	Oxycephalosporin	Latamoxef	Can lead to thrombin deficiency, thrombocytopenia, and dysfunction leading to bleeding	
	Aminoglycoside	Neomycin; paromomycin	Due to the high toxicity (nephrotoxicity, ototoxicity, and neuromuscular blockade), it is only used orally or topically	Should not be used with other strong diuretics
	Tetracycline	Tetracycline	Can cause tooth staining, enamel dysplasia, and liver damage	
	Carbapenem	Imipenem; meropenem; panipenem	Nausea, vomiting, diarrhea, etc.	Combination with valproic acid or divalproic acid is not recommended
MRGN (multidrug-resistant gram-negative) bacteria	Carbapenem	Imipenem; meropenem; panipenem	Nausea, vomiting, diarrhea, etc.	Combination with valproic acid or divalproic acid is not recommended
	Ceftolozane	Tazobactam	Contraindicated for penicillin allergy	Complex abdominal infection (cIAI) or complex urinary tract infection (cUTI) are not recommended
	β-lactamase inhibitor	Sulbactam	Contraindicated for penicillin allergy	Can used in conjunction with other drugs

Table 2 Cases of biomaterial-associated infections in various body sites

Body site	Implant or device	Cause of infection	Prevention strategy	References
Bone	Hip/knee arthroplasty	*Staphylococcus aureus*	Good aseptic technique and procedures in the operating room	[33–36]
		α-toxin and clumping factor A		
	Nasal implants	Pharyngeal anaerobic bacteria (e.g., digestive streptococci)	Surgical debridement	
	Chin augmentation implants		Targeted therapy	
	Cerebrospinal fluid shunts		Impregnated implant materials incorporated with antimicrobial agents	
	Dental implants		Antibiotic-loaded spacer	
			First- and second-generation cephalosporins, metronidazole, clindamycin, gentamicin	
			Peptide	
Soft tissue	Mammary prosthesis	Brucella species	Surface coating	[37–40]
	Abdominal wall patches	Diabetes mellitus (glycated hemoglobin level)	Reduction of surgical time	
	Penile prostheses	*Escherichia coli (E. coli)*, *Staphylococcus* species, and *Candida* species	Tobramycin or levofloxacin	
	Intraocular lenses		Penicillin	
Subcutaneous	Tissue expanders	*Staphylococcus aureus*, coagulase-negative staphylococci, streptococcus	First- and second-generation cephalosporins	[41]
	Pins in external fracture fixation		Penicillin	
Intraperitoneal	Peritoneal dialysis catheters	Gram-negative bacilli	First- and second-generation cephalosporins, fluoroquinolone	[42]
Circulatory system	Cardiac pacemaker	MRSA	Vancomycin	[28, 29, 31, 32, 43–45]
	Foley catheter	Uropathogenic *E. coli*	Norvancomycin	
	Arterial catheters		Topical antimicrobial agents	
	Prosthetic heart valve		Clindamycin	
	Peripheral inserted venous catheters		Rifampin	
	Aortoiliofemoral bypasses		Tetracycline	
	Intravascular catheters		Trimethoprim/sulfamethoxazole	

Low toxicity, nonallergenic, and low inflammatory reaction should be tested as a biocompatibility indicator. Biomaterials with sufficient in vivo stability (corrosion resistance and abrasion resistance) are required. Biomaterials used for implants, implant device, or catheters that penetrate the skin that is in contact with tissue or bone area must have interface compatibility and firm connectivity. Adhesion property is also required to be considered in order to avoid the invasion space of bacteria. In contact with tissue, biomaterials may trigger the surrounding tissue cells to generate extracellular matrix (ECM) components contained in serum. Adsorption of biomolecules onto the surface of implanted biomaterials is followed by cell adhesion behavior as well as immune responses (cell migration, proliferation, differentiation). For instance, if cell adhesion molecules such as fibronectin are adsorbed on the surface of the biomaterial before bacteria colonization, the adhesion between the implanted biomaterial and surrounding tissue cells increases.

The process of biomaterial–cell adhesion within the implant's surrounding tissue takes place through a series of events as follows: (1) physical adsorption of ECM to the surface of the material; (2) binding between the ECM and the cell membrane protein (integrin) and the adhesion spot associated protein; (3) binding between the adhesion complex protein and the cytoskeleton, that is, the binding proteins penetrate cell membrane in form of chains. In this state, when a shearing force (a force parallel to the adhesion interface) is applied to the cells, the material–cell adhesion breaks at the weakest part, and the cells are detached. It has been reported that the weakest binding point is actually inside the cells rather than between the surface of the substrate and the ECM. As for the improvement of biomaterial design strategies, it is important to facilitate the adsorption step of cell adhesion with the surface of biomaterials, while ensuring that minimum shearing force is applied, to break the material–cell adhesion interface binding. The strength of deformation force is generated at the interface. In each biomaterial–cell/tissue interface, the binding breaking force is different. In addition to controlling the biomaterial adsorption behavior on the material surface, it is important to match the mechanical properties between the material and the biological tissue, in order to maintain the intermolecular binding properties. Ideal biomaterials with intelligence should be able to generate self-organizing and self-governing functionality at their interface with surrounding host tissues. Activation of host tissue–biomaterial interaction and long-term functional retention are also key performance indicators.

Besides mechanical properties, examination of the intracellular interactions between the biomaterial implants and the surrounding tissue cells also requires biochemical analysis. Infection-reducing components must not interfere with the physicochemical properties of the biomaterial. On the other hand, the biomaterial activities should not be inactivated by the patient's innate immune response. Therefore, elucidation of various biomarkers for performance evaluation is needed. The recent development of nucleic acid-based microarray analysis has made it possible to examine in a timely manner the gene expression level of surrounding cells interacting with the biomaterial. However, with the emerging research in regenerative medicine and tissue engineering, at present, the correlation between the gene expression profile of cultured cells in vitro and the gene expression of implantation

surrounding tissues in vivo of the patient's body has not been confirmed. At the same time, many studies have reported the optimal culture conditions for inducing functional expression of cells on scaffold biomaterials. In order to resolve the occurrence of infections after long-term implantation, recent reports have shown that it is possible to examine the effectiveness of infection-reducing agents in biomaterials by various tests. The problem that remains to be addressed is the need for conducting an evaluation of the infection-reducing properties not only at the time of manufacture but also after long-term implantation. Current methods for evaluating long-term exposure to the in vivo environment and the long-lasting infection-reducing activity of the biomaterial after implantation have still not been fully studied. As the nature of the interface determines the function of the biomaterial to a large extent, strategic designing of interface with more advanced functions such as sensing or exerting bioactivity and stimuli responsiveness is needed. The strategic design of interface properties and functionalities between biomaterials and surrounding tissue cells is considered to be a major development, namely, the intelligentization of the interface.

Approach from various disciplines could be employed for the design of infection-reducing biomaterials, including chemical and physical methods for alteration of material composition, surface treatment; biomedical methods such as construction of drug releasing materials; molecular biology approach such as using functional proteins. Recent reports have shown that biomaterials releasing drugs such as bisphosphonates, statins, and parathyroid hormone could facilitate bone metabolism.

Summary and Outlook

The reliability of retrospective studies on the rate of infection after prosthesis implantations might be compromised because of individual variability among patients and differences in other aspects (operative time, surgical techniques, blood transfusions, operating room, etc.), which are factors that have a major impact on the infection process. For the same reason, the analysis of implantation registration center data may also lead to biased conclusions given the lack of information about biomaterial-associated infections. It is clear that we need to find a more scientific method to assess the capacity of biomaterials' resilience to infection. In the future, for long-term implantation with intelligent biomaterials, multidisciplinary collaborations of epidemiology, etiology, surgery, microbiology, infectious disease, and pharmacology should be promoted to conduct in-depth research on the diagnosis and treatment of biomaterial-associated infections and to fully combine the expertise of materials chemistry and physics research with that of industry.

Acknowledgments This work was financially supported by the National Key R&D Program of China (2018YFC1105402 and 2017YFA0207202), the National Natural Science Foundation of China (21875189 and 81601553), Key R&D Program of Jiangsu Province (BE2017740), the Natural Science Foundation of Zhejiang Province (LGF19H200005), and the Fundamental Research Funds for the Central Universities.

References

1. Biomaterial industry in China. CCM Data & Business Intelligence 2016
2. Nana A, Nelson SB, McLaren A, Chen AF (2016) What's new in musculoskeletal infection: update on biofilms. JBJS 98(14):1226–1234
3. Europe, Health & Medicine, Pharmaceuticals & Biotech (2019) Asia pacific orthopedic biomaterial market 2019, industry analysis, size, share, trends, product price, profit, forecast to 2023
4. Patent application trend survey report summary (artificial organs). https://www.jpo.go.jp/shiryou/pdf/gidou-houkoku/h28/28_06.pdf. Accessed 8 May 2019
5. Kheir MM, Tan TL, Shohat N, Foltz C, Parvizi J (2018) Routine diagnostic tests for periprosthetic joint infection demonstrate a high false-negative rate and are influenced by the infecting organism. JBJS 100(23):2057–2065
6. US Disease Control and Prevention (CDC). Appropriate antibiotic use in hospitals and long-term care. https://www.cdc.gov/antibiotic-use/healthcare/index.html. Accessed 26 April 2019
7. Use of International Standard ISO 10993-1. Biological evaluation of medical devices—part 1: evaluation and testing within a risk management process. https://www.iso.org/standard/44908.html. Accessed 26 April 2019
8. Holzapfel BM, Reichert JC, Schantz JT, Gbureck U, Rackwitz L, Noth U, Jakob F, Rudert M, Groll J, Hutmacher DW (2013) How smart do biomaterials need to be? A translational science and clinical point of view. Adv Drug Deliv Rev 65(4):581–603
9. Singh B (2018) NanoBioMaterials: nanobiomaterials. CRC Press
10. Yamamoto R (2017) Changes and prospects of implant materials from the viewpoint of biocompatibility. Materia 56(3):225–228. (in Japanese)
11. Gristina AG, Naylor P, Myrvik Q (1989) Infections from biomaterials and implants-a race for the surface. Med Prog Technol 14(3–4):205–224
12. Busscher HJ, van der Mei HC, Subbiahdoss G, Jutte PC, van den Dungen JJ, Zaat SA, Schultz MJ, Grainger DW (2012) Biomaterial-associated infection: locating the finish line in the race for the surface. Sci Transl Med 4(153):153rv10
13. Kapadia BH, Berg RA, Daley JA, Fritz J, Bhave A, Mont MA (2016) Periprosthetic joint infection. Lancet 387(10016):386–394
14. Kannan MB, Walter R, Yamamoto A (2015) Biocompatibility and in vitro degradation behavior of magnesium–calcium alloy coated with calcium phosphate using an unconventional electrolyte. ACS Biomater Sci Eng 2(1):56–64
15. Baidya AK, Bhattacharya S, Dubey GP, Mamou G, Ben-Yehuda S (2018) Bacterial nanotubes: a conduit for intercellular molecular trade. Curr Opin Microbiol 42:1–6
16. Bryers JD (2018) Modeling biofilm accumulation. Physiological models in microbiology volume II, Chapter 11
17. de la Fuente-Nunez C, Reffuveille F, Fernandez L, Hancock REW (2013) Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. Curr Opin Microbiol 16(5):580–589
18. Flemming HC, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8(9):623–633
19. Romling U, Balsalobre C (2012) Biofilm infections, their resilience to therapy and innovative treatment strategies. J Intern Med 272(6):541–561
20. Sasaki K (2016) Polysaccharide intercellular adhesion (PIA) process also happens which involves staphylococcal surface protein. J Jpn Soc Poweder Powder Metallurgy 63(8). (in Japanese)
21. Guidelines for Biomaterial Research That Supports Medical Care (2017) Annual meeting of Japan biomaterial engineering committee. (in Japanese)
22. Wolfmeier H, Pletzer D, Mansour SC, Hancock REW (2018) New perspectives in biofilm eradication. ACS Infect Dis 4(2):93–106
23. Guilhen C, Forestier C, Balestrino D (2017) Biofilm dispersal: multiple elaborate strategies for dissemination of bacteria with unique properties. Mol Microbiol 105(2):188–210

24. Guo Y, Shao HY. Characteristics and challenges of human implant infection (in Chinese). Beijing-Hong Kong infection Forum 2018
25. Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, Rao N, Hanssen A, Wilson WR (2013) Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 56(1):e1–e25
26. Elkins JM, Kates S, Lange J, Lange J, Lichstein P, Otero J, Soriano A, Wagner C, Wouthuyzen-Bakker M (2019) General assembly, diagnosis, definitions: proceedings of international consensus on orthopedic infections. J Arthroplast 34(2):S181–S185
27. Parvizi J, Della Valle CJ (2010) AAOS Clinical Practice guideline: diagnosis and treatment of periprosthetic joint infections of the hip and knee. J Am Acad Orthop Surg 18(12):771–772
28. Drago L, Lidgren L, Bottinelli E, Villafañe JH, Berjano P, Banfi G, Romanò CL, Sculco TP (2016) Mapping of microbiological procedures by the members of the International Society of Orthopaedic Centers (ISOC) for diagnosis of periprosthetic infections. J Clin Microbiol 54(5):1402–1403
29. Higuera CA, Zmistowski B, Malcom T, Barsoum WK, Sporer SM, Mommsen P, Kendoff D, Della Valle CJ, Parvizi J (2017) Synovial fluid cell count for diagnosis of chronic periprosthetic hip infection. JBJS 99(9):753–759
30. Johns Hopkins ABX Guide. 2018
31. Guidelines for clinical application of antimicrobial agents (2015 edition, in Chinese). National Health and Family Planning Commission
32. Thoendel M, Jeraldo P, Greenwood-Quaintance KE, Chia N, Abdel MP, Steckelberg JM, Osmon DR, Patel R (2017) A novel prosthetic joint infection pathogen, Mycoplasma salivarium, identified by metagenomic shotgun sequencing. Clin Infect Dis 65(2):332–335
33. Cabrita HB, Croci AT, de Camargo OP, de Lima A (2007) Prospective study of the treatment of infected hip arthroplasties with or without the use of an antibiotic-loaded cement spacer. Clinics 62(2):99–108
34. Del Pozo JL, Patel R (2009) Infection associated with prosthetic joints. N Engl J Med 361(8):787–794
35. Wang Y, Cheng LI, Helfer DR, Ashbaugh AG, Miller RJ, Tzomides AJ, Thompson JM, Ortines RV, Tsai AS, Liu H, Dillen CA, Archer NK, Cohen TS, Tkaczyk C, Stover CK, Sellman BR, Miller LS (2017) Mouse model of hematogenous implant-related Staphylococcus aureus biofilm infection reveals therapeutic targets. Proc Natl Acad Sci U S A 114(26):E5094–E5102
36. Wang Z, de la Fuente-Nunez C, Shen Y, Haapasalo M, Hancock REW (2015) Treatment of oral multispecies biofilms by an anti-biofilm peptide. PLoS One 10(7):e0132512
37. Gao Q, Yu M, Su Y, Xie M, Zhao X, Li P, Ma PX (2017) Rationally designed dual functional block copolymers for bottlebrush-like coatings: in vitro and in vivo antimicrobial, antibiofilm, and antifouling properties. Acta Biomater 51:112–124
38. Gross MS, Phillips EA, Carrasquillo RJ, Thornton A, Greenfield JM, Levine LA, Alukal JP, Conners WP, Glina S, Tanrikut C, Honig SC, Becher EF, Bennett NE, Wang R, Perito PE, Stahl PJ, Gaya MR, Barbara MR, Cedeno JD, Gheiler EL, Kalejaiye O, Ralph DJ, Kohler TS, Stember DS, Carrion RE, Maria PP, Brant WO, Bickell MW, Garber BB, Pineda M, Burnett AL, Eid JF, Henry GD, Munarriz RM (2017) Multicenter investigation of the micro-organisms involved in penile prosthesis infection: an analysis of the efficacy of the AUA and EAU guidelines for penile prosthesis prophylaxis. J Sex Med 14(3):455–463
39. Habous M, Tal R, Tealab A, Soliman T, Nassar M, Mekawi Z, Mahmoud S, Abdelwahab O, Elkhouly M, Kamr H, Remeah A, Binsaleh S, Ralph D, Mulhall J (2018) Defining a glycated haemoglobin (HbA1c) level that predicts increased risk of penile implant infection. BJU Int 121(2):293–300
40. Scholz HC, Nockler K, Gollner C, Bahn P, Vergnaud G, Tomaso H, Al Dahouk S, Kampfer A, Cloeckaert M, Maquart MS, Zygmunt AM, Whatmore M, Pfeffer B, Huber P, Busse HJ, De BK (2010) Brucella inopinata sp nov., isolated from a breast implant infection. Int J Syst Evol Microbiol 60:801–808

41. Hoque J, Haldar J (2017) Direct synthesis of dextran-based antibacterial hydrogels for extended release of biocides and eradication of topical biofilms. ACS Appl Mater Interfaces 9(19):15975–15985
42. Mao X, Cheng R, Zhang H, Bae J, Cheng L, Zhang L, Deng L, Cui W, Zhang Y, Santos HA, Sun X (2019) Self-healing and injectable hydrogel for matching skin flap regeneration. Adv Sci (Weinh) 6(3):1801555
43. Hoque J, Adhikary U, Yadav V, Samaddar S, Konai MM, Prakash RG, Pararnanandham K, Shome BR, Sanyal K, Haldar J (2016) Chitosan derivatives active against multidrug-resistant bacteria and pathogenic fungi: in vivo evaluation as topical antimicrobials. Mol Pharm 13(10):3578–3589
44. Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, Talan DA, Grp EMINS (2006) Methicillin-resistant S-aureus infections among patients in the emergency department. N Engl J Med 355(7):666–674
45. Scott VCS, Haake DA, Churchill BM, Justice SS, Kim JH (2015) Intracellular bacterial communities: a potential etiology for chronic lower urinary tract symptoms. Urology 86(3):425–431
46. Minassian AM, Newnham R, Kalimeris E, Bejon P, Atkins BL, Bowler IC (2014) Use of an automated blood culture system (BD BACTEC™) for diagnosis of prosthetic joint infections: easy and fast. BMC Infect Dis 14(1):233
47. Heudorf U, Albert-Braun S, Hunfeld KP, Birne FU, Schulze J, Strobel K, Petscheleit K, Kempf VA, Brandt C (2016) Multidrug-resistant organisms in refugees: prevalences and impact on infection control in hospitals. GMS Hyg Infect Control 11
48. US Public Law 105-230 105th Congress, Biomaterials Access Assurance Act of 1998. https://www.biomaterials.org/sites/default/files/docs/2014/biomaterials_access_assurance_act_of_1998.pdf. Accessed 26 April 2019

Perspectives on and Need to Develop New Infection Control Strategies

Yong Liu, Linqi Shi, Henny C. van der Mei, Weihui Wu, Yijin Ren, and Henk J. Busscher

Abstract Bacterial infections by antimicrobial-resistant pathogens threaten to become the number one cause of death in 2050. Therewith the optimism about infection control that arose after the discovery of antibiotics has come to an end and new infection control strategies are direly needed. Development of new antibiotics is generally considered unlikely. In this chapter, a likelihood perspective is given, for the possibilities offered by combination and smart encapsulation of existing antibiotics, use of probiotics and phage therapy, antimicrobial peptides and nanotechnology-based antimicrobials. Combination of existing antibiotics with probiotics, antimicrobial peptides, or nanotechnology-based antimicrobials may also have good perspectives for clinical infection control, also when caused by antimicrobial-resistant strains. Therewith, existing antibiotics may still be useful for several decades to come despite the occurrence of antibiotic resistance, provided further research and development of the above strategies are focused on their downward clinical translation, carried out collaboratively within academia and industry, rather than on developing and publishing yet another, new antimicrobial compound.

Y. Liu · H. C. van der Mei · H. J. Busscher (✉)
Department of Biomedical Engineering, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
e-mail: h.j.busscher@umcg.nl

L. Shi
State Key Laboratory of Medicinal Chemical Biology, Key Laboratory of Functional Polymer Materials, Ministry of Education, Institute of Polymer Chemistry, College of Chemistry, Nankai University, Tianjin, PR China
e-mail: shilinqi@nankai.edu.cn

W. Wu
State Key Laboratory of Medicinal Chemical Biology, Key Laboratory of Molecular Microbiology and Technology of the Ministry of Education, Department of Microbiology, College of Life Sciences, Nankai University, Tianjin, PR China

Y. Ren
Department of Orthodontics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_5

Keywords Antimicrobial resistance · Antimicrobial delivery · Nanocarriers · Biofilm · Nano-antimicrobials · Probiotics · Phage therapies · Intracellular pathogens · Antimicrobial peptides

Introduction: Historical Perspective and Outlook

Long before the first microscopic observation of infectious bacteria, mankind has been struggling to effectively combat bacterial infections. Infection control strategies have for many centuries consisted of low potency antimicrobials, such as herbs, honey, old bread, and heavy metal salts, which were already used in ancient Roman, Chinese, and Egyptian cultures to cure infections. In 1640, Parkington found that molds were effective in curing wound infection (in: Wainwright [1]), while around the same time Van Leeuwenhoek [2] described the "*small animals on our teeth*" that we now call bacteria. In 1877, Pasteur found *Penicillium notatum* is harmful for the growth of *Bacillus anthracis*. Lactic acid producing bacteria were suggested by Döderlein [3] as early as in 1892 for the control of urogenital infections in women, while others made similar suggestions for intestinal infections [4–6].

In 1908, Nobel prize laureate Metchnikov proposed that longevity of Caucasian peasants was related to the high intake of fermented milk products. In his landmark paper "*On the prolongation of life*," Metchnikov described that ageing was caused by toxic bacteria in the gut and that the consumption of lactic acid bacteria in sour milk could elongate life. He was the first to allude to "*probiotic*" bacteria, by suggesting that harmful intestinal bacteria could be replaced by useful ones. In 2013, the World Health Organization recognized probiotics as "*live microorganisms that, when administered in adequate amounts, confer a health benefit on the host*" (in: Hill et al. [7]).

Around the same period that Metchnikov published his groundbreaking work on probiotics, Twort in 1915 demonstrated that bacteriophages could be targeted to and kill specific bacteria (in: Levin and Bull [8]). The first therapeutic use of phages by d'Herelle was reported in 1919, approximately a decade before Fleming's discovery of penicillin (in: Chan et al. [9]). In the 1940s, phages were marketed in the USA by Eli Lilly to treat a range of bacterial infections. However, further development of both probiotics and phage therapy for infection control were arrested by the hopeful discovery of penicillin by Fleming, except in the former Soviet Union where phage therapy was continued to be further developed and successfully applied during WWII to the aid of wounded soldiers (in: Wittebole et al. [10]).

Fleming incidentally observed the antibiotic effects of penicillin in 1923 [11]. Penicillin was first isolated in 1939 and its potency was unprecedented at the time. Penicillin was brought to clinical application in a record time, as accelerated by the need to help the many wounded soldiers in WWII. In 1943, penicillin was first tested on soldiers and in 1945 more than seven billion units were produced for

military use, in which year Flemming the name is Fleming was also awarded the Nobel Prize.

Many new antibiotics have been developed since and for several decades there was great optimism with respect to the control of bacterial infections: "*One day we could not save lives, or hardly any lives; on the very next day we could do so across a wide spectrum of diseases*" (in: McDermott and Rogers [12]). Antibiotics saved millions of lives, but at the same time their abuse and overuse stimulated the development of resistant bacteria. Although the first reports on bacterial resistance stem from the early 1940s [13, 14], it was still foreseen by some in 1986 that "*a manpower reduction of 36% in the number of fellows in infectious disease may be just about right*" (in: Petersdorf [15]).

Nowadays, the timeline of antibiotic discovery to observation of antibiotic resistance shows that in general antibiotic resistance occurs faster and faster after first discovery (Fig. 1). It is estimated that at least 700,000 people per year die from infections caused by antimicrobial-resistant pathogens and this number will rise to 10 million per year by 2050 (Fig. 2), overwhelming the current number of deaths caused by cancers [16].

Therewith, now that available antibiotics seem to reach their end-phase of efficacy, infection control is back to square one and the tide has changed again to pessimism, following the optimism stimulated by the discoveries of probiotics and phage therapy, both left largely unexplored. This somber outlook can only be reverted to a favorable change of the tides by rapid development and clinical translation of new infection control strategies, that we here briefly summarize and place in a likelihood perspective.

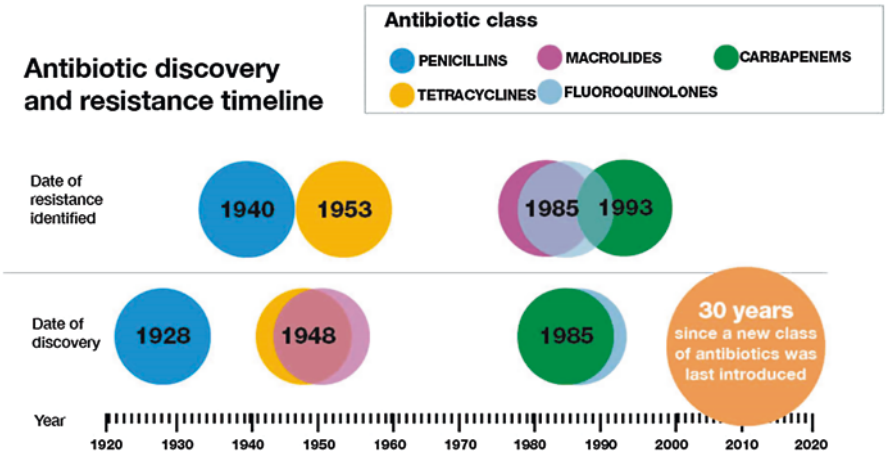


Fig. 1 Timeline of antibiotic discovery to observation of antibiotic resistance. (Downloaded on 10 Jan 2019 from: https://desdaughter.com/2016/02/14/antibiotic-discovery-and-resistance-timeline/)

New Strategies for Infection Control: A Likelihood Perspective

The timeline of antibiotic discovery to observation of antimicrobial resistance (Fig. 1) has greatly discouraged development of new antibiotics [17, 18]: "*Low hanging fruits have been plucked, economically it is not a good investment and research and development is too risky and expensive due to regulatory requirements*." Yet, with the outlook of the numbers of death by antimicrobial-resistant bacterial infections overwhelming the number of deaths caused by cancer in 2050 (Fig. 2 and [16]), new strategies for bacterial infection control are direly needed. As a consequence, "*old-fashioned*" strategies like probiotic and phage therapies are currently experiencing renewed interest. Biomimetic strategies, including application of antimicrobial peptides, are considered as well, while hopes are high with respect to nanotechnology-based antimicrobial strategies. In this section, we will briefly summarize new strategies considered nowadays and place them in a likelihood perspective.

Antibiotics

Whereas development of new antibiotics is considered unlikely for reasons mentioned above, this does not necessarily imply that the "age of antibiotics" has definitely come to a halt. Since the first reports on antibiotic resistance, many mechanisms of bacterial recalcitrance to antibiotic treatment have been revealed

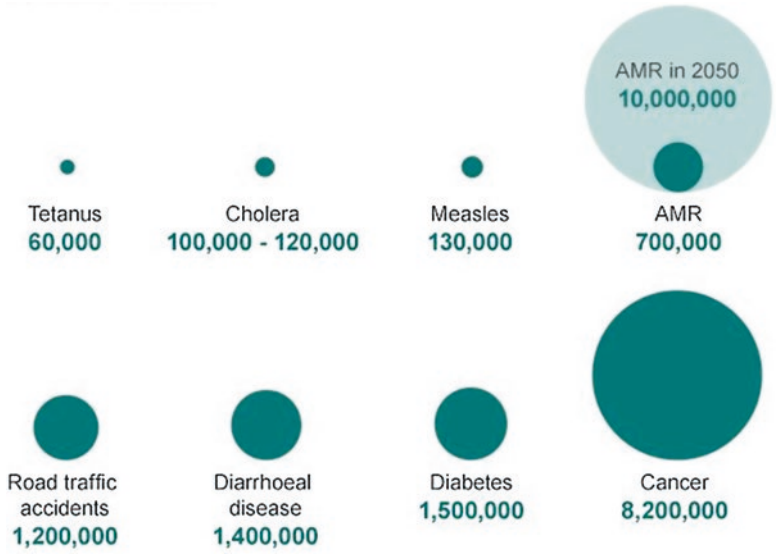


Fig. 2 Current annual numbers of deaths attributable to antimicrobial resistance and other diseases and the projected number of deaths attributable to antimicrobial-resistant (AMR) infection in the year 2050. (Downloaded on 10 Jan 2019 from: https://www.bbc.com/news/health-30416844)

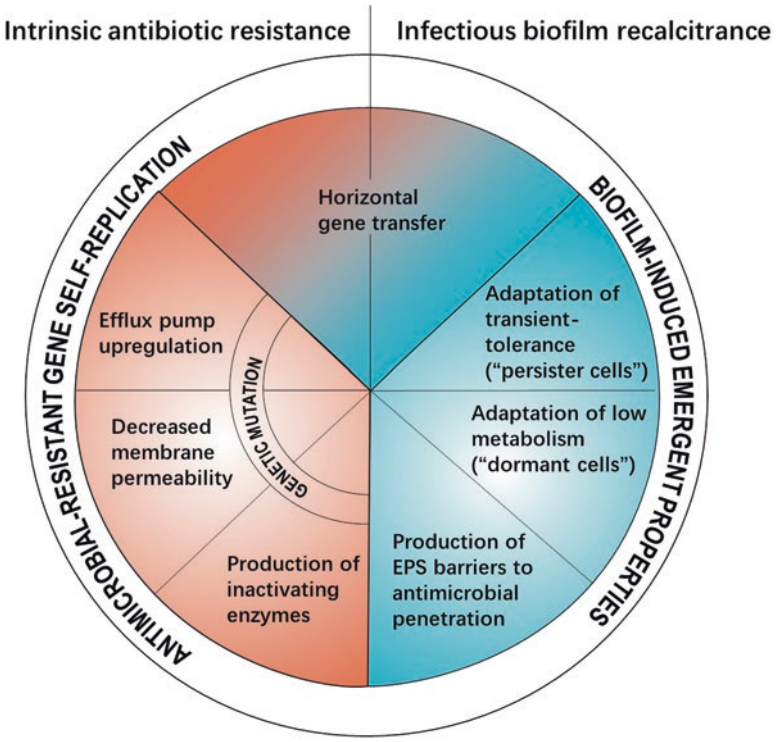


Fig. 3 Important mechanisms of bacterial recalcitrance to antimicrobial treatment, distinguishing factors related to intrinsic antibiotic resistance of the infecting bacterium and emergent properties of bacteria in their biofilm mode of growth, as is characteristic to the majority of bacterial infections [23]

(Fig. 3), that are either intrinsic [19–21] to the infecting bacterium or related to emergent properties [22] due to the biofilm mode of bacterial growth, in which the majority of bacterial infection present themselves [23]. In addition to the mechanisms summarized in Fig. 3, bacterial pathogens seek shelter in mammalian cells, that are difficult to penetrate by most existing antibiotics [24]. Despite these recalcitrance mechanisms, even intrinsically antibiotic-resistant bacteria have difficulties evading treatment by multiple, existing antibiotics at the same time and dual-antibiotic treatment can be effective against multidrug-resistant bacterial infections [25]. Alternatively, existing antibiotics can be administered together with protected (encapsulated, see below) probiotic bacteria or other new antimicrobial strategies for enhanced, synergistic action. Also, smart encapsulation of existing antibiotics with responsive and targeting features allows to establish higher local concentrations near or in an infection site than can be achieved through conventional administration (see also below). These developments imply that (existing) antibiotics when applied differently, may remain to be useful in bacterial infection control for several decades despite antibiotic resistance, even though the development of new antibiotics may have come to a halt.

Probiotics

Ever since Metchnikov suggested the use of probiotics for replacement of toxic bacteria in the gut and prolongation of life, mechanisms of probiotic action have become more clear (Fig. 4). The idea of establishing a healthy oral, gastrointestinal, urogenital, or skin microbiome, in which recognized probiotic bacteria like lactobacilli or bifidobacteria play a dominant role, is large scale applied in over-the-counter or web-order products as lifestyle drugs to prevent infection. Scientifically founded, benefit demonstration of prevention efficacy by probiotics is cumbersome, while in vitro the relatively low bactericidal potency of probiotics compared with the one of antibiotics impedes extensive downward clinical translation for therapeutic use. Moreover, many probiotics have difficulties surviving and permanently installing themselves in their host target site. Encapsulation of probiotic bacteria by functionalized, nano-engineered shells to enhance installation and protect them against the often, hostile environment of their host target site, constitutes a possible route to solve this problem [26, 27]. Importantly, protective biofilm-inspired alginate shells have been demonstrated to allow survival of probiotic lactobacilli in the presence of tobramycin, while encapsulated lactobacilli applied in combination with tobramycin

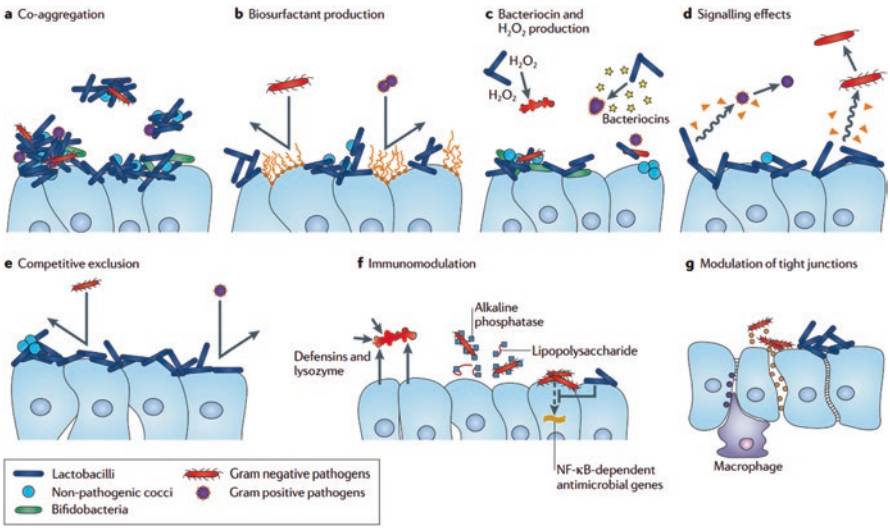


Fig. 4 Mechanisms of the restoration of the microbiota. (**a**) Co-aggregation of probiotic bacteria and pathogens interferes with the ability of the pathogenic species to infect the host. (**b**) Biosurfactants produced by probiotic bacteria help prevent pathogen adhesion to host surfaces. (**c**) Bacteriocins and hydrogen peroxide produced by probiotic bacteria can inhibit or kill pathogens. (**d**) Signaling between bacteria can lead to downregulation of toxin production in pathogens. (**e**) Probiotic bacteria can competitively exclude pathogens from host surfaces. (**f**) Probiotic bacteria can regulate immune responses, resulting production of, e.g., antimicrobial peptides. (**g**) Upregulation of tight junction proteins to limit the damage caused to host epithelia by pathogenic bacteria. (Reprinted with permission from [29], copyright at Nature Publishing Group)

had the ability to eradicate methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* in vitro [28]. These new developments warrant research investments in probiotic use, of which the likelihood of dual administration of encapsulated probiotics combined with existing antibiotics to become clinically applied, may be considered quite large.

Phage Therapy

Phage therapy was never truly abandoned in former Soviet Union countries and rediscovered worldwide in the 1980s, in order to face the rising threat of antibiotic resistance [10]. Phage therapy is especially applied in Georgia, part of the former Soviet Union, for the treatment of antibiotic-resistant infections. A drawback of phage therapy is the high specificity of phages applied for a specific bacterial strain, which sometimes necessitates culturing of effective phages or the use of "phage cocktails" [9]. Phage therapy is not without risks and patients may suffer a septic shock due to bacterial endotoxins released from bacteria when they are broken up by phages [30]. Thus although in certain patients, phage therapy may have demonstrated efficacy in eradicating bacterial infection, including infections due to antibiotic-resistant strains [10], poorly understood complications [31] have obstructed regulatory approval in many countries worldwide. Nevertheless, its likelihood perspective is not to be underestimated as a strategy for infection control.

Antimicrobial Peptides

Antimicrobial peptides are part of the innate immune system and have emerged in synthetic form as novel antimicrobials to treat bacterial infections [32]. Antimicrobial peptides are positively charged, amphiphilic molecules that kill bacteria through membrane disruption and pore formation, but are prone to hydrolytic and proteolytic breakdown [32]. Antimicrobial peptides have been around since the onset of human existence without stimulating bacterial resistance and therefore, natural development of bacterial resistance against antimicrobial peptides seems unlikely [33]. Yet, others anticipate bacterial strategies of resistance to antimicrobial peptides to arise, especially if and when used large scale in clinical infection treatment [34, 35]. So far, clinical application of antimicrobial peptides is limited to address surface infections such as in chronic wound healing, as antimicrobial peptides do not specifically target bacterial cell membranes, but possibly also mammalian ones. Use of low concentration administration of antimicrobial peptides may prevent mammalian cell membrane damage, but lowers antimicrobial efficacy, which stimulated dual administration with existing antibiotics. Also specific targeting of bacterial cell membranes by in-tandem administration with nanoparticles might solve this problem [36–39].

While peptides may be synthesized in the future that effectively address these problems, manufacturing is expensive (around $100–$600 per gram using solid-phase chemical synthesis [40]). Therewith, the likelihood perspective of antimicrobial peptides to present a clinical alternative to antibiotics is hard to estimate.

Nanotechnology-Based Strategies

Intrinsic antibiotic resistance and poor penetration of antimicrobials into infectious biofilms form the two main reasons for the recalcitrance of infection to antimicrobial treatment (Fig. 3). Metal-based nanoparticles either on their own or in synergy with existing antibiotics can kill multidrug-resistant bacterial strains through ion release, (photoactivated) release of reactive-oxygen species, damage to intracellular proteins and DNA, membrane puncture or photothermal effects. Existing antibiotics can also be encapsulated to allow targeting and stealth penetration in infectious biofilms, while pH responsive features of such nanocarriers can create electrostatic double-layer attraction with bacteria inside infectious biofilms to prevent their washout. Magnetic nanoparticles are also investigated for their potential to become targeted in infectious biofilms. In this way, higher concentrations of existing antibiotics can be achieved in biofilms than with the use of antibiotics on their own.

Nanotechnology-based antimicrobial strategies closely follow developments in tumor treatment, that have further advanced to clinical application than antimicrobial strategies [41]. Likelihood perspectives of nanotechnology-based antimicrobials are good, as they may offer the possibility to make longer use of existing antibiotics and at the same time provide bacterial killing based on mechanisms to which bacteria may not be easily able to build up resistance mechanisms.

Conclusion

The war of mankind against antimicrobial-resistant pathogens may go on forever, with chances of winning fluctuating over the times from one side to the other. With the increasing number of bacterial strains and species resistant against all known antibiotics, bacterial pathogens appear on the winning side, for the first time since the discovery of antibiotics. Human defeat is well possible, since the likelihood of developing new antibiotics is low. Yet, existing antibiotics have not become useless, and combinations of existing antibiotics with probiotics, antimicrobial peptides, or nanotechnology-based antimicrobials yield good perspectives for clinical infection control, also when caused by antimicrobial-resistant strains. However, the complex regulatory landscape and need for commercially feasible strategies requires close collaboration between academia and industry in order to bring new strategies to clinical application. The need to develop yet another, new antimicrobial compound may be less urgent than the need to focus on downward clinical translation of available strategies, so far only published upon in scientific journals.

Acknowledgments This work was financially supported by the National Natural Science Foundation of China (21620102005, 91527306, 51390483). HJB is director-owner of a consulting company, SASA BV. The authors declare no potential conflicts of interest with respect to authorship and/or publication of this chapter. The authors also gratefully acknowledge the helpful comments and suggestions of the reviewers, which have improved the presentation.

References

1. Wainwright M (1989) Moulds in folk medicine. Folklore 100(2):162–166. https://doi.org/10.1080/0015587x.1989.9715763
2. Van Leewenhoek A (1684) Some microscopical observations, about animals in the scurf of the teeth. Philos Trans R Soc B Biol Sci 14:568–574. https://doi.org/10.1098/rstl.1684.0030
3. Döderlein A (1892) Das Scheidensekret und seine Bedeutung für das Puerperalfieber (The vaginal transsudate and its significance for childbed fever). Centralblatt für Bacteriologie 11:699–700. (in German)
4. Beijerinck MW (1901) Sur les ferments de lactique de l'industrie. (Lactic acid bacteria of the industry). Arch Neerland des sciences exactes et naturelles 6:212–243. (in French)
5. Cahn DR (1901) Über die nach Gram färbbaren Bacillen des Säulingsstuhles (Bacilli of infant stools stainable according to Gram). Centralblatt für Bakteriologie I. Abteilung Originale 30:721–726. (in German)
6. Moro E (1900) Über den *Bacillus acidophilus* n. spec. Ein Beitrag zur Kenntnis der normalen Darmbacterien des Säuglings (*Bacillus acidophilus* n. spec.). (A contribution to the knowledge of the normal intestinal bacteria of infants). Jahrbuch fär Kinderheilkunde 52:38–55. (in German)
7. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME (2014) The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11:506–514. https://doi.org/10.1038/nrgastro.2014.66
8. Levin BR, Bull JJ (2004) Population and evolutionary dynamics of phage therapy. Nat Rev Microbiol 2(2):166–173. https://doi.org/10.1038/nrmicro822
9. Chan BK, Abedon ST, Loc-Carrillo C (2013) Phage cocktails and the future of phage therapy. Future Microbiol 8(6):769–783. https://doi.org/10.2217/fmb.13.47
10. Wittebole X, De Roock S, Opal SM (2014) A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. Virulence 5:209–218. https://doi.org/10.4161/viru.25991
11. Fleming A (1929) On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of *B. influenzae*. Br J Exp Pathol 10(3):226. https://doi.org/10.1038/146837a0.
12. McDermott W, Rogers DE (1982) Social ramifications of control of microbial disease. Johns Hopkins Med J 151(6):302–312
13. Abraham EP, Chain E (1940) An enzyme from bacteria able to destroy penicillin. Rev Infect Dis 10:677–678
14. Luria SE, Delbrück M (1943) Mutations of bacteria from virus sensitivity to virus resistance. Genetics 28(6):491–511
15. Petersdorf RG (1986) Whither infectious diseases? Memories, manpower, and money. J Infect Dis 153(2):189–195. https://doi.org/10.1093/infdis/153.2.189
16. O'Neill J (2014) Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Rev Antimicrob Resist 20:1–16
17. Gaynes R (2017) The discovery of penicillin—new insights after more than 75 years of clinical use. Emerg Infect Dis 23(5):849. https://doi.org/10.3201/eid2305.161556

18. Spellberg B (2014) The future of antibiotics. Crit Care 18:228. https://doi.org/10.1186/cc13948
19. Peterson E, Kaur P (2018) Antibiotic resistance mechanisms in bacteria: Relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. Front Microbiol 9:2928. https://doi.org/10.3389/fmicb.2018.02928
20. Sultan I, Rahman S, Jan AT, Siddiqui MT, Mondal AH, Haq QMR (2018) Antibiotics, resistome and resistance mechanisms: a bacterial perspective. Front Microbiol 9:2066. https://doi.org/10.3389/fmicb.2018.02066
21. Sun D (2018) Pull in and push out: mechanisms of horizontal gene transfer in bacteria. Front Microbiol 9:2154. https://doi.org/10.3389/fmicb.2018.02154
22. Flemming H-C, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S (2016) Biofilms: an emergent form of bacterial life. Nat Rev Microbiol 14(9):563–575. https://doi.org/10.1038/nrmicro.2016.94
23. Kester JC, Fortune SM (2014) Persisters and beyond: mechanisms of phenotypic drug resistance and drug tolerance in bacteria. Crit Rev Biochem Mol Biol 49:91–101. https://doi.org/10.3109/10409238.2013.869543
24. Lehar SM, Pillow T, Xu M, Staben L, Kajihara KK, Vandlen R, DePalatis L, Raab H, Hazenbos WL, Hiroshi Morisaki J et al (2015) Novel antibody-antibiotic conjugate eliminates intracellular *S. aureus*. Nature 527(7578):323–328. https://doi.org/10.1038/nature16057.
25. Klahn P, Brönstrup M (2017) Bifunctional antimicrobial conjugates and hybrid antimicrobials. Nat Prod Rep 34:832–885. https://doi.org/10.1039/c7np00006e
26. Cook MT, Tzortzis G, Charalampopoulos D, Khutoryanskiy VV (2012) Microencapsulation of probiotics for gastrointestinal delivery. J Control Release 162(1):56–67. https://doi.org/10.1016/j.jconrel.2012.06.003
27. De Vos P, Faas MM, Spasojevic M, Sikkema J (2010) Encapsulation for preservation of functionality and targeted delivery of bioactive food components. Int Dairy J 20(4):292–302. https://doi.org/10.1016/j.idairyj.2009.11.008
28. Li Z, Behrens AM, Ginat N, Tzeng SY, Lu X, Sivan S, Langer R, Jaklenec A (2018) Biofilm-inspired encapsulation of probiotics for the treatment of complex infections. Adv Mater 30:1803925. https://doi.org/10.1002/adma.201803925
29. Reid G, Younes JA, Van der Mei HC, Gloor GB, Knight R, Busscher HJ (2011) Microbiota restoration: natural and supplemented recovery of human microbial communities. Nat Rev Microbiol 9(27). https://doi.org/10.1007/978-3-7908-2355-4
30. Abedon ST, Garcia P, Mullany P, Aminov R (2017) Editorial: phage therapy: past, present and future. Front Microbiol 8:981. https://doi.org/10.3389/fmicb.2017.00981.
31. Skurnik M, Pajunen M, Kiljunen S (2007) Biotechnological challenges of phage therapy. Biotechnol Lett 29:995–1003. https://doi.org/10.1007/s10529-007-9346-1
32. Andersson DI, Hughes D, Kubicek-Sutherland JZ (2016) Mechanisms and consequences of bacterial resistance to antimicrobial peptides. Drug Resist Updat 26:43–57. https://doi.org/10.1016/j.drup.2016.04.002
33. Pasupuleti M, Schmidtchen A, Malmsten M (2012) Antimicrobial peptides: key components of the innate immune system. Crit Rev Biotechnol 32(2):143–171. https://doi.org/10.3109/07388551.2011.594423
34. Joo HS, Fu CI, Otto M (2016) Bacterial strategies of resistance to antimicrobial peptides. Philos Trans R Soc B Biol Sci 371:20150292. https://doi.org/10.1098/rstb.2015.0292.
35. Maria-Neto S, De Almeida KC, Macedo MLR, Franco OL (2015) Understanding bacterial resistance to antimicrobial peptides: from the surface to deep inside. Biochim Biophys Acta Biomembr 1848(11):3078–3088. https://doi.org/10.1016/j.bbamem.2015.02.017
36. Duncan B, Li XN, Landis RF, Kim ST, Gupta A, Wang LS, Ramanathan R, Tang R, Boerth JA, Rotello VM (2015) nanoparticle-stabilized capsules for the treatment of bacterial biofilms. ACS Nano 9:7775–7782
37. Kwon EJ, Skalak M, Bertucci A, Braun G, Ricci F, Ruoslahti E, Sailor MJ, Bhatia SN (2017) Porous silicon nanoparticle delivery of tandem peptide anti-infectives for the treatment of *Pseudomonas aeruginosa* lung infection. Adv Mater 29:1701527. https://doi.org/10.1002/adma.201701527.

38. Liu Y-H, Kuo S-C, Yao B-Y, Fang Z-S, Lee Y-T, Chang Y-C, Chen T-L, Hu CMJ (2018) Colistin nanoparticle assembly by coacervate complexation with polyanionic peptides for treating drug-resistant gram-negative bacteria. Acta Biomater 82:133–142. https://doi.org/10.1016/j.actbio.2018.10.013
39. Liu Y, Shi L, Su L, Van der Mei HC, Jutte PC, Ren Y, Busscher HJ (2019) Nanotechnology-based antimicrobials and delivery systems for biofilm-infection control. Chem Soc Rev 48(2):428–446. https://doi.org/10.1039/c7cs00807d
40. Hancock REW, Sahl H-G (2006) Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol 24(12):1551. https://doi.org/10.1038/nbt1267
41. Gupta A, Mumtaz S, Li C-H, Hussain I, Rotello VM (2019) Combatting antibiotic-resistant bacteria using nanomaterials. Chem Soc Rev 48(2):415–427. https://doi.org/10.1039/C7CS00748E

Part II
Pathogenesis of Infection

Pathogenesis of Biomaterial-Associated Infection

S. T. Jerry Tsang and A. Hamish R. W. Simpson

Abstract Biomaterial infections associated with indwelling surgical devices are responsible for ~50% of all nosocomial infections. The development of orthopedic biomaterial-associated infections comes at great physical and emotional cost to patients, resulting in substantial economic costs to healthcare providers. Understanding of its pathogenesis has progressed greatly since the biofilm hypothesis was first proposed. However, the biofilm hypothesis only partially elucidates the pathogenesis of these infections. A greater appreciation of the mechanisms underpinning immune evasion by common pathogens has highlighted a previous underestimation of the role this behavior has in the development of these troublesome infections. Recognition of the importance of the immune system interaction in the pathogenesis of biomaterial-associated infections will not only update our paradigm of this condition but also help to identify and develop potential therapeutic targets. This review aims to provide an overview of the pathogenesis of biomaterial-associated infections. It focuses primarily on the development of bacterial biofilms and the immune-evasive behavior of the most common orthopedic pathogens.

Keywords Nosocomial infection · Surgical site infection · Pathogenesis · Biofilms · Persister cells · Immunomodulation · Immune evasion · Intracellular pathogens · Antimicrobial resistance · Antimicrobial tolerance · *Staphylococcus aureus* · *Staphylococcus epidermidis*

Introduction

Biomaterial infections related to medical devices are responsible for ~50% of all nosocomial infections [1, 2]. Although infections related to surgical devices are less common than those associated with intravascular and intraurethral catheters, surgical

S. T. J. Tsang (✉) · A. H. R. W. Simpson
Department of Orthopaedics, University of Edinburgh, Edinburgh, UK
e-mail: Jerry.Tsang@ed.ac.uk; Hamish.Simpson@ed.ac.uk

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_6

biomaterial-associated infections often require prolonged antibiotic therapy and multiple invasive procedures [2]. Orthopedic surgery is a primary focus of surgical site infection surveillance given the high rates of implanted biomaterial use. The development of orthopedic biomaterial-associated infections has been shown to have major implications on patient-reported quality of life and function, healthcare costs, and medicolegal costs [1]. Prosthetic joint infections (PJI) are associated with a 3-month mortality ~2% for patients around 65 years of age [3], rising to a 21% 5-year mortality following prosthetic hip infections requiring staged revision surgery [4]. The 5-year mortality of PJI has been reported to be greater than four of the five most commonly diagnosed cancers in the USA [5]. National surveillance programs of orthopedic practice estimate the prevalence of PJI to range between 0.2 and 5% [6–9]. Similar estimates of infection prevalence have been reported for fracture-related infections (0.7–5%) [10–12]), with open fractures being disproportionately affected [13]. Combined total treatment costs for orthopedic device-related infections in North America and Europe are estimated to be ~$4 billion per annum [6, 14–16].

Etiology

For orthopedic surgery gram-positive organisms account for the majority of biomaterial-associated infections [9]. Bacteria reported to cause monomicrobial biomaterial-associated infections include: Coagulase-negative *Staphylococci* (~40%), *Staphylococcus aureus* (*S. aureus*) (~20%), *Streptococci* (~10%), *Enterococci* (~5%), gram-negative organisms (~5%), and anaerobes (~3%) [17]. In polymicrobial cases gram-positive organisms are implicated in 70–80% of cases [9]. However, historic estimates obtained from microbiology culture results are likely to be misleading, as some species, such as *Cutibacterium acnes* (*C. acnes*) (formerly *Propionibacterium acnes*), were previously considered to be nonpathogenic or "weakly" pathogenic, and often dismissed as contaminants. The true prevalence of *C. acnes* infection has been demonstrated through the application of more robust sampling and detection methods [18–20].

Pathogenesis

The pathogenesis of orthopedic biomaterial-associated infection is a complex interaction between the host, microorganism, and the surgical device [21]. Contact between the potential pathogen and the device often results in the formation of a bacterial biofilm, which influences interactions between host defense mechanisms (+/− antimicrobial management) and the microorganism (Fig. 1). This overview will focus primarily on the pathogenesis of gram-positive bacteria, particularly *Staphylococci*, as they are the most common pathogens in orthopedic biomaterial-associated infections.

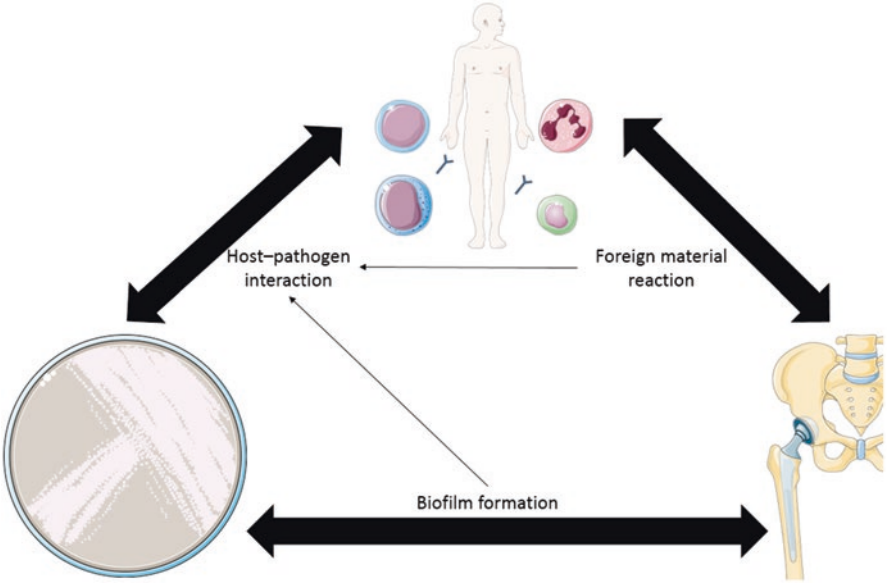


Fig. 1 Host–bacteria–medical device interactions in the pathogenesis of biomaterial-associated infections

Routes of Infection

Biomaterials can be colonized through the following mechanisms: (1) direct seeding from external contaminants or contiguous spreading, (2) hematogenous spreading from distant body sites, and (3) recurrent infection. Direct seeding may originate either during or immediately after implantation if the device is placed within a contaminated surgical bed or left exposed (e.g., open fracture with delayed soft tissue coverage). A meta-analysis of clinical studies reported that a prior diagnosis of septic arthritis was associated with up to an eightfold increase in risk of PJI following same site joint replacement surgery [22].

Susceptibility to infection is increased by the presence of biomaterials. In vivo animal studies have shown that the bacterial concentration needed to induce an infection is reduced by more than 100,000 times in the presence of foreign material [23]. Furthermore, the interaction of neutrophils with foreign material can induce neutrophil depletion and exhaustion, which enhances infection susceptibility [24]. Using a small animal model Zimmerli et al. [25] demonstrated that hematogenous seeding of biomaterials occurred between 100 and 1000 CFU/mL blood. Therefore, episodes of bacteremia associated with dental procedures do not compromise implanted biomaterials, as the density of bacteremia during these activities does not exceed 1–28 CFU/mL [23, 26]. Furthermore, the interaction of neutrophils with foreign material can induce neutrophil depletion and exhaustion, which enhances infection susceptibility [24]. Using a small animal model, Zimmerli et al. [25]

demonstrated that hematogenous seeding of biomaterials occurred between 100 and 1000 CFU/mL blood. Therefore episodes of bacteremia associated with dental procedures do not compromise implanted biomaterials, as the density of bacteremia during these activities does not exceed 1–28 CFU/mL [26].

Biofilms

Bacterial biofilms are reported to be the leading cause of recalcitrant and recurrent infections of orthopedic biomaterials. Up to 80% of pathogens that form biofilms are associated with persistent infections [27]. Their ability to develop tolerance to a diverse range of antimicrobial compounds threatens to halt elective orthopedic biomaterial-associated procedures [28, 29]. The formation of biofilms by bacteria has evolved as an adaptation to austere environments (Figs. 2 and 3). By forming multicellular communities it allowed single-celled bacteria to survive in hostile conditions [30]. Costerton et al. [31] were the first to describe the biofilm hypothesis, stating that "bacteria in all nutrient sufficient ecosystems grow predominantly in matrix-enclosed surface-associated communities, within which they are protected from a wide variety of antibacterial factors." Using transmission electron micrograph imaging, Costerton et al. [32] reported the first direct observation that clinical isolates of *Escherichia coli* (*E. coli*) had a thick glycocalyx that was almost nonexistent in high-passage reference strains. The biofilm hypothesis prompted a paradigm shift in medical microbiology, with the first reported case of a clinical biofilm

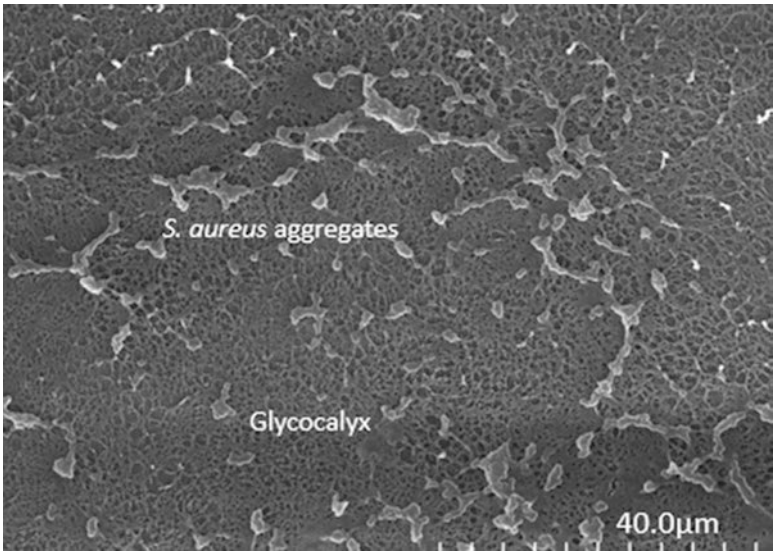


Fig. 2 A scanning electron cryomicroscopy image showing the biomaterial in the background with the glycocalyx secreted by and encasing the colonies of *S. aureus*

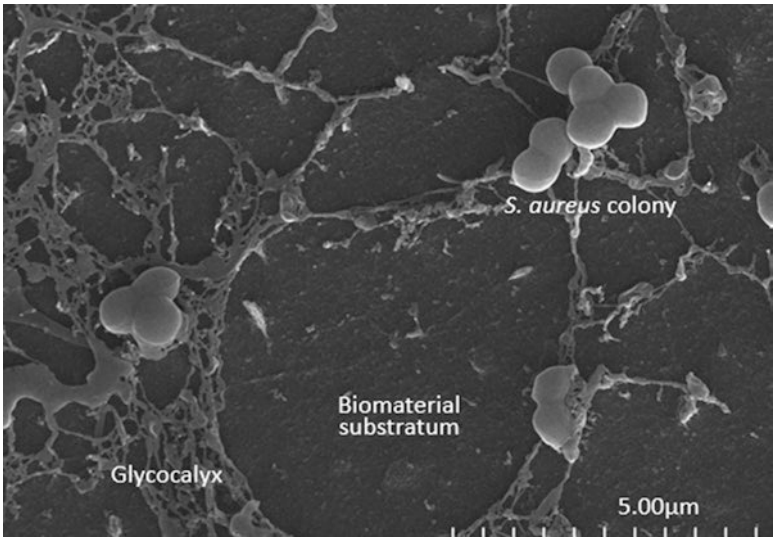


Fig. 3 A scanning electron cryomicroscopy image of a *S. aureus* biofilm adherent to a biomaterial surface

infection published in 1982. It described the presence of a *S. aureus* biofilm on a cardiac pacemaker lead, which had formed following hematogenous spread from an infected olecranon bursitis [33]. The sessile cells within this biofilm were found to be recalcitrant to a 6-week course of high dose antibiotics, with the bacterial biofilm serving as a nidus for recurrent bacteremia each time therapy was discontinued. The first reported application of the biofilm hypothesis to orthopedic biomaterial-associated infections was in 1984, providing a framework to explain the pathogenesis of these, often chronic and recalcitrant, infections [34]. As with all biofilm infections, orthopedic biomaterial-associated infections can develop over months or years with few signs of inflammation and usually remained localized to the colonized implant [35]. It was thought that traditional antimicrobial therapy simply addressed the symptoms triggered by floating single planktonic cells shed from these biofilms. However, the stationary sessile cells locked in the glycocalyx were not affected by systemic concentrations of antibacterial agents, allowing the infection to persist [36]. Biofilm formation is now recognized as one of the key virulence factors in biomaterial-associated infections. Recalcitrance is not primarily due to resistance conferred by genetic mutation, although the high cell density has been shown to promote the transfer of resistance genes; instead the bacteria within biofilms develop antimicrobial tolerance through the promotion of cell dormancy and persistence [37]. Recent work has gone on to show that concentrations required to adequately eradicate biofilm colonies (i.e., the minimum biofilm eradication concentration (MBEC)) are generally 100–1000 times greater than the inhibitory concentrations for planktonic cells (i.e., the minimum inhibitory concentration (MIC)) [38, 39]. Even when host immunity is functioning, orthopedic biomaterial-associated

infections rarely resolve spontaneously. It has been shown that biofilm formation induces the expression of virulence factors which confer inactivation of both passive [40] and adaptive immunity [41].

Biofilm Development

Biofilm development is believed to be a cycle with four distinct phases: (1) attachment, (2) microcolony formation, (3) biofilm maturation, and (4) dispersion [42] (Fig. 4). The different biofilm phases include physiological and phenotypical responses that represent a unique biofilm biology which are not displayed by planktonic bacteria. The switch from a solitary planktonic state to the communal biofilm state involves a phenotypical change to initiate the production of adhesins and extracellular matrix compounds which interconnect the cells. The extracellular biofilm matrix (i.e., glycocalyx) serves as a scaffold that has an essential physiological and structural function in biofilms, and influences a number of processes including cell attachment, cell-to-cell interactions, and antimicrobial tolerance [43–45]. The glycocalyx is composed of extracellular polysaccharides, phospholipids, proteins, and DNA [46], providing stability, mediating surface adhesion, and serving as a scaffold for further cellular attachment [47–50]. It accounts for ~90% of biofilm mass. Biofilm formation begins with initial weak interactions between individual bacterial cells and the surface, followed by a strong adhesion step. The cells begin to secrete the component biomolecules of the glycocalyx as they grow and divide. The biofilm reaches a maximum size and enters its maturation phase. Finally cells detach from the biofilm and disperse [51]. In an alternative developmental model, there is an early dispersal stage at ~6 h following formation, during which a small population

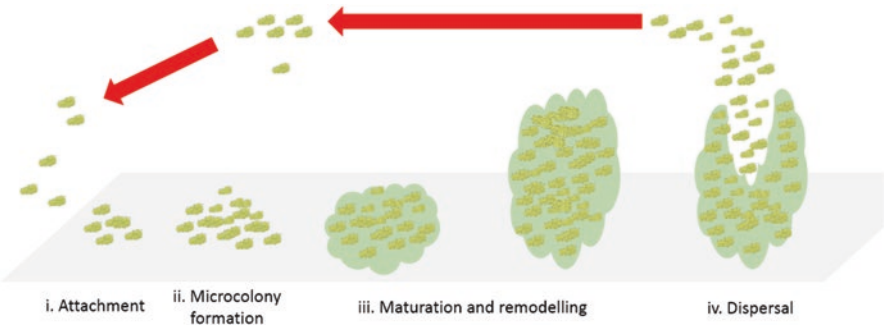


Fig. 4 The biofilm cycle. (**i**) **Attachment**—free-floating/planktonic bacteria encounter the conditioned biomaterial surface and become attached within minutes. (**ii**) **Microcolony formation**—adherent bacteria switch to the biofilm phenotype with glycocalyx production. (**iii**) **Maturation and remodeling**—biofilm formation develops with greater intercellular interactions, encouraging further bacterial attachment and colony growth. (**iv**) **Dispersal**—detachment of bacterial cells either in large clumps or by releasing individual cells allow bacteria to reattach to a surface or another biofilm colony downstream of the original community

of cells returns to a planktonic state prior to biofilm maturation [42, 52] (Fig. 4). The different biofilm phases include physiological and phenotypical responses that represent a unique biofilm biology which are not displayed by planktonic bacteria. The switch from a solitary planktonic state to the communal biofilm state involves a phenotypical change to initiate the production of adhesins and extracellular matrix compounds which interconnect the cells. The extracellular biofilm matrix (i.e., glycocalyx) serves as a scaffold that has an essential physiological and structural function in biofilms, and influences a number of processes including cell attachment, cell-to-cell interactions, and antimicrobial tolerance [43–45]. The glycocalyx is composed of extracellular polysaccharides, phospholipids, proteins, and DNA [46], providing stability, mediating surface adhesion, and serving as a scaffold for further cellular attachment [47–50]. It accounts for ~90% of biofilm mass. Biofilm formation begins with initial weak interactions between individual bacterial cells and the surface, followed by a strong adhesion step. The cells begin to secrete the component biomolecules of the glycocalyx as they grow and divide. The biofilm reaches a maximum size and enters its maturation phase. Finally cells detach from the biofilm and disperse [51]. In an alternative developmental model, there is an early dispersal stage at ~6 h following formation, during which a small population of cells returns to a planktonic state prior to biofilm maturation [52].

Bacterial Motion

Before a cell can attach itself to a surface, it must first locate and initiate contact. Motility is therefore a critical part of this initial process. Bacterial motion can either be passive (nonmotile), dependent on the local environment to generate propulsion; or active (motile), using energy-dependent cellular mechanisms to direct motion [53]. Traditionally passive motion includes sliding, darting, Brownian motion, or carriage by fluid [54, 55]. Sliding is the radially movement of bacterial cells from their inoculation site across a surface driven by colony growth, which forms a monolayer of densely packed cells [54]. Spreading is a variant of sliding where multiple disorganized layers are formed: a phenomenon observed in both *S. aureus* and *Staphylococcus epidermidis* [56]. *S. aureus* achieves this variant form of sliding through the secretion of the surfactants known as phenol-soluble modulins [57]. This family of amphipathic, alpha helical peptides are now known to be key virulence factors in staphylococcal pathogenicity. Phenol-soluble modulins not only inhibit immune and inflammatory responses but also contribute to biofilm development [57]. Their production is controlled by the accessory gene regulator (*agr*) [58, 59]. This regulatory system governs the cellular response to bacterial density and biofilm formation though the expression of virulence factors [60]. During the initiation of spreading, the production of surfactants scatters individual cells, preventing attachment of the growing bacteria. During maturation the colony forms multiple layers where the bacteria physically drive themselves [53]. Darting occurs when growing bacteria overcome intercellular adhesion and sporadically eject themselves forward [55]. Brownian motion is the random, uncoordinated movement of particles and

cells in a liquid caused by molecular collisions [61]. Carriage by fluid describes the phenomenon whereby bacteria are transported within a flowing medium such as blood or synovial fluid. Passive motion can either be in a random and limited fashion (e.g., darting and Brownian motion), or synchronous (e.g., sliding and carriage by fluid). Traditionally *S. aureus* has been thought of as a nonmotile organism [62]; however, recent work has shown that it displays active motility under certain conditions [53, 63]. Traditionally passive motion included sliding, darting, Brownian motion, or carriage by fluid [54, 55]. Sliding is the radially movement of bacterial cells from their inoculation site across a surface driven by colony growth, which forms a monolayer of densely packed cells [54]. Spreading is a variant of sliding where multiple disorganized layers are formed: a phenomenon observed in both *S. aureus* and *Staphylococcus epidermidis* (*S. epidermidis*) [56]. *S. aureus* achieves this variant form of sliding through the secretion of the surfactants known as phenol-soluble modulins [57]. This family of amphipathic, alpha helical peptides are now known to be key virulence factors in staphylococcal pathogenicity. Phenol-soluble modulins not only inhibit immune and inflammatory responses, but also contribute to biofilm development [57]. Their production is controlled by the accessory gene regulator (*agr*), [58, 59]. This regulatory system governs the cellular response to bacterial density and biofilm formation though the expression of virulence factors [60]. During the initiation of spreading, the production of surfactant is scatters individual cells, preventing attachment of the growing bacteria. During maturation the colony forms multiple layers where the bacteria physically drive themselves [53]. Darting occurs when growing bacteria overcome intercellular adhesion and sporadically eject themselves forward [55]. Brownian motion is the random, uncoordinated movement of particles and cells in a liquid caused by molecular collisions [61]. Carriage by fluid describes the phenomenon whereby bacteria are transported within a flowing medium such as blood or synovial fluid. Passive motion can either be in a random and limited fashion (e.g., darting and Brownian motion), or synchronous (e.g., sliding and carriage by fluid). Traditionally *S. aureus* has been thought of as a nonmotile organism [62]; however, recent work has shown that it displays active motility under certain conditions [63].

Examples of active bacterial motion include swimming, swarming, gliding, and twitching [54]. Swimming and swarming are both dependent on the action of flagella. Flagella are rotating, flexible, filament-shaped appendages that are used by bacteria to propel themselves. A flagellum is typically formed of three components: a body, a hook, and a flexible filament [64]. The flexible filament is made up of flagellin subunits [65]. The flagellar filament is connected to the basal body via a rigid hook. The body harbors the biological motor that produces flagellar rotation [66–68]. Most bacteria use energy generated from the electron transport chain, in the form of proton potential, to drive the flagellum [69]. By controlling the direction of flagellar rotation, the bacterial cells are able to direct the course of travel. Swimming is characterized by bacteria moving in an individual and random manner, and takes place when there is sufficient fluid film associated with the surface [54]. Swarming occurs when flagellated bacteria, such as *Pseudomonas aeruginosa* (*P. aeruginosa*), move in a grouped and organized manner. Twitching is dependent

on the coordinated movement of specialized appendages known type IV pili, which result in an intermittent and jerky pattern of locomotion. Type IV pili are protein polymers present on the surfaces of mostly gram-negative bacteria. There are present in a select number of gram-positive species but not *Staphylococci* [70]. Gliding motility has evolved in a diverse range of bacterial genera [71, 72], including *S. aureus* [63]. The exact mechanics underpinning gliding are still unknown, possibilities include: slime secretion, focal adhesion complexes, cell twisting, type IV pili retraction, or membrane deformation [72–74]. *S. aureus* have been observed to display characteristics of gliding when moving across soft agar media as clusters forming "comets" and "dendrites." The absence of such motion in *agr*-deficient *S. aureus* strains suggests that this process is driven by the production of the surfactant peptide, phenol-soluble modulin [63].

The physical properties of the local environment are critical in determining bacterial movement and adherence. Bacteria have been shown to move toward a surface in response to environmental cues: concentration gradients, heat, light, and oxygen tension [75]. There are reported to be three signaling pathways that control bacterial motion: (1) chemotaxis, (2) the secondary signaling molecule bis-(3′–5′)-cyclic dimeric guanosine monophosphate (c-di-GMP), and (3) quorum sensing [76].

Most chemosensory systems rely on transmembrane chemoreceptors that bind distinct chemical ligands, which induce cytoplasmic signaling cascades [77]. For example, the reduction of *E. coli* chemoattractants in a media leads to the activation of the CheA-CheY cascade, which affects the direction of the flagellar motor. By switching the direction of travel, the CheA-CheY system can control bacterial motion until the cell encounters high concentrations of the chemoattractant it is seeking [78]. In other species CheY activation results in molecular braking, which allows the bacteria to engage a surface and initiate attachment [78].

The secondary messenger c-di-GMP has also been shown to reduce flagellar motion. It is thought to be a key factor in the switch from a motile to sessile phenotype and in doing so it is thought to promote biofilm formation in many gram-negative bacteria [79]. Generally, high levels of c-di-GMP are required for the formation of biofilms. However, in *E. coli* the degradation of c-di-GMP by the phosphodiesterase, *YhjH*, is critical in the development of biofilms [80]. During the exponential phase of planktonic growth *YhjH* is known to be active, but its expression decreases as the planktonic cells enter their stationary phase [80]. "It has been shown that the promotion of intracellular c-di-GMP can be achieved through the inhibition of YhjH expression, which facilitates interaction of c-di-GMP with the "molecular brake" (YcgR); slowing flagellar rotation and promoting surface adhesion [81].

Quorum sensing is a form of bacterial communication, with particular importance in biofilm cell signaling [76]. Through the production and release of chemical signals, known as autoinducers, bacteria are able to communicate fluctuations in biofilm cell density. The extracellular autoinducer concentration rises in proportion with the population. When a threshold is reached, the autoinducer reenters the bacterial cells. The interaction of the autoinducer with specific transcriptional regulators

or transduction systems alters gene expression and phenotypic changes, such as biomaterial attachment, biofilm formation, or cellular invasion (see section "Quorum Sensing" for further details) [82–86].

Adhesion

Adhesion is advantageous for bacteria, as it provides access to nutrients precipitated on surfaces, as well as protection from predators and environmental hazards [87, 88]. Bacterial adhesion to surfaces starts with initial weak attractions, which are reversible and can be broken relatively easily. Bacterial adhesion has two phases, primary unspecific reversible attachment and secondary irreversible attachment. In general, the initial adhesion to biomaterial surfaces is unspecific, in contrast, adhesion to organic surfaces involves binding to specific adhesion molecules [89]. Indwelling biomaterial surfaces are quickly coated ("conditioned") by proteins, and immune components to form a matrix for attachment [90, 91]. This process has been shown to take place within nanoseconds [90] and is determined by the surface properties such as topography and hydrophobicity [92, 93]. Common biomaterial pathogens, such as *Staphylococci*, have multiple known virulent processes related to attachment and biofilm formation that drive the pathogenesis of orthopedic device-related infections [94–96].; with adhesins being the primary molecular anchor for many bacterial genera [97].

When bacteria approach the surface, the likelihood of adhesion is determined by the sum of attractive and repulsive forces [98]. Along with surface properties, other factors such as the nutrient availability and oxygen tension of the bulk liquid can also influence bacterial adhesion [99, 100]. Initial bacterial attachment to biomaterial surfaces is thought to be determined by nonspecific forces (e.g., Lifshitz–van der Waal's, Lewis acid–base, and electrostatic forces) [101, 102]. This model of adhesion (the extended Derjaguin–Landau–Verwey–Overbeek theory, XDLVO theory) assumes that bacteria behave like colloidal microparticles [103]. It predicts adhesion through the interaction of van der Waal's forces, electrostatic charges, and hydrophobic forces present on the surfaces of the biomaterial and the bacterium [98]. The chemical properties of the bacterial cell membrane and envelope can show variation [104, 105], but most pathogens, including *Staphylococci*, are negatively charged [106, 107]. Teichoic acids embedded in the peptidoglycan wall of gram-positive bacteria give the cell a net negative charge, while the lipopolysaccharides on the outer membrane of gram-negative bacteria are responsible for their net negative surface charge. Therefore, surface materials with positive or neutral net charges are preferentially colonized over those with a net negative charge [108].

However, the bacterial cell envelope can adjust its charge and hydrophobicity in response to growth conditions [109]. Heterogeneity of the bacterial cell envelope has been found to be present even between individual cells within a clonal population [110]. Bacterial filamentous cell appendages, such as nanofibers also function as adhesins [111]. Some mediate cell adhesion to biomaterial surfaces and are involved in biofilm formation [112]; others have been found to bind to surface mol-

ecules and/or extracellular matrix components, such as fibronectin on host cells [113]. Species-specific proteins, such as the autolysins (e.g., AtlE and AtlA) also facilitate biomaterial surface adhesion. AtlE has been shown to facilitate the adherence of *S. epidermidis* to both abiotic and biotic surfaces [114]. AtlA binds *S. aureus* to matrix proteins, such as fibrinogen, fibronectin, and vitronectin [115, 116]. It is the primary mediator of adhesion of *S. aureus* to conditioned biomaterials [117] and also contributes to biofilm regulation through its autolytic activity in *S. aureus* [117].

The surface properties of the device also influence the likelihood of bacterial adhesion. Surface topography at the micrometer and nanometer scale can be manipulated to alter its hydrophobic properties, a crucial parameter for bacterial adhesion [118]. Patterning at the microscale (micropatterning) not only maximizes the available contact area for bacterial cells but it also reduces the shear stress experienced by attached cells [119, 120]. The influence of nanopatterning, however, has yet to be fully elucidated [121, 122]. For example, common biomaterial pathogens (*E. coli*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis*) have been found in some studies to be inhibited by patterning which are smaller than the size of the bacterium [120–122]. However, earlier studies reported that the inhibitory effect of nanopatterning only affected *Staphylococci*, with no loss of adhesion by *P. aeruginosa* or *E. coli* [123, 124]. This difference was attributed to cell shape (spherical *Staphylococci* versus rod-shaped *P. aeruginosa* and *E. coli*) [124] and the composition of the cell envelope (gram-positive *Staphylococci* versus gram-negative *P. aeruginosa* and *E. coli*) [123].

During biomaterial conditioning, a range of particles and molecules adsorb onto its surface, modifying its charge, potential and surface tension. This results in a heterogeneous surface with varying adhesion efficiency [125]. The composition of conditioning films varies greatly as the adsorbed molecules come from the local environment and bacteria that are present, either in bulk liquid or already attached to the surface [125]. Adsorbed molecules can either inhibit cell surface binding sites or can act as ligands, forming adhesive bonds [89, 125, 126].

In recent years, attempts have been made to develop superhydrophobic surfaces [127]. Novel surfaces inspired by nature (lotus leaves, dragonfly wings, and shark skin [127]) with nanopatterned structures and very low affinity for water have been developed to prevent colonization against a variety of bacterial species [122, 128].

Surface Sensing and Strengthening the Initial Attachment

Having overcome the repulsive forces at the biomaterial surface, bacteria are able to secure a permanent attachment by consolidating initial adhesive bonds. Surface-sensing following initial contact triggers a cascade of conformational changes through mechanotransduction that result in the production of surface structures and adhesion molecules [129]. The biofilm development cycle can only begin following the formation of irreversible adhesion by a single bacterium.

Initially permanent adhesion is mediated by the cell envelope (+/− appendages), namely, hydrogen bond interactions between the cell and surface [130]. However, bacterial adhesion to conditioned surfaces is governed by specific binding between bacterial adhesins (piliated and non-piliated) and host proteins [97]. The cell wall of

Staphylococci, like most gram-positive bacteria, contains teichoic acids. They are covalently bonded in the cell wall to peptidoglycan and the cytoplasmic membrane as wall teichoic acid or lipoteichoic acid, respectively [131]. Teichoic acids have been shown to have an important role in *S. aureus* and *S. epidermidis* adherence to biomaterials and in biofilm formation [132]. Wall teichoic acids have been reported have an important role in the adhesion of *S. epidermidis* to conditioned biomaterials as they preferential bind to adsorbed fibronectin [133].

Polysaccharide intercellular adhesin is one of the main components in the glycocalyx of *Staphylococci*. It was one of the first adhesin molecules reported to have a key role in staphylococcal biofilm pathogenicity [134]. It is a partially deacetylated, positively charged molecule (poly-β(1-6)-*N*-acetylglucosamine) whose synthesis is controlled by the *icaADBC* gene locus [135]. Its expression is thought to be closely related with staphylococcal virulence [136]. A study of *S. epidermidis* isolates from orthopedic biomaterial infections found that polysaccharide intercellular adhesin producers exhibited higher tolerance to aminoglycosides than non-producers [137], with *icaADBC* knockout strains found to be susceptible to a broad range of antibiotics [137]. Furthermore the production of polysaccharide intercellular adhesin has been found to form part of the *S. epidermidis* stress response [138]; however, the exact mechanisms are thought to be stressor-specific. The induction of polysaccharide intercellular adhesin synthesis by sodium chloride/high osmolality is dependent on *rsbU*, which encodes an activator of the *sigB* operon [139]. *sigB* is found in a number of the gram-positive genera and has been shown to have a key role in response to hostile environments [140]. Conversely ethanol stress has been shown to induce polysaccharide intercellular adhesin synthesis and biofilm formation in a *rsbU*-independent manner [139]. Polysaccharide intercellular adhesin production has been shown to be also increased during iron limitation, reduced oxygen tensions, and high shear stress [141]. *S. epidermidis* colonies isolated from high-shear environments have been found to produce polysaccharide intercellular adhesin-containing biofilms whereas the adhesin molecule was absent in the biofilms of isolates collected from sites of low shear (e.g., joint replacements and intraocular lenses) [141]. *S. epidermidis* controls the synthesis of polysaccharide intercellular adhesin through induced insertion and excision of the sequence *IS256* into and from *icaC* [142]. The presence of *IS256* in the *icaADBC* sequence of *S. epidermidis* isolates from biomaterial infections is associated with multidrug tolerance [143]. The polysaccharide intercellular adhesin-mediated stress response, whether innate or obtained by horizontal gene transfer, is a key factor in the adaptation of *S. epidermidis* to the biomaterial niche [97].

Non-polysaccharide adhesion molecules, known as adhesins, also have a role in bacterial attachment. *S. aureus* have a rich armamentarium of bacterial adhesins [91, 144–146]. Methicillin-resistant *S. aureus*, both hospital-associated and community-associated strains, are dependent on proteinaceous adhesins, rather than polysaccharides, during glycocalyx formation [147–149]. Adhesins, not only mediate adhesion to extracellular matrix proteins, but have been found to modulate host immune activity and trigger host cell invasion [146]. Collagen, fibronectin, and fibrinogen are the primary ligands for bacterial adhesins [146]. Collagen represents a class of over 20 matrix proteins with a structural role in specialized connective

tissues. Type I collagen (along with bone sialoprotein) is the most abundant bone matrix protein. Unsurprisingly, the ability of common orthopedic pathogens, such as *S. aureus*, to bind components of the bone matrix is a key virulence trait [150]. Fibronectins are homodimeric glycoproteins with disulfide bonds at the carboxyl terminus that are secreted primarily by hepatocytes into the plasma. Secondarily, and more importantly in the context of orthopedic biomaterial-associated infections, fibroblasts also secrete fibronectin into the interstitial space. Fibronectins attach to fibrous collagens, promoting adhesion and spreading of host cells, as well as regulating cell morphology by influencing cytoskeleton assembly [151]. Using dynamic force spectroscopy, the binding of *S. epidermidis* to fibronectin has been observed at a molecular level. *S. epidermidis* binds to the carboxyl-terminal domain of fibronectin, with this interaction inhibited by heparin [152]. Fibrinogen, also synthesized by hepatocytes, has been shown to have a key role in catheter-related staphylococcal infections rather than in the pathogenesis of orthopedic device infections [153]. Cell-wall anchored proteins, covalently bound to peptidoglycans, are a further class of adhesins known to be important in *S. aureus* attachment [154]. Up to 24 proteins are thought to belong to this class of bacterial surface components. They are classified according to the presence of unique structural motifs: (1) the microbial surface component recognizing adhesive matrix molecule (MSCRAMM), (2) the near-iron transporter (NEAT) motif, (3) the three-helical bundle, and (4) the G5-E repeat [154, 155]. The MSCRAMM family of cell wall-anchored proteins contributes to *S. aureus* osteomyelitis and septic arthritis by facilitating irreversible attachment to host plasma proteins [144, 154, 155] and tissue matrices [144, 156]. Bacteria can possess multiple ligand-specific MSCRAMMs. For example, *S. aureus* have been found to form the following MSCRAMMs: collagen-binding adhesin, fibronectin-binding proteins, and fibrinogen-binding proteins [157]. These adhesins have been shown to be key *S. aureus* virulence factors, for example the collagen adhesin molecule is critical for adhesion in septic arthritis but not osteomyelitis [158]. The Clf-Sdr class, a subcollection of MSCRAMMs, includes clumping factor proteins (e.g., ClfA and ClfB), and surface-anchored proteins (e.g., SdrC, SdrD, and SdrE). They are known to facilitate *S. aureus* biofilm formation on conditioned biomaterials [154, 159–161]. Wang et al. reported that ClfA was a key virulence factor of hematogenous *S. aureus* in a rodent implant-infection model [162]. This study demonstrated that *S. aureus* biofilm formation could be inhibited by introducing antibodies against ClfA and α-hemolysin [162]. In vitro analysis of *S. aureus* isolates from nasal epithelium of humans have found high-level expression of ClfB. It has been shown that ClfB binds to the squamous cell membranes via keratin [163]. The NEAT motif family of cell wall-anchored proteins has been found to induce biofilm formation during iron deprivation [164]. They are also critical in *S. aureus* immune evasion [165–167]. An important member of the three-helical bundle group is staphylococcal protein A (SpA). It is often used for strain typing due to its universal expression and hypervariability in *S. aureus*. It has been shown to be critical for *S. aureus* nasal colonization and pathogenesis in humans [168–174]. SpA is responsible for *S. aureus* accumulation and intercellular adhesion contributing to biofilm development and abscess formation [175]. There is an asso-

ciation between *SpA* expression and osteomyelitis severity. It is thought that SpA modulates osteoclast activation and differentiation, as well as cortical bone destruction via cytokine signaling pathways [176–179]. Although the role of adhesion molecules in maintaining biofilm structure and function at the population level has been established [180], the role of these molecules in the transition to irreversible adhesion at the single-cell level has yet to be fully elucidated. The advent of modern single-cell techniques, such as atomic force microscopy, may facilitate further understanding of the role adhesion molecules have in single-cell adherence [181].

DNA is a non-proteinaceous, non-polysaccharide adhesion molecule that has been shown to contribute to bacterial adherence to biomaterial surfaces. In *S. aureus* extracellular DNA is a critical component of the glycocalyx. It is released by autolysis [182–184], with a role in both surface adhesion [185, 186] and glycocalyx maturation [43]. Extracellular DNA changes the hydrophobicity of the bacterium, altering its interactions with biomaterial surfaces [187]. Das et al. [186] reported that the presence of extracellular DNA on bacterial cell surfaces enhanced adhesion and surface aggregation due to the involvement of acid-base interactions. They also reported that extracellular DNA of *Staphylococci* created favorable conditions for bacterial adhesion even to hydrophobic surfaces.

Bacteria have been found to use different adhesin combinations in response to environmental factors, including the surface properties of the biomaterial. However, stochastic heterogeneity within the biofilm population makes it difficult to characterize and manipulate for therapeutic applications [188–192].

Reversible and Irreversible Attachment

The ability of bacteria to sense and respond to surface attachment, known as contact-dependent signal transduction, is a form of mechanosensing and is critical in the formation of irreversible attachment [129]. The transition of the attached single cell into a multicellular biofilm community requires a switch of phenotype [180]. The biofilm population is dynamic with a continual turn-over of cells, through cell reproduction and death, as well as dispersion [193, 194]. However, not every attached cell makes the switch to the biofilm phenotype; some adhere to a surface, divide, and disperse without interacting with other bacteria. It is not known whether this is a regulated behavior or stochastic phenomenon. It has been recently suggested that transient surface interaction forms an adhesion memory and modulates future attachment behavior of the individual bacterium [195].

If a bacterial cell undergoes contact mechanotransduction it continues to strengthen its initial attachment (as described in section “Surface Sensing and Strengthening the Initial Attachment”) with irreversible bonds and forms a glycocalyx. The glycocalyx immobilizes cells onto the surface, provides a robust shield against environmental and mechanical stresses, and creates a close network that facilitates intercellular communication (quorum sensing) [48]. The structure of the matrix is dynamic and its composition and morphology varies greatly between bacterial species [48]. The macroscopic morphology of the biofilm has been shown to be dependent on the nutrient source, as it influences clonal growth and the arrangement of cells within the biofilms

[196]. The glycocalyx assists in the recruitment of nearby bacteria by acting as a point of adhesion. Through cellular growth and reproduction, the biofilm self-regulates and forms a highly structured and organized multicellular population. The maturation of biofilms is controlled by several components within the glycocalyx, such as exopolysaccharides, adhesin proteins, and extracellular DNA [197].

The relative contributions of active bacterial responses (e.g., gene expression of adhesion factors) and passive factors (e.g., environmental conditions) in the formation and development of the biofilm are yet to be fully elucidated [198, 199]. Although cell signaling has been shown to control in vitro biofilm differentiation [200], its influence can be mitigated by growth conditions [201–203].

Race to the Surface

The concept of the "race to the surface" was created to describe the process encountered when host cells compete with contaminating bacteria to colonize biomaterial surfaces [204, 205]. It is thought that the odds of success can be stacked against the potential pathogens by facilitating timely integration of biomaterials into host tissues [206]. Often orthopedic devices rely on osseointegration for fixation, that is, bone tissue growing around and onto the biomaterial leading to the integration of the implant. In vitro studies have shown that biomaterials with pre-colonized bacteria greatly impair the ability of host cells to attach to the surface and to clear the colonizing bacteria [102, 207]. Although in vitro models have provided mechanistic insights into the interactions of bacteria with host cells and biomaterial surfaces, they are limited by the use of single cell cultures and short periods of observation. Therefore, further development of relevant, reproducible, observable in vivo infection models is still required [208].

Biofilm Formation

Biofilm formation can be described either as a developmental process or as a process governed by adaptive responses of individual bacterium. Biofilm formation as seen through the prism of a developmental process involves biofilm-specific genes that are part of hierarchically ordered pathways dedicated to controlling the transition through specific biofilm stages. Biofilm formation governed by adaptive responses is dependent on the ability of the individual bacterium to regulate intercellular adhesion and matrix formation in response to local environmental cues. The developmental and adaptive response hypotheses are evolutionarily distinct as the former involves selection of a given trait because of its benefit to the group, whereas the latter involves a selection of a given trait because of its benefit to the individual bacterium. The counter-intuitive cooperative and altruistic traits observed in biofilms can in many cases be rationalized by the fitness advantages that these behaviors confer on the supposedly self-sacrificing bacterium [209]. The production of the intercellular adhesion may be viewed as a biological cost each bacterium pays to contribute to the mutually beneficial creation of a protective glycocalyx, or it may

be considered a means that increases the adhesiveness of an individual cell, facilitating its persistence in a specific environment. Additionally, quorum-sensing could be viewed as either a means to regulate production of specific factors at the population level, or simply as diffusion sensing that enables the individual bacterium to determine molecular concentration gradients; thus allowing the cell to regulate the production of degradation enzymes to minimize nutritional losses [210].

The Biofilm Matrix

The formation of the glycocalyx is a critical step in biofilm maturation. Its formation facilitates the bacterial switch to the biofilm phenotype [48]. In most biofilms the glycocalyx accounts for over 90% of its dry mass [180]. It is the extracellular matrix in which the bacteria reside and is composed predominately of components produced by the organisms. Ultimately, it is responsible for surface adhesion and intercellular cohesion in the biofilm.

The glycocalyx not only forms a physical barrier, but also facilitates diffusion-limited transport of chemicals throughout the biofilm, thus allowing for intra-biofilm interactions and regulation. The glycocalyx can dynamically modulate metabolite gradients and mitigate hostile environmental conditions, such as low pH and hypoxia. It also has an influence on bacterial virulence through the facilitation of antimicrobial tolerance and modulation of host immunity [180, 211]. The glycocalyx also helps to recycle the cellular components of lysed bacteria by preventing their diffusion away from the biofilm. It also serves as a source of nutrition, although some components of the glycocalyx require the presence of degradation enzymes to become available to the resident bacteria [180]. The glycocalyx also protects organisms against a host of physical and biological insults, such as desiccation, biocides, antibiotics protozoa, and host immune defenses [180, 211, 212]. The glycocalyx also helps to recycle the cellular components of lysed bacteria by preventing their diffusion away from the biofilm. It also serves as a source of nutrition, although some components of the glycocalyx require the presence of degradation enzymes to become available to the resident bacteria [180]. The glycocalyx also protects organisms against a host of physical and biological insults, such as desiccation, biocides, antibiotics protozoa, and host immune defenses [212].

The composition of the glycocalyx varies across genera, species, and strains, responding to specific environmental cues such as temperature, shear, and nutrient availability [213–216]. It is thought that interactions between the glycocalyx components within the biofilm matrix drive adaptation during the various stages of the biofilm life cycle [217–220]. The glycocalyx typically contains a combination of water, polysaccharides, proteins, nucleic acids, and lipids [44, 48, 185, 221–231]. *S. aureus* biofilms have been found to contain predominantly water, extracellular DNA, polysaccharides, and proteins, including adhesins and amyloid fibers [134, 146, 213, 232–234].

Water is the major component of the glycocalyx, which provides a buffer against fluctuations in water potential within the local environment. Glycocalyces generally

retain water entropically rather than through specific binding mechanisms [180]. It is the presence of polymeric substances within the glycocalyx that help to maintain water homeostasis within the biofilm. Bacteria have been shown to actively respond to desiccation by producing a glycocalyx [235]. Desiccation concentrates the glycocalyx, increasing the number of extracellular polymeric substance interactions and reducing biofilm volume [48]. The glycocalyx has also been shown to act as a molecular sieve sequestering ionic and apolar compounds and particles. Due to this mix of apolar regions, cations, and anions there is a heterogeneous physicochemical "stickiness" within the glycocalyx. Particles and nanoparticles can therefore be trapped and accumulate in a spatially variable manner [48, 236]. For example, heavy metals tend to bind cell walls of bacteria, whereas lipids are dispersed throughout the matrix [237].

Polysaccharides are a predominating glycocalyx polymer in many species [238, 239]. Gram-positive pathogens produce a number of exopolysaccharides, such as polysaccharide intercellular adhesin, which are incorporated within their glycocalyces [134, 240–243]. It is thought that the exopolysaccharide component of the glycocalyx is the primary mechanical stabilizer in gram-positive biofilms, particularly *S. epidermidis* [48].

Extracellular proteins present in *S. aureus* glycocalyx have been shown to promote biofilm maturation [48, 233]. Several are enzymatic, such as the autolysins (e.g., AtlA and AtlE), and others are structural, such as biofilm-associated surface protein (Bap). The production of degradation enzymes converts the glycocalyx into an external digestive system that increases the bioavailability of polymers that can be utilized as carbon and energy sources. Furthermore, some degradation enzymes are utilized by bacteria to facilitate the detachment and dispersal from biofilms. The nonenzymatic proteins in the matrix are involved in stabilization of the glycocalyx. Bap and the Bap-like proteins promote biofilm formation in several bacterial species, including *S. aureus* [244]. Other ubiquitous proteinaceous components of the matrix are amyloid polymers. These stable β-sheet polymers form ordered, self-propagating fibers that enhance biofilm stability [245–247]. Bacterial amyloids are now recognized to be a key constituent of glycocalyces in many staphylococcal and non-staphylococcal species [246, 248–252]. The innate resistance of amyloid to enzymatic degradation helps to reinforce and protect the glycocalyx [247]. Amyloid fibers produced by *S. aureus* biofilms are composed of phenol-soluble modulins [234, 253], which are cytotoxic, modulating neutrophil chemotaxis and cytolysis [254], as well as having a role in biofilm development and colony spreading through their surfactant action [58, 217, 254–256]. It has been reported that the formation of amyloid fibers from phenol-soluble modulin peptides is stimulated by the presence of extracellular DNA in *S. aureus* biofilms [257].

Extracellular DNA has been reported to be a matrix component of many single- and multi-species biofilms, including gram-positive and -negative species [48, 187]. It can be actively secreted by viable cells or scavenged from lysed bacteria to bolster biofilm maturation [48]. It is a major structural component in the glycocalyx of *S. aureus*, but is a relatively insignificant component of *S. epidermidis* biofilms [227]. Extracellular DNA was initially thought to be residual waste product from

lysed cells but it is now recognized as an integral structural and functional component of the glycocalyx [258]. Extracellular DNA has been shown to (1) stabilize and strengthen biofilms [259], (2) confer antibiotic resistance, (3) modulate the innate immune response [260], (4) provide a nutrient source during limited nutrient availability [261], (5) promote colony spreading and structuring, and (6) provide a gene pool for horizontal gene transfer [262, 263]. In *S. aureus* biofilms, extracellular DNA is produced predominately by the induced autolysis of a subpopulation [231, 264], which is regulated by the *cidA* gene [232]. Autolysis is mediated through the activity of cell wall hydrolases, AtlA and AtlE [117, 265, 266]. The physiological role of AtlA is to maintain cell wall metabolism during cell division and growth [267, 268], with its upregulation resulting in autolysis [117]. AtlA knockout mutants in vitro display reduced levels of extracellular DNA and decreased biofilm formation [114, 269]. *S. aureus* biofilm cells are viewed by some as altruists and survivors, with the former undergoing induced cell lysis for the sake of the community [264] and the latter inducing fratricide killing [259]. In addition, it has been hypothesized that induced cell lysis, not only provides a source of extracellular DNA and carbon metabolites, but the subsequent local degradation of the surrounding glycocalyx create microchannels [270], which are thought to improve oxygen and substrate transportation throughout the biofilm [271].

Lipids are also found in the glycocalyx of some species, though to a lesser degree in staphylococcal biofilms [272]. Lipopolysaccharides are crucial for the adherence of some gram-negative organisms [273, 274]. Surface-active surfactants, such as surfactin and viscosin, disperse biofilm-associated lipids and make them available as a carbon source. These biosurfactants also have antimicrobial properties and are used by some bacteria to inhibit colonization by competing microorganisms [275]. It has been proposed that they have a role in initial microcolony formation, preventing colonization of microchannels, and inducing cellular dispersion [276].

In general, biofilms display viscoelastic properties, that is, nonlinear, time-dependent, and anisotropic responses to deforming forces, resulting in the mechanical properties of creep, stress relaxation, and hysteresis [277]. It has been suggested that this behavior in response to physical and chemical challenges could partially explain recalcitrance to antimicrobial agents and physical disruption, preventing drug penetration and removal with low-pressure irrigation systems, respectively [277]. Using a combination of in vitro and in silico techniques the rheological properties of biofilms have been examined at the microscale level. Compression experiments using *P. aeruginosa* biofilms have shown that in response to increasing pressure, the glycocalyx initially undergoes an initial phase of elastic deformation until its ultimate tensile strength is reached, after which it responds like a viscous fluid [278]. It has been suggested that this is due to the presence of fluctuating adhesion points between matrix components that are kept together by weak physicochemical forces such as hydrogen bonds. In addition it has also been shown that entanglement of biopolymers conferred stability to the matrix [278]. It has been observed that staphylococcal biofilms display properties of strain hardening, that is, increased resistance to deformation with continued application of shear stress [279]. *S. aureus* biofilms show both elastic and viscous-fluid responses depending

on the time-scale of the force being applied [55]. The result is a dynamic adjustment of the biofilm to mitigate external shear stresses. On an intermediate timescale, biofilms are able to increase the strength of its glycocalyx in response to mechanical stresses by increasing matrix production [280]. The interaction of inorganic ions with the glycocalyx can also confer structural integrity, for example the presence of Ca^{2+} has been found to increase the mechanical stability of *P. aeruginosa* biofilms as it cross-links negatively charged alginate molecules within the matrix [278].

The influence of glycocalyx components on biofilm development in different bacterial species has yet to be clarified. For example, the roles of the matrix polymers polysaccharide intercellular adhesin and extracellular DNA have contrasting significance in staphylococcal biofilms [227]. With polysaccharide intercellular adhesin being integral to matrix stability in *S. epidermidis*, whereas extracellular DNA is the critical component in *S. aureus* biofilms. Furthermore, the exact composition and timing of glycocalyx formation for in vivo biomaterial-associated biofilms remain unclear [180].

Maturation

In the early stages of biofilm development, the glycocalyx is less stable and more susceptible to physical, chemical, and immunological insult [281]; however, as the biofilm matures, the embedded bacteria display a tolerance to many antimicrobials and immune responses. Biofilm formation and maturation is dependent on intercellular interactions, which are regulated by cellular networks such as the Agr system. Biomaterial colonization and biofilm formation have been found to be associated with changes in gene expression. Genomic studies have found the following virulence factor sequences to be upregulated in *S. epidermidis* biofilm formation: *atlE* (autolysin adhesion molecule), *aap* (accumulation associated protein), *agrBDCA* (Agr quorum sensing system), and *icaADBC* genes (polysaccharide intercellular adhesin), suggesting that these genes are important for biomaterial colonization and subsequent infections [282, 283]. The accumulation associated protein is an independent mediator of *S. epidermidis* adhesion [284], as well as a cell wall receptor for polysaccharide intercellular adhesin [285]. Cell signaling networks alter gene expression and subsequent cell phenotype in response to environmental stresses and cell density. The Agr quorum sensing system regulates the expression of numerous virulence factors in the pathogenesis of staphylococcal biomaterial infections [286].

Quorum Sensing

The high cell density and limited diffusion through the biofilm creates an environment conducive to local intercellular communication. Quorum sensing is a mechanism that many micro-organisms use to co-ordinate behavior within the population

in response to local environmental conditions, including cell density and nutrient availability [287]. The quorum sensing system for most bacteria consists of the following: (1) proteins involved in producing and transporting the signaling molecule, (2) the signaling molecule itself, (3) the receptor for the signaling molecule and, (4) additional regulatory proteins (in some systems) [287]. The most-studied systems are those that use *N*-acyl homoserine lactones (AHL) as signalling molecules (present in many gram-negative bacteria, including *P. aeruginosa*) and the autoinducing peptide-Agr (AIP-Agr) system in *S. aureus* [287].

S. aureus sense fluctuations in population density through the production and release of autoinducers. *S. aureus* secretes autoinducing peptides which are detected through transmembrane transduction systems or are actively transported via ATP-binding cassette transporters to engage cytoplasmic regulators, such as Agr [83, 288]. In general density-dependent pathogenicity is regulated by *RNAIII* and the Agr system [289, 290]. Autoinducing peptides of *S. aureus* are highly variable with their specificity determined by their interactions with AgrC kinase sensors (Fig. 5) Thus, *S. aureus* strains can be categorized into four distinct groups based on the specificity of their autoinducing peptide. A notable feature of the *S. aureus* quorum sensing system is that each autoinducing peptide not only initiates analogous Agr virulence cascades, but can inhibit dissimilar systems [291–293]. It has been hypothesized that this interference of cross-strain virulence allows the primary invading strain to out-compete secondary *S. aureus* pathogens [83].

Further quorum sensing systems shown to regulate staphylococcal biofilm formation include S-ribosylhomocysteine lyase (LuxS) and autoinducer 2. In many organisms, biofilm formation is co-regulated by quorum sensing, making it a potential target for novel therapeutics. In addition, quorum sensing has also been found to be involved in the development of antimicrobial and immune tolerance [294, 295]. However, the contribution of each system has yet to be fully elucidated [296, 297].

Dispersal

Dispersal is the final stage of the biofilm life cycle. Biofilm disassembly reverts bacteria to its previous planktonic state, allowing it to reestablish further colonies locally or to metastasize to distant sites [298]. Dispersal can be a result of (1) external perturbations, such as increased fluid shear [299], (2) external environmental cues, such as nutrient limitation or reduced oxygen tension, or (3) the release of glycocalyx- or surface-binding proteins [300–302]. In some species, dispersal is an active process to allow colonization of new biotic and abiotic niches [42]. Three distinct biofilm dispersal strategies have been described: (1) “swarming/seeding” (release of individual cells into the bulk fluid or substratum), (2) “clumping” (shedding of cell aggregates), and (3) “surface” (biofilm structures move across surfaces). *S. aureus* biofilms in vitro have been shown to resist external perturbations by displaying viscoelasticity. In response to fluid shear, *S. aureus* microcolonies have been observed, in microfluidic studies, to roll downstream along the walls of a glass microchannel,

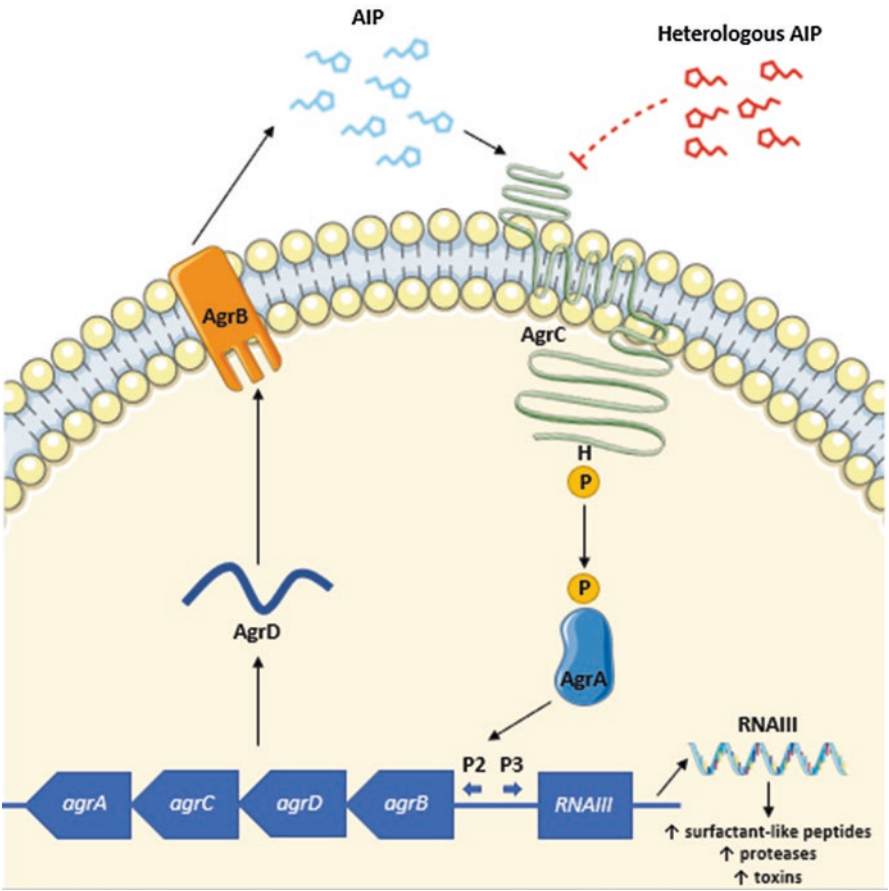


Fig. 5 Autoinducing peptide-accessory gene regulatory (AIP-Agr) system autoactivation. Autoinducing peptide (AIP) activates the transmembrane receptor domain of AgrC to induce phosphorylation of the histidine (H)-protein-kinase domain within the cytoplasm. The subsequent transportation of phosphate (P) to AgrA activates expression of the *agr* promoter regions (P2 and P3). The P2 promoter drives the autoactivation circuit via AgrD expression. The P3 promoter activates expression of *RNAIII*, which is the regulatory effector of the AIP-Agr system and leads the production of virulence factors including phenol-soluble modulin

rather than completely detaching. This behavior is thought to be controlled by viscoelastic tethers [55].

The nutritional status of the local environment also dictates biofilm dispersal. Fluctuations in nutrient availability within the local environment can lead to biofilm dispersal. In flow chamber models *P. aeruginosa* biofilms have been shown to disperse within 15 min of nutrient limitation [303]. For other bacterial species, an increase in extra-biofilm nutrients induces biofilm dispersal. The addition of carbon and nitrogen-based nutrients in minimal medium has been found to be associated

with biofilm dissolution [304]. It has been suggested that biofilm formation is favored by bacteria only within a specific window of nutrient concentrations [86]. Microelectrodes studies have shown that there is limited oxygen penetration though bacterial biofilms [305, 306]. Cells residing with the deepest layers of the biofilm have been shown to rely on anaerobic respiration. Both oxygen depletion and the metabolites of anaerobic respiration have been shown to induce biofilm dispersal [86]. Anaerobic respiration produces reactive nitrogen intermediates in non-staphylococcal species, which cause cellular damage and dispersal in mature *P. aeruginosa* biofilms [307].

Endogenous dispersal in staphylococcal biofilms is driven by the production of extracellular enzymes and phenol-soluble modulins (bacterial toxins with detergent properties) [308]. It is regulated through quorum sensing, namely, the AIP-Agr system [298, 309–311] (Fig. 5). Extracellular autoinducing peptide (AIP) accumulates in proportion with a rising population density, when it exceeds a threshold concentration it begins to bind with the membrane receptor AgrC. This interaction initiates a signalling cascade that results in the expression of *RNAIII*, which is the effector gene of the Agr system. The final step of the AIP-Agr system is the production of virulence factors [286]. Surfactant-like peptides are one group of virulence factors expressed by the AIP-Agr system. They not only help break down the glycocalyx contributing to disassembly, but also modulate host immune processes [97, 269, 298]; examples include staphopain cysteine protease, V8 glutamyl endopeptidase, and staphylococcal nuclease [97]. The relative importance of each enzyme in biofilm dispersion is dependent on the strain-specific composition of the glycocalyx [312].

As described earlier in this review, phenol-soluble modulin production is also controlled by the AIP-Agr system. In a response to increasing population density phenol-soluble modulins are produced. The subsequent disruption of the glycocalyx by the surfactant-like properties of phenol-soluble modulins creates microchannels which facilitate nutrient delivery within the biofilm. More recent studies have reported a secondary role for phenol-soluble modulins in the dispersion and metastasis of biofilm colonies [313]. During initial biofilm development, *agr* expression and subsequent virulence factor production is downregulated, with increased production of adhesion factors. The importance of phenol-soluble modulin expression in AIP-Agr regulation of biofilm structure and disassembly has been demonstrated using *agr* mutant phenotypes [217]. Isogenic *agr* knockout staphylococcal strains have been used to demonstrate upregulated glycocalyx production and reduce the ability to metastasize from in vivo murine orthopedic device infections [314–317]. Other AIP-Agr system regulated genes, besides phenol-soluble modulins, are also thought to have a role in biofilm persistence through modulation of the innate immune system. *S. aureus* biofilms in a murine PJI model have been found to inhibit host cell phagocytosis via Agr-mediated toxin production, namely, α-hemolysin and leukocidin AB [318].

Tolerance Mechanisms

Various mechanisms for the development of antimicrobial tolerance in bacterial biofilms have been hypothesized (Fig. 6). One mechanism is the incomplete penetration of some antimicrobials through the biofilm due to the presence of the glycocalyx [27]. Secondly, the nutrient-deplete environment induces a stress response in bacteria, leading to a substantial reduction in cellular growth and metabolic activity in comparison to planktonic phenotypes [319]. Reduced cellular growth and metabolism lead to the development of tolerance as antimicrobials generally exploit targets involved in bacterial reproduction and cell maintenance. This "biofilm phenotype" has been compared to the sessile state seen in persister cells [320]. Reduced antimicrobial susceptibility has been observed in biofilms formed by common biomaterial pathogens, such as *S. aureus* [321].

Impaired Penetration

It is thought that the glycocalyx inhibits passive diffusion of some antimicrobials, with the resulting reduction in antimicrobial concentration within the biofilm the primary cause of tolerance [27]. In silico biofilm models have been used to demonstrate that limited antimicrobial diffusion leads to death of the outer layer of bacteria but stimulates adaptive changes in the subpopulation of bacteria buried deeper within the glycocalyx, resulting in the development of antimicrobial tolerance [322].

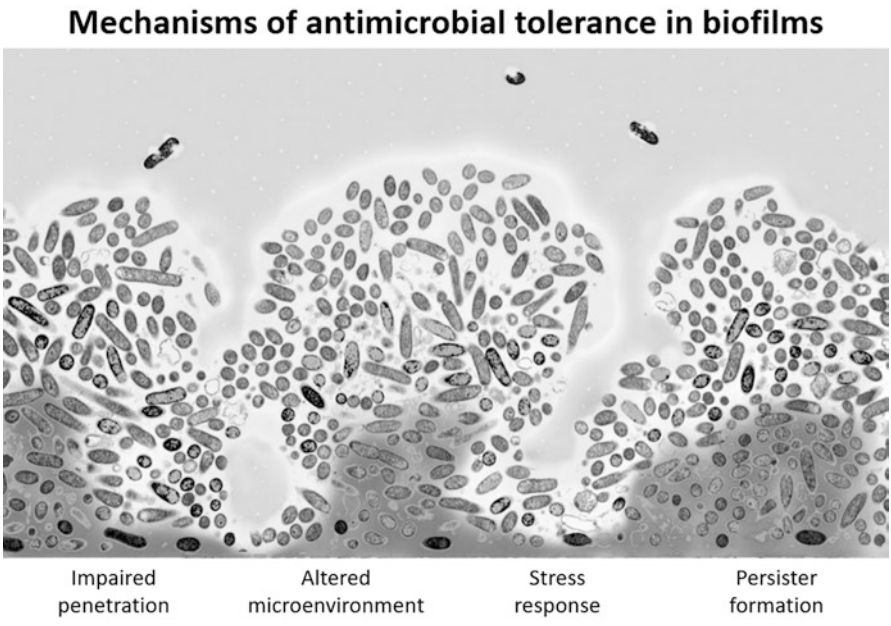


Fig. 6 Summary of antimicrobial tolerance mechanisms within bacterial biofilms

This has been supported by in vitro studies which have found that the glycocalyx limited passive diffusion of some antimicrobials. Alginate, a major component of *P. aeruginosa* biofilms, has been studied intensely in the context of antimicrobial tolerance. The anionic nature of alginate is thought to be critical in limiting penetration of cationic molecules. Alginate solutions have been shown to denature and aggregate cationic antimicrobial peptides inhibiting their action against membrane vesicles. Alginate has also been shown to protect biofilm-associated bacteria from cationic biocides such as chlorhexidine gluconate [323]. Furthermore, alginate has also been shown to bind cationic antimicrobials, such as aminoglycosides (gentamicin and tobramycin), inhibiting their activity [324], as well as toxic cationic heavy metals. Metal chelation has been demonstrated for secreted polymeric molecules from a number of different gram-positive and gram-negative biofilms [325–329]. However, limited antimicrobial penetration is not a universal trait of biofilms. Indeed gentamicin, ciprofloxacin [330], daptomycin [331], rifampicin [332], linezolid [333], and clindamycin [334] can adequately penetrate staphylococcal biofilms while only vancomycin, oxacillin, and cefotaxime (both β-lactams) have been shown to be significantly hampered by the glycocalyx [330, 333]. In the majority of studies, bacteria at the periphery of the biofilms experienced small reductions, but the presence of antimicrobials [27]. In silico biofilm models have been used to demonstrate that limited antimicrobial diffusion leads to death of the outer layer of bacteria but stimulates adaptive changes in the subpopulation of bacteria buried deeper within the glycocalyx, resulting in the development of antimicrobial tolerance [322].
This has been supported by in vitro studies which have found that the glycocalyx limited passive diffusion of some antimicrobials. Alginate, a major component of *P. aeruginosa* biofilms, has been studied intensely in the context of antimicrobial tolerance. The anionic nature of alginate is thought to be critical in limiting penetration of cationic molecules. Alginate solutions have been shown to denature and aggregate cationic antimicrobial peptides inhibiting their action against membrane vesicles. Alginate has also been shown to protect biofilm-associated bacteria from cationic biocides such as chlorhexidine gluconate [323]. Furthermore, alginate has also been shown to bind cationic antimicrobials, such as aminoglycosides (gentamicin and tobramycin), inhibiting their activity [324], as well as toxic cationic heavy metals. Metal chelation has been demonstrated for secreted polymeric molecules from a number of different gram-positive and gram-negative biofilms [325–329]. However, limited antimicrobial penetration is not a universal trait of biofilms. Indeed gentamicin, ciprofloxacin [330], daptomycin [331], rifampicin [332], linezolid [333], and clindamycin [334] can adequately penetrate staphylococcal biofilms, while only vancomycin, oxacillin, and cefotaxime (both β-lactams) have been shown to be significantly hampered by the glycocalyx [330, 333]. In the majority of studies, bacteria at the periphery of the biofilms experienced small reductions, but the presence of antimicrobial throughout the community did not drastically impact cell viability.

It is thought that cellular rather than the glycocalyx permeability may be a mechanism that further explains antimicrobial tolerance within biofilms. Lipids are the primary components of the bacterial cytoplasmic membrane, which is critical for

cellular viability and growth. Lipids allow for passive permeability of hydrophobic molecules, such as antimicrobial agents, disinfectants, and antimicrobial peptides, as well as modulating the function of membrane-associated proteins. The bacterial membrane fluidity can be modulated by modifying phospholipid synthesis and acyl chain configuration [335]. Increased rigidity of the lipid bilayer has been shown to restrict the passive penetration of exogenous hydrophobic compounds through the membrane [336]. For example, when exposed to environmental disturbances, such as presence of toxic compounds at sublethal doses, growing bacterial cells tend to produce fatty acids that help in decreasing their membrane fluidity, thus limiting exchanges and saving energy [337]. To maintain optimal membrane fluidity in response to environmental disturbance bacterial cells can alter the acyl chain configuration of membrane glycerophospholipids by manipulating the following: (1) the levels of fatty acid saturation and branching, (2) acyl chain length, and (3) the synthesis of cyclopropane fatty acids [337, 338]. Lipidomic studies have reported that profiles of biofilm-associated cells contain significantly higher proportions of saturated fatty acids compared to planktonic counterparts. In biofilms, gram-positive bacteria, including *S. aureus*, demonstrate a concomitant increase in saturated fatty acids and acyl chain cross-linking with decreased branched chain content. These changes increase the rigidity of the membrane and restrict the diffusion of hydrophobic antimicrobial molecules, such as aminoglycosides (e.g., gentamicin), macrolides (e.g., erythromycin), and rifampicin [337, 339].

For some species, subinhibitory antibiotic concentrations enhance biofilm formation [340, 341]. Subinhibitory concentrations of tetracycline and erythromycin have been shown to activate the production of polysaccharide intercellular adhesin in *Staphylococci*, promoting glycocalyx formation [342]. As outlined earlier in this review, polysaccharide intercellular adhesin is an integral structural, adhesion, and pathogenicity factor in staphylococcal biofilm formation and biomaterial-associated infection.

Altered Microenvironment

A heterogeneous environment develops within the biofilm as it grows and matures. Bacterial cells at the periphery of the biofilm disproportionately consume nutrients and oxygen, driving centrally located bacteria toward cellular dormancy [27, 89, 305, 343]. Nutrient limitation and other physical stressors, such as low pH, cause bacteria to enter a persister-like state [344]. Given that most agents typically act on pathways involved in cellular growth, reproduction, and cell metabolism; slow or stationary phase microorganisms are relatively protected from antimicrobials. Persister and small-colony variants within biofilms have been shown to survive exposure to supra-therapeutic concentrations of antimicrobials due to their dormancy [345]. Even in broth cultures, deprivation of oxygen and nutrients results in the development of antimicrobial tolerance [305, 346].

The relatively hypoxic and nutrient limited zones in the deeper regions of the biofilm have been demonstrated using microelectrode measurements combined with genomic [347] and proteomic analysis [306, 343, 348]. In the proteomic

studies, protein production ceased when a critical threshold in oxygen tension was breached, suggesting that decreased metabolic activity was directly related to oxygen tension within the biofilm [306, 348]. Similarly, limited diffusion of glucose has also been found to correspond to both a reduction in bacterial growth and the development of penicillin tolerance [305]. Fluoroquinolone tolerance in *E. coli* biofilms has also been observed using in vitro starvation models [349]. In both these studies, the antimicrobial of interest was shown to freely diffuse throughout the biofilm, but bacterial killing was observed only in the periphery of the biofilm [305, 306]. Even stationary phase cultures of planktonic bacteria have also been shown to display antimicrobial tolerance [350–353]. Stationary phase cells are in a limited-growth or non-growth state, similar to biofilm cells and persister cells. Cells in this dormant state are inherently more tolerant to antimicrobial drugs that target metabolically active cells. For example, beta-lactams are ineffective against dormant cells that are not actively replicating or producing peptidoglycan [354]. Stationary phase cultures exhibit high cell density colonies as seen in biofilms. In this environment, cells, deprived of both nutrients and oxygen, produce less ATP. It has been shown that intracellular ATP concentration is a major determinant of survival to antimicrobial challenges in stationary phase and persister cells [353, 355].

Metabolomic techniques have been used to highlight the metabolic changes of *S. aureus* in the biofilm and planktonic states. Sun et al., using untargeted nuclear magnetic resonance spectroscopy, found that *S. aureus* consume predominantly glucose, arginine, and threonine during biofilm development. Meanwhile, they accumulated 2-aminobutyrate, ornithine, acetate, lactate, ethanol, and isopropanol [356]. This has been independently corroborated using an alternative metabolomic technique, targeted mass spectrometry [357–359]. Stipetic et al. [360] reported that arginine metabolism, specifically the urea cycle, is significantly altered during *S. aureus* biofilm development. It has been suggested that *S. aureus* generates ammonia to restore pH balance in response to anaerobic respiration found within the deeper layers of the biofilm [361]. This hypothesis is also supported by genomic, transcriptomic, and proteomic studies which suggest that amino acid catabolism is crucial for biofilm pH balance [362–364]. Thomas et al. demonstrated that overflow carbon pathways are also important in *S. aureus* biofilm development. These pathways have been shown to have a role in the potentiating programmed cell death (i.e., apoptosis) in bacteria by modulation of acetate metabolism [365]. Previously, it was thought that only eukaryotic cells had the ability to undergo programmed cell death [366]. It has been hypothesized that the metabolite, acetate, acts as a physiological trigger for apoptosis, controlling the biofilm colony population. The final step of acetate-induced programmed cell death in *S. aureus* biofilms involves reactive oxygen species formation and subsequent DNA damage, which are both critical features seen in apoptosis [365]. There is a growing body of evidence to support the claim that the metabolic activity of bacteria within biofilms is significantly altered and limited. The reversal of this limited trophic state is an aspect of the biofilm phenotype that could be targeted for therapeutic use.

Stress Response

It has been suggested that the biofilm phenotype is a variant of the persister or stationary-phase state [305]. Persistence has been linked to the activation of cellular stress responses [353]. In addition, the presence of environmental stresses have been shown to increase the rates of horizontal gene transfer and de novo mutations, resulting in the rapid acquisition of antibiotic resistance sequences [367]. This has led to the investigation of stress-response gene expression within biofilms.

The primary mediator of the stringent response in persister cells is ppGpp [368, 369]. It has been hypothesized that strategies leading to reduced levels of stringent response mediators could help fight persistence. Using the known *S. aureus* stringent response inhibitor, peptide 1018, a direct antibiofilm action against in vitro biofilms formed by common biomaterial pathogens (e.g., *P. aeruginosa* and *S. aureus*) has been observed. Direct biofilm disruption by peptide 1018 has been shown to be induced through ppGpp degradation [370], as well as having a potentiating effect on ciprofloxacin [371].

A genomic feature of stationary-phase bacteria is the upregulation of *rpoS*, which is a key regulatory gene in the bacterial stress response [372]. Genomic analysis of *E. coli* biofilms revealed the upregulation of half of all *rpoS*-regulated genes, with *rpoS* knockout mutants failing to form biofilms [347]. Further studies with other bacterial species have also highlighted the role stress response factors have in biofilm formation and development of antimicrobial tolerance. Analysis of tobramycin-treated wild type *P. aeruginosa* biofilms showed upregulation of *groES* and *dnaK*, which are key stress response factors [373]. Upregulation of catalase expression in *Klebsiella pneumoniae* (*K. pneumoniae*) is a feature of persister cells, stationary-phase bacteria and in biofilms, but is absent in the planktonic phenotype [305]. Catalase is a key enzyme in the breakdown of hydrogen peroxide and protects against antimicrobial-mediated inactivation. The same protective mechanism against hydrogen peroxide has also shown to be present in *P. aeruginosa* biofilms [374]. It has been postulated that the final common pathway for all bactericidal drugs is lethal oxidative damage secondary to the generation of hydroxyl radicals [375, 376]. Therefore, the tolerance to bactericidal antimicrobials seen in *K. pneumoniae* and *P. aeruginosa* biofilms may have been developed originally as a response to hydrogen peroxide stress. In *S. aureus* biofilm formation the stress-response regulatory system, GraRS, plays an important role in its tolerance to antimicrobial peptides and cationic antimicrobial agents, such as gentamicin, daptomycin, and vancomycin [377]. *Staphylococci* have a diverse network of stress response regulators that confer protection against a host of environmental stressors, including antimicrobial peptides and antimicrobials. In response to cellular stress, *Staphylococci* are able to alter the proportion of the anionic polysaccharide intercellular adhesin and cationic teichoic acids in their extracellular polymeric matrix and cell membrane, respectively, through the GraRS system [378]. This envelope stress response confers significant tolerance to both antimicrobial peptides and cationic antimicrobials by counteracting cell wall and membrane perturbations [379, 380]. In vitro

investigations of antibiotic combinations used to treat staphylococcal biofilms have found that combinations of bactericidal cell wall targeting antimicrobials such as daptomycin, gentamicin, and vancomycin display synergism [376, 381]. It has been suggested that overactivation of the staphylococcal envelope stress response to dual antimicrobial peptides and/or cell wall targeting antimicrobials may account for this observed synergistic effect [376, 382–384].

It has been shown that stress responses can confer antimicrobial tolerance in persister cells and stationary-phase planktonic bacteria. There is now emerging evidence to support that they are also upregulated in the biofilm phenotype. Further understanding of the activation and regulatory pathways involved in these stress responses within bacterial biofilms will reveal the presence of novel therapeutic targets [372].

Persister Formation

Bacterial cell persistence was first described in the mid-1940s. Subpopulations of penicillin-sensitive bacteria were found to survive exposures to supratherapeutic concentrations of penicillin. The surviving subpopulation were called “persisters” [385]. They are characterized by transient resistance with susceptibility returning following subsequent culture [386]. In the persister theory, this subpopulation, whether in biofilms or planktonic culture, differentiates into dormant, spore-like cells [387]. This differentiation is driven by phenotypic variation rather than genetic modification. Persister cells that neither grow nor die in the presence of bactericidal antibiotics are important in the tolerance of traditional antimicrobial agents within biofilms [388, 389]. Insufficient eradication of persister cells has been shown to contribute to the recalcitrance and recurrence of biomaterial-associated infections [390]. The physiology of biofilm cells is remarkably similar to that of persister cells. Cells that detach from antimicrobial-tolerant biofilms and grown planktonically have been shown to revert to an antimicrobial-susceptible state [286, 391].

Persister cell formation is initiated by stress signaling and activation of toxin–antitoxin systems [387]. Features of biomaterial-associated infections, such as biofilm development, a hostile host environment, and sublethal concentrations of antibiotics are important activators of bacterial stress responses [392]. Biofilm formation is viewed by some as a bacterial response to environmental stress that not only induces glycocalyx production but also selects for dormant persister cells that can survive environmental stressors, including antimicrobial challenges [386, 393]. Toxin–antitoxin modules induce cellular dormancy by inhibiting the activity of specific organelles, such as the ribosome. Evidence for this induced dormant state comes from studies in which toxin–antitoxin module-expressing bacteria were initially shown to be viable but non-culturable; however, colony growth could be restored by the induced transcription of the corresponding antitoxin [394]. The induction of toxin–antitoxin systems have also been suggested as a mechanism for antimicrobial tolerance [395]. It is believed that this tolerance may be reversed by the induction of programmed persister cell death (i.e., “death in sleep”) or awakening the dormant cells [372]. One approach has been to explore the effect of

manipulating toxin and antitoxin levels in persisters, as well as biofilm-associated cells as a means to induce either of these antimicrobial susceptible states [396, 397].

Transcriptomic studies have been used to show that persisters have a different phenotype from planktonic bacteria [398]. While no comparison was made to biofilms, it has been proposed that the persister phenotype is similar to the biofilm state [398]. In silico modelling has been used to predict that there is a steady accumulation of persisters during biofilm maturation [399]. Thus, it has been suggested that persister formation is a key mechanism of antimicrobial tolerance in biofilms [372]. The innate accumulation of persisters during biofilm formation might represent a common mechanism for the emergence of antimicrobial tolerance in biomaterial-associated infections. However, while persisters may be phenotypically similar to biofilm cells, the specific genetic elements required to form the persister state have yet to be elucidated in biofilm-associated bacteria [400].

Immune Evasion

The ability of some pathogens, such as *S. aureus*, to colonize specialized niches such as biomaterials and connective tissue is attributed to their extensive repertoire of virulence factors that allow them to evade, inactivate, and manipulate the host immune system. They have been shown to inhibit elements of both innate and acquired immunity [401–403].

Innate Immunity

Professional phagocytes, such as neutrophils and macrophages, are a cornerstone of the innate immunity that must be inactivated for pathogens to successfully establish infection. For example, *S. aureus*, one of the most prevalent biomaterial pathogens, produces an armamentarium of cytotoxins aimed at professional phagocytes [404–407]. However, the host immune defenses not only respond to the pathogen but also the biomaterial. This foreign body reaction initially triggers an acute inflammatory response, which results in neutrophil recruitment and activation [90].

Neutrophils have evolved a number antimicrobial strategies: (1) phagocytosis, (2) antimicrobial peptides, (3) generation of reactive oxygen species, (4) secretion of pro-inflammatory cytokines and chemokines, and (5) extracellular traps [408, 409]. Biomaterial-induced neutrophil activation causes prolonged oxidative bursts, leading eventually to cellular exhaustion and depletion of intracellular reactive oxygen species. This state of depletion reduces the bactericidal capacity of neutrophils [23, 317], and has been implicated in recalcitrance biomaterial-associated infections [90].

Bacteria in both their planktonic and biofilm states have evolved their own strategies to counter neutrophil function. For example *S. aureus* produce a host of proteins to facilitate innate immune evasion and pathogenesis [145, 267, 410–413].

Hemolysins, such as α-hemolysin, β-hemolysin, and ϒ-hemolysin, are pore-forming toxins. They are able to lyse eukaryotic cells and also modulate cytokine signaling pathways [410, 414, 415]. Leukocidins (e.g., LukAB and Panton–Valentine leukocidin) are pore-forming toxins, which target white blood cells [411, 412]. Superantigenic exotoxins (e.g., Toxic shock syndrome toxin) can inhibit host immunity in osteomyelitis and even activate bone resorption via T-cell stimulation [412]. *S. aureus* wall teichoic acid can modulate complement activity and confer resistance to lysosome killing during phagocytosis [413]. Chemotaxis inhibitory proteins are known to disrupt neutrophil activation and recruitment, as well as interfering with complement activation [416]. Membrane-bound pigments, such as staphyloxanthin (responsible for the golden appearance of *S. aureus* cell walls) act as reactive oxygen species scavengers helping to combat against oxidative bursts and phagocytosis in neutrophil-mediated bacterial eradication [417, 418].

Many key factors in biofilm formation and maintenance have also been shown to have immune modulatory effects. Autolysins (e.g., AtlA and AtlE) not only are responsible for bacterial cell autolysis but have been shown to alter the peptidoglycan cell wall to confer resistance to lysozymes, achieved through the action of peptidoglycan-specific O-acetyltransferase (OatA) [419, 420]. Phenol-soluble modulins (e.g., PSM α1–α4) are not only critical for biofilm dispersion but have also been found to lyse neutrophils [58]. *S. aureus* biofilms upregulate the AIP-Agr quorum-sensing system in the presence of a neutrophilic challenge. The resultant increase in phenol-soluble modulin production is thought to be critical in the ability of *S. aureus* biofilm to resist neutrophil killing and phagocytosis [421]. *S. aureus* nucleases, used to digest extracellular DNA in biofilms, also degrade neutrophil extracellular traps (NETs) to deoxyadenosine; not only this inactivates the NETs, but the resultant substrate is also known to be cytotoxic to macrophages in abscess environments [422]. This wide array of anti-immune virulence factors allows *S. aureus* to survive phagocytosis and persist intracellularly. Hijacked phagocytes are then used by *S. aureus* as vehicles to further disseminate while being protected from further immune interaction [423].

M1-polarized (classically activated) macrophages are evoked by toll-like receptor activation and secrete pro-inflammatory cytokines to promote bacterial clearance. Staphylococcal biofilms actively evade recognition by toll-like receptors and skew macrophage responses toward an M2-polarized (alternatively activated) anti-inflammatory state [260, 424, 425]. M2-polarized macrophages display inefficient phagocytosis and promote fibrosis, further stimulating glycocalyx formation [421, 425]. Impaired monocyte and granulocyte differentiation, resulting in the formation of myeloid-derived suppressor cells, is also integral to the pathogenesis of biomaterial-related *S. aureus* biofilm infections [426, 427]. Using a mouse PJI model it was found that myeloid-derived suppressor cells actively inhibited T-cell recruitment and pro-inflammatory cytokine production, which led to *S. aureus* biofilm recalcitrance. The myeloid-derived suppressor cells have also been shown to inhibit acute inflammation within the biofilm by differentiating into M2 macrophages and producing anti-inflammatory mediators, such as IL-10 [428–430].

S. epidermidis has fewer anti-immune factors. Although it also produces phenol-soluble modulins like *S. aureus* [308], they are only expressed at low levels [431]. Yet despite the lack of known virulence factors *S. epidermidis* causes a disproportionate number of orthopedic biomaterial-associated infections [137]. Its pathogenesis is thought to be dependent predominately on biofilm formation [432]. The contrasting importance of passive immune evasive between the two most common staphylococcal pathogens should be considered in the development of novel preventative and therapeutic antistaphylococcal infection strategies.

Adaptive Immunity

The relative immunodeficient environment within biofilms was initially thought to be solely due to the inability of immune cells to penetrate the glycocalyx, "a mature biofilm has a dense polymeric matrix that is difficult to engulf by macrophages, which results in 'frustrated phagocytosis' [260]." This concept was thought to be analogous to the ineffective response of phagocytes during asbestosis [433]. However, in vitro studies have shown that biofilms are not impenetrable to human leukocytes [434], including professional phagocytes [435]. Cellular and humoral responses against bacterial infection are predominately dependent on $CD4^+$ T helper-1 (Th1) cells and T helper-2 (Th2) cells to facilitate adaptive immunity, respectively; with Th1 cells promoting cellular immunity against intracellular bacteria, while Th2 cells facilitate humoral immunity against predominately extracellular pathogens [428, 436]. Typically the Th1 response is coupled with Th17 activation to clear intracellular bacteria; however, *S. aureus* biofilms are able to evoke Th1/Th17 responses even in the presence of extracellular pathogens [435, 437]. Furthermore Th1/Th17 responses in biomaterial-associated infections have been shown to promote chronic inflammation and fibrosis, further facilitating biofilm proliferation [438]. This Th1/Th17 bias has been further demonstrated using Th1/Th17-biased genetically modified mice. These mice were unable to clear *S. aureus* biofilm-associated chronic osteomyelitis, while Th2-skewed modified mice successfully eradicated the same infection [439]. This Th1/Th17 biased-response has also been observed in non-staphylococcal biofilms [424, 440–442].

Host Cell Invasion

A further mechanism in the pathogenesis of biomaterial-associated bacterial infections is the ability of some pathogens, such as *S. aureus,* to invade host cells. Once considered to be a strict extracellular pathogen it is now accepted that *S. aureus* can survive within eukaryotic cells, in both professional phagocytes [443–446] and non-professional phagocytes such as epithelial cells, keratinocytes, and endothelial cells [444, 447–449].

The role of professional phagocytes, such as macrophages and neutrophils, in the innate immune response is to isolate and inactivate invading microbes. They are able to perform this role through phagocytosis, a receptor-driven process that results in the creation of a vacuole (known as a phagosome during phagocytosis) which envelopes the offending pathogen [450]. The phagosome is initially benign but is transformed, following the [450]. The phagosome is initially benign but is transformed, following the interaction with the endolysosomal network, into a degradative organelle known as a phagolysosome [451].

S. aureus is able to resist phagocyte ingestion by producing proteins, such as protein A and Efb, that neutralize host opsonins, thereby limiting antibody- and complement-mediated phagocytosis [452, 453]. Intracellular persistence is driven by a variety of AIP-Agr regulated virulence factors such as α-hemolysin and phenol-soluble modulin α, plus the ability to form small colony variants [444, 447]. A unified theory of the interaction between host cells and *S. aureus* has yet to be formulated, which may be a consequence of variations between in vitro models, experimental methodologies, and the bacterial strains examined [444, 446, 454, 455]. Some of these in vitro studies have shown that *S. aureus* persist intracellularly for up to 7 days before bacterial proliferation and host cell lysis [444, 446]. During this intracellular phase *S. aureus* remains within the confines of the phagolysosome where survival is dependent of the expression of the lytic toxin, α-hemolysin [444]. This virulence factor has been shown to alter vacuole membrane integrity, which prevents phagolysosome acidification [455]. Other studies have suggested that phagocytosed *S. aureus* express phenol-soluble modulin α, which induces lysis of the phagolysosome. *S. aureus* are then subsequently able to reside within the cytoplasm with bacterial growth and reproduction resuming within hours of phagocytosis [455–457]. Similarly, the fate of the host macrophage following intracellular *S. aureus* persistence has yet to be resolved, with some studies reporting that intracellular pathogens can induce an antiapoptotic program, with others reporting evidence of phagocyte inactivation [407, 454, 455, 458]. A more recent in vitro study reported that a failure of phagolysosomal maturation and acidification was associated with an absence of apoptosis-associated bacterial killing in macrophages. This allowed viable bacteria to accumulate because of ongoing phagocytosis and form an intracellular pool that was maintained through cycles of cell lysis and phagocytosis by other macrophages [459]. It has been proposed that this population of bacteria may be a potential therapeutic target in limiting the recalcitrant and metastatic nature of *S. aureus* biomaterial-associated infections [459, 460].

It has also been shown that *S. aureus* cells also have the ability to invade and persist within non-professional phagocytes, such as osteoblasts [461]. Inside the cell, *S. aureus* avoid antimicrobial exposure and activated professional phagocytes. The intracellular *S. aureus* subsequently induce apoptosis of the host cell, allowing it to colonize biomaterial surfaces and connective tissue niches [462]. Internalization of *S. aureus* into osteoblasts is mediated by fibronectin, which acts as a ligand between the staphylococcal fibronectin-binding protein and the osteoblast integrin, α5β1 [463]. This interaction triggers tumor necrosis factor-induced apoptosis via activation of caspase-8, which results in osteoblast apoptosis and bone destruction [464].

Internalized bacteria adopt a persister phenotype, as has been observed in biofilm-associated bacteria [465]. The persister phenotype enables the internalized bacteria to prolong survival [466, 467]. The role of intracellular *S. aureus* and persistence within non-professional phagocytic host cells has been demonstrated in vivo and has been shown to have a role in the development of primary and secondary osteomyelitis, as well as biomaterial-associated infections [468–470]. For example, when rat osteoblasts were infected ex vivo with *S. aureus*, the internalized bacteria initiated infection in vivo when reintroduced to an open fracture in a secondary rat [471]. In addition to osteoblast internalization, *S. aureus* can also reside within the canaliculi of bone, in which they can form biofilms [472]. Both *S. aureus* [461, 473] and *Staphylococcus pseudintermedius* [473] have been shown to have the ability to invade osteoblasts, whereas clinical isolates of other staphylococcal species, such as *S. epidermidis* and *Staphylococcus lugdunensis* [461, 473] have been found to have lower intracellular invasion rates than *S. aureus*.

With ever emerging evidence highlighting the ability of biomaterial-associated pathogens to seek refuge in specialized connective tissue, strategies to overcome this feature of pathogenesis should be a current focus of therapeutic development.

Conclusion

Understanding of the pathogenesis in biomaterial-associated infections has progressed greatly since the biofilm hypothesis was first proposed almost 40 years ago. However, the biofilm hypothesis only partially elucidates the pathogenesis of these highly morbid infections. A greater appreciation of the mechanisms underpinning immune evasion by common pathogens, particularly immune cell manipulation and intracellular dormancy, has highlighted a previous underestimation of the role this behavior has on the development of biomaterial-associated infections. A more rounded understanding of its pathogenesis will not only help to develop potential therapeutic targets (Table 1) but will update the paradigm for biomaterial-associated infections.

Further understanding of the initial routes of transmission can be gained through the application of next generation sequencing in clonal studies of bacteria [474, 475]. Elaborating transmission pathways will allow more effective targeting of existing and novel preventative strategies, such as preoperative skin and nasal decolonization [476–478] and intraoperative ambient phototherapy [479, 480], respectively.

Anti-adhesion strategies aimed at preventing bacteria from adhering to biomaterial surfaces to prevent biofilm formation, also has the potential for development. This could be accomplished by changing the physical properties of biomaterial surfaces (e.g., hydrophobicity, topography, and electrical charge) [481, 482], facilitating the attachment of host cells to competitively inhibit bacterial adhesion [483], or integrating antimicrobial agents (e.g., nanoparticles and antimicrobial peptides) [484, 485].

Table 1 Potential therapeutic targets in the prevention and management of biomaterial-associated infections

Pathophysiological target	Potential therapy	References
Route of transmission	Preoperative decolonization	[476–478]
	Intraoperative bactericidal phototherapy	[479, 480]
Attachment	Biomaterial surface modification	[481–483]
	Surface-bound antimicrobials	[484, 485]
Dormancy	Metabolic stimulation	[396, 486–488]
	Stress response inhibition/manipulation	[370, 371, 378]
Dispersal	Enzyme therapy	[298, 490, 491]
	Passive immunization	[162, 492, 493]
	Physical therapies	[494–498]
	Quorum sensing manipulation	[500–504]
Cell internalization	Cell penetrating peptide addition	[507, 508]
	Liposome encapsulation	[509, 510]

Particularly intriguing is the relationship between antimicrobial tolerance and the biofilm phenotype [482]. There has been a focus on strategies to reverse the cell dormancy associated with the persister and biofilm phenotypes. With growing evidence that one of the main factors leading to persister formation and dormancy is nutritional stress [368, 369], preclinical investigations have focused on the inhibition and manipulation of the cellular stress responses [370, 371, 378], as well as metabolic stimulation [396, 486–488].

A further area of research has been on the induction of biofilm dispersal, as antimicrobial tolerance has been shown to be reversed following dispersion [489]. Early efforts have focused on the utilization of: enzyme therapies [298, 490, 491]; passive immunotherapy, which utilize monoclonal antibodies against components of the glycocalyx [162, 492, 493]; and physical modalities, such as ultrasound [494] and pulsed electromagnetic fields [495–498] to disperse the bacteria from the biofilm. A further method of dispersion which has been investigated more recently is based on the manipulation of quorum sensing mechanisms [499]. Targeting of quorum sensing pathways has been shown to prevent biofilm formation and increase antimicrobial susceptibility through induction of phenol-soluble modulin-mediated dispersal mechanisms [500–504].

Internalization of bacteria by mammalian cells is now being accepted as an important feature of recalcitrance in biomaterial-associated infections [469, 505]. Attempts to modify antimicrobial agents to target intracellular infections have been reported. Through the addition of cell penetrating peptides, antimicrobial molecules and larger (nano)particles are able to penetrate eukaryotic cells [506], facilitating mammalian cell internalization, and thereby co-localizing the antimicrobial agent with the pathogen [507, 508]. A further approach is the development of liposome nanocarriers. Liposomes are phospholipid vesicles that are able to penetrate biofilms and mammalian cells. They are compatible with a wide range of antimicrobials commonly used against biomaterial-associated infections [509, 510]. Liposomes are also able to

prevent the degradation of antimicrobials by glycocalyx-associated enzymes, host components, and virulence factors [510].

Despite the progress made in the understanding of the pathophysiology of biomaterial-associated infections and the identification of potential therapeutic targets, very few non-drug antimicrobial therapies and strategies have progressed beyond preclinical investigation. Conventional clinical treatment of biomaterial-associated infections has shown little progress beyond the traditional tenets of surgical debridement, irrigation +/− excision, plus local and systemic antimicrobial drug therapy. The urgency to bridge this lag in the translation of basic science understanding to clinical therapies is greater than ever, especially in light of the looming global crisis of antimicrobial resistance [511, 512], which threatens to halt elective biomaterial-associated procedures [28, 29].

References

1. Whitehouse JD, Friedman ND, Kirkland KB et al (2002) The impact of surgical-site infections following orthopedic surgery at a community hospital and a university hospital: adverse quality of life, excess length of stay, and extra cost. Infect Control Hosp Epidemiol 23:183–189. https://doi.org/10.1086/502033
2. Darouiche RO (2004) Treatment of infections associated with surgical implants. N Engl J Med 350:1422–1429. https://doi.org/10.1056/NEJMra035415
3. Fisman DN, Reilly DT, Karchmer AW, Goldie SJ (2001) Clinical effectiveness and cost-effectiveness of 2 management strategies for infected total hip arthroplasty in the elderly. Clin Infect Dis 32:419–430. https://doi.org/10.1086/318502
4. Natsuhara KM, Shelton TJ, Meehan JP, Lum ZC (2019) Mortality during total hip periprosthetic joint infection. J Arthroplast 34(7S):S337–S342. https://doi.org/10.1016/j.arth.2018.12.024
5. Stanton T (2017) PJI and cancer: more similar than different? American Association of Orthopaedic Surgeons
6. British Orthopaedic Association (2015) Getting it right first time.. https://www.boa.ac.uk/pro-practice/getting-it-right-first-time/.
7. Kamath AF, Ong KL, Lau E et al (2015) Quantifying the burden of revision total joint arthroplasty for periprosthetic infection. J Arthroplast 30:1492–1497. https://doi.org/10.1016/J.ARTH.2015.03.035
8. Bozic KJ, Ries MD (2005) The impact of infection after total hip arthroplasty on hospital and surgeon resource utilization. J Bone Joint Surg Am 87:1746–1751. https://doi.org/10.2106/JBJS.D.02937
9. Public Health England (2016) Surveillance of surgical site infections in NHS hospitals in England. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/577418/Surgical_site_infections_NHS_hospitals_2015_to_2016.pdf. Accessed 2 Feb 2017
10. Trampuz A, Widmer AF (2006) Infections associated with orthopedic implants. Curr Opin Infect Dis 19(4):349–356
11. Mills LA, Aitken SA, Simpson AHRW (2017) The risk of non-union per fracture: current myths and revised figures from a population of over 4 million adults. Acta Orthop 88:434–439. https://doi.org/10.1080/17453674.2017.1321351
12. Tsang S, Aitken SA, Golay SK et al (2014) When does hip fracture surgery fail? Injury 45:1059–1065. https://doi.org/10.1016/j.injury.2014.03.019

13. Trampuz A, Zimmerli W (2006) Diagnosis and treatment of infections associated with fracture-fixation devices. Injury 37:S59–S66. https://doi.org/10.1016/J.INJURY.2006.04.010
14. Kurtz SM, Lau E, Schmier J et al (2008) Infection burden for hip and knee arthroplasty in the United States. J Arthroplast 23(7):984–991. https://doi.org/10.1016/j.arth.2007.10.017
15. Kurtz S, Ong K, Lau E et al (2007) Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Jt Surg 89:780–785. https://doi.org/10.2106/JBJS.F.00222
16. Miller AO, Henry MW, Brause BD (2017) 1—Prevention of joint infections. Management of Periprosthetic Joint Infections (PJIs), pp 3–23
17. Pandey R, Berendt AR, Athanasou NA (2000) Histological and microbiological findings in non-infected and infected revision arthroplasty tissues. The OSIRIS Collaborative Study Group. Oxford Skeletal Infection Research and Intervention Service. Arch Orthop Trauma Surg 120:570–574
18. Athwal GS, Holmes S, Diaz AP et al (2017) A rapid detection method for Propionibacterium acnes from surgical biopsies of the shoulder. J Shoulder Elb Surg 26:e162. https://doi.org/10.1016/j.jse.2016.12.037
19. Achermann Y, Vogt M, Leunig M et al (2010) Improved diagnosis of periprosthetic joint infection by multiplex PCR of sonication fluid from removed implants. J Clin Microbiol 48:1208–1214. https://doi.org/10.1128/JCM.00006-10
20. Bossard DA, Ledergerber B, Zingg PO et al (2016) Optimal length of cultivation time for isolation of Propionibacterium acnes in suspected bone and joint infections is more than 7 days. J Clin Microbiol 54(12):3043–3049. https://doi.org/10.1128/JCM.01435-16
21. Darouiche RO, Darouiche RO (2001) Device-associated infections: a macroproblem that starts with microadherence. Clin Infect Dis 33:1567–1572. https://doi.org/10.1086/323130
22. Aalirezaie A, Anoushiravani A, Cashman J et al (2019) General assembly, prevention, host risk mitigation—local factors: proceedings of international consensus on orthopedic infections. J Arthroplast 34(2):S37–S41. https://doi.org/10.1016/j.arth.2018.09.051
23. Zimmerli W, Waldvogel FA, Vaudaux P, Nydegger UE (1982) Pathogenesis of foreign body infection: description and characteristics of an animal model. J Infect Dis 146:487–497
24. Zimmerli W, Lew PD, Waldvogel FA (1984) Pathogenesis of foreign body infection. Evidence for a local granulocyte defect. J Clin Invest 73:1191–1200. https://doi.org/10.1172/JCI111305
25. Zimmerli W, Zak O, Vosbeck K (1985) Experimental hematogenous infection of subcutaneously implanted foreign bodies. Scand J Infect Dis 17:303–310
26. Lucas VS, Lytra V, Hassan T et al (2002) Comparison of lysis filtration and an automated blood culture system (BACTEC) for detection, quantification, and identification of odontogenic bacteremia in children. J Clin Microbiol 40:3416–3420. https://doi.org/10.1128/JCM.40.9.3416-3420.2002
27. Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15:167–193
28. O'Neill J (2014) Antimicrobial resistance: tackling a crisis for the health and wealth of nations. https://amr-review.org/sites/default/files/AMR Review Paper - Tackling a crisis for the health and wealth of nations_1.pdf. Accessed 25-01-2019
29. Davies S (2013) Antimicrobial resistance poses 'catastrophic threat', says Chief Medical Officer—GOV.UK. In: Dep. Heal. Soc. Care. https://www.gov.uk/government/news/antimicrobial-resistance-poses-catastrophic-threat-says-chief-medical-officer%2D%2D2. Accessed 11 Mar 2019
30. Hall-Stoodley L, Costerton JW, Stoodley P (2004) Bacterial biofilms: from the Natural environment to infectious diseases. Nat Rev Microbiol 2:95–108. https://doi.org/10.1038/nrmicro821
31. Costerton JW, Geesey GG, Cheng KJ (1978) How bacteria stick. Sci Am 238:86–95
32. Costerton JW, Irvin RT, Cheng KJ (1981) The bacterial glycocalyx in nature and disease. Annu Rev Microbiol 35:299–324. https://doi.org/10.1146/annurev.mi.35.100181.001503
33. Marrie TJ, Nelligan J, Costerton JW (1982) A scanning and transmission electron microscopic study of an infected endocardial pacemaker lead. Circulation 66:1339–1341

34. Gristina AG, Costerton JW (1984) Bacterial adherence and the glycocalyx and their role in musculoskeletal infection. Orthop Clin North Am 15:517–535
35. Khoury A, Lam K, Ellis B, Costerton J (1992) Prevention and control of bacterial infections associated with medical devices. ASAIO J 38:174–178
36. Nickel JC, Ruseska I, Wright JB, Costerton JW (1985) Tobramycin resistance of Pseudomonas aeruginosa cells growing as a biofilm on urinary catheter material. Antimicrob Agents Chemother 27:619–624
37. Koo H, Allan RN, Howlin RP et al (2017) Targeting microbial biofilms: current and prospective therapeutic strategies. Nat Rev Microbiol 15:740–755. https://doi.org/10.1038/nrmicro.2017.99
38. Olson ME, Ceri H, Morck DW et al (2002) Biofilm bacteria: formation and comparative susceptibility to antibiotics. Can J Vet Res 66:86–92
39. Girard LP, Ceri H, Gibb AP et al (2010) MIC versus MBEC to determine the antibiotic sensitivity of staphylococcus aureus in peritoneal dialysis peritonitis. Perit Dial Int 30:652–656. https://doi.org/10.3747/pdi.2010.00010
40. Jensen ET, Kharazmi A, Lam K et al (1990) Human polymorphonuclear leukocyte response to Pseudomonas aeruginosa grown in biofilms. Infect Immun 58:2383–2385
41. Pier GB, Coleman F, Grout M et al (2001) Role of alginate O acetylation in resistance of mucoid Pseudomonas aeruginosa to opsonic phagocytosis. Infect Immun 69:1895–1901. https://doi.org/10.1128/IAI.69.3.1895-1901.2001
42. Sauer K, Camper AK, Ehrlich GD et al (2002) Pseudomonas aeruginosa displays multiple phenotypes during development as a biofilm. J Bacteriol 184:1140–1154. https://doi.org/10.1128/JB.184.4.1140-1154.2002
43. Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS (2002) Extracellular DNA required for bacterial biofilm formation. Science 295:1487–1487. https://doi.org/10.1126/science.295.5559.1487
44. Friedman L, Kolter R (2004) Genes involved in matrix formation in Pseudomonas aeruginosa PA14 biofilm. Mol Microbiol 51:675–690. https://doi.org/10.1046/j.1365-2958.2003.03877.x
45. Ma L, Jackson KD, Landry RM et al (2006) Analysis of Pseudomonas aeruginosa conditional psl variants reveals roles for the psl polysaccharide in adhesion and maintaining biofilm structure postattachment. J Bacteriol 188:8213–8221. https://doi.org/10.1128/JB.01202-06
46. Costerton JW (1999) Introduction to biofilm. Int J Antimicrob Agents 11:217–221. https://doi.org/10.1016/S0924-8579(99)00018-7
47. Stewart PS, Costerton JW (2001) Antibiotic resistance of bacteria in biofilms. Lancet 358:135–138
48. Flemming H-C, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8:623–633. https://doi.org/10.1038/nrmicro2415
49. Mann EE, Wozniak DJ (2012) Pseudomonas biofilm matrix composition and niche biology. FEMS Microbiol Rev 36:893–916. https://doi.org/10.1111/j.1574-6976.2011.00322.x
50. Beloin C, Renard S, Ghigo J-M, Lebeaux D (2014) Novel approaches to combat bacterial biofilms. Curr Opin Pharmacol 18:61–68. https://doi.org/10.1016/J.COPH.2014.09.005
51. Tandogan N, Abadian PN, Huo B, Goluch ED (2017) Characterization of bacterial adhesion and biofilm formation. In: Antimicrobial coatings and modifications on medical devices. Springer, Cham, pp 67–95
52. Moormeier DE, Bayles KW (2017) *Staphylococcus aureus* biofilm: a complex developmental organism. Mol Microbiol 104(3):365–376. https://doi.org/10.1111/mmi.13634
53. Pollitt EJG, Diggle SP (2017) Defining motility in the Staphylococci. Cell Mol Life Sci 74:2943–2958. https://doi.org/10.1007/s00018-017-2507-z
54. Henrichsen J (1972) Bacterial surface translocation: a survey and a classification. Bacteriol Rev 36:478–503
55. Rupp CJ, Fux CA, Stoodley P (2005) Viscoelasticity of Staphylococcus aureus biofilms in response to fluid shear allows resistance to detachment and facilitates rolling migration. Appl Environ Microbiol 71:2175–2178. https://doi.org/10.1128/AEM.71.4.2175-2178.2005
56. Kaito C, Sekimizu K (2007) Colony spreading in Staphylococcus aureus. J Bacteriol 189:2553–2557. https://doi.org/10.1128/JB.01635-06

57. Cheung GYC, Joo H-S, Chatterjee SS, Otto M (2014) Phenol-soluble modulins—critical determinants of staphylococcal virulence. FEMS Microbiol Rev 38:698–719. https://doi.org/10.1111/1574-6976.12057
58. Wang R, Braughton KR, Kretschmer D et al (2007) Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. Nat Med 13:1510–1514. https://doi.org/10.1038/nm1656
59. Queck SY, Jameson-Lee M, Villaruz AE et al (2008) RNAIII-independent target gene control by the agr quorum-sensing system: insight into the evolution of virulence regulation in Staphylococcus aureus. Mol Cell 32:150–158. https://doi.org/10.1016/j.molcel.2008.08.005
60. Novick RP (2003) Autoinduction and signal transduction in the regulation of staphylococcal virulence. Mol Microbiol 48:1429–1449. https://doi.org/10.1046/j.1365-2958.2003.03526.x
61. Mitchell JG, Kogure K (2006) Bacterial motility: links to the environment and a driving force for microbial physics. FEMS Microbiol Ecol 55:3–16. https://doi.org/10.1111/j.1574-6941.2005.00003.x
62. Baird-Parker AC (1990) The staphylococci: an introduction. J Appl Bacteriol 69:1S–8S. https://doi.org/10.1111/j.1365-2672.1990.tb01793.x
63. Pollitt EJG, Crusz SA, Diggle SP (2016) Staphylococcus aureus forms spreading dendrites that have characteristics of active motility. Sci Rep 5:17698. https://doi.org/10.1038/srep17698
64. Berg HC, Anderson RA (1973) Bacteria swim by rotating their flagellar filaments. Nature 245:380–382. https://doi.org/10.1038/245380a0
65. Macnab RM (2003) How bacteria assemble flagella. Annu Rev Microbiol 57:77–100. https://doi.org/10.1146/annurev.micro.57.030502.090832
66. Macnab RM, Ornston MK (1977) Normal-to-curly flagellar transitions and their role in bacterial tumbling. Stabilization of an alternative quaternary structure by mechanical force. J Mol Biol 112:1–30
67. Silverman M, Simon M (1974) Flagellar rotation and the mechanism of bacterial motility. Nature 249:73–74. https://doi.org/10.1038/249073a0
68. Turner L, Ryu WS, Berg HC (2000) Real-time imaging of fluorescent flagellar filaments. J Bacteriol 182:2793–2801. https://doi.org/10.1128/JB.182.10.2793-2801.2000
69. Berg HC (2003) The rotary motor of bacterial flagella. Annu Rev Biochem 72:19–54. https://doi.org/10.1146/annurev.biochem.72.121801.161737
70. Melville S, Craig L (2013) Type IV pili in Gram-positive bacteria. Microbiol Mol Biol Rev 77:323–341. https://doi.org/10.1128/MMBR.00063-12
71. Reichenbach H (1981) Taxonomy of the gliding bacteria. Annu Rev Microbiol 35:339–364. https://doi.org/10.1146/annurev.mi.35.100181.002011
72. McBride MJ (2001) Bacterial gliding motility: multiple mechanisms for cell movement over surfaces. Annu Rev Microbiol 55:49–75. https://doi.org/10.1146/annurev.micro.55.1.49
73. Jarrell KF, McBride MJ (2008) The surprisingly diverse ways that prokaryotes move. Nat Rev Microbiol 6:466–476. https://doi.org/10.1038/nrmicro1900
74. Nan B, McBride MJ, Chen J et al (2014) Bacteria that glide with helical tracks. Curr Biol 24:R169–R173. https://doi.org/10.1016/J.CUB.2013.12.034
75. Schweinitzer T, Josenhans C (2010) Bacterial energy taxis: a global strategy? Arch Microbiol 192:507–520. https://doi.org/10.1007/s00203-010-0575-7
76. Floyd KA, Eberly AR, Hadjifrangiskou M (2017) 3—Adhesion of bacteria to surfaces and biofilm formation on medical devices. Biofilms and Implantable Medical Devices, pp 47–95
77. Briegel A, Ortega DR, Tocheva EI et al (2009) Universal architecture of bacterial chemoreceptor arrays. Proc Natl Acad Sci U S A 106:17181–17186. https://doi.org/10.1073/pnas.0905181106
78. Porter SL, Wadhams GH, Armitage JP (2011) Signal processing in complex chemotaxis pathways. Nat Rev Microbiol 9:153–165. https://doi.org/10.1038/nrmicro2505
79. Povolotsky TL, Hengge R (2015) Genome-based comparison of cyclic Di-GMP signaling in pathogenic and commensal Escherichia coli strains. J Bacteriol 198:111–126. https://doi.org/10.1128/JB.00520-15

80. Pesavento C, Becker G, Sommerfeldt N et al (2008) Inverse regulatory coordination of motility and curli-mediated adhesion in Escherichia coli. Genes Dev 22:2434–2446. https://doi.org/10.1101/gad.475808
81. Fang X, Gomelsky M (2010) A post-translational, c-di-GMP-dependent mechanism regulating flagellar motility. Mol Microbiol 76:1295–1305. https://doi.org/10.1111/j.1365-2958.2010.07179.x
82. Marles-Wright J, Lewis RJ (2007) Stress responses of bacteria. Curr Opin Struct Biol 17:755–760. https://doi.org/10.1016/j.sbi.2007.08.004
83. Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55:165–199. https://doi.org/10.1146/annurev.micro.55.1.165
84. Henke JM, Bassler BL (2004) Bacterial social engagements. Trends Cell Biol 14:648–656. https://doi.org/10.1016/j.tcb.2004.09.012
85. Waters CM, Bassler BL (2005) Quorum sensing: cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol 21:319–346. https://doi.org/10.1146/annurev.cellbio.21.012704.131001
86. Karatan E, Watnick P (2009) Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiol Mol Biol Rev 73:310–347. https://doi.org/10.1128/MMBR.00041-08
87. Busscher HJ, van der Mei HC (2012) How do bacteria know they are on a surface and regulate their response to an adhering state? PLoS Pathog 8:e1002440. https://doi.org/10.1371/journal.ppat.1002440
88. Tuson HH, Weibel DB (2013) Bacteria-surface interactions. Soft Matter 9:4368–4380. https://doi.org/10.1039/C3SM27705D
89. Dunne WM (2002) Bacterial adhesion: Seen any good biofilms lately? Clin Microbiol Rev 15:155–166
90. Franz S, Rammelt S, Scharnweber D, Simon JC (2011) Immune responses to implants—a review of the implications for the design of immunomodulatory biomaterials. Biomaterials 32:6692–6709. https://doi.org/10.1016/J.BIOMATERIALS.2011.05.078
91. Speziale P, Pietrocola G, Rindi S et al (2009) Structural and functional role of *Staphylococcus aureus* surface components recognizing adhesive matrix molecules of the host. Future Microbiol 4:1337–1352. https://doi.org/10.2217/fmb.09.102
92. Wilson CJ, Clegg RE, Leavesley DI, Pearcy MJ (2005) Mediation of biomaterial-cell interactions by adsorbed proteins: a review. Tissue Eng 11:1–18. https://doi.org/10.1089/ten.2005.11.1
93. Banerjee I, Pangule RC, Kane RS (2011) Antifouling coatings: recent developments in the design of surfaces that prevent fouling by proteins, bacteria, and marine organisms. Adv Mater 23:690–718. https://doi.org/10.1002/adma.201001215
94. Arciola CR, Campoccia D, Speziale P et al (2012) Biofilm formation in Staphylococcus implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials. Biomaterials 33:5967–5982. https://doi.org/10.1016/j.biomaterials.2012.05.031
95. Otto M (2009) Staphylococcus epidermidis—the "accidental" pathogen. Nat Rev Microbiol 7:555–567. https://doi.org/10.1038/nrmicro2182
96. Paharik AE, Horswill AR (2016) The staphylococcal biofilm: adhesins, regulation, and host response. Microbiol Spectr 4(2). https://doi.org/10.1128/microbiolspec.VMBF-0022-2015
97. Arciola CR, Campoccia D, Montanaro L (2018) Implant infections: adhesion, biofilm formation and immune evasion. Nat Rev Microbiol 16:397–409. https://doi.org/10.1038/s41579-018-0019-y
98. Ren Y, Wang C, Chen Z et al (2018) Emergent heterogeneous microenvironments in biofilms: substratum surface heterogeneity and bacterial adhesion force-sensing. FEMS Microbiol Rev 42:259–272. https://doi.org/10.1093/femsre/fuy001
99. Costerton JW, Lewandowski Z, Caldwell DE et al (1995) Microbial biofilms. Annu Rev Microbiol 49:711–745. https://doi.org/10.1146/annurev.mi.49.100195.003431
100. Petrova OE, Sauer K (2012) Sticky situations: key components that control bacterial surface attachment. J Bacteriol 194:2413–2425. https://doi.org/10.1128/JB.00003-12

101. Bos R, van der Mei HC, Busscher HJ (1999) Physico-chemistry of initial microbial adhesive interactions—its mechanisms and methods for study. FEMS Microbiol Rev 23:179–230. https://doi.org/10.1111/j.1574-6976.1999.tb00396.x
102. Ribeiro M, Monteiro FJ, Ferraz MP (2012) Infection of orthopedic implants with emphasis on bacterial adhesion process and techniques used in studying bacterial-material interactions. Biomatter 2:176–194. https://doi.org/10.4161/biom.22905
103. Hermansson M (1999) The DLVO theory in microbial adhesion. Colloids Surf B 14:105–119. https://doi.org/10.1016/S0927-7765(99)00029-6
104. Nguyen VT, Chia TWR, Turner MS et al (2011) Quantification of acid–base interactions based on contact angle measurement allows XDLVO predictions to attachment of Campylobacter jejuni but not Salmonella. J Microbiol Methods 86:89–96. https://doi.org/10.1016/J.MIMET.2011.04.005
105. Perni S, Preedy EC, Prokopovich P (2014) Success and failure of colloidal approaches in adhesion of microorganisms to surfaces. Adv Colloid Interf Sci 206:265–274. https://doi.org/10.1016/J.CIS.2013.11.008
106. Silhavy TJ, Kahne D, Walker S (2010) The bacterial cell envelope. Cold Spring Harb Perspect Biol 2:a000414. https://doi.org/10.1101/cshperspect.a000414
107. Gannon JT, Manilal VB, Alexander M (1991) Relationship between cell surface properties and transport of bacteria through soil. Appl Environ Microbiol 57:190–193
108. An YH, Friedman RJ (1998) Concise review of mechanisms of bacterial adhesion to biomaterial surfaces. J Biomed Mater Res 43:338–348. https://doi.org/10.1002/(SICI)1097-4636(199823)43:3<338::AID-JBM16>3.0.CO;2-B
109. Dufrêne YF, Martínez-Martín D, Medalsy I et al (2013) Multiparametric imaging of biological systems by force-distance curve–based AFM. Nat Methods 10:847–854. https://doi.org/10.1038/nmeth.2602
110. Martins BM, Locke JC (2015) Microbial individuality: how single-cell heterogeneity enables population level strategies. Curr Opin Microbiol 24:104–112. https://doi.org/10.1016/J.MIB.2015.01.003
111. Kline KA, Fälker S, Dahlberg S et al (2009) Bacterial adhesins in host-microbe interactions. Cell Host Microbe 5:580–592. https://doi.org/10.1016/J.CHOM.2009.05.011
112. Mandlik A, Swierczynski A, Das A, Ton-That H (2008) Pili in Gram-positive bacteria: assembly, involvement in colonization and biofilm development. Trends Microbiol 16:33–40. https://doi.org/10.1016/j.tim.2007.10.010
113. Hori K, Matsumoto S (2010) Bacterial adhesion: from mechanism to control. Biochem Eng J 48:424–434. https://doi.org/10.1016/J.BEJ.2009.11.014
114. Heilmann C, Hussain M, Peters G, Gotz F (1997) Evidence for autolysin-mediated primary attachment of Staphylococcus epidermidis to a polystyrene surface. Mol Microbiol 24:1013–1024. https://doi.org/10.1046/j.1365-2958.1997.4101774.x
115. Hirschhausen N, Schlesier T, Schmidt MA et al (2010) A novel staphylococcal internalization mechanism involves the major autolysin Atl and heat shock cognate protein Hsc70 as host cell receptor. Cell Microbiol 12:1746–1764. https://doi.org/10.1111/j.1462-5822.2010.01506.x
116. Foster SJ (1995) Molecular characterization and functional analysis of the major autolysin of Staphylococcus aureus 8325/4. J Bacteriol 177:5723–5725
117. Bose JL, Lehman MK, Fey PD, Bayles KW (2012) Contribution of the Staphylococcus aureus Atl AM and GL murein hydrolase activities in cell division, autolysis, and biofilm formation. PLoS One 7:e42244. https://doi.org/10.1371/journal.pone.0042244
118. Palmer J, Flint S, Brooks J (2007) Bacterial cell attachment, the beginning of a biofilm. J Ind Microbiol Biotechnol 34:577–588. https://doi.org/10.1007/s10295-007-0234-4
119. Renner LD, Weibel DB (2011) Physicochemical regulation of biofilm formation. MRS Bull 36:347–355. https://doi.org/10.1557/mrs.2011.65
120. Helbig R, Günther D, Friedrichs J et al (2016) The impact of structure dimensions on initial bacterial adhesion. Biomater Sci 4:1074–1078. https://doi.org/10.1039/C6BM00078A
121. James SA, Hilal N, Wright CJ (2017) Atomic force microscopy studies of bioprocess engineering surfaces—imaging, interactions and mechanical properties mediating bacterial adhesion. Biotechnol J 12:1600698. https://doi.org/10.1002/biot.201600698

122. Hizal F, Rungraeng N, Lee J et al (2017) Nanoengineered superhydrophobic surfaces of aluminum with extremely low bacterial adhesivity. ACS Appl Mater Interfaces 9:12118–12129. https://doi.org/10.1021/acsami.7b01322
123. Bagherifard S, Hickey DJ, de Luca AC et al (2015) The influence of nanostructured features on bacterial adhesion and bone cell functions on severely shot peened 316L stainless steel. Biomaterials 73:185–197. https://doi.org/10.1016/J.BIOMATERIALS.2015.09.019
124. Ivanova EP, Truong VK, Webb HK et al (2011) Differential attraction and repulsion of Staphylococcus aureus and Pseudomonas aeruginosa on molecularly smooth titanium films. Sci Rep 1:165. https://doi.org/10.1038/srep00165
125. Geng J, Henry N (2011) Short time-scale bacterial adhesion dynamics. In: Linke D, Goldman A (eds) Bacterial adhesion. Springer, pp 315–331
126. Friedlander RS, Vlamakis H, Kim P et al (2013) Bacterial flagella explore microscale hummocks and hollows to increase adhesion. Proc Natl Acad Sci U S A 110:5624–5629. https://doi.org/10.1073/pnas.1219662110
127. Jaggessar A, Shahali H, Mathew A, Yarlagadda PKDV (2017) Bio-mimicking nano and micro-structured surface fabrication for antibacterial properties in medical implants. J Nanobiotechnol 15:64. https://doi.org/10.1186/s12951-017-0306-1
128. Bruzaud J, Tarrade J, Celia E et al (2017) The design of superhydrophobic stainless steel surfaces by controlling nanostructures: a key parameter to reduce the implantation of pathogenic bacteria. Mater Sci Eng C 73:40–47. https://doi.org/10.1016/J.MSEC.2016.11.115
129. Ellison C, Brun YV (2015) Mechanosensing: a regulation sensation. Curr Biol 25:R113–R115. https://doi.org/10.1016/j.cub.2014.12.026
130. Persat A (2017) Bacterial mechanotransduction. Curr Opin Microbiol 36:1–6. https://doi.org/10.1016/J.MIB.2016.12.002
131. Xia G, Kohler T, Peschel A (2010) The wall teichoic acid and lipoteichoic acid polymers of Staphylococcus aureus. Int J Med Microbiol 300:148–154. https://doi.org/10.1016/J.IJMM.2009.10.001
132. Fedtke I, Mader D, Kohler T et al (2007) A Staphylococcus aureus ypfP mutant with strongly reduced lipoteichoic acid (LTA) content: LTA governs bacterial surface properties and autolysin activity. Mol Microbiol 65:1078–1091. https://doi.org/10.1111/j.1365-2958.2007.05854.x
133. Sabaté Brescó M, Harris LG, Thompson K et al (2017) Pathogenic mechanisms and host interactions in staphylococcus epidermidis device-related infection. Front Microbiol 8:1401. https://doi.org/10.3389/fmicb.2017.01401
134. Gotz F (2002) Staphylococcus and biofilms. Mol Microbiol 43:1367–1378. https://doi.org/10.1046/j.1365-2958.2002.02827.x
135. Arciola CR, Campoccia D, Ravaioli S, Montanaro L (2015) Polysaccharide intercellular adhesin in biofilm: structural and regulatory aspects. Front Cell Infect Microbiol 5:7. https://doi.org/10.3389/fcimb.2015.00007
136. Vuong C, Voyich JM, Fischer ER et al (2004) Polysaccharide intercellular adhesin (PIA) protects Staphylococcus epidermidis against major components of the human innate immune system. Cell Microbiol 6:269–275
137. Arciola CR, Campoccia D, Gamberini S et al (2005) Antibiotic resistance in exopolysaccharide-forming Staphylococcus epidermidis clinical isolates from orthopaedic implant infections. Biomaterials 26:6530–6535. https://doi.org/10.1016/J.BIOMATERIALS.2005.04.031
138. Vuong C, Kidder JB, Jacobson ER et al (2005) Staphylococcus epidermidis polysaccharide intercellular adhesin production significantly increases during tricarboxylic acid cycle stress. J Bacteriol 187:2967–2973. https://doi.org/10.1128/JB.187.9.2967-2973.2005
139. Knobloch JK, Bartscht K, Sabottke A et al (2001) Biofilm formation by Staphylococcus epidermidis depends on functional RsbU, an activator of the sigB operon: differential activation mechanisms due to ethanol and salt stress. J Bacteriol 183:2624–2633. https://doi.org/10.1128/JB.183.8.2624-2633.2001
140. Ferreira A, Gray M, Wiedmann M, Boor KJ (2004) Comparative genomic analysis of the sigB operon in Listeria monocytogenes and in other Gram-positive bacteria. Curr Microbiol 48:39–46

141. Schaeffer CR, Hoang T-MN, Sudbeck CM et al (2016) Versatility of biofilm matrix molecules in *Staphylococcus epidermidis* clinical isolates and importance of polysaccharide intercellular adhesin expression during high shear stress. mSphere 1(5). https://doi.org/10.1128/mSphere.00165-16
142. Ziebuhr W, Krimmer V, Rachid S et al (1999) A novel mechanism of phase variation of virulence in Staphylococcus epidermidis: evidence for control of the polysaccharide intercellular adhesin synthesis by alternating insertion and excision of the insertion sequence element IS256. Mol Microbiol 32:345–356. https://doi.org/10.1046/j.1365-2958.1999.01353.x
143. Kozitskaya S, Cho S-H, Dietrich K et al (2004) The bacterial insertion sequence element IS256 occurs preferentially in nosocomial Staphylococcus epidermidis isolates: association with biofilm formation and resistance to aminoglycosides. Infect Immun 72:1210–1215. https://doi.org/10.1128/IAI.72.2.1210-1215.2004
144. Patti JM, Allen BL, McGavin MJ, Hook M (1994) MSCRAMM-mediated adherence of microorganisms to host tissues. Annu Rev Microbiol 48:585–617. https://doi.org/10.1146/annurev.mi.48.100194.003101
145. Chavakis T, Wiechmann K, Preissner KT, Herrmann M (2005) Staphylococcus aureus interactions with the endothelium. The role of bacterial "Secretable Expanded Repertoire Adhesive Molecules" (SERAM) in disturbing host defense systems. Thromb Haemost 94:278–285. https://doi.org/10.1160/TH05-05-0306
146. Foster TJ, Geoghegan JA, Ganesh VK, Höök M (2014) Adhesion, invasion and evasion: the many functions of the surface proteins of Staphylococcus aureus. Nat Rev Microbiol 12:49–62. https://doi.org/10.1038/nrmicro3161
147. O'Neill E, Pozzi C, Houston P et al (2008) A novel Staphylococcus aureus biofilm phenotype mediated by the fibronectin-binding proteins, FnBPA and FnBPB. J Bacteriol 190:3835–3850. https://doi.org/10.1128/JB.00167-08
148. Geoghegan JA, Monk IR, O'Gara JP, Foster TJ (2013) Subdomains N2N3 of fibronectin binding protein A mediate Staphylococcus aureus biofilm formation and adherence to fibrinogen using distinct mechanisms. J Bacteriol 195:2675–2683. https://doi.org/10.1128/JB.02128-12
149. Lauderdale KJ, Boles BR, Cheung AL, Horswill AR (2009) Interconnections between Sigma B, agr, and proteolytic activity in Staphylococcus aureus biofilm maturation. Infect Immun 77:1623–1635. https://doi.org/10.1128/IAI.01036-08
150. Campoccia D, Speziale P, Ravaioli S et al (2009) The presence of both bone sialoprotein-binding protein gene and collagen adhesin gene as a typical virulence trait of the major epidemic cluster in isolates from orthopedic implant infections. Biomaterials 30:6621–6628. https://doi.org/10.1016/J.BIOMATERIALS.2009.08.032
151. Mao Y, Schwarzbauer JE (2005) Fibronectin fibrillogenesis, a cell-mediated matrix assembly process. Matrix Biol 24:389–399. https://doi.org/10.1016/J.MATBIO.2005.06.008
152. Arciola CR, Bustanji Y, Conti M et al (2003) Staphylococcus epidermidis–fibronectin binding and its inhibition by heparin. Biomaterials 24:3013–3019. https://doi.org/10.1016/S0142-9612(03)00133-9
153. Arciola CR, Campoccia D, Gamberini S et al (2004) Presence of fibrinogen-binding adhesin gene in Staphylococcus epidermidis isolates from central venous catheters-associated and orthopaedic implant-associated infections. Biomaterials 25:4825–4829. https://doi.org/10.1016/J.BIOMATERIALS.2003.11.056
154. Geoghegan JA, Foster TJ (2015) Cell wall-anchored surface proteins of Staphylococcus aureus: many proteins, multiple functions. In: Staphylococcus aureus. Springer, Cham, pp 95–120
155. Foster TJ (2016) The remarkably multifunctional fibronectin binding proteins of Staphylococcus aureus. Eur J Clin Microbiol Infect Dis 35:1923–1931. https://doi.org/10.1007/s10096-016-2763-0
156. Vengadesan K, Narayana SVL (2011) Structural biology of Gram-positive bacterial adhesins. Protein Sci 20:759–772. https://doi.org/10.1002/pro.613
157. Clarke SR, Foster SJ (2006) Surface adhesins of Staphylococcus aureus. Adv Microb Physiol 51:187–224

158. Patti JM, Bremell T, Krajewska-Pietrasik D et al (1994) The Staphylococcus aureus collagen adhesin is a virulence determinant in experimental septic arthritis. Infect Immun 62:152–161
159. Ní Eidhin D, Perkins S, Francois P et al (1998) Clumping factor B (ClfB), a new surface-located fibrinogen-binding adhesin of *Staphylococcus aureus*. Mol Microbiol 30:245–257. https://doi.org/10.1046/j.1365-2958.1998.01050.x
160. Herman-Bausier P, Formosa-Dague C, Feuillie C et al (2017) Forces guiding staphylococcal adhesion. J Struct Biol 197:65–69. https://doi.org/10.1016/J.JSB.2015.12.009
161. Farnsworth CW, Schott EM, Jensen SE et al (2017) Adaptive upregulation of Clumping Factor A (ClfA) by Staphylococcus aureus in the obese, type 2 diabetic host mediates increased virulence. Infect Immun 85(6):e01005-16. https://doi.org/10.1128/IAI.01005-16
162. Wang Y, Cheng LI, Helfer DR et al (2017) Mouse model of hematogenous implant-related Staphylococcus aureus biofilm infection reveals therapeutic targets. Proc Natl Acad Sci U S A 114:E5094–E5102. https://doi.org/10.1073/pnas.1703427114
163. Wertheim HFL, Walsh E, Choudhurry R et al (2008) Key role for clumping factor B in Staphylococcus aureus nasal colonization of humans. PLoS Med 5:e17. https://doi.org/10.1371/journal.pmed.0050017
164. Hammer ND, Skaar EP (2011) Molecular mechanisms of Staphylococcus aureus iron acquisition. Annu Rev Microbiol 65:129–147. https://doi.org/10.1146/annurev-micro-090110-102851
165. Clarke SR, Mohamed R, Bian L et al (2007) The Staphylococcus aureus surface protein IsdA mediates resistance to innate defenses of human skin. Cell Host Microbe 1:199–212. https://doi.org/10.1016/J.CHOM.2007.04.005
166. Zapotoczna M, Jevnikar Z, Miajlovic H et al (2013) Iron-regulated surface determinant B (IsdB) promotes *Staphylococcus aureus* adherence to and internalization by non-phagocytic human cells. Cell Microbiol 15:1026–1041. https://doi.org/10.1111/cmi.12097
167. Missineo A, Di Poto A, Geoghegan JA et al (2014) IsdC from Staphylococcus lugdunensis induces biofilm formation under low-iron growth conditions. Infect Immun 82:2448–2459. https://doi.org/10.1128/IAI.01542-14
168. Shopsin B, Gomez M, Montgomery SO et al (1999) Evaluation of protein A gene polymorphic region DNA sequencing for typing of Staphylococcus aureus strains. J Clin Microbiol 37:3556–3563
169. Muthukrishnan G, Quinn GA, Lamers RP et al (2011) Exoproteome of *Staphylococcus aureus* reveals putative determinants of nasal carriage. J Proteome Res 10:2064–2078. https://doi.org/10.1021/pr200029r
170. Cole AL, Muthukrishnan G, Chong C et al (2016) Host innate inflammatory factors and staphylococcal protein A influence the duration of human Staphylococcus aureus nasal carriage. Mucosal Immunol 9:1537–1548. https://doi.org/10.1038/mi.2016.2
171. Gómez MI, Lee A, Reddy B et al (2004) Staphylococcus aureus protein A induces airway epithelial inflammatory responses by activating TNFR1. Nat Med 10:842–848. https://doi.org/10.1038/nm1079
172. Silverman GJ, Cary S, Graille M et al (2000) A B-cell superantigen that targets B-1 lymphocytes. Curr Top Microbiol Immunol 252:251–263
173. Graille M, Stura EA, Corper AL et al (2000) Crystal structure of a Staphylococcus aureus protein A domain complexed with the Fab fragment of a human IgM antibody: structural basis for recognition of B-cell receptors and superantigen activity. Proc Natl Acad Sci U S A 97:5399–5404
174. Ricklin D, Tzekou A, Garcia BL et al (2009) A molecular insight into complement evasion by the staphylococcal complement inhibitor protein family. J Immunol 183:2565–2574. https://doi.org/10.4049/jimmunol.0901443
175. Merino N, Toledo-Arana A, Vergara-Irigaray M et al (2009) Protein A-mediated multicellular behavior in Staphylococcus aureus. J Bacteriol 191:832–843. https://doi.org/10.1128/JB.01222-08
176. Mendoza Bertelli A, Delpino MV, Lattar S et al (2016) Staphylococcus aureus protein A enhances osteoclastogenesis via TNFR1 and EGFR signaling. Biochim Biophys Acta 1862:1975–1983. https://doi.org/10.1016/J.BBADIS.2016.07.016

177. Claro T, Widaa A, McDonnell C et al (2013) Staphylococcus aureus protein A binding to osteoblast tumour necrosis factor receptor 1 results in activation of nuclear factor kappa B and release of interleukin-6 in bone infection. Microbiology 159:147–154. https://doi.org/10.1099/mic.0.063016-0
178. Claro T, Widaa A, O'Seaghdha M et al (2011) Staphylococcus aureus protein A binds to osteoblasts and triggers signals that weaken bone in osteomyelitis. PLoS One 6:e18748. https://doi.org/10.1371/journal.pone.0018748
179. Widaa A, Claro T, Foster TJ et al (2012) Staphylococcus aureus protein A plays a critical role in mediating bone destruction and bone loss in osteomyelitis. PLoS One 7:e40586. https://doi.org/10.1371/journal.pone.0040586
180. Flemming HC, Wingender J, Szewzyk U et al (2016) Biofilms: an emergent form of bacterial life. Nat Rev Microbiol 14(9):563–575. https://doi.org/10.1038/nrmicro.2016.94
181. Formosa-Dague C, Feuillie C, Beaussart A et al (2016) Sticky matrix: adhesion mechanism of the staphylococcal polysaccharide intercellular adhesin. ACS Nano 10:3443–3452. https://doi.org/10.1021/acsnano.5b07515
182. Petersen FC, Tao L, Scheie AA (2005) DNA binding-uptake system: a link between cell-to-cell communication and biofilm formation. J Bacteriol 187:4392–4400. https://doi.org/10.1128/JB.187.13.4392-4400.2005
183. Qin Z, Ou Y, Yang L et al (2007) Role of autolysin-mediated DNA release in biofilm formation of Staphylococcus epidermidis. Microbiology 153:2083–2092. https://doi.org/10.1099/mic.0.2007/006031-0
184. Steinberger RE, Holden PA (2005) Extracellular DNA in single- and multiple-species unsaturated biofilms. Appl Environ Microbiol 71:5404–5410. https://doi.org/10.1128/AEM.71.9.5404-5410.2005
185. Vilain S, Pretorius JM, Theron J, Brözel VS (2009) DNA as an adhesin: bacillus cereus requires extracellular DNA to form biofilms. Appl Environ Microbiol 75:2861–2868. https://doi.org/10.1128/AEM.01317-08
186. Das T, Sharma PK, Busscher HJ et al (2010) Role of extracellular DNA in initial bacterial adhesion and surface aggregation. Appl Environ Microbiol 76:3405–3408. https://doi.org/10.1128/AEM.03119-09
187. Okshevsky M, Meyer RL (2015) The role of extracellular DNA in the establishment, maintenance and perpetuation of bacterial biofilms. Crit Rev Microbiol 41:341–352. https://doi.org/10.3109/1040841X.2013.841639
188. Absolom DR, Lamberti FV, Policova Z et al (1983) Surface thermodynamics of bacterial adhesion. Appl Environ Microbiol 46:90–97
189. Dickson JS, Koohmaraie M (1989) Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. Appl Environ Microbiol 55:832–836
190. van Loosdrecht MC, Lyklema J, Norde W et al (1987) Electrophoretic mobility and hydrophobicity as a measured to predict the initial steps of bacterial adhesion. Appl Environ Microbiol 53:1898–1901
191. Arnold JW, Bailey GW (2000) Surface finishes on stainless steel reduce bacterial attachment and early biofilm formation: scanning electron and atomic force microscopy study. Poult Sci 79:1839–1845
192. Das T, Sharma PK, Krom BP et al (2011) Role of eDNA on the adhesion forces between *Streptococcus mutans* and substratum surfaces: influence of ionic strength and substratum hydrophobicity. Langmuir 27:10113–10118. https://doi.org/10.1021/la202013m
193. Petrova OE, Sauer K (2016) Escaping the biofilm in more than one way: desorption, detachment or dispersion. Curr Opin Microbiol 30:67–78. https://doi.org/10.1016/j.mib.2016.01.004
194. Berne C, Ducret A, Hardy GG, Brun YV (2015) Adhesins involved in attachment to abiotic surfaces by gram-negative bacteria. Microbiol Spectr 3(4). https://doi.org/10.1128/microbiolspec.MB-0018-2015
195. Lee CK, de Anda J, Baker AE et al (2018) Multigenerational memory and adaptive adhesion in early bacterial biofilm communities. Proc Natl Acad Sci U S A 115:4471–4476. https://doi.org/10.1073/pnas.1720071115

196. Klausen M, Heydorn A, Ragas P et al (2003) Biofilm formation by Pseudomonas aeruginosa wild type, flagella and type IV pili mutants. Mol Microbiol 48:1511–1524
197. Houry A, Briandet R, Aymerich S, Gohar M (2010) Involvement of motility and flagella in Bacillus cereus biofilm formation. Microbiology 156:1009–1018. https://doi.org/10.1099/mic.0.034827-0
198. Hall-Stoodley L, Stoodley P (2002) Developmental regulation of microbial biofilms. Curr Opin Biotechnol 13:228–233
199. Kjelleberg S, Molin S (2002) Is there a role for quorum sensing signals in bacterial biofilms? Curr Opin Microbiol 5:254–258
200. Davies DG, Parsek MR, Pearson JP et al (1998) The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science 280:295–298. https://doi.org/10.1126/SCIENCE.280.5361.295
201. Heydorn A, Ersbøll B, Kato J et al (2002) Statistical analysis of Pseudomonas aeruginosa biofilm development: impact of mutations in genes involved in twitching motility, cell-to-cell signaling, and stationary-phase sigma factor expression. Appl Environ Microbiol 68:2008–2017. https://doi.org/10.1128/AEM.68.4.2008-2017.2002
202. Purevdorj B, Costerton JW, Stoodley P (2002) Influence of hydrodynamics and cell signaling on the structure and behavior of Pseudomonas aeruginosa biofilms. Appl Environ Microbiol 68:4457–4464. https://doi.org/10.1128/AEM.68.9.4457-4464.2002
203. Stoodley P, Jørgensen F, Williams P, Lappin-Scott HM (1999) Biofilms: the good, the bad, and the ugly. BioLine, Cardiff, UK
204. Gristina AG, Naylor P, Myrvik Q (1988) Infections from biomaterials and implants: a race for the surface. Med Prog Technol 14:205–224
205. Subbiahdoss G, Kuijer R, Grijpma DW et al (2009) Microbial biofilm growth vs. tissue integration: "the race for the surface" experimentally studied. Acta Biomater 5:1399–1404. https://doi.org/10.1016/J.ACTBIO.2008.12.011
206. Stones DH, Krachler AM (2016) Against the tide: the role of bacterial adhesion in host colonization. Biochem Soc Trans 44:1571–1580. https://doi.org/10.1042/BST20160186
207. Gristina AG (1987) Biomaterial-centered infection: microbial adhesion versus tissue integration. Science 237:1588–1595. https://doi.org/10.1126/science.3629258
208. Roberts AEL, Kragh KN, Bjarnsholt T, Diggle SP (2015) The limitations of in vitro experimentation in understanding biofilms and chronic infection. J Mol Biol 427:3646–3661. https://doi.org/10.1016/j.jmb.2015.09.002
209. Tolker-Nielsen T (2015) Biofilm development. Microbiol Spectr 3(2):MB-0001-2014. https://doi.org/10.1128/microbiolspec.MB-0001-2014
210. Redfield RJ (2002) Is quorum sensing a side effect of diffusion sensing? Trends Microbiol 10:365–370. https://doi.org/10.1016/S0966-842X(02)02400-9
211. Hobley L, Harkins C, MacPhee CE, Stanley-Wall NR (2015) Giving structure to the biofilm matrix: an overview of individual strategies and emerging common themes. FEMS Microbiol Rev 39:649–669. https://doi.org/10.1093/femsre/fuv015
212. Xavier JB, Foster KR (2007) Cooperation and conflict in microbial biofilms. Proc Natl Acad Sci U S A 104:876–881. https://doi.org/10.1073/pnas.0607651104
213. Boles BR, Thoendel M, Roth AJ, Horswill AR (2010) Identification of genes involved in polysaccharide-independent Staphylococcus aureus biofilm formation. PLoS One 5:e10146. https://doi.org/10.1371/journal.pone.0010146
214. Moormeier DE, Endres JL, Mann EE et al (2013) Use of microfluidic technology to analyze gene expression during Staphylococcus aureus biofilm formation reveals distinct physiological niches. Appl Environ Microbiol 79:3413–3424. https://doi.org/10.1128/AEM.00395-13
215. Landini P (2009) Cross-talk mechanisms in biofilm formation and responses to environmental and physiological stress in Escherichia coli. Res Microbiol 160:259–266. https://doi.org/10.1016/J.RESMIC.2009.03.001
216. Beenken KE, Spencer H, Griffin LM, Smeltzer MS (2012) Impact of extracellular nuclease production on the biofilm phenotype of Staphylococcus aureus under in vitro and in vivo conditions. Infect Immun 80:1634–1638. https://doi.org/10.1128/IAI.06134-11

217. Periasamy S, Joo H-S, Duong AC et al (2012) How Staphylococcus aureus biofilms develop their characteristic structure. Proc Natl Acad Sci 109(4):1281–1286. https://doi.org/10.1073/pnas.1115006109
218. Huseby MJ, Kruse AC, Digre J et al (2010) Beta toxin catalyzes formation of nucleoprotein matrix in staphylococcal biofilms. Proc Natl Acad Sci U S A 107:14407–14412. https://doi.org/10.1073/pnas.0911032107
219. Pavlovsky L, Younger JG, Solomon MJ (2013) In situ rheology of Staphylococcus epidermidis bacterial biofilms. Soft Matter 9:122–131. https://doi.org/10.1039/C2SM27005F
220. Ganesan M, Stewart EJ, Szafranski J et al (2013) Molar mass, entanglement, and associations of the biofilm polysaccharide of Staphylococcus epidermidis. Biomacromolecules 14:1474–1481. https://doi.org/10.1021/bm400149a
221. Steinberg N, Kolodkin-Gal I (2015) The matrix reloaded: probing the extracellular matrix synchronizes bacterial communities. J Bacteriol 197:2092–2103. https://doi.org/10.1128/JB.02516-14
222. Zogaj X, Nimtz M, Rohde M et al (2001) The multicellular morphotypes of Salmonella typhimurium and Escherichia coli produce cellulose as the second component of the extracellular matrix. Mol Microbiol 39:1452–1463
223. Hung C, Zhou Y, Pinkner JS et al (2013) Escherichia coli biofilms have an organized and complex extracellular matrix structure. MBio 4:e00645–e00613. https://doi.org/10.1128/mBio.00645-13
224. McCrate OA, Zhou X, Reichhardt C, Cegelski L (2013) Sum of the parts: composition and architecture of the bacterial extracellular matrix. J Mol Biol 425:4286–4294. https://doi.org/10.1016/j.jmb.2013.06.022
225. Sutherland IW (2001) Biofilm exopolysaccharides: a strong and sticky framework. Microbiology 147:3–9. https://doi.org/10.1099/00221287-147-1-3
226. Branda SS, Vik A, Friedman L, Kolter R (2005) Biofilms: the matrix revisited. Trends Microbiol 13:20–26. https://doi.org/10.1016/j.tim.2004.11.006
227. Izano EA, Amarante MA, Kher WB, Kaplan JB (2008) Differential roles of poly-N-acetylglucosamine surface polysaccharide and extracellular DNA in Staphylococcus aureus and Staphylococcus epidermidis biofilms. Appl Environ Microbiol 74:470–476. https://doi.org/10.1128/AEM.02073-07
228. Tetz GV, Artemenko NK, Tetz VV (2009) Effect of DNase and antibiotics on biofilm characteristics. Antimicrob Agents Chemother 53:1204–1209. https://doi.org/10.1128/AAC.00471-08
229. Koo H, Xiao J, Klein MI, Jeon JG (2010) Exopolysaccharides produced by Streptococcus mutans glucosyltransferases modulate the establishment of microcolonies within multispecies biofilms. J Bacteriol 192:3024–3032. https://doi.org/10.1128/JB.01649-09
230. Bokranz W, Wang X, Tschäpe H, Römling U (2005) Expression of cellulose and curli fimbriae by Escherichia coli isolated from the gastrointestinal tract. J Med Microbiol 54:1171–1182. https://doi.org/10.1099/jmm.0.46064-0
231. Thomas VC, Hiromasa Y, Harms N et al (2009) A fratricidal mechanism is responsible for eDNA release and contributes to biofilm development of Enterococcus faecalis. Mol Microbiol 72:1022–1036. https://doi.org/10.1111/j.1365-2958.2009.06703.x
232. Rice KC, Mann EE, Endres JL et al (2007) The cidA murein hydrolase regulator contributes to DNA release and biofilm development in Staphylococcus aureus. Proc Natl Acad Sci U S A 104:8113–8118. https://doi.org/10.1073/pnas.0610226104
233. Foulston L, Elsholz AKW, DeFrancesco AS, Losick R (2014) The extracellular matrix of Staphylococcus aureus biofilms comprises cytoplasmic proteins that associate with the cell surface in response to decreasing pH. MBio 5:e01667–e01614. https://doi.org/10.1128/mBio.01667-14
234. Schwartz K, Syed AK, Stephenson RE et al (2012) Functional amyloids composed of phenol soluble modulins stabilize Staphylococcus aureus biofilms. PLoS Pathog 8:e1002744. https://doi.org/10.1371/journal.ppat.1002744

235. Roberson EB, Firestone MK (1992) Relationship between desiccation and exopolysaccharide production in a soil Pseudomonas sp. Appl Environ Microbiol 58:1284–1291
236. van Hullebusch ED, Zandvoort MH, Lens PNL (2003) Metal immobilisation by biofilms: mechanisms and analytical tools. Rev Environ Sci Bio/Technol 2:9–33. https://doi.org/10.1023/B:RESB.0000022995.48330.55
237. Wuertz S, Spaeth R, Hinderberger A et al (2001) A new method for extraction of extracellular polymeric substances from biofilms and activated sludge suitable for direct quantification of sorbed metals. Water Sci Technol 43:25–31
238. Frølund B, Palmgren R, Keiding K, Nielsen PH (1996) Extraction of extracellular polymers from activated sludge using a cation exchange resin. Water Res 30:1749–1758. https://doi.org/10.1016/0043-1354(95)00323-1
239. Wingender J, Strathmann M, Rode A et al (2001) Isolation and biochemical characterization of extracellular polymeric substances from Pseudomonas aeruginosa. Methods Enzymol 336:302–314. https://doi.org/10.1016/S0076-6879(01)36597-7
240. Maira-Litrán T, Kropec A, Abeygunawardana C et al (2002) Immunochemical properties of the staphylococcal poly-N-acetylglucosamine surface polysaccharide. Infect Immun 70:4433–4440. https://doi.org/10.1128/IAI.70.8.4433-4440.2002
241. Gökçen A, Vilcinskas A, Wiesner J (2013) Methods to identify enzymes that degrade the main extracellular polysaccharide component of Staphylococcus epidermidis biofilms. Virulence 4:260. https://doi.org/10.4161/VIRU.23560
242. Lin MH, Shu JC, Lin LP et al (2015) Elucidating the crucial role of poly N-acetylglucosamine from Staphylococcus aureus in cellular adhesion and pathogenesis. PLoS One 10:e0124216. https://doi.org/10.1371/journal.pone.0124216
243. Cerca F, França Â, Guimarães R et al (2011) Modulation of poly-N-acetylglucosamine accumulation within mature Staphylococcus epidermidis biofilms grown in excess glucose. Microbiol Immunol 55:673–682. https://doi.org/10.1111/j.1348-0421.2011.00368.x
244. Lasa I, Penadés JR (2006) Bap: a family of surface proteins involved in biofilm formation. Res Microbiol 157:99–107. https://doi.org/10.1016/J.RESMIC.2005.11.003
245. Schwartz K, Boles BR (2013) Microbial amyloids—functions and interactions within the host. Curr Opin Microbiol 16:93–99. https://doi.org/10.1016/j.mib.2012.12.001
246. DePas WH, Chapman MR (2012) Microbial manipulation of the amyloid fold. Res Microbiol 163:592–606. https://doi.org/10.1016/j.resmic.2012.10.009
247. Shewmaker F, McGlinchey RP, Wickner RB (2011) Structural insights into functional and pathological amyloid. J Biol Chem 286:16533–16540. https://doi.org/10.1074/jbc.R111.227108
248. Chapman MR, Robinson LS, Pinkner JS et al (2002) Role of Escherichia coli curli operons in directing amyloid fiber formation. Science 295:851–855. https://doi.org/10.1126/science.1067484
249. Dueholm MS, Petersen SV, Sønderkaer M et al (2010) Functional amyloid in Pseudomonas. Mol Microbiol 77(4):1009–1020. https://doi.org/10.1111/j.1365-2958.2010.07269.x
250. Bieler S, Estrada L, Lagos R et al (2005) Amyloid formation modulates the biological activity of a bacterial protein. J Biol Chem 280:26880–26885. https://doi.org/10.1074/jbc.M502031200
251. Oli MW, Otoo HN, Crowley PJ et al (2012) Functional amyloid formation by Streptococcus mutans. Microbiology 158:2903–2916. https://doi.org/10.1099/mic.0.060855-0
252. Alteri CJ, Xicohténcatl-Cortes J, Hess S et al (2007) Mycobacterium tuberculosis produces pili during human infection. Proc Natl Acad Sci U S A 104:5145–5150. https://doi.org/10.1073/pnas.0602304104
253. Schwartz K, Sekedat MD, Syed AK et al (2014) The AgrD N-terminal leader peptide of Staphylococcus aureus has cytolytic and amyloidogenic properties. Infect Immun 82:3837–3844. https://doi.org/10.1128/IAI.02111-14
254. Otzen D, Nielsen PH (2008) We find them here, we find them there: Functional bacterial amyloid. Cell Mol Life Sci 65:910–927. https://doi.org/10.1007/s00018-007-7404-4

255. Wang R, Khan BA, Cheung GYC et al (2011) Staphylococcus epidermidis surfactant peptides promote biofilm maturation and dissemination of biofilm-associated infection in mice. J Clin Invest 121:238–248. https://doi.org/10.1172/JCI42520
256. Tsompanidou E, Sibbald MJJB, Chlebowicz MA et al (2011) Requirement of the agr locus for colony spreading of Staphylococcus aureus. J Bacteriol 193:1267–1272. https://doi.org/10.1128/JB.01276-10
257. Schwartz K, Ganesan M, Payne DE et al (2016) Extracellular DNA facilitates the formation of functional amyloids in Staphylococcus aureus biofilms. Mol Microbiol 99:123–134. https://doi.org/10.1111/mmi.13219
258. Wingender J, Neu TR, Flemming H-C (1999) Microbial extracellular polymeric substances: characterization, structure, and function. Springer, Heidelberg
259. Montanaro L, Poggi A, Visai L et al (2011) Extracellular DNA in Biofilms. Int J Artif Organs 34:824–831. https://doi.org/10.5301/ijao.5000051
260. Thurlow LR, Hanke ML, Fritz T et al (2011) Staphylococcus aureus biofilms prevent macrophage phagocytosis and attenuate inflammation in vivo. J Immunol 186:6585–6596. https://doi.org/10.4049/jimmunol.1002794
261. Vorkapic D, Pressler K, Schild S (2016) Multifaceted roles of extracellular DNA in bacterial physiology. Curr Genet 62(1):71–79
262. Chiang W-C, Nilsson M, Jensen PØ et al (2013) Extracellular DNA shields against aminoglycosides in Pseudomonas aeruginosa biofilms. Antimicrob Agents Chemother 57:2352–2361. https://doi.org/10.1128/AAC.00001-13
263. Gloag ES, Turnbull L, Huang A et al (2013) Self-organization of bacterial biofilms is facilitated by extracellular DNA. Proc Natl Acad Sci U S A 110:11541–11546. https://doi.org/10.1073/pnas.1218898110
264. Thomas VC, Hancock LE (2009) Suicide and fratricide in bacterial biofilms. Int J Artif Organs 32:537–544
265. Nedelcu AM, Driscoll WW, Durand PM et al (2011) On the paradigm of alturistic suicide in the unicellular world. Evolution (N Y) 65:3–20. https://doi.org/10.1111/j.1558-5646.2010.01103.x
266. Molin S, Tolker-Nielsen T (2003) Gene transfer occurs with enhanced efficiency in biofilms and induces enhanced stabilisation of the biofilm structure. Curr Opin Biotechnol 14:255–261. https://doi.org/10.1016/S0958-1669(03)00036-3
267. Biswas R, Voggu L, Simon UK et al (2006) Activity of the major staphylococcal autolysin Atl. FEMS Microbiol Lett 259:260–268. https://doi.org/10.1111/j.1574-6968.2006.00281.x
268. Oshida T, Sugai M, Komatsuzawa H et al (1995) A Staphylococcus aureus autolysin that has an N-acetylmuramoyl-L-alanine amidase domain and an endo-beta-N-acetylglucosaminidase domain: cloning, sequence analysis, and characterization. Proc Natl Acad Sci U S A 92:285–289
269. Mann EE, Rice KC, Boles BR et al (2009) Modulation of eDNA release and degradation affects Staphylococcus aureus biofilm maturation. PLoS One 4:e5822. https://doi.org/10.1371/journal.pone.0005822
270. Webb JS, Thompson LS, James S et al (2003) Cell death in Pseudomonas aeruginosa biofilm development. J Bacteriol 185:4585–4592. https://doi.org/10.1128/JB.185.15.4585-4592.2003
271. Stoodley P, Debeer D, Lewandowski Z (1994) Liquid flow in biofilm systems. Appl Environ Microbiol 60:2711–2716
272. Conrad A, Kontro M, Keinänen MM et al (2003) Fatty acids of lipid fractions in extracellular polymeric substances of activated sludge flocs. Lipids 38:1093–1105. https://doi.org/10.1007/s11745-006-1165-y
273. Matsuyama T, Nakagawa Y (1996) Surface-active exolipids: analysis of absolute chemical structures and biological functions. J Microbiol Methods 25:165–175. https://doi.org/10.1016/0167-7012(95)00109-3
274. Sand W, Gehrke T (2006) Extracellular polymeric substances mediate bioleaching/biocorrosion via interfacial processes involving iron(III) ions and acidophilic bacteria. Res Microbiol 157:49–56. https://doi.org/10.1016/J.RESMIC.2005.07.012

275. Ron EZ, Rosenberg E (2001) Natural roles of biosurfactants. minireview. Environ Microbiol 3:229–236. https://doi.org/10.1046/j.1462-2920.2001.00190.x
276. Boles BR, Thoendel M, Singh PK (2004) Self-generated diversity produces "insurance effects" in biofilm communities. Proc Natl Acad Sci 101(47):16630–16635. https://doi.org/10.1073/pnas.0407460101
277. Peterson BW, He Y, Ren Y et al (2015) Viscoelasticity of biofilms and their recalcitrance to mechanical and chemical challenges. FEMS Microbiol Rev 39:234–245. https://doi.org/10.1093/femsre/fuu008
278. Körstgens V, Flemming HC, Wingender J, Borchard W (2001) Influence of calcium ions on the mechanical properties of a model biofilm of mucoid Pseudomonas aeruginosa. Water Sci Technol 43:49–57
279. Hohne DN, Younger JG, Solomon MJ (2009) Flexible microfluidic device for mechanical property characterization of soft viscoelastic solids such as bacterial biofilms. Langmuir 25:7743–7751. https://doi.org/10.1021/la803413x
280. Shaw T, Winston M, Rupp CJ et al (2004) Commonality of elastic relaxation times in biofilms. Phys Rev Lett 93:098102. https://doi.org/10.1103/PhysRevLett.93.098102
281. Høiby N, Ciofu O, Johansen HK et al (2011) The clinical impact of bacterial biofilms. Int J Oral Sci 3:55–65. https://doi.org/10.4248/IJOS11026
282. Patel JD, Colton E, Ebert M, Anderson JM (2012) Gene expression during *S. epidermidis* biofilm formation on biomaterials. J Biomed Mater Res Part A 100A:2863–2869. https://doi.org/10.1002/jbm.a.34221
283. Vandecasteele SJ, Peetermans WE, Merckx R, Eldere JV (2003) Expression of biofilm-associated genes in *Staphylococcus epidermidis* during in vitro and in vivo foreign body infections. J Infect Dis 188:730–737. https://doi.org/10.1086/377452
284. Rohde H, Burdelski C, Bartscht K et al (2005) Induction of *Staphylococcus epidermidis* biofilm formation via proteolytic processing of the accumulation-associated protein by staphylococcal and host proteases. Mol Microbiol 55:1883–1895. https://doi.org/10.1111/j.1365-2958.2005.04515.x
285. Hussain M, Herrmann M, von Eiff C et al (1997) A 140-kilodalton extracellular protein is essential for the accumulation of Staphylococcus epidermidis strains on surfaces. Infect Immun 65:519–524
286. Boles BR, Horswill AR (2008) agr-mediated dispersal of Staphylococcus aureus biofilms. PLoS Pathog 4:e1000052. https://doi.org/10.1371/journal.ppat.1000052
287. LaSarre B, Federle MJ (2013) Exploiting quorum sensing to confuse bacterial pathogens. Microbiol Mol Biol Rev 77:73–111. https://doi.org/10.1128/MMBR.00046-12
288. Kleerebezem M, Quadri LE, Kuipers OP, de Vos WM (1997) Quorum sensing by peptide pheromones and two-component signal-transduction systems in Gram-positive bacteria. Mol Microbiol 24:895–904
289. Janzon L, Arvidson S (1990) The role of the delta-lysin gene (hld) in the regulation of virulence genes by the accessory gene regulator (agr) in Staphylococcus aureus. EMBO J 9:1391–1399
290. Morfeldt E, Taylor D, von Gabain A, Arvidson S (1995) Activation of alpha-toxin translation in Staphylococcus aureus by the trans-encoded antisense RNA, RNAIII. EMBO J 14:4569–4577
291. Otto M, Süßmuth R, Vuong C et al (1999) Inhibition of virulence factor expression in *Staphylococcus aureus* by the *Staphylococcus epidermidis* agr pheromone and derivatives. FEBS Lett 450:257–262. https://doi.org/10.1016/S0014-5793(99)00514-1
292. Mayville P, Ji G, Beavis R et al (1999) Structure-activity analysis of synthetic autoinducing thiolactone peptides from Staphylococcus aureus responsible for virulence. Proc Natl Acad Sci U S A 96:1218–1223
293. Ji G, Beavis R, Novick RP (1997) Bacterial interference caused by autoinducing peptide variants. Science 276:2027–2030. https://doi.org/10.1126/SCIENCE.276.5321.2027
294. Bjarnsholt T, Jensen PØ, Burmølle M et al (2005) Pseudomonas aeruginosa tolerance to tobramycin, hydrogen peroxide and polymorphonuclear leukocytes is quorum-sensing dependent. Microbiology 151:373–383. https://doi.org/10.1099/mic.0.27463-0

295. Hassett DJ, Ma J-F, Elkins JG et al (1999) Quorum sensing in Pseudomonas aeruginosa controls expression of catalase and superoxide dismutase genes and mediates biofilm susceptibility to hydrogen peroxide. Mol Microbiol 34:1082–1093. https://doi.org/10.1046/j.1365-2958.1999.01672.x
296. Xue T, Ni J, Shang F et al (2015) Autoinducer-2 increases biofilm formation via an ica- and bhp-dependent manner in Staphylococcus epidermidis RP62A. Microbes Infect 17:345–352. https://doi.org/10.1016/J.MICINF.2015.01.003
297. Yu D, Zhao L, Xue T, Sun B (2012) Staphylococcus aureus autoinducer-2 quorum sensing decreases biofilm formation in an icaR-dependent manner. BMC Microbiol 12:288. https://doi.org/10.1186/1471-2180-12-288
298. Boles BR, Horswill AR (2011) Staphylococcal biofilm disassembly. Trends Microbiol 19(9):449–455
299. Stoodley P, Cargo R, Rupp CJ et al (2002) Biofilm material properties as related to shear-induced deformation and detachment phenomena. J Ind Microbiol Biotechnol 29:361–367. https://doi.org/10.1038/sj.jim.7000282
300. Boyd A, Chakrabarty AM (1994) Role of alginate lyase in cell detachment of Pseudomonas aeruginosa. Appl Environ Microbiol 60:2355–2359
301. Kaplan JB, Ragunath C, Ramasubbu N, Fine DH (2003) Detachment of Actinobacillus actinomycetemcomitans biofilm cells by an endogenous beta-hexosaminidase activity. J Bacteriol 185:4693–4698. https://doi.org/10.1128/JB.185.16.4693-4698.2003
302. Lee SF, Li YH, Bowden GH (1996) Detachment of Streptococcus mutans biofilm cells by an endogenous enzymatic activity. Infect Immun 64:1035–1038
303. Gjermansen M, Nilsson M, Yang L, Tolker-Nielsen T (2010) Characterization of starvation-induced dispersion in *Pseudomonas putida* biofilms: genetic elements and molecular mechanisms. Mol Microbiol 75:815–826. https://doi.org/10.1111/j.1365-2958.2009.06793.x
304. Sauer K, Cullen MC, Rickard AH et al (2004) Characterization of nutrient-induced dispersion in Pseudomonas aeruginosa PAO1 biofilm. J Bacteriol 186:7312–7326. https://doi.org/10.1128/JB.186.21.7312-7326.2004
305. Anderl JN, Zahller J, Roe F, Stewart PS (2003) Role of nutrient limitation and stationary-phase existence in Klebsiella pneumoniae biofilm resistance to ampicillin and ciprofloxacin. Antimicrob Agents Chemother 47:1251–1256. https://doi.org/10.1128/AAC.47.4.1251-1256.2003
306. Walters MC, Roe F, Bugnicourt A et al (2003) Contributions of antibiotic penetration, oxygen limitation, and low metabolic activity to tolerance of Pseudomonas aeruginosa biofilms to ciprofloxacin and tobramycin. Antimicrob Agents Chemother 47:317–323. https://doi.org/10.1128/aac.47.1.317-323.2003
307. Barraud N, Hassett DJ, Hwang S-H et al (2006) Involvement of nitric oxide in biofilm dispersal of Pseudomonas aeruginosa. J Bacteriol 188:7344–7353. https://doi.org/10.1128/JB.00779-06
308. Peschel A, Otto M (2013) Phenol-soluble modulins and staphylococcal infection. Nat Rev Microbiol 11:667–673. https://doi.org/10.1038/nrmicro3110
309. Novick RP, Ross HF, Projan SJ et al (1993) Synthesis of staphylococcal virulence factors is controlled by a regulatory RNA molecule. EMBO J 12:3967–3975
310. Lina G, Jarraud S, Ji G et al (1998) Transmembrane topology and histidine protein kinase activity of AgrC, the agr signal receptor in Staphylococcus aureus. Mol Microbiol 28:655–662
311. Novick RP, Geisinger E (2008) Quorum sensing in staphylococci. Annu Rev Genet 42:541–564. https://doi.org/10.1146/annurev.genet.42.110807.091640
312. Lister JL, Horswill AR, Geoghegan JA (2014) Staphylococcus aureus biofilms: recent developments in biofilm dispersal. Front Cell Infect Microbiol 4:1–9. https://doi.org/10.3389/fcimb.2014.00178
313. Le KY, Dastgheyb S, Ho TV et al (2014) Molecular determinants of staphylococcal biofilm dispersal and structuring. Front Cell Infect Microbiol 4:1–7. https://doi.org/10.3389/fcimb.2014.00167

314. Vuong C, Saenz HL, Götz F, Otto M (2000) Impact of the *agr* quorum-sensing system on adherence to polystyrene in *Staphylococcus aureus*. J Infect Dis 182:1688–1693. https://doi.org/10.1086/317606
315. Vuong C, Kocianova S, Yao Y et al (2004) Increased colonization of indwelling medical devices by quorum-sensing mutants of *Staphylococcus epidermidis* in vivo. J Infect Dis 190(8):1498–1505. https://doi.org/10.1086/424487
316. Balasubramanian D, Ohneck EA, Chapman J et al (2016) Staphylococcus aureus coordinates leukocidin expression and pathogenesis by sensing metabolic fluxes via RpiRc. MBio 7(3). https://doi.org/10.1128/mBio.00818-16
317. Patel JD, Krupka T, Anderson JM (2007) iNOS-mediated generation of reactive oxygen and nitrogen species by biomaterial-adherent neutrophils. J Biomed Mater Res Part A 80A:381–390. https://doi.org/10.1002/jbm.a.30907
318. Scherr TD, Hanke ML, Huang O et al (2015) Staphylococcus aureus biofilms induce macrophage dysfunction through leukocidin AB and alpha-toxin. MBio 6:e01021–e01015. https://doi.org/10.1128/mBio.01021-15
319. Mah TFC, O'Toole GA (2001) Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 9:34–39
320. Lewis K (2001) Riddle of biofilm resistance. Antimicrob Agents Chemother 45:999–1007. https://doi.org/10.1128/AAC.45.4.999-1007.2001
321. Chuard C, Vaudaux PE, Proctor RA, Lew DP (1997) Decreased susceptibility to antibiotic killing of a stable small colony variant of Staphylococcus aureus in fluid phase and on fibronectin-coated surfaces. J Antimicrob Chemother 39:603–608
322. Szomolay B, Klapper I, Dockery J, Stewart PS (2005) Adaptive responses to antimicrobial agents in biofilms. Environ Microbiol 7:1186–1191. https://doi.org/10.1111/j.1462-2920.2005.00797.x
323. Campanac C, Pineau L, Payard A et al (2002) Interactions between biocide cationic agents and bacterial biofilms. Antimicrob Agents Chemother 46:1469–1474. https://doi.org/10.1128/AAC.46.5.1469-1474.2002
324. Nichols WW, Dorrington SM, Slack MP, Walmsley HL (1988) Inhibition of tobramycin diffusion by binding to alginate. Antimicrob Agents Chemother 32:518
325. Kaplan D, Christiaen D, Arad SM (1987) Chelating properties of extracellular polysaccharides from Chlorella spp. Appl Environ Microbiol 53:2953–2956
326. McLean RJ, Beauchemin D, Clapham L, Beveridge TJ (1990) Metal-binding characteristics of the gamma-glutamyl capsular polymer of Bacillus licheniformis ATCC 9945. Appl Environ Microbiol 56:3671–3677
327. Mittelman MW, Geesey GG (1985) Copper-binding characteristics of exopolymers from a freshwater-sediment bacterium. Appl Environ Microbiol 49:846–851
328. Teitzel GM, Parsek MR (2003) Heavy metal resistance of biofilm and planktonic Pseudomonas aeruginosa. Appl Environ Microbiol 69:2313–2320. https://doi.org/10.1128/AEM.69.4.2313-2320.2003
329. Pepi M, Borra M, Tamburrino S et al (2016) A Bacillus sp. isolated from sediments of the Sarno River mouth, Gulf of Naples (Italy) produces a biofilm biosorbing Pb(II). Sci Total Environ 562:588–595. https://doi.org/10.1016/j.scitotenv.2016.04.097
330. Singh R, Ray P, Das A, Sharma M (2010) Penetration of antibiotics through Staphylococcus aureus and Staphylococcus epidermidis biofilms. J Antimicrob Chemother 65:1955–1958. https://doi.org/10.1093/jac/dkq257
331. Stewart PS, Davison WM, Steenbergen JN (2009) Daptomycin rapidly penetrates a Staphylococcus epidermidis biofilm. Antimicrob Agents Chemother 53:3505–3507. https://doi.org/10.1128/AAC.01728-08
332. Zheng Z, Stewart PS (2002) Penetration of rifampin through Staphylococcus epidermidis biofilms. Antimicrob Agents Chemother 46:900–903. https://doi.org/10.1128/AAC.46.3.900-903.2002
333. Mathur T, Singhal S, Khan S et al (2005) Adverse effect of staphylococci slime on in vitro activity of glycopeptides. Jpn J Infect Dis 58:353–357

334. Farber BF, Kaplan MH, Clogston AG (1990) Staphylococcus epidermidis extracted slime inhibits the antimicrobial action of glycopeptide antibiotics. J Infect Dis 16121517:37–40
335. Parsons JB, Rock CO (2013) Bacterial lipids: metabolism and membrane homeostasis. Prog Lipid Res 52:249–276. https://doi.org/10.1016/j.plipres.2013.02.002
336. Dubois-Brissonnet F (2019) Characterization of bacterial membrane fatty acid profiles for biofilm cells. In: Bridier A (ed) Foodborne bacterial pathogens: methods and protocols. Humana Press, New York, NY, pp 165–170
337. Denich T, Beaudette L, Lee H, Trevors J (2003) Effect of selected environmental and physico-chemical factors on bacterial cytoplasmic membranes. J Microbiol Methods 52:149–182. https://doi.org/10.1016/S0167-7012(02)00155-0
338. Loffhagen N, Härtig C, Geyer W et al (2007) Competition betweencis, trans and cyclopropane fatty acid formation and its impact on membrane fluidity. Eng Life Sci 7:67–74. https://doi.org/10.1002/elsc.200620168
339. Delcour AH (2009) Outer membrane permeability and antibiotic resistance. Biochim Biophys Acta 1794:808–816. https://doi.org/10.1016/j.bbapap.2008.11.005
340. Sailer FC, Meberg BM, Young KD (2003) beta-Lactam induction of colanic acid gene expression in Escherichia coli. FEMS Microbiol Lett 226:245–249
341. Bagge N, Schuster M, Hentzer M et al (2004) Pseudomonas aeruginosa biofilms exposed to imipenem exhibit changes in global gene expression and beta-lactamase and alginate production. Antimicrob Agents Chemother 48:1175–1187. https://doi.org/10.1128/AAC.48.4.1175-1187.2004
342. Rachid S, Ohlsen K, Witte W et al (2000) Effect of subinhibitory antibiotic concentrations on polysaccharide intercellular adhesin expression in biofilm-forming Staphylococcus epidermidis. Antimicrob Agents Chemother 44:3357–3363. https://doi.org/10.1128/AAC.44.12.3357-3363.2000
343. Borriello G, Richards L, Ehrlich GD, Stewart PS (2006) Arginine or nitrate enhances antibiotic susceptibility of Pseudomonas aeruginosa in biofilms. Antimicrob Agents Chemother 50:382–384. https://doi.org/10.1128/AAC.50.1.382-384.2006
344. Wood TK, Knabel SJ, Kwan BW (2013) Bacterial persister cell formation and dormancy. Appl Environ Microbiol 79:7116–7121. https://doi.org/10.1128/AEM.02636-13
345. McConoughey SJ, Howlin R, Granger JF et al (2014) Biofilms in periprosthetic orthopedic infections. Future Microbiol 9:987–1007. https://doi.org/10.2217/FMB.14.64
346. Field TR, White A, Elborn JS, Tunney MM (2005) Effect of oxygen limitation on the in vitro antimicrobial susceptibility of clinical isolates of Pseudomonas aeruginosa grown planktonically and as biofilms. Eur J Clin Microbiol Infect Dis 24:677–687. https://doi.org/10.1007/s10096-005-0031-9
347. Schembri MA, Kjaergaard K, Klemm P (2003) Global gene expression in Escherichia coli biofilms. Mol Microbiol 48:253–267
348. Xu KD, Stewart PS, Xia F et al (1998) Spatial physiological heterogeneity in Pseudomonas aeruginosa biofilm is determined by oxygen availability. Appl Environ Microbiol 64:4035–4039
349. Bernier SP, Lebeaux D, DeFrancesco AS et al (2013) Starvation, together with the SOS response, mediates high biofilm-specific tolerance to the fluoroquinolone ofloxacin. PLoS Genet 9:e1003144. https://doi.org/10.1371/journal.pgen.1003144
350. Conlon BP, Nakayasu ES, Fleck LE et al (2013) Activated ClpP kills persisters and eradicates a chronic biofilm infection. Nature 503:365–370. https://doi.org/10.1038/nature12790
351. Conlon BP, Rowe SE, Lewis K (2015) Persister cells in biofilm associated infections. In: Advances in experimental medicine and biology. Springer, Cham, pp 1–9
352. Ling LL, Schneider T, Peoples AJ et al (2015) A new antibiotic kills pathogens without detectable resistance. Nature 517:455–459. https://doi.org/10.1038/nature14098
353. Conlon BP, Rowe SE, Gandt AB et al (2016) Persister formation in Staphylococcus aureus is associated with ATP depletion. Nat Microbiol 1:16051. https://doi.org/10.1038/nmicrobiol.2016.51

354. Peacock SJ, Paterson GK (2015) Mechanisms of methicillin resistance in *Staphylococcus aureus*. Annu Rev Biochem 84:577–601. https://doi.org/10.1146/annurev-biochem-060614-034516
355. Lobritz MA, Belenky P, Porter CBM et al (2015) Antibiotic efficacy is linked to bacterial cellular respiration. Proc Natl Acad Sci U S A 112:8173–8180. https://doi.org/10.1073/pnas.1509743112
356. Sun J, Chen X, Chen J, Han B (2017) Metabolic profile of biofilm-coated Staphylococcus aureus. J Chinese Inst Food Sci Technol 17: 258–264
357. Schelli K, Rutowski J, Roubidoux J, Zhu J (2017) Staphylococcus aureus methicillin resistance detected by HPLC-MS/MS targeted metabolic profiling. J Chromatogr B Analyt Technol Biomed Life Sci 1047:124–130. https://doi.org/10.1016/j.jchromb.2016.05.052
358. Zhong F, Xu M, Bruno RS et al (2017) Targeted high performance liquid chromatography tandem mass spectrometry-based metabolomics differentiates metabolic syndrome from obesity. Exp Biol Med 242:773–780. https://doi.org/10.1177/1535370217694098
359. Xu M, Zhong F, Zhu J (2017) Evaluating metabolic response to light exposure in Lactobacillus species via targeted metabolic profiling. J Microbiol Methods 133:14–19. https://doi.org/10.1016/J.MIMET.2016.12.008
360. Stipetic LH, Dalby MJ, Davies RL et al (2016) A novel metabolomic approach used for the comparison of Staphylococcus aureus planktonic cells and biofilm samples. Metabolomics 12:75. https://doi.org/10.1007/s11306-016-1002-0
361. Resch A, Rosenstein R, Nerz C, Götz F (2005) Differential gene expression profiling of Staphylococcus aureus cultivated under biofilm and planktonic conditions. Appl Environ Microbiol 71:2663–2676. https://doi.org/10.1128/AEM.71.5.2663-2676.2005
362. Beenken KE, Dunman PM, McAleese F et al (2004) Global gene expression in Staphylococcus aureus biofilms. J Bacteriol 186:4665–4684. https://doi.org/10.1128/JB.186.14.4665-4684.2004
363. Resch A, Leicht S, Saric M et al (2006) Comparative proteome analysis of Staphylococcus aureus biofilm and planktonic cells and correlation with transcriptome profiling. Proteomics 6:1867–1877. https://doi.org/10.1002/pmic.200500531
364. Zhu Y, Weiss EC, Otto M et al (2007) Staphylococcus aureus biofilm metabolism and the influence of arginine on polysaccharide intercellular adhesin synthesis, biofilm formation, and pathogenesis. Infect Immun 75:4219–4226. https://doi.org/10.1128/IAI.00509-07
365. Thomas VC, Sadykov MR, Chaudhari SS et al (2014) A central role for carbon-overflow pathways in the modulation of bacterial cell death. PLoS Pathog 10:e1004205. https://doi.org/10.1371/journal.ppat.1004205
366. Raff MC (1992) Social controls on cell survival and cell death. Nature 356:397–400. https://doi.org/10.1038/356397a0
367. Savage VJ, Chopra I, O'Neill AJ (2013) Staphylococcus aureus biofilms promote horizontal transfer of antibiotic resistance. Antimicrob Agents Chemother 57:1968–1970. https://doi.org/10.1128/AAC.02008-12
368. Nguyen D, Joshi-Datar A, Lepine F et al (2011) Active starvation responses mediate antibiotic tolerance in biofilms and nutrient-limited bacteria. Science 334:982–986. https://doi.org/10.1126/science.1211037
369. Amato SM, Fazen CH, Henry TC et al (2014) The role of metabolism in bacterial persistence. Front Microbiol 5:70. https://doi.org/10.3389/fmicb.2014.00070
370. de la Fuente-Núñez C, Reffuveille F, Haney EF et al (2014) Broad-spectrum anti-biofilm peptide that targets a cellular stress response. PLoS Pathog 10(5):e1004152. https://doi.org/10.1371/journal.ppat.1004152
371. Reffuveille F, de la Fuente-Núñez C, Mansour S, Hancock REW (2014) A broad-spectrum antibiofilm peptide enhances antibiotic action against bacterial biofilms. Antimicrob Agents Chemother 58:5363–5371. https://doi.org/10.1128/AAC.03163-14
372. Anderson GG, O'Toole GA (2008) Innate and induced resistance mechanisms of bacterial biofilms. Curr Top Microbiol Immunol 322:85–105

373. Whiteley M, Bangera MG, Bumgarner RE et al (2001) Gene expression in Pseudomonas aeruginosa biofilms. Nature 413:860–864. https://doi.org/10.1038/35101627
374. Elkins JG, Hassett DJ, Stewart PS et al (1999) Protective role of catalase in Pseudomonas aeruginosa biofilm resistance to hydrogen peroxide. Appl Environ Microbiol 65:4594–4600
375. Kohanski MA, Dwyer DJ, Hayete B et al (2007) A common mechanism of cellular death induced by bactericidal antibiotics. Cell 130:797–810. https://doi.org/10.1016/j.cell.2007.06.049
376. Dall GF, Tsang STJ, Gwynne PJ et al (2018) Unexpected synergistic and antagonistic antibiotic activity against Staphylococcus biofilms. J Antimicrob Chemother 73:1830–1840. https://doi.org/10.1093/jac/dky087
377. Herbert S, Bera A, Nerz C et al (2007) Molecular basis of resistance to muramidase and cationic antimicrobial peptide activity of lysozyme in staphylococci. PLoS Pathog 3:e102. https://doi.org/10.1371/journal.ppat.0030102
378. Weidenmaier C, Peschel A, Kempf VAJ et al (2005) DltABCD- and MprF-mediated cell envelope modifications of Staphylococcus aureus confer resistance to platelet microbicidal proteins and contribute to virulence in a rabbit endocarditis model. Infect Immun 73:8033–8038. https://doi.org/10.1128/IAI.73.12.8033-8038.2005
379. Kelley WL, Lew DP, Renzoni A (2012) Antimicrobial peptide exposure and reduced susceptibility to daptomycin: insights into a complex genetic puzzle. J Infect Dis 206:1153–1156. https://doi.org/10.1093/infdis/jis485
380. Cui L, Lian J-Q, Neoh H-M et al (2005) DNA microarray-based identification of genes associated with glycopeptide resistance in Staphylococcus aureus. Antimicrob Agents Chemother 49:3404–3413. https://doi.org/10.1128/AAC.49.8.3404-3413.2005
381. Tsuji BT, Rybak MJ (2005) Short-course gentamicin in combination with daptomycin or vancomycin against Staphylococcus aureus in an in vitro pharmacodynamic model with simulated endocardial vegetations. Antimicrob Agents Chemother 49:2735–2745. https://doi.org/10.1128/AAC.49.7.2735-2745.2005
382. Yang S-JS-J, Bayer AS, Mishra NN et al (2012) The Staphylococcus aureus two-component regulatory system, GraRS, senses and confers resistance to selected cationic antimicrobial peptides. Infect Immun 80:74–81. https://doi.org/10.1128/IAI.05669-11
383. Mataraci E, Dosler S (2012) In vitro activities of antibiotics and antimicrobial cationic peptides alone and in combination against methicillin-resistant Staphylococcus aureus biofilms. Antimicrob Agents Chemother 56:6366–6371. https://doi.org/10.1128/AAC.01180-12
384. Dosler S, Mataraci E (2013) In vitro pharmacokinetics of antimicrobial cationic peptides alone and in combination with antibiotics against methicillin resistant Staphylococcus aureus biofilms. Peptides 49:53–58. https://doi.org/10.1016/J.PEPTIDES.2013.08.008
385. Bigger J (1944) Treatment of staphylococcal infections with penicillin by intermittent sterilisation. Lancet 244:497–500. https://doi.org/10.1016/S0140-6736(00)74210-3
386. Rowe SE, Conlon BP, Keren I, Lewis K (2016) Persisters: methods for isolation and identifying contributing factors—a review. In: Bacterial persistence. Humana Press, New York, NY, pp 17–28
387. Lewis K (2005) Persister cells and the riddle of biofilm survival. Biochemistry (Mosc) 70:267–274. https://doi.org/10.1007/s10541-005-0111-6
388. Lewis K (2010) Persister cells. Annu Rev Microbiol 64:357–372. https://doi.org/10.1146/annurev.micro.112408.134306
389. Fisher RA, Gollan B, Helaine S (2017) Persistent bacterial infections and persister cells. Nat Rev Microbiol 15:453–464. https://doi.org/10.1038/nrmicro.2017.42
390. Michiels JE, Van den Bergh B, Verstraeten N, Michiels J (2016) Molecular mechanisms and clinical implications of bacterial persistence. Drug Resist Updat 29:76–89. https://doi.org/10.1016/j.drup.2016.10.002
391. França A, Carvalhais V, Vilanova M et al (2016) Characterization of an in vitro fed-batch model to obtain cells released from S. epidermidis biofilms. AMB Express 6:23. https://doi.org/10.1186/s13568-016-0197-9

392. Brauner A, Fridman O, Gefen O, Balaban NQ (2016) Distinguishing between resistance, tolerance and persistence to antibiotic treatment. Nat Rev Microbiol 14:320–330. https://doi.org/10.1038/nrmicro.2016.34
393. Waters EM, Rowe SSE, O'Gara JPJ et al (2016) Convergence of Staphylococcus aureus persister and biofilm research: can biofilms be defined as communities of adherent persister cells? PLoS Pathog 12:e1006012. https://doi.org/10.1371/journal.ppat.1006012
394. Pedersen K, Christensen SK, Gerdes K (2002) Rapid induction and reversal of a bacteriostatic condition by controlled expression of toxins and antitoxins. Mol Microbiol 45:501–510
395. Keren I, Shah D, Spoering A et al (2004) Specialized persister cells and the mechanism of multidrug tolerance in Escherichia coli. J Bacteriol 186:8172–8180. https://doi.org/10.1128/JB.186.24.8172-8180.2004
396. Allison KR, Brynildsen MP, Collins JJ (2011) Metabolite-enabled eradication of bacterial persisters by aminoglycosides. Nature 473:216–220. https://doi.org/10.1038/nature10069
397. Mina EG, Marques CNH (2016) Interaction of *Staphylococcus aureus* persister cells with the host when in a persister state and following awakening. Sci Rep 6:31342. https://doi.org/10.1038/srep31342
398. Shah D, Zhang Z, Khodursky A et al (2006) Persisters: a distinct physiological state of E. coli. BMC Microbiol 6:53. https://doi.org/10.1186/1471-2180-6-53
399. Roberts ME, Stewart PS (2005) Modelling protection from antimicrobial agents in biofilms through the formation of persister cells. Microbiology 151:75–80. https://doi.org/10.1099/mic.0.27385-0
400. Keren I, Kaldalu N, Spoering A et al (2004) Persister cells and tolerance to antimicrobials. FEMS Microbiol Lett 230:13–18
401. Bartlett AH, Hulten KG (2010) Staphylococcus aureus pathogenesis: secretion systems, adhesins, and invasins. Pediatr Infect Dis J 29:860–861. https://doi.org/10.1097/INF.0b013e3181ef2477
402. Otto M (2010) Basis of virulence in community-associated methicillin-resistant *Staphylococcus aureus*. Annu Rev Microbiol 64:143–162. https://doi.org/10.1146/annurev.micro.112408.134309
403. Yoong P, Torres VJ (2013) The effects of Staphylococcus aureus leukotoxins on the host: cell lysis and beyond. Curr Opin Microbiol 16:63–69. https://doi.org/10.1016/j.mib.2013.01.012
404. DuMont AL, Yoong P, Day CJ et al (2013) Staphylococcus aureus LukAB cytotoxin kills human neutrophils by targeting the CD11b subunit of the integrin Mac-1. Proc Natl Acad Sci U S A 110:10794–10799. https://doi.org/10.1073/pnas.1305121110
405. Reyes-Robles T, Alonzo F, Kozhaya L et al (2013) Staphylococcus aureus leukotoxin ED targets the chemokine receptors CXCR1 and CXCR2 to kill leukocytes and promote infection. Cell Host Microbe 14:453–459. https://doi.org/10.1016/j.chom.2013.09.005
406. Spaan AN, Vrieling M, Wallet P et al (2014) The staphylococcal toxins γ-haemolysin AB and CB differentially target phagocytes by employing specific chemokine receptors. Nat Commun 5:5438. https://doi.org/10.1038/ncomms6438
407. Melehani JH, James DBA, DuMont AL et al (2015) Staphylococcus aureus leukocidin A/B (LukAB) kills human monocytes via host NLRP3 and ASC when extracellular, but not intracellular. PLoS Pathog 11:e1004970. https://doi.org/10.1371/journal.ppat.1004970
408. Rigby KM, DeLeo FR (2012) Neutrophils in innate host defense against Staphylococcus aureus infections. Semin Immunopathol 34:237–259. https://doi.org/10.1007/s00281-011-0295-3
409. Papayannopoulos V, Zychlinsky A (2009) NETs: a new strategy for using old weapons. Trends Immunol 30:513–521. https://doi.org/10.1016/j.it.2009.07.011
410. Cassat JE, Hammer ND, Campbell JP et al (2013) A secreted bacterial protease tailors the Staphylococcus aureus virulence repertoire to modulate bone remodeling during osteomyelitis. Cell Host Microbe 13:759–772. https://doi.org/10.1016/j.chom.2013.05.003
411. Brady RA, Leid JG, Camper AK et al (2006) Identification of Staphylococcus aureus proteins recognized by the antibody-mediated immune response to a biofilm infection. Infect Immun 74:3415–3426. https://doi.org/10.1128/IAI.00392-06

412. Flammier S, Rasigade J-P, Badiou C et al (2016) Human monocyte-derived osteoclasts are targeted by staphylococcal pore-forming toxins and superantigens. PLoS One 11:e0150693. https://doi.org/10.1371/journal.pone.0150693
413. Weidenmaier C, Peschel A (2008) Teichoic acids and related cell-wall glycopolymers in Gram-positive physiology and host interactions. Nat Rev Microbiol 6:276–287. https://doi.org/10.1038/nrmicro1861
414. Vandenesch F, Lina G, Henry T (2012) Staphylococcus aureus hemolysins, bi-component leukocidins, and cytolytic peptides: a redundant arsenal of membrane-damaging virulence factors? Front Cell Infect Microbiol 2:12. https://doi.org/10.3389/fcimb.2012.00012
415. Gouaux E, Hobaugh M, Song L (1997) Alpha-Hemolysin, gamma-hemolysin, and leukocidin from Staphylococcus aureus: distant in sequence but similar in structure. Protein Sci 6:2631–2635. https://doi.org/10.1002/pro.5560061216
416. de Haas CJC, Veldkamp KE, Peschel A et al (2004) Chemotaxis inhibitory protein of Staphylococcus aureus, a bacterial antiinflammatory agent. J Exp Med 199:687–695. https://doi.org/10.1084/jem.20031636
417. Liu GY, Nizet V (2009) Color me bad: microbial pigments as virulence factors. Trends Microbiol 17:406–413. https://doi.org/10.1016/j.tim.2009.06.006
418. Clauditz A, Resch A, Wieland K-P et al (2006) Staphyloxanthin plays a role in the fitness of Staphylococcus aureus and its ability to cope with oxidative stress. Infect Immun 74:4950–4953. https://doi.org/10.1128/IAI.00204-06
419. Bera A, Herbert S, Jakob A et al (2005) Why are pathogenic staphylococci so lysozyme resistant? The peptidoglycan O-acetyltransferase OatA is the major determinant for lysozyme resistance of Staphylococcus aureus. Mol Microbiol 55:778–787. https://doi.org/10.1111/j.1365-2958.2004.04446.x
420. Moynihan PJ, Clarke AJ (2011) O-Acetylated peptidoglycan: controlling the activity of bacterial autolysins and lytic enzymes of innate immune systems. Int J Biochem Cell Biol 43:1655–1659. https://doi.org/10.1016/J.BIOCEL.2011.08.007
421. Scherr TD, Roux CM, Hanke ML et al (2013) Global transcriptome analysis of Staphylococcus aureus biofilms in response to innate immune cells. Infect Immun 81:4363–4376. https://doi.org/10.1128/IAI.00819-13
422. Thammavongsa V, Missiakas D, Schneewind O (2013) Staphylococcus aureus degrades neutrophil extracellular traps to promote immune cell death. Science 342(6160):863–866. https://doi.org/10.1126/science.1240667
423. Cheng AG, DeDent AC, Schneewind O, Missiakas D (2011) A play in four acts: Staphylococcus aureus abscess formation. Trends Microbiol 19:225–232. https://doi.org/10.1016/J.TIM.2011.01.007
424. Gries CM, Kielian T (2017) Staphylococcal biofilms and immune polarization during prosthetic joint infection. J Am Acad Orthop Surg 25:20–24. https://doi.org/10.5435/JAAOS-D-16-00636
425. Scherr TD, Heim CE, Morrison JM, Kielian T (2014) Hiding in plain sight: interplay between staphylococcal biofilms and host immunity. Front Immunol 5:37. https://doi.org/10.3389/fimmu.2014.00037
426. Heim CE, Vidlak D, Odvody J et al (2018) Human prosthetic joint infections are associated with myeloid-derived suppressor cells (MDSCs): implications for infection persistence. J Orthop Res 36(6):1605–1613. https://doi.org/10.1002/jor.23806
427. Heim CE, Vidlak D, Scherr TD et al (2014) Myeloid-derived suppressor cells contribute to Staphylococcus aureus orthopedic biofilm infection. J Immunol 192(8):3778–3792. https://doi.org/10.4049/jimmunol.1303408
428. Heim CE, Vidlak D, Scherr TD et al (2015) IL-12 promotes myeloid-derived suppressor cell recruitment and bacterial persistence during *Staphylococcus aureus* orthopedic implant infection. J Immunol 194(8):3861–3872. https://doi.org/10.4049/jimmunol.1402689
429. Heim CE, Vidlak D, Kielian T (2015) Interleukin-10 production by myeloid-derived suppressor cells contributes to bacterial persistence during *Staphylococcus aureus* orthopedic biofilm infection. J Leukoc Biol 98:1003–1013. https://doi.org/10.1189/jlb.4VMA0315-125RR

430. Peng K-T, Hsieh C-C, Huang T-Y et al (2017) Staphylococcus aureus biofilm elicits the expansion, activation and polarization of myeloid-derived suppressor cells in vivo and in vitro. PLoS One 12:e0183271. https://doi.org/10.1371/journal.pone.0183271
431. Cheung GYC, Rigby K, Wang R et al (2010) Staphylococcus epidermidis strategies to avoid killing by human neutrophils. PLoS Pathog 6(10):e1001133. https://doi.org/10.1371/journal.ppat.1001133
432. Foster TJ (2005) Immune evasion by staphylococci. Nat Rev Microbiol 3(12):948–958
433. Donaldson K, Murphy FA, Duffin R, Poland CA (2010) Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. Part Fibre Toxicol 7:5
434. Leid JG, Shirtliff ME, Costerton JW, Stoodley P (2002) Human leukocytes adhere to, penetrate, and respond to *Staphylococcus aureus* biofilms. Infect Immun 70(11):6339–6345. https://doi.org/10.1128/IAI.70.11.6339-6345.2002
435. Hanke ML, Angle A, Kielian T (2012) MyD88-dependent signaling influences fibrosis and alternative macrophage activation during Staphylococcus aureus biofilm infection. PLoS One 7:e42476. https://doi.org/10.1371/journal.pone.0042476
436. Bröker B, Mrochen D, Péton V et al (2016) The T cell response to Staphylococcus aureus. Pathogens 5:31. https://doi.org/10.3390/pathogens5010031
437. Jensen LK, Jensen HE, Koch J et al (2015) Specific antibodies to Staphylococcus aureus biofilm are present in serum from pigs with osteomyelitis. In Vivo 29:555–560
438. Peck A, Mellins ED (2010) Precarious balance: Th17 cells in host defense. Infect Immun 78(1):32–38
439. Prabhakara R, Harro JM, Leid JG et al (2011) Suppression of the inflammatory immune response prevents the development of chronic biofilm infection due to methicillin-resistant Staphylococcus aureus. Infect Immun 79(12):5010–5018. https://doi.org/10.1128/IAI.05571-11
440. Hanke ML, Kielian T (2012) Deciphering mechanisms of staphylococcal biofilm evasion of host immunity. Front Cell Infect Microbiol 2:62. https://doi.org/10.3389/fcimb.2012.00062
441. Benoit M, Desnues B, Mege J-L (2008) Macrophage polarization in bacterial infections. J Immunol 181:3733–3739. https://doi.org/10.4049/JIMMUNOL.181.6.3733
442. Mbalaviele G, Novack DV, Schett G, Teitelbaum SL (2017) Inflammatory osteolysis: a conspiracy against bone. J Clin Invest 127:2030–2039. https://doi.org/10.1172/JCI93356
443. Schnaith A, Kashkar H, Leggio SA et al (2007) Staphylococcus aureus subvert autophagy for induction of caspase-independent host cell death. J Biol Chem 282:2695–2706. https://doi.org/10.1074/jbc.M609784200
444. Kubica M, Guzik K, Koziel J et al (2008) A potential new pathway for Staphylococcus aureus dissemination: the silent survival of S. aureus phagocytosed by human monocyte-derived macrophages. PLoS One 1:1–16
445. Tuchscherr L, Medina E, Hussain M et al (2011) Staphylococcus aureus phenotype switching: an effective bacterial strategy to escape host immune response and establish a chronic infection. EMBO Mol Med 3:129–141. https://doi.org/10.1002/emmm.201000115
446. Hamza T, Li B (2014) Differential responses of osteoblasts and macrophages upon Staphylococcus aureus infection. BMC Microbiol 14:207. https://doi.org/10.1186/s12866-014-0207-5
447. Vesga O, Groeschel MC, Otten MF et al (1996) Staphylococcus aureus small colony variants are induced by the endothelial cell intracellular milieu. J Infect Dis 173:739–742
448. von Eiff C, Becker K, Metze D et al (2001) Intracellular persistence of Staphylococcus aureus small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with Darier's disease. Clin Infect Dis 32:1643–1647. https://doi.org/10.1086/320519
449. Clement S, Vaudaux P, Francois P et al (2005) Evidence of an intracellular reservoir in the nasal mucosa of patients with recurrent *Staphylococcus aureus* rhinosinusitis. J Infect Dis 192:1023–1028. https://doi.org/10.1086/432735
450. Flannagan RS, Jaumouillé V, Grinstein S (2012) The cell biology of phagocytosis. Annu Rev Pathol Mech Dis 7:61–98. https://doi.org/10.1146/annurev-pathol-011811-132445

451. Fairn GD, Grinstein S (2012) How nascent phagosomes mature to become phagolysosomes. Trends Immunol 33(8):397–405
452. Dossett JH, Kronvall G, Williams RC, Quie PG (1969) Antiphagocytic effects of staphylococcal protein A. J Immunol 103:1405–1410
453. Ko Y-P, Kuipers A, Freitag CM et al (2013) Phagocytosis escape by a Staphylococcus aureus protein that connects complement and coagulation proteins at the bacterial surface. PLoS Pathog 9:e1003816. https://doi.org/10.1371/journal.ppat.1003816
454. Müller S, Faulhaber A, Sieber C et al (2014) The endolysosomal cysteine cathepsins L and K are involved in macrophage-mediated clearance of Staphylococcus aureus and the concomitant cytokine induction. FASEB J 28:162–175. https://doi.org/10.1096/fj.13-232272
455. Grosz M, Kolter J, Paprotka K et al (2014) Cytoplasmic replication of Staphylococcus aureus upon phagosomal escape triggered by phenol-soluble modulin α. Cell Microbiol 16:451–465. https://doi.org/10.1111/cmi.12233
456. Strobel M, Pförtner H, Tuchscherr L et al (2016) Post-invasion events after infection with Staphylococcus aureus are strongly dependent on both the host cell type and the infecting S. aureus strain. Clin Microbiol Infect 22:799–809. https://doi.org/10.1016/j.cmi.2016.06.020
457. Giese B, Glowinski F, Paprotka K et al (2011) Expression of δ-toxin by Staphylococcus aureus mediates escape from phago-endosomes of human epithelial and endothelial cells in the presence of β-toxin. Cell Microbiol 13:316–329. https://doi.org/10.1111/j.1462-5822.2010.01538.x
458. Koziel J, Maciag-Gudowska A, Mikolajczyk T et al (2009) Phagocytosis of Staphylococcus aureus by macrophages exerts cytoprotective effects manifested by the upregulation of antiapoptotic factors. PLoS One 4:e5210. https://doi.org/10.1371/journal.pone.0005210
459. Jubrail J, Morris P, Bewley MA et al (2016) Inability to sustain intraphagolysosomal killing of Staphylococcus aureus predisposes to bacterial persistence in macrophages. Cell Microbiol 18:80–96. https://doi.org/10.1111/cmi.12485
460. Noore J, Noore A, Li B (2013) Cationic antimicrobial peptide LL-37 Is effective against both extra- and intracellular Staphylococcus aureus. Antimicrob Agents Chemother 57:1283–1290. https://doi.org/10.1128/AAC.01650-12
461. Campoccia D, Testoni F, Ravaioli S et al (2016) Orthopedic implant infections: incompetence of Staphylococcus epidermidis, Staphylococcus lugdunensis, and Enterococcus faecalis to invade osteoblasts. J Biomed Mater Res Part A 104(3):788–801. https://doi.org/10.1002/jbm.a.35564
462. Josse J, Velard F, Gangloff SC (2015) Staphylococcus aureus vs. osteoblast: relationship and consequences in osteomyelitis. Front Cell Infect Microbiol 5:85. https://doi.org/10.3389/fcimb.2015.00085
463. Fowler T, Wann ER, Joh D et al (2000) Cellular invasion by Staphylococcus aureus involves a fibronectin bridge between the bacterial fibro-nectin-binding MSCRAMMs and host cell b1 integrins. Eur J Cell Biol 79(10):672–679
464. Alexander EH, Rivera A, Marriott I et al (2003) Staphylococcus aureus-induced tumor necrosis factor-related apoptosis-inducing ligand expression mediates apoptosis and caspase-8 activation in infected osteoblasts. BMC Microbiol 3:1–11
465. Bui LMGG, Conlon BPP, Kidd SPP (2017) Antibiotic tolerance and the alternative lifestyles of Staphylococcus aureus. Essays Biochem 61:71–79. https://doi.org/10.1042/EBC20160061
466. Proctor RA, von Eiff C, Kahl BC et al (2006) Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol 4:295–305. https://doi.org/10.1038/nrmicro1384
467. Tuchscherr L, Heitmann V, Hussain M et al (2010) *Staphylococcus aureus* small-colony variants are adapted phenotypes for intracellular persistence. J Infect Dis 202:1031–1040. https://doi.org/10.1086/656047
468. Sendi P, Rohrbach M, Graber P et al (2006) Staphylococcus aureus small colony variants in prosthetic joint infection. Clin Infect Dis 43:961–967. https://doi.org/10.1086/507633
469. Garzoni C, Kelley WL (2009) Staphylococcus aureus: new evidence for intracellular persistence. Trends Microbiol 17:59–65. https://doi.org/10.1016/J.TIM.2008.11.005

470. Reilly SS, Hudson MC, Kellam JF, Ramp WK (2000) In vivo internalization of Staphylococcus aureus by embryonic chick osteoblasts. Bone 26(1):63–70. https://doi.org/10.1016/S8756-3282(99)00239-2
471. Hamza T, Dietz M, Pham D et al (2013) Intra-cellular Staphylococcus aureus alone causes infection in vivo. Eur Cell Mater 25:341–350; discussion 350
472. de Mesy Bentley KL, Trombetta R, Nishitani K et al (2017) Evidence of Staphylococcus aureus deformation, proliferation, and migration in canaliculi of live cortical bone in murine models of osteomyelitis. J Bone Miner Res 32:985–990. https://doi.org/10.1002/jbmr.3055
473. Maali Y, Martins-Simões P, Valour F et al (2016) Pathophysiological mechanisms of Staphylococcus non-aureus bone and joint infection: Interspecies homogeneity and specific behavior of S. pseudintermedius. Front Microbiol 7:1063. https://doi.org/10.3389/fmicb.2016.01063
474. Haddad FS (2018) Next generation sequencing: is this the moment? Bone Joint J 100–B:125–126. https://doi.org/10.1302/0301-620X.100B2.BJJ-2018-0057
475. Tarabichi M, Shohat N, Goswami K, Parvizi J (2018) Can next generation sequencing play a role in detecting pathogens in synovial fluid? Bone Joint J 100–B:127–133. https://doi.org/10.1302/0301-620X.100B2.BJJ-2017-0531.R2
476. Allegranzi B, Bischoff P, de Jonge S et al (2016) New WHO recommendations on preoperative measures for surgical site infection prevention: an evidence-based global perspective. Lancet Infect Dis 11:1–12. https://doi.org/10.1016/S1473-3099(16)30398-X
477. Tsang STJ, McHugh MP, Guerendiain D et al (2018) Evaluation of Staphylococcus aureus eradication therapy in orthopaedic surgery. J Med Microbiol 67(6):893–901. https://doi.org/10.1099/jmm.0.000731
478. Bode LGM, Kluytmans JAJW, Wertheim HFL et al (2010) Preventing surgical-site infections in nasal carriers of Staphylococcus aureus. N Engl J Med 362:9–17. https://doi.org/10.1056/NEJMoa0808939
479. Maclean M, Booth M, Anderson J et al (2013) Continuous decontamination of an intensive care isolation room during patient occupancy using 405 nm light technology. J Infect Prev 14:176–181. https://doi.org/10.1177/1757177413483646
480. Gwynne PJ, Gallagher MP (2018) Light as a broad-spectrum antimicrobial. Front Microbiol 9:119. https://doi.org/10.3389/fmicb.2018.00119
481. Hook AL, Chang C-Y, Yang J et al (2012) Combinatorial discovery of polymers resistant to bacterial attachment. Nat Biotechnol 30:868–875. https://doi.org/10.1038/nbt.2316
482. Bhattacharya M, Wozniak DJ, Stoodley P, Hall-Stoodley L (2015) Prevention and treatment of Staphylococcus aureus biofilms. Expert Rev Anti-Infect Ther 13:1499. https://doi.org/10.1586/14787210.2015.1100533
483. Kucharíková S, Gerits E, De Brucker K et al (2016) Covalent immobilization of antimicrobial agents on titanium prevents *Staphylococcus aureus* and *Candida albicans* colonization and biofilm formation. J Antimicrob Chemother 71:936–945. https://doi.org/10.1093/jac/dkv437
484. Knetsch MLW, Koole LH (2011) New strategies in the development of antimicrobial coatings: the example of increasing usage of silver and silver nanoparticles. Polymers (Basel) 3:340–366. https://doi.org/10.3390/polym3010340
485. Alt V (2017) Antimicrobial coated implants in trauma and orthopaedics—a clinical review and risk-benefit analysis. Injury 48:599–607. https://doi.org/10.1016/J.INJURY.2016.12.011
486. Barraud N, Buson A, Jarolimek W, Rice SA (2013) Mannitol enhances antibiotic sensitivity of persister bacteria in Pseudomonas aeruginosa biofilms. PLoS One 8:e84220. https://doi.org/10.1371/journal.pone.0084220
487. Lebeaux D, Chauhan A, Létoffé S et al (2014) pH-Mediated potentiation of aminoglycosides kills bacterial persisters and eradicates in vivo biofilms. J Infect Dis 210:1357–1366. https://doi.org/10.1093/infdis/jiu286
488. Prax M, Mechler L, Weidenmaier C, Bertram R (2016) Glucose augments killing efficiency of daptomycin challenged Staphylococcus aureus persisters. PLoS One 11:e0150907. https://doi.org/10.1371/journal.pone.0150907

489. Königs AM, Flemming H-C, Wingender J (2015) Nanosilver induces a non-culturable but metabolically active state in Pseudomonas aeruginosa. Front Microbiol 06:395. https://doi.org/10.3389/fmicb.2015.00395
490. Hogan S, O'Gara JP, O'Neill E (2018) Novel treatment of Staphylococcus aureus device-related infections using fibrinolytic agents. Antimicrob Agents Chemother 62:e02008–e02017. https://doi.org/10.1128/AAC.02008-17
491. Hogan S, Zapotoczna M, Stevens NT et al (2017) Potential use of targeted enzymatic agents in the treatment of Staphylococcus aureus biofilm-related infections. J Hosp Infect 96:177–182. https://doi.org/10.1016/J.JHIN.2017.02.008
492. Ricciardi BF, Muthukrishnan G, Masters E et al (2018) Staphylococcus aureus evasion of host immunity in the setting of prosthetic joint infection: biofilm and beyond. Curr Rev Musculoskelet Med 11(3):389–400. https://doi.org/10.1007/s12178-018-9501-4
493. Estellés A, Woischnig AK, Liu K et al (2016) A high-affinity native human antibody disrupts biofilm from Staphylococcus aureus bacteria and potentiates antibiotic efficacy in a mouse implant infection model. Antimicrob Agents Chemother 60(4):2292–2301. https://doi.org/10.1128/AAC.02588-15
494. Pitt WG, McBride MO, Lunceford JK et al (1994) Ultrasonic enhancement of antibiotic action on gram-negative bacteria. Antimicrob Agents Chemother 38:2577–2582. https://doi.org/10.1128/AAC.38.11.2577
495. Del Pozo JL, Rouse MS, Euba G et al (2009) The electricidal effect is active in an experimental model of Staphylococcus epidermidis chronic foreign body osteomyelitis. Antimicrob Agents Chemother 53:4064–4068. https://doi.org/10.1128/AAC.00432-09
496. Del Pozo JL, Rouse MS, Patel R (2008) Bioelectric effect and bacterial biofilms. A systematic review. Int J Artif Organs 31:786–795
497. del Pozo JL, Rouse MS, Mandrekar JN et al (2009) The electricidal effect: reduction of Staphylococcus and pseudomonas biofilms by prolonged exposure to low-intensity electrical current. Antimicrob Agents Chemother 53:41–45. https://doi.org/10.1128/AAC.00680-08
498. Pickering SAW, Bayston R, Scammell BE (2003) Electromagnetic augmentation of antibiotic efficacy in infection of orthopaedic implants. J Bone Joint Surg Br 85B:588–593. https://doi.org/10.1302/0301-620x.85b4.12644
499. Lauderdale KJ, Malone CL, Boles BR et al (2009) Biofilm dispersal of community-associated methicillin-resistant *Staphylococcus aureus* on orthopedic implant material. J Orthop Res 28(1):55–61. https://doi.org/10.1002/jor.20943
500. Brackman G, Cos P, Maes L et al (2011) Quorum sensing inhibitors increase the susceptibility of bacterial biofilms to antibiotics in vitro and in vivo. Antimicrob Agents Chemother 55:2655–2661. https://doi.org/10.1128/AAC.00045-11
501. Brackman G, Coenye T (2015) Inhibition of quorum sensing in Staphylococcus spp. Curr Pharm Des 21:2101–2108. https://doi.org/10.2174/1381612821666150310101014
502. Francolini I, Norris P, Piozzi A et al (2004) Usnic acid, a natural antimicrobial agent able to inhibit bacterial biofilm formation on polymer surfaces. Antimicrob Agents Chemother 48:4360–4365. https://doi.org/10.1128/AAC.48.11.4360-4365.2004
503. Balaban N, Cirioni O, Giacometti A et al (2007) Treatment of Staphylococcus aureus biofilm infection by the quorum-sensing inhibitor RIP. Antimicrob Agents Chemother 51:2226–2229. https://doi.org/10.1128/AAC.01097-06
504. O'Loughlin CT, Miller LC, Siryaporn A et al (2013) A quorum-sensing inhibitor blocks Pseudomonas aeruginosa virulence and biofilm formation. Proc Natl Acad Sci 110(44):17981–17986. https://doi.org/10.1073/pnas.1316981110
505. Dusane DH, Kyrouac D, Petersen I et al (2017) Targeting intracellular *Staphylococcus aureus* to lower recurrence of orthopaedic infection. J Orthop Res 36:1086–1092. https://doi.org/10.1002/jor.23723
506. Zahid M, Robbins P (2015) Cell-Type Specific Penetrating Peptides: Therapeutic Promises and Challenges. Molecules 20:13055–13070. https://doi.org/10.3390/molecules200713055
507. Donovan D (2013) Fusion of peptidoglycan hydrolase enzymes to a protein tranduction domain allow eradication of broth extracellular and intracellular Gram positive pathogens.

Patent No: US 8,383,102 B2 https://patents.google.com/patent/US8383102B2/en. Accessed 25-01-2019
508. Donovan DM, Dong S, Garrett W et al (2006) Peptidoglycan hydrolase fusions maintain their parental specificities. Appl Environ Microbiol 72:2988–2996. https://doi.org/10.1128/AEM.72.4.2988-2996.2006
509. Rukavina Z, Vanić Ž (2016) Current trends in development of liposomes for targeting bacterial biofilms. Pharmaceutics 8(2). https://doi.org/10.3390/pharmaceutics8020018
510. Forier K, Raemdonck K, De Smedt SC et al (2014) Lipid and polymer nanoparticles for drug delivery to bacterial biofilms. J Control Release 190:607–623. https://doi.org/10.1016/J.JCONREL.2014.03.055
511. WHO (2014) Surveillance of antimicrobial resistance for local and global action. http://www.who.int/drugresistance/events/SwedenMeeting/en/. Accessed 1 Dec 2014
512. Li B, Webster TJ (2018) Bacteria antibiotic resistance: new challenges and opportunities for implant-associated orthopedic infections. J Orthop Res 36:22–32. https://doi.org/10.1002/jor.23656

Device-Related Infections

Paul Renick and Liping Tang

Abstract Device-related infection is responsible for a quarter of all health care-associated infections and can even compromise device function. These infections are caused by the colonization of microorganisms during the implantation processes. Unfortunately, the treatment option for device-related infection is limited. To make the situation worse, some of these organisms form biofilms that cover the device surface notably weakening the effectiveness of antimicrobial treatments. This chapter summarizes our current understanding of the pathogenesis of device-related infection. It also discusses our knowledge of the processes governing the formation, regulation, and resistance of biofilms. Finally, we introduce several new methods developed for diagnosing and treating biofilm infections on medical devices.

Keywords Medical device · Infection · Extracellular polymeric substances · Biofilm · Protein · Biomaterials · Fibrinogen · Implants · Hydrophobic · Quorum sensing · Surface-active compounds · Diagnosis

Introduction

Medical devices have transformed health care significantly improving the lives of patients. The incorporation of medical devices for treatment have restored mobility, regulated or restored body functions, and permitted easy and relatively painless drug delivery. Examples of these devices include: cardiac implants (pacemakers, vascular grafts, cardiac valves, etc.); central and peripheral vascular catheters; endotracheal tubes; contact lenses; tissue fillers/breast implants; orthopedic and prosthetic implants; and urinary catheters [1]. Unfortunately, implanting devices can result in the introduction of normally benign flora or pathogenic organisms resulting in infection and compromising device function. This represents a significant burden on the health care system and causes significant morbidity and mortality.

P. Renick
Department of Biology, University of Texas at Arlington, Arlington, TX, USA

L. Tang (✉)
Department of Bioengineering, University of Texas at Arlington, Arlington, TX, USA
e-mail: ltang@uta.edu

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_7

Device-related infections account for 25.6% of all health care-associated infections in the USA [2] and a 6.4% prevalence in England with 1,000,000 reported per year [3]. The routes of infection include surgical implantation procedures, placement of devices in extended contact with mucous membranes and hematogenous seeding [4, 5]. Causative organisms include Gram-positive bacteria such as *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), Coagulase-negative *Staphylococci (CoNS), Streptococcal* species, *Enterococcus faecalis (E. faecalis)*, and Enterococcal species. *S. aureus* and S. *epidermidis* are known to make up the majority of prosthetic implant infections [2, 3]. Commonly isolated Gram-negative species include *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli (E. coli), Proteus miriabilis (P. miriabilis)*, and *Klebsiella pneumoniae (K. pneumoniae)* [1, 3, 6]. In addition to bacteria, yeasts, especially the Candida species, can play a role in these infections [6].

Further complicating device infection is the formation of biofilms by the infecting organisms. A biofilm is a highly organized aggregate of bacteria (or yeast) attached to a surface or each other that secretes hydrated extracellular polymeric substances (EPS). The EPS is composed of polysaccharides, extracellular DNA, and proteins. Biofilms are known to exhibit community behavior, communicating and regulating gene expression in the biofilm by quorum sensing molecules. The biofilm aggregate represents a defense against hostile environments (chemotherapy, immune response, and predation) enabling the survival of the microorganisms in the biofilm [7–14]. Biofilm formation on devices occurs in several steps: attachment to conditioned implant surfaces, microcolony formation, maturation and dispersal [2, 6, 8, 10, 11, 15–18]. A graphical summary of these traits is shown in Fig. 1.

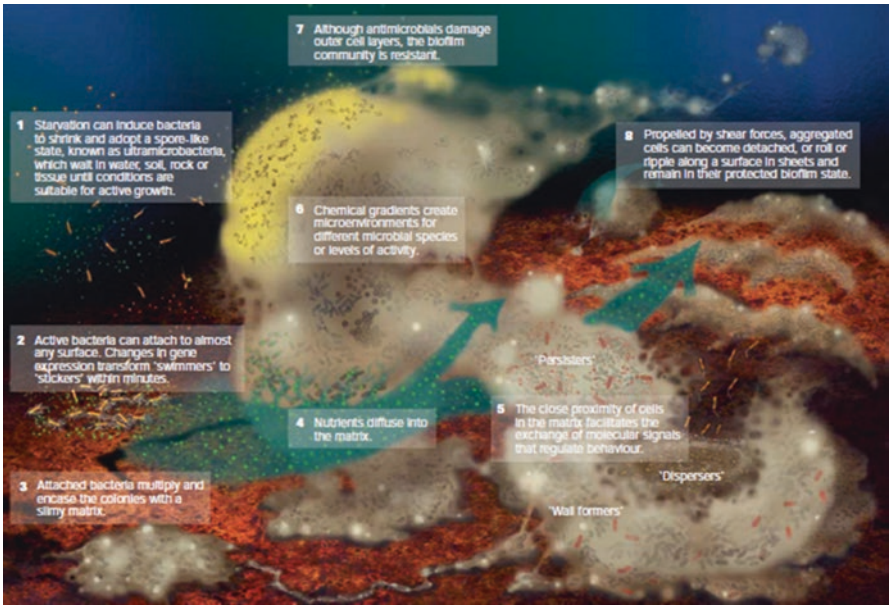


Fig. 1 A summary of the complexity of biofilms illustrating the colonial and organized nature of this type of infection. Reprinted with permission from Springer Nature, Nature Reviews Microbiology, Hall-Stoodley et al. (2004) [11] Copyright 2004

Implant Surface Conditioning

Immediately after implantation, medical devices are rapidly coated with host proteins, specifically plasma proteins that condition the surfaces of the implants. A majority of the implant devices attract hydrophobic proteins like albumin, immunoglobulin IgG, and fibrinogen. Once in contact with these surfaces, the proteins can either maintain a configuration similar to the configuration in the liquid phase or, due to conditions in the local environment, unfold and denature exposing occult epitopes to the immune system enhancing inflammation at the implant site [19]. An example of this is the binding and conformational change of the serum protein fibrinogen. In Tang et al. [20], it was demonstrated that fibrinogen underwent a time-dependent conformational change, exposing the occult sequences P1 and P2. These epitopes enhanced the recruitment of phagocytic cells to the implant, increasing levels of inflammatory cytokines, suggesting that these two epitopes are linked to fibrotic reactions [20]. In this background of inflammation and surface coating, bacteria have developed a means to exploit and bind to these host proteins that coat implanted materials. Figure 2 shows an image of *S. aureus* biofilm that has formed on a host-conditioned catheter segment.

Bacterial Adhesion to Surfaces

Bacterial adhesion is a two-step process with a primary adhesion step ("docking") and a secondary adhesion step ("locking") [16]. The first stage of adhesion is random with the organism arriving at the surface by chance. This process occurs by physiochemical interactions (hydrophobic, electrostatic, van der Waals forces, temperature, and hydrodynamic forces). These interactions are reversible and can be altered by environmental conditions and depend on the net sum of attractive and repulsive forces over a critical proximity to the surface [16]. Overall, electrostatic interactions favor repulsion based on bacterial and surface-negative charges, while hydrophobic interactions drive primary adhesion [16, 21]. The secondary adhesion

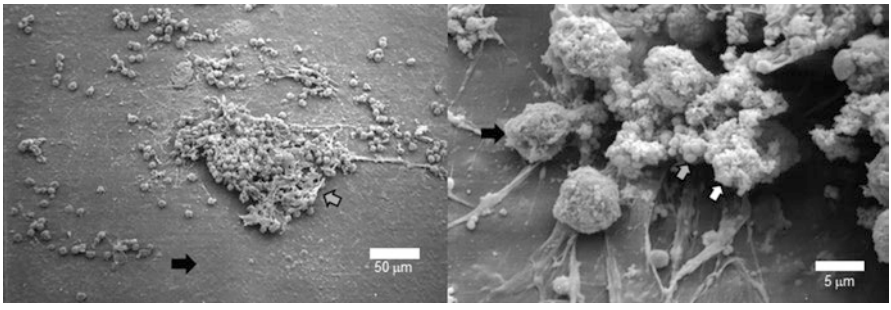


Fig. 2 SEM images of *Staphylococcus aureus* biofilms on the surface of Teflon-coated catheters established in a mouse model of biofilm infection. The panel on the left shows the biofilm (gray arrow) adhered to the catheter surface (black arrow). The right-hand image shows the individual staphylococci (gray arrow), matrix (white arrow), and host immune cells (black arrow)

or "Locking" is facilitated by receptor–ligand reactions between the bacteria and the surface. This step of adhesion is permanent unless disrupted by mechanical and physical means. Once this secondary binding is complete, the process of biofilm formation begins [2, 8, 16]. Figure 2 shows a scanning electron microscopy (SEM) established biofilm on a Teflon-coated catheter segment.

Binding to abiotic surfaces by bacteria is driven by nonspecific means such as electrostatic, hydrophobic and hydrophilic interactions mentioned above but different mechanisms come into play on conditioned surfaces [2, 8, 16, 21, 22]. In the case of device-related infections, the bacteria encounter surfaces that are preconditioned by host proteins. Bacteria have developed a wide array of adhesion that can exploit collagen, fibronectin, fibrinogen, and lectin and can express a variety of surface-active compounds (SACs) to aid in attachment [16, 21, 23]. *S. epidermidis* has been demonstrated to have competitive binding for fibronectin with heparin [22] and the ability of *S. aureus* to bind to a variety of epitopes including fibrinogen, collagen, and bone sialoprotein is well documented [23–26]. There is evidence that suggests *E. coli* and *Pseudomonas aeruginosa* can alter their surface hydrophobicity by the secretion of SACs [21]. *Pseudomonas aeruginosa* expresses PA-IL and PA-IIL which recognize host glycans [27]. Other bacteria cell surface features that initiate or aid in binding include flagella, lipopolysaccharides (LPS), fimbrae, mycolic acids and lipopolysaccharides [8]. Additionally, their context and environmental conditions can result in distinct adhesion coming into play to aid with surface attachment. The El Tor strain of *Vibrio cholera* when in contact with borosilicate uses a mannose-sensitive hemagglutinin not associated with pathogenicity to bind to these surfaces. In contrast with this, when the bacteria comes in contact to chitin, a virulence-associated toxin-coregulated pilus is used to attach and begin biofilm formation [28]. Another feature of some of these adhesions is that they are transcriptionally regulated and are expressed either during the planktonic or sessile phases of life. Polysaccharide intercellular adhesion (PIA) expressed by *S. epidermidis* is an example of these transcriptionally regulated inhibitors. Interruption of the icxADBC operon controlling the expression of PIA results in impaired adherence mutants, while expression in a deficient strain enables attachment to surfaces [16, 29–31]. The binding of organisms to surfaces can also promote the adhesion of other organisms to the surface and each other [32]. For example, Leung et al. demonstrated in an in vitro biofilm model that colonization of biliary stents by *E. coli* enhances the binding of *Enterococcus* [32].

Biofilm Formation

After adhesion to the surface, bacteria form microcolonies composed of single and multiple species of bacteria, alter their phenotypes to a sessile existence, and begin to express EPS. The maturing biofilm develops stratified structures with nutrient channels and differing zones of metabolic rates and genomic expression giving rise to a situation analogous to tissues in higher organisms [15, 33]. As the high densities

of cells limit the rate of growth and nutrients [15], biofilms display altruistic and cooperative properties [34]. In multispecies biofilms, different species can utilize alternative catabolic pathways and feed off the metabolites of other species [34, 35]. The resulting microenvironments with the developing biofilm result in different growth responses and gene expression by the bacteria ultimately resulting in structurally complex mature biofilms [33]. Environmental stresses placed on the forming biofilm can speed the development of the biofilm. In both *S. aureus* and *S. epidermidis*, the main polysaccharide in the matrix is PIA, which is expressed via the icxADBC operon. In response to environmental stresses such as antibiotic treatment, osmolarity, alcohols, low oxygen, low nutrients, and heat lead to increased expression of PIA and more rapid matrix development [2, 29, 30]. The rate of liquid flow and sheer stress also can result in modifications to the amount of the matrix produced depending on the vascularization and location in the body. Increased levels of PIA are present in *S. epidermidis* catheter infections compared to other lower shear environments [2]. After maturation, complex signaling within biofilms can result in the dispersal of planktonic bacteria and can occur actively or passively [11, 15, 17]. Passive dispersal of biofilms occurs because of abrasion, fluid shear (erosion and sloughing), predator grazing, and medical intervention [15, 17, 33]. Active dispersal is initiated by the biofilms in response to environmental or signaling cues. These cues include changes in nutrient levels, quorum sensing molecules, chemical signals, and cyclic dimeric guanosine monophosphate (GMP) [17]. Active biofilm dispersion allows the bacteria to colonize other surfaces and serves as a survival mechanism [15, 17, 33].

Quorum Sensing and Biofilm Regulation

Bacteria regulate physical processes and cooperative efforts via small molecule autoinducers that are expressed at a basal level during growth in a process known as quorum sensing (QS) [8, 11, 18, 36, 37]. These molecules allow coordination of a response in a population-dependent manner by the activation or repression of gene expression. The localized QS molecules are directly related to the population density and only induce behavior in locally high concentrations of bacteria [18, 36, 38]. Currently, there are three classes of QS molecules with example systems and functions showing in Table 1. For a more comprehensive review of these systems, see references (18, 36–39).

Table 1 The three classes of quorum sensing molecules used by bacteria

Bacteria	Signaling molecule	Example system	Function
Gram-negative	Acyl Homoserine Lactones	*LuxI/LuxR*	Bioluminescence
Gram-positive	Small peptides	*Agr*	Virulence factors
Both Gram-negative and Gram-positive	AI-2	*LuxS*	Interspecies communication

The AI-2 signaling molecules are unique in that they allow for cross-species communication

QS molecules are known to play a role in biofilm formation and regulate societal traits such as competence, sporulation, virulence factors, structural formations, dispersion, antimicrobial expression, fratricide, bioluminescence, and symbiosis [18, 39]. QS molecules are reported to be involved in altruistic cooperative group benefits even when confronted with other bacteria that would exploit this altruism. An example would be a trade-off in growth rates where slow rates with a high yield are ultimately better for the population than a fast growth rate with low yield. The higher yields suggest a more efficient use of resources even at the expense of individual bacteria [18, 34]. The modulation of virulence factors by QS molecules implicates them in the biofilm formation and infection processes. Multiple species of bacteria do not express virulence factors until a critical concentration of bacteria is reached allowing them to collectively avoid the host immune system [39–42]. While QS systems can be extremely precise, there is also a certain degree of leakiness in these communication systems allowing cross talk between species [36]. In cystic fibrosis infections, *P. aeruginosa* can upregulate virulence factors in response to intercepting AI-2 signals from nonpathogenic oropharyngeal flora [41]. Another cystic fibrosis pathogen, *Burkholderia cepacia*, can intercept *P. aeruginosa* QS signals and upregulate its virulence factors to establish infection [37, 41]. Species cross talk between *Haemophilus influenzae* and *Moraxella catarrhalis* can help establish chronic infections and resistance in polymicrobial otitis media [43].

The EPS Matrix

The essential part of the biofilm is the production of an EPS matrix which comprises roughly 90% of the biomass of the biofilms [44]. The EPS represents both a habitat and a fortress for the bacteria encased within. The organization of the matrix depends on the structural components within the matrix and the metabolic activity occurring within the biofilm [10]. The largest component of the matrix is water comprising up to 97% of the matrix with the remaining bulk of the materials being composed of soluble components like polysaccharides, proteins, and eDNA. Insoluble matrix components include amyloids, cellulose, pili, flagella, and fimbriae [9]. The physical distances between microcolonies during the initial formation result in voids that ultimately become pore and channels which facilitate nutrient and liquid transport within the biofilm [9, 10]. The formation of the matrix results in emergent properties that help the biofilms survive in the environment. The matrix provides localized gradients allowing for different populations of bacteria to survive various niches and utilize different metabolic pathways for survival. The material of the matrix also functions to absorb resources from the surrounding environment. The matrix also serves to sequester secreted enzymes resulting in a de facto external digestive system. This environment enables social behavior between bacterial species, both cooperative and competitive [10, 18, 35]. Since the matrix is a semisolid gel, the matrix can also form a skin and retain water protecting the biofilm from dehydration. Its gel-like nature also allows the migration of bacteria in the

biofilm and in some cases can represent population efforts that parallel the division of labor [9, 10]. A key advantage of matrix formation is tolerance and resistance from chemotherapy, host defenses, and predation by *Protista* [45].

Biofilm Resistance

One of the prime advantages of the EPS matrix is the protection from antimicrobials, the immune system, and predators. In some cases, it has been noted that to affect biofilms sometimes up to 1000-fold or more, antibiotics are required to kill the planktonic form of the same bacteria [46]. Biofilm resistance is a multifactorial process involving the biology, chemistry, and physics of the biofilm [12]. The factors that have been associated with the increase in antibiotic resistance are gradients (oxygen, nutrients, slowed agent diffusion, etc.) stress responses, gene expression (resistance factors), dormancy, and tolerance [3, 7, 8, 10–13, 15, 47, 48]. Gradients present in the biofilm can result from the diffusion of agents into the biofilm resulting in sublethal concentrations of antibiotics selecting for resistance. Gradients in nutrients and oxygen lead to zones of decreased metabolism and dormant bacteria [10, 48]. The slowing metabolism of these phenotypes can affect antimicrobials that require active cellular metabolism for efficacy [49–51]. The enzyme sequestering effects of the matrix can lead to antimicrobial deactivation and the matrix components can complex with antimicrobials leading to chelation and precipitation of these agents [10]. Close proximity of bacteria in a biofilm facilitate horizontal gene transfer of resistance mechanisms, especially under conditions of environmental stress [2, 7, 52]. In addition, preexisting drug resistance could be present in biofilms. One of the most commonly used agents to treat biofilm infections is the ansamycin antibiotic rifampicin. While highly efficacious, this RNA synthesis targeting agent requires a single mutation in the *rpoB* gene to confer resistance. In vitro resistance determination studies have found that the frequency of mutation conferring rifampin resistance is between 10^{-7} and 10^{-8} [53–55]. Base on this frequency, if the biofilm being treated has a population of 10^9 cfu, then by random chance there are approximately 10–100 bacteria that have the mutation conferring rifampicin resistance. Thus, monotherapy treatment with antibiotics will result in enrichment of the mutant population and addressing this requires extended therapy with drug cocktails to avoid this enrichment [56–58]. A final source of biofilm resistance is the subpopulation of persister cells that develop in biofilms. This cell phenotype can survive high levels of antibacterials while lacking any specific resistance mechanisms [7, 47, 59]. These dormant cells can survive blocking the activity of antibacterials by depriving them of targets through metabolic inactivity and remain dormant [47]. Eventually when environmental conditions permit, these cells will emerge from dormancy and proliferate. The exact mechanisms of persister formation are unknown but current theories center on toxin and antitoxin systems (TA) [47, 60]. The five classes of TA systems are composed of a stable protein toxin that disrupts an essential metabolic function and a labile antitoxin which is coded in an operon (see Table 2).

Table 2 The five toxin–antitoxin systems with their regulatory elements and mechanisms of actions [61]

Type	Regulatory element	Mechanism of action
I	sRNA	Binding to toxin mRNA preventing ribosome binding
II	Protein	DNA binding that suppresses toxin transcription
III	RNA–protein complex	Toxin function inhibited by interaction with pseudoknots antitoxin RNA
IV	Protein	Blocking of toxin target site on cytoskeletal proteins
V	Endoribonuclease	Cleavage of toxin mRNA

All of these systems are believed to play an active role in the generation of persister populations in biofilms

This arrangement results in tight co-transcription and translation [60, 61]. One of the key drivers of persister formation is environmental stress, especially antibiotic treatment. It is believed that the TA system activity is modulated by the (p)ppGpp signaling nucleotide and that persister cells can spontaneously form in bacterial populations [61].

Biofilms are also highly resistant to clearance by the immune system. When a device is implanted, especially internal implants, the procedure can result in localized acute and chronic inflammation which can lead to a foreign body reaction. The implantation results in localized acute and chronic inflammation plus a foreign body reaction to the implant [2, 19, 20]. Ultimately a fibrous capsule forms around the implant resulting in a zone of suppressed immune response know as a *locus minoris resistentiae* which can increase the chance of infection and biofilm formation [2, 62, 63]. Studies have also uncovered that biomaterial implants can also alter immune cell responses. The implanted biomaterial can activate the complement system, platelets, and neutrophils. Chronic inflammatory responses may lead to neutrophil exhaustion, depletion of oxidative species and "frustrated phagocytosis" while other demonstrate that leukocytes can react and then penetrate the biofilm [2]. Studies performed in animal models with *S. aureus* suggest that the immune response may skew from the traditional pro-inflammatory response to a pro-fibrotic response. The *S. aureus* biofilm was able to alter macrophage responses toward an anti-inflammatory response with significant reductions in IL-1β, TNF-α, CXCL2, and CCL2 expression [2, 14]. In addition to dampening the inflammation, *S. aureus* biofilms have been shown to change macrophage responses to the M2 phenotype and immune suppressive T cell response by increased expression of *Arg1* [64, 65]. *S. aureus* can also induce dysfunction and death in macrophages via various toxins, including Leukocidin [64]. *Pseudomonas aeruginosa* biofilms have been found to suppress neutrophils disrupting the response and reducing neutrophil oxidation potential [66].

Diagnosis of Biofilm Infections on Medical Devices

Diagnosis of infections on biofilm-infected devices is commonly determined using traditional microbial growth means. For orthopedic devices, the device itself is sampled with three to six biopsies of the surrounding tissues [67]. Sonication of the

devices or samples to remove the adherent bacteria has proven to be superior to identifying delayed and late infections compared to a tissue sample, histology, and synovial culture [2, 3, 46, 68, 69]. In most cases, removal of the device or sampling of the surface and associate materials (respiratory secretions, urine samples, etc.) are used to confirm the presence of a biofilm [67]. These conventional methods are not without drawback. The main challenge is that it is difficult to survey the presence of small colony variants in biofilms on different regions of medical implants. To overcome such limitations, several new methods have been investigated in recent years. For example, indirect methods of diagnosis have been successfully used to confirm implant infection including immunoglobulin assays, the inflammatory marker C-reactive protein, and histopathological evaluation of samples [70]. Other diagnostic methods include PCR (which can also screen for drug resistance markers) [3, 70], next-generation sequencing, fluorescent in situ hybridization (FISH), Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF) mass spectroscopy, and assay of α-defensin levels in the synovial fluid [2, 67].

Of recent interest has been the incorporation of nuclear medicine in visualization of infected implanted devices and foci of infection. These methods have included computerized tomography (CT) magnetic resonance imaging (MRI), ultrasound, and radionucleotide methods such as Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET). Current applications include combinations of both screening modalities to generate anatomical information via CT scanning with the labeling data from either SPECT or PET (SPECT/CT or PET/CT) [71–73]. CT imaging utilizes X-rays to generate three-dimensional slices of the target while SPECT incorporates the gamma ray emissions from a radioisotope to show specific areas of interest via the radiolabel's interactions with the target (see Fig. 3). PET looks for the localization of specific radioisotope accumulation and measures the emission of gamma photons from positron annihilations at 511 KeV which results in these photons moving in opposite directions. The impact of these photons on detectors results in a simultaneous detection event that can be used to construct a three-dimensional image of the areas where the radioisotopes have accumulated. Many of the combination systems are already available from commercial vendors and have seen use clinically [71, 72, 74–76]. SPECT/CT has been used to visualize a wide variety of infections including osteomyelitis, prosthetic joint infections, mixed infections, infectious endocarditis, and infected cardiac implant devices [72]. Currently, PET has been used to image tumors in cancer patients indirectly by using radiolabels on metabolites that have enhanced uptake in tumors or white blood cells (WBCs) migrating to the site of the tumor (see Table 3) [77–80]. While these methods have been shown to work, many rely on indirect measurements looking at a paired response to infection, such as metabolite update and infiltration of immune cells and modulators [79, 81, 82]. More direct approaches have recently been successfully attempted using tagged antibodies, antimicrobials, and molecules that are utilized by the infecting pathogens including the differentiation between Gram-positive and Gram-negative infections [75, 83–90]. While targeting by antimicrobials and immune cells is a proven approach, it must be considered that labeled agents of this type could generate a skewed or no signal based on killing of the target. An approach taken by Ordonez et al. [91] has used in silico screening to identify radiolabeled molecules that are

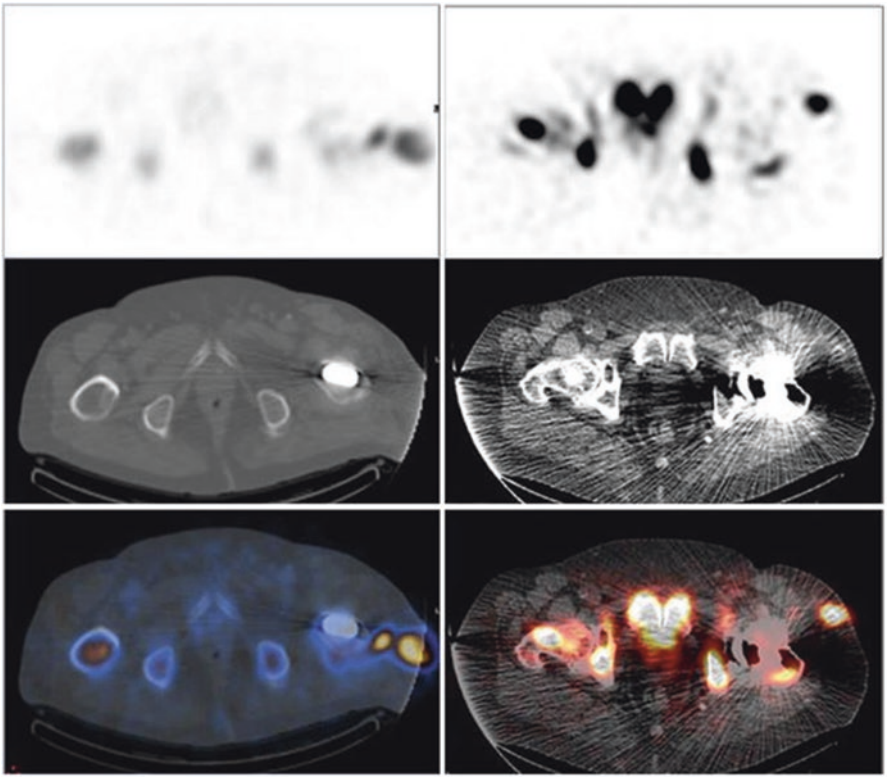


Fig. 3 SPECT/CT images of infections in two patients with prosthetic hip infections using SPECT/CT. The upper panels show the emission while the middle panels show the CT images while the bottom panels show the superimposed images of 99mTC-HMPAO WBC uptake. Reprinted with permission from Springer Nature, Clinical and Translational Imaging, Erba and Israel [72]

Table 3 PET radiolabels used to detect inflammation and infection

Tracer	Abbreviation	Diagnosis	Reference
^{18}F-fluorodeoxyglucose (FDG)	^{18}F-FDG	Tuberculosis *S. aureus* biofilm infection Bacterial infection	Ankrah et al. [83], Neumann et al. [85], Ordonez and Jain [87], Palestro and Love [74] and Signore et al. [76]
^{18}F-labeled glutamate analogs	BAY 94-9392 BAY 85-8050	Cancer	Koglin et al. [77] and Krasikova et al. [78]
Labeled white blood cells	^{111}In-WBCs ^{99m}Tc-WBC	Bacterial infection	Neumann et al. [85], Signore et al. [76] and Erba and Israel [72]
D-[methyl-^{11}C]-methionine	[^{11}C]-D-Met	Bacterial infection	Neumann et al. [85]
^{68}Ga-labled phage display peptides	^{68}Ga-A9-K-DOTA	*S. aureus* biofilm	Nielsen et al. [86]
[^{18}F]-fluoropropyl-trimethoprim	[^{18}F]-FPTMP	Bacterial infection	Sellmyer et al. [75]
2-[^{18}F]-fluorodeoxysorbital	^{18}F-FDS	Bacterial infection	Weinstein et al. [90]

These have been used successfully to identify tumors or infection

specifically taken up by bacteria and are not antimicrobial. There results identified ten promising leads that identified three lead candidates (Para-aminobenzoic acid or PABA, D-mannitol, and D-sorbital) that were successful in in vivo testing, specifically identifying infection sites in a murine model of myositis.

Both the SPECT and PET methods have limitations to their use that must be accounted for in the final interpretation of the results and to prevent misdiagnosis. With the indirect visualization of infection, distinctions between sterile inflammation and actual infection must be made with the approach of infection-specific tracers allowing this differentiation [75, 85, 89]. In the cases of combined systems (SPEC/CT and PET/CT) allowances must be made for the proximity of the two independent screening modalities in the physical design of the device [92] and CT measurements have to take into account photon attenuation and correction for scattering. An example of successful imaging is shown in the SPECT/CT scan in Fig. 3 from two different patients with suspected prosthetic hip infections. The top image is the emission of the tracer administered to both patients while the second image is the traditional CT scan. The final set of images is the superposition of both of the SPECT and CT images. By the combination of these results, the clinicians were able to specifically identify that the infection was limited to either the soft tissue and posterior aspect of the prothesis or the peri-prosthetic soft tissue. This fusion of the imaging technology has further allowed the identification of the cortical, corticomedullary, and subperiosteal foci of chronic osteomyelitis with a specificity value of 89% and a sensitivity of 100% [72, 93]. These results would allow for a targeted intervention if surgery and debridement would be required or allow noninvasive monitoring of efficacy of pharmaceutical treatment.

Treatment of Biofilm Infections on Medical Devices

Treatment for device-related infections varies with the type of device and the location. In the case of peripheral devices, the easiest course is to remove the device and treat the infection with antibiotics [67]. In some cases, central venous catheters can be kept in place and treated using antimicrobial lock therapy typically with combinations of disinfectants and antibiotic at elevated levels above the minimum inhibitory concentration (MIC). With implanted devices such as prosthetic joints, the timing of the detection is critical. Infections occurring within 3 weeks of surgery can be treated with antibiotic therapy with a 70–90% success rate. For delayed or late infections, the device is usually removed to ensure that the biofilm is eradicated. The gold standard treatment is a two-stage surgical procedure where the infected device is removed, and the devitalized tissue is debrided. An antibiotic-impregnated filler is placed in the wound and at least 6 weeks of antimicrobial therapy is carried out [94]. At the completion of antibiotic therapy, the new sterile device is implanted. The success rate for the two-stage procedure is 93–100% [2, 46, 70]. Antibiotic therapy for the treatment of these infections is typically a combination therapy of rifampin, a fluoroquinolone followed by a glycopeptide [2, 70, 94, 95]. Other options in the combination therapy include daptomycin, linezolid,

tigecycline, cephalosporins and carbapenems [67], amoxicillin and trimethoprim-sulfamethoxazole [46].

Due to increasing rates of antimicrobial resistance mechanisms and the inherent resistance of biofilms, some novel approaches to dealing with biofilm infection are being explored. Therapy using bacteriophages and cocktails of bacteriophages are being used against biofilms including phages that lyse the target bacteria and phage-encoded enzymes to dissolve the EPS matrix [96]. Phages were used as successful therapeutic agents by the former Soviet Union and Eastern European countries [97]. In 2017, a personalized cocktail targeting drug resistant *Acinetobacter baumannii* successfully cleared a persistent infection in a clinical setting illustrating the utility of this therapeutic approach [98]. The incorporation of phage therapy also has been reported to enhance the efficacy of antibiotics against *S. aureus* biofilms in vitro [99]. Another novel therapeutic approach being explored is the use of antimicrobial peptides (AMPs). AMPs are small positively charged peptides secreted by virtually every type of organisms to combat pathogens [100–102]. The AMP Database as of 2019 contains a total of 3055 entries from all the kingdoms of life (Protista, Archaebacteria, Eubacteria, Plants, Fungi, and Animals) [103]. The mode of action of these ubiquitous agents is through membrane disruption and depolarization but recently evidence has been mounting that there are additional targets within bacteria such as translation, transcription, and replication that are affected by these peptides [100, 101, 104]. Currently, there are several classes of AMPs used clinically as systemic and topical agents including colistin, polymyxin B, nisin and bacitracin in addition to synthetic AMPs in development [104–106].

A challenge posed by is the modification of the environment around the wound is that it typically becomes anoxic and mildly acidic (pH 5.0). Acidic pH values can both enhance or inhibit the activity of antibiotics [107, 108]. The MICs for gentamicin against *S. aureus* increase as pH decreases while the opposite holds true for oxacillin [109]. In purulent wounds, the bactericidal activity of ciprofloxacin and imipenem is inhibited [110]. A novel approach to adapting agents to this acidic environment is the design targeted delivery systems that only activate in these mildly acidic conditions. A pH activated targeted delivery system has been tried using poly(D,L,-lactic-co-glycolic acid) (PGLA) nanoparticles that were laced with PEG to prevent nonspecific interactions. To provide specificity to the target bacteria, a poly-L-lysine was incorporated that becomes a positively charged cationic moiety by gaining electrons at an acidic pH. This technology was successfully used to deliver vancomycin to *S. aureus* in an in vitro system [110]. The targeted delivery concept has also been applied to AMPs. Modification of the Cardin and Weintraub heparin-binding sequences (AKKARA and ARKKAAKA) with histidines yielded membrane damaging antimicrobials that only were activated under acidic conditions and were active against Gram-negative, Gram-positive, and yeast [111]. A similar approach has shown in vivo efficacy against *H. pylori* infection, a causative organism in the generation of stomach ulcers. This pH responsive polypeptide AMP was designed with a random distribution of positive and negative residues which, under a physiological pH adopted a nontoxic, inactive random configuration. When exposed to acidic conditions, the AMP transitioned to the antimicrobial helical configuration [112].

Conclusion and Summary

Device-related infection remains to be a major burden on the health care system. With the recent improved knowledge on the pathogenesis of bacterial infection, we may be able to develop new methods for the detection of bacterial activities and eradication of biofilm-encapsulated microorganisms surrounding implanted medical devices. Equally important is the need for more studies to explore the possibility of designing medical device surfaces that can reduce bacterial colonization while restoring "normal" antimicrobial responses of immune cells. It is our belief that such a biological response-oriented approach will help in the creation of next-generation medical devices with significantly improved safety and functionality.

References

1. Lebeaux D, Ghigo J-M, Beloin C (2014) Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiol Mol Biol Rev 78:510–543
2. Arciola CR, Campoccia D, Montanaro L (2018) Implant infections: adhesion, biofilm formation and immune evasion. Nat Rev Microbiol 16:397–409
3. Percival SL, Suleman L, Vuotto C, Donelli G (2015) Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. J Med Microbiol 64:323–334
4. Akgün D, Müller M, Perka C, Winkler T (2018) An often-unrecognized entity as a cause of recurrent infection after successfully treated two-stage exchange arthroplasty: hematogenous infection. Arch Orthop Trauma Surg 138:1199–1206
5. Dennison T, Alentorn-Geli E, Assenmacher AT, Sperling JW, Sánchez-Sotelo J, Cofield RH (2017) Management of acute or late hematogenous infection after shoulder arthroplasty with irrigation, débridement, and component retention. J Shoulder Elbow Surg 26:73–78
6. Donlan RM (2001) Biofilms and device-associated infections. Emerg Infect Dis 7:277
7. Davies D (2003) Understanding biofilm resistance to antibacterial agents. Nat Rev Drug Discov 2:114–122
8. Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8:881–890
9. Flemming H-C, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8:623–633
10. H-c F, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S (2016) Biofilms: an emergent form of bacterial life. Nat Rev Microbiol 14:563–575
11. Hall-Stoodley L, Costerton JW, Stoodley P (2004) Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2:95
12. Stewart P (2018) How bacteria in biofilms withstand antibiotics. Montana Biofilm Science and Technology Meeting, 2018
13. Stewart PS, Franklin MJ, Williamson KS, Folsom JP, Boegli L, James GA (2015) Contribution of stress responses to antibiotic tolerance in Pseudomonas aeruginosa biofilms. Antimicrob Agents Chemother 59:3838–3847
14. Thurlow LR, Hanke ML, Fritz T, Angle A, Aldrich A, Williams SH, Engebretsen IL, Bayles KW, Horswill AR, Kielian T (2011) Staphylococcus aureus biofilms prevent macrophage phagocytosis and attenuate inflammation in vivo. J Immunol 186:6585–6596
15. Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science 284:1318–1322
16. Dunne WM Jr (2002) Bacterial adhesion: seen any good biofilms lately? Clin Microbiol Rev 15:155–166

17. Kaplan JB (2010) Biofilm dispersal: mechanisms, clinical implications, and potential therapeutic uses. J Dent Res 89:205–218
18. Li Y-H, Tian X (2012) Quorum sensing and bacterial social interactions in biofilms. Sensors 12:2519–2538
19. Liping T, Paul T, Wenjing H (2008) Surface chemistry influences implant biocompatibility. Curr Top Med Chem 8:270–280
20. Tang L, Hu W (2005) Molecular determinants of biocompatibility. Expert Rev Med Devices 2:493–500
21. Neu TR (1996) Significance of bacterial surface-active compounds in interaction of bacteria with interfaces. Microbiol Rev 60:151–166
22. Arciola CR, Bustanji Y, Conti M, Campoccia D, Baldassarri L, Samorì B, Montanaro L (2003) Staphylococcus epidermidis–fibronectin binding and its inhibition by heparin. Biomaterials 24:3013–3019
23. Patti JM, Allen BL, McGavin MJ, Höök M (1994) MSCRAMM-mediated adherence of microorganisms to host tissues. Annu Rev Microbiol 48:585–617
24. Herrmann M, Vaudaux PE, Pittet D, Auckenthaler R, Lew PD, Schumacher-Perdreau F, xe o, Peters G, Waldvogel FA (1988) Fibronectin, fibrinogen, and laminin act as mediators of adherence of clinical staphylococcal isolates to foreign material. J Infect Dis 158:693–701
25. Foster TJ, Geoghegan JA, Ganesh VK, Höök M (2014) Adhesion, invasion and evasion: the many functions of the surface proteins of Staphylococcus aureus. Nat Rev Microbiol 12:49–62
26. Herman-Bausier P, El-Kirat-Chatel S, Foster TJ, Geoghegan JA, Dufrêne YF (2015) Staphylococcus aureus fibronectin-binding protein A mediates cell-cell adhesion through low-affinity homophilic bonds. MBio 6:e00413–e00415
27. Imberty A, Wimmerová M, Mitchell EP, Gilboa-Garber N (2004) Structures of the lectins from Pseudomonas aeruginosa: insights into the molecular basis for host glycan recognition. Microbes Infect 6:221–228
28. Watnick PI, Fullner KJ, Kolter R (1999) A role for the mannose-sensitive hemagglutinin in biofilm formation by Vibrio cholerae El Tor. J Bacteriol 181:3606–3609
29. Mack D, Riedewald J, Rohde H, Magnus T, Feucht HH, Elsner HA, Laufs R, Rupp ME (1999) Essential functional role of the polysaccharide intercellular adhesin of Staphylococcus epidermidis in hemagglutination. Infect Immun 67:1004–1008
30. Mack D, Nedelmann M, Krokotsch A, Schwarzkopf A, Heesemann J, Laufs R (1994) Characterization of transposon mutants of biofilm-producing Staphylococcus epidermidis impaired in the accumulative phase of biofilm production: genetic identification of a hexosamine-containing polysaccharide intercellular adhesin. Infect Immun 62:3244–3253
31. Li H, Xu L, Wang J, Wen Y, Vuong C, Otto M, Gao Q (2005) Conversion of Staphylococcus epidermidis strains from commensal to invasive by expression of the ica locus encoding production of biofilm exopolysaccharide. Infect Immun 73:3188–3191
32. Leung JW, Liu YL, Desta T, Libby E, Inciardi JF, Lam K (1998) Is there a synergistic effect between mixed bacterial infection in biofilm formation on biliary stents? Gastrointest Endosc 48:250–257
33. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM (1995) Microbial biofilms. Annu Rev Microbiol 49:711–745
34. Kreft J-U (2004) Biofilms promote altruism. Microbiology 150:2751–2760
35. Kuramitsu HK, He X, Lux R, Anderson MH, Shi W (2007) Interspecies interactions within oral microbial communities. Microbiol Mol Biol Rev 71:653–670
36. Hawver LA, Jung SA, Ng W-L, Shen A (2016) Specificity and complexity in bacterial quorum-sensing systems. FEMS Microbiol Rev 40:738–752
37. de Kievit TR, Iglewski BH (2000) Bacterial quorum sensing in pathogenic relationships. Infect Immun 68:4839–4849
38. Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55:165
39. Le KY, Otto M (2015) Quorum-sensing regulation in staphylococci—an overview. Front Microbiol 6:1174

40. Antunes LCM, Ferreira RBR, Buckner MMC, Finlay BB (2010) Quorum sensing in bacterial virulence. Microbiology 156:2271–2282
41. Juhas M, Eberl L, Tümmler B (2005) Quorum sensing: the power of cooperation in the world of Pseudomonas. Environ Microbiol 7:459–471
42. Sircili MP, Walters M, Trabulsi LR, Sperandio V (2004) Modulation of enteropathogenic Escherichia coli virulence by quorum sensing. Infect Immun 72:2329–2337
43. Armbruster CE, Hong W, Pang B, Weimer KED, Juneau RA, Turner J, Swords WE (2010) Indirect pathogenicity of Haemophilus influenzae and Moraxella catarrhalis in polymicrobial otitis media occurs via interspecies quorum signaling. MBio 1:e00102–e00110
44. Kostakioti M, Hadjifrangiskou M, Hultgren SJ (2013) Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. Cold Spring Harb Perspect Med 3:a010306–a010306
45. Matz C, McDougald D, Moreno AM, Yung PY, Yildiz FH, Kjelleberg S (2005) Biofilm formation and phenotypic variation enhance predation-driven persistence of Vibrio cholerae. Proc Natl Acad Sci U S A 102:16819–16824
46. Hogan S, Stevens NT, Humphreys H, O'Gara JP, O;Neill E (2015) Current and future approaches to the prevention and treatment of staphylococcal medical device-related infections. Curr Pharm Des 21:100
47. Lewis K (2007) Persister cells, dormancy and infectious disease. Nat Rev Microbiol 5:48–56
48. Roberts ME, Stewart PS (2004) Modeling antibiotic tolerance in biofilms by accounting for nutrient limitation. Antimicrob Agents Chemother 48:48–52
49. English BK (2014) Limitations of beta-lactam therapy for infections caused by susceptible Gram-positive bacteria. J Infect 69:S5–S9
50. Hausler WJ (1996) Antibiotics in laboratory medicine, vol 29, 4th edn. Wilkins & Wilkins, Baltimore, MD
51. Stevens DL, Gibbons AE, Bergstrom R, Winn V (1988) The Eagle Effect revisited: efficacy of clindamycin, erythromycin, and penicillin in the treatment of Streptococcal myositis. J Infect Dis 158:23–28
52. Savage VJ, Chopra I, O'Neill AJ (2013) Staphylococcus aureus biofilms promote horizontal transfer of antibiotic resistance. Antimicrob Agents Chemother 57:1968–1970
53. Curry SR, Marsh JW, Shutt KA, Muto CA, O'Leary MM, Saul MI, Pasculle AW, Harrison LH (2009) High frequency of rifampin resistance identified in an epidemic Clostridium difficile clone from a large teaching hospital. Clin Infect Dis 48:425–429
54. Morosini M-I, Baquero M-R, Sánchez-Romero JM, Negri M-C, Galán J-C, Campo RD, Pérez-Díaz JC, Baquero F (2003) Frequency of mutation to rifampin resistance in *Streptococcus pneumoniae* clinical strains: *hexA* and *hexB* polymorphisms do not account for hypermutation. Antimicrob Agents Chemother 47:2064–2064
55. O'Neill AJ, Chopra I, Cove JH (2001) Mutation frequencies for resistance to fusidic acid and rifampicin in Staphylococcus aureus. J Antimicrob Chemother 47:647–650
56. Croes S, Beisser PS, Neef C, Bruggeman CA, Stobberingh EE (2010) Unpredictable effects of rifampin as an adjunctive agent in elimination of rifampin-susceptible and -resistant Staphylococcus aureus strains grown in biofilms. Antimicrob Agents Chemother 54:3907–3912
57. Floss HG, Yu T-W (2005) Rifamycin-mode of action, resistance, and biosynthesis. Chem Rev 105:621
58. Wichelhaus TA, Böddinghaus B, Besier S, Schäfer V, Brade V, Ludwig A (2002) Biological cost of rifampin resistance from the perspective of Staphylococcus aureus. Antimicrob Agents Chemother 46:3381–3385
59. Singh R, Ray P, Das A, Sharma M (2009) Role of persisters and small-colony variants in antibiotic resistance of planktonic and biofilm-associated Staphylococcus aureus: an in vitro study. J Med Microbiol 58:1067–1073
60. Wood TK, Knabel SJ, Kwan BW (2013) Bacterial persister cell formation and dormancy. Appl Environ Microbiol 79:7116–7121

61. Wen Y, Behiels E, Devreese B (2014) Toxin–antitoxin systems: their role in persistence, biofilm formation, and pathogenicity. Pathog Dis 70:240–249
62. Bouvresse S, Chiras J, Bricaire F, Bossi P (2006) Pott's disease occurring after percutaneous vertebroplasty: an unusual illustration of the principle of locus minoris resistentiae. J Infect 53:e251–e253
63. Chan ED, Po-Marn K, Kevin F, Anthony PD, Iseman MD (2001) Vertebral osteomyelitis due to infection with Nontuberculous mycobacterium species after blunt trauma to the back: 3 examples of the principle of Locus Minoris Resistentiae. Clin Infect Dis 32:1506–1510
64. Scherr T, Heim C, Morrison J, Kielian T (2014) Hiding in plain sight: interplay between Staphylococcal biofilms and host immunity. Front Immunol 5:37
65. Prabhakara R, Harro JM, Leid JG, Harris M, Shirtliff ME (2011) Murine immune response to a chronic Staphylococcus aureus biofilm infection. Infect Immun 79:1789
66. Jesaitis AJ, Franklin MJ, Berglund D, Sasaki M, Lord CI, Bleazard JB, Duffy JE, Beyenal H, Lewandowski Z (2003) Compromised host defense on Pseudomonas aeruginosa biofilms: characterization of neutrophil and biofilm interactions. J Immunol 171:4329–4339
67. Høiby N, Bjarnsholt T, Moser C, Bassi GL, Coenye T, Donelli G, Hall-Stoodley L, Holá V, Imbert C, Kirketerp-Møller K, Lebeaux D, Oliver A, Ullmann AJ, Williams C, Biofilms ESGf, Consulting External Expert Werner Z (2015) ESCMID guideline for the diagnosis and treatment of biofilm infections 2014. Clin Microbiol Infect 21:S1–S25
68. Vasoo S (2018) Improving the diagnosis of orthopedic implant-associated infections: optimizing the use of tools already in the box. J Clin Microbiol 56:e01379–e01318
69. Xu Y, Larsen LH, Lorenzen J, Hall-Stoodley L, Kikhney J, Moter A, Thomsen TR (2017) Microbiological diagnosis of device-related biofilm infections. APMIS 125:289–303
70. Parikh MS, Antony S (2015) A comprehensive review of the diagnosis and management of prosthetic joint infections in the absence of positive cultures. J Infect Public Health 9:545–556
71. Ady J, Fong Y (2014) Imaging for infection: from visualization of inflammation to visualization of microbes. Surg Infect (Larchmt) 15:700–707
72. Erba PA, Israel O (2014) SPECT/CT in infection and inflammation. Clin Transl Imaging 2:519–535
73. Granov A, Tiutin L, Schwarz T (2013) Positron Emission Tomography, 1. Aufl., 1st edn. Springer, Heidelberg
74. Palestro CJ, Love C (2017) Role of nuclear medicine for diagnosing infection of recently implanted lower extremity arthroplasties. Semin Nucl Med 47:630–638
75. Sellmyer MA, Lee I, Hou C, Weng C-C, Li S, Lieberman BP, Zeng C, Mankoff DA, Mach RH (2017) Bacterial infection imaging with [^{18}F]fluoropropyl-trimethoprim. Proc Natl Acad Sci 114:8372–8377
76. Signore A, Glaudemans AWJM, Gheysens O, Lauri C, Catalano OA (2017) Nuclear medicine imaging in pediatric infection or chronic inflammatory diseases. Semin Nucl Med 47:286–303
77. Koglin N, Mueller A, Berndt M, Schmitt-Willich H, Toschi L, Stephens AW, Gekeler V, Friebe M, Dinkelborg LM (2011) Specific PET imaging of x_c^-transporter activity using a 18F-labeled glutamate derivative reveals a dominant pathway in tumor metabolism. Clin Cancer Res 17:6000–6011
78. Krasikova RN, Kuznetsova OF, Fedorova OS, Belokon YN, Maleev VI, Mu L, Ametamey S, Schubiger PA, Friebe M, Berndt M, Koglin N, Mueller A, Graham K, Lehmann L, Dinkelborg LM (2011) 4-[18F]Fluoroglutamic Acid (BAY 85-8050), a new amino acid radiotracer for PET imaging of tumors: synthesis and in vitro characterization. J Med Chem 54:406–410
79. Vallabhajosula S, Solnes L, Vallabhajosula B (2011) A broad overview of positron emission tomography radiopharmaceuticals and clinical applications: what is new? Semin Nucl Med 41:246–264
80. Wang L, Zha Z, Qu W, Qiao H, Lieberman BP, Plössl K, Kung HF (2012) Synthesis and evaluation of 18F labeled alanine derivatives as potential tumor imaging agents. Nucl Med Biol 39:933–943

81. Shukla AK, Kumar U (2006) Positron emission tomography: an overview. J Med Phys 31:13–21
82. Vaquero JJ, Kinahan P (2015) Positron emission tomography: current challenges and opportunities for technological advances in clinical and preclinical imaging systems. Annu Rev Biomed Eng 17:385–414
83. Ankrah AO, Glaudemans AWJM, Maes A, Van de Wiele C, Dierckx RAJO, Vorster M, Sathekge MM (2018) Tuberculosis. Semin Nucl Med 48:108–130
84. Kouijzer IJE, Mulders-Manders CM, Bleeker-Rovers CP, Oyen WJG (2018) Fever of unknown origin: the value of FDG-PET/CT. Semin Nucl Med 48:100–107
85. Neumann KD, Villanueva-Meyer JE, Mutch CA, Flavell RR, Blecha JE, Kwak T, Sriram R, VanBrocklin HF, Rosenberg OS, Ohliger MA, Wilson DM (2017) Imaging active infection in vivo using D-amino acid derived pet radiotracers. Sci Rep 7:7903
86. Nielsen KM, Kyneb MH, Alstrup AKO, Jensen JJ, Bender D, Schønheyder HC, Afzelius P, Nielsen OL, Jensen SB (2016) 68Ga-labeled phage-display selected peptides as tracers for positron emission tomography imaging of Staphylococcus aureus biofilm-associated infections: selection, radiolabelling and preliminary biological evaluation. Nucl Med Biol 43:593–605
87. Ordonez AA, Jain SK (2018) Pathogen-specific bacterial imaging in nuclear medicine. Semin Nucl Med 48:182–194
88. Rice SL, Roney CA, Daumar P, Lewis JS (2011) The next generation of positron emission tomography radiopharmaceuticals in oncology. Semin Nucl Med 41:265–282
89. Salmanoglu E, Kim S, Thakur ML (2018) Currently available radiopharmaceuticals for imaging infection and the Holy Grail. Semin Nucl Med 48:86–99
90. Weinstein EA, Ordonez AA, DeMarco VP, Murawski AM, Pokkali S, MacDonald EM, Klunk M, Mease RC, Pomper MG, Jain SK (2014) Imaging Enterobacteriaceae infection in vivo with ^{18}F-fluorodeoxysorbitol positron emission tomography. Sci Transl Med 6:259ra146–259ra146
91. Ordonez AA, Weinstein EA, Bambarger LE, Saini V, Chang YS, DeMarco VP, Klunk MH, Urbanowski ME, Moulton KL, Murawski AM, Pokkali S, Kalinda AS, Jain SK (2018) A systematic approach for developing bacteria-specific imaging tracers. J Nucl Med 58:144–150
92. Livieratos L (2015) Technical pitfalls and limitations of SPECT/CT. Semin Nucl Med 45:530–540
93. Horger M, Eschmann SM, Pfannenberg C, Storek D, Dammann F, Vonthein R, Claussen CD, Bares R (2003) The value of SPET/CT in chronic osteomyelitis. Eur J Nucl Med Mol Imaging 30:1665–1673
94. Chaussade H, Uçkay I, Vuagnat A, Druon J, Gras G, Rosset P, Lipsky BA, Bernard L (2017) Antibiotic therapy duration for prosthetic joint infections treated by debridement and implant retention (DAIR): similar long-term remission for 6 weeks as compared to 12 weeks. Int J Infect Dis 63:37–42
95. Sendi P, Zimmerli W (2012) Antimicrobial treatment concepts for orthopaedic device-related infection. Clin Microbiol Infect 18:1176–1184
96. Chan BK, Abedon ST (2015) Bacteriophages and their enzymes in biofilm control. Curr Pharm Des 21:85
97. Sulakvelidze A, Alavidze Z, Morris JG Jr (2001) Bacteriophage therapy. Antimicrob Agents Chemother 45:649–659
98. Schooley RT, Biswas B, Gill JJ, Hernandez-Morales A, Lancaster J, Lessor L, Barr JJ, Reed SL, Rohwer F, Benler S, Segall AM, Taplitz R, Smith DM, Kerr K, Kumaraswamy M, Nizet V, Lin L, McCauley MD, Strathdee SA, Benson CA, Pope RK, Leroux BM, Picel AC, Mateczun AJ, Cilwa KE, Regeimbal JM, Estrella LA, Wolfe DM, Henry MS, Quinones J, Salka S, Bishop-Lilly KA, Young R, Hamilton T (2017) Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant Acinetobacter baumannii infection. Antimicrob Agents Chemother 61:e00954–e00917

99. Dickey J, Perrot V (2019) Adjunct phage treatment enhances the effectiveness of low antibiotic concentration against Staphylococcus aureus biofilms in vitro. PLoS One 14:e0209390
100. Strempel N, Strehmel J, Overhage J (2015) Potential application of antimicrobial peptides in the treatment of bacterial biofilm infections. Curr Pharm Des 21:67
101. Jenssen H, Hamill P, Hancock REW (2006) Peptide antimicrobial agents. Clin Microbiol Rev 19:491–511
102. Zasloff M (2002) Antimicrobial peptides of multicellular organisms. Nature 415:389–395
103. Li X, Wang Z, Wang G (2015) APD3: the antimicrobial peptide database as a tool for research and education. Nucleic Acids Res 44:D1087–D1093
104. Li Y, Xiang Q, Zhang Q, Huang Y, Su Z (2012) Overview on the recent study of antimicrobial peptides: origins, functions, relative mechanisms and application. Peptides 37:207–215
105. Hermsen ED, Sullivan CJ, Rotschafer JC (2003) Polymyxins: pharmacology, pharmacokinetics, pharmacodynamics, and clinical applications, vol 17. Elsevier, New York, pp 545–562
106. Landman D, Georgescu C, Martin DA, Quale J (2008) Polymyxins revisited. Clin Microbiol Rev 21:449–465
107. Thomas J, Linton S, Corum L, Slone W, Okel T, Percival SL (2012) The affect of pH and bacterial phenotypic state on antibiotic efficacy. Int Wound J 9:428–435
108. Yang L, Wang K, Li H, Denstedt JD, Cadieux PA (2014) The influence of urinary pH on antibiotic efficacy against bacterial uropathogens. Urology 84:731.e1–731.e7
109. Baudoux P, Bles N, Lemaire S, Mingeot-Leclercq M-P, Tulkens PM, Van Bambeke F (2007) Combined effect of pH and concentration on the activities of gentamicin and oxacillin against Staphylococcus aureus in pharmacodynamic models of extracellular and intracellular infections. J Antimicrob Chemother 59:246–253
110. Bryant RE, Mazza JA (1989) Effect of the abscess environment on the antimicrobial activity of ciprofloxacin. Am J Med 87:S23–S27
111. Kacprzyk L, Rydengård V, Mörgelin M, Davoudi M, Pasupuleti M, Malmsten M, Schmidtchen A (2007) Antimicrobial activity of histidine-rich peptides is dependent on acidic conditions. Biochim Biophys Acta Biomembranes 1768:2667–2680
112. Xiong M, Bao Y, Xu X, Wang H, Han Z, Wang Z, Liu Y, Huang S, Song Z, Chen J, Peek RM Jr, Yin L, Chen L-F, Cheng J (2017) Selective killing of Helicobacter pylori with pH-responsive helix–coil conformation transitionable antimicrobial polypeptides. Proc Natl Acad Sci U S A 114:12675–12680

Insights into the Emergence, Clinical Prevalence, and Significance of *Staphylococcus aureus* Small Colony Variants

Derek E. Andreini, Christopher D. Bell, Malcolm Xing, and Bingyun Li

Abstract Small colony variants (SCVs) of *Staphylococcus aureus* (*S. aureus*), one of the most commonly observed pathogens, have been observed in clinical patients for more than half a century in a variety of infectious diseases (e.g., osteomyelitis, cystic fibrosis, endocarditis, skin infections, and abscess). The presence of *S. aureus* SCVs in patients has been rising and recent clinical studies have raised concerns about their potential roles in chronic and persistent infections. In this chapter, the emergency and clinical prevalence of *S. aureus* SCVs are examined; their characteristics and types of samples and techniques studied are discussed; and perspectives and recommendations for their diagnosis, pathogenesis, and treatment are proposed. Clinical cases involving *S. aureus* infections lasting for weeks or longer, or when pinpoint colonies are noted on routine cultures, should be screened for *S. aureus* SCVs and, if present, antibiotics that are effective in eliminating *S. aureus* SCVs must be considered.

Keywords Small colony variant · *Staphylococcus aureus* · Chronic infection · Persistent infection · Intracellular disease

Introduction

Staphylococcus aureus (*S. aureus*) is found to be most prevalent in inpatient specimens and second most prevalent in outpatient specimens [1]. Small colony variants (SCVs) of *S. aureus* have been reported in persistent and chronic infections as well

D. E. Andreini · C. D. Bell · B. Li (✉)
Department of Orthopaedics, School of Medicine, West Virginia University, Morgantown, WV, USA
e-mail: bili@hsc.wvu.edu

M. Xing
Department of Mechanical Engineering, University of Manitoba, Winnipeg, MB, Canada

The Children's Hospital Research Institute of Manitoba, Winnipeg, MB, Canada

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_8

as in secondary infections in genetic and acquired diseases. They have been observed in infections of implants (prosthetic joint, pacemaker), skin (cutaneous abscesses), tissue (muscular abscesses), bone (osteomyelitis), blood (bacterial sepsis), sinuses (sinusitis), and airways (cystic fibrosis or CF) [2–14].

The clinical presence of *S. aureus* SCVs has been documented since the 1950s when a dwarf colony was isolated from a skin abscess [15]. This subpopulation of *S. aureus* produced colonies that were noted to be nonpigmented and nonhemolytic [16]. In 1978, using eight clinical samples, *S. aureus* SCVs were acquired from blood, osteomyelitis, subcutaneous abscess, and cerebrospinal fluid [17]. These clinical isolates displayed delayed growth and reverted to the parent strain (normal-type or wild-type) when supplemented with certain nutritional needs [16]. Quite a few recent clinical reports have raised concerns about the roles of *S. aureus* SCVs in the occurrence and persistence of infections. In 2013, Yagci et al. examined 123 CF patients (out of a total of 248 patients) with persistent airway infections and, of these patients, 16% presented with *S. aureus* SCVs [18]. In 2014, Tande et al. retrospectively examined 35 patients (among a total of 134 infected patients) with periprosthetic joint infections caused by *S. aureus*; 28.6% of these patients were found to possess *S. aureus* SCVs [19].

Infections associated with *S. aureus* SCVs seem to be chronic and usually persist even after long antimicrobial therapies. SCVs, compared to their normal-type, are more resistant to traditional antibiotics and, because of their location within the cells and their reduced uptake of antimicrobial agents, they persist [20, 21]. Certain subtypes are also associated with different treatment types, such as menadione; hemin-dependent subtypes are probably associated with the use of aminoglycoside antibiotics (e.g., kanamycin, tobramycin, gentamicin, streptomycin, and others) [22].

To deal with established infections, delivering an effective and early antimicrobial treatment works the best. In the early stages of infection, localized antimicrobial applications are preferred because they act in the location they are needed, and do not cause as many side effects as the systemic circulation is bypassed. However, the use of antibiotics likely has contributed to the appearance of *S. aureus* SCVs [4, 23, 24]. Strategies for reducing or eliminating *S. aureus* SCVs in infections have yet to be developed, but the clinical implications of SCVs are apparent. Understanding the roles and mechanisms of *S. aureus* SCVs in infection seems to be important to chronic and recurrent infections, and putting an effective therapeutic strategy in place to decrease the chance of chronic and recurrent infections will save patients' frustration, time, and money.

What Are *S. aureus* SCVs and Their Characteristics?

S. aureus may be phagocytized by professional phagocytes (e.g., macrophages) and a fraction of the phagocytized bacteria may survive and reside intracellularly. Alarmingly, bacteria like *S. aureus* have also been found to enter human cells (nonprofessional phagocytes) that do not typically phagocytize foreign materials [25].

Adherence of *S. aureus* onto such host cell surfaces is a prerequisite for such invasions. *S. aureus* is known to express an array of adhesins on their surfaces including the microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). *S. aureus* utilizes these MSCRAMMs to adhere directly and efficiently to host cells or via bridging ligands with host proteins (e.g., fibronectin-binding proteins). Next, the attachment of *S. aureus* to the host cell surfaces can induce changes in the host cells' cytoskeleton which leads to the phagocytosis of *S. aureus* into the host cells [26]. Upon phagocytosis, in general, it requires several characteristics of *S. aureus* in order for them to survive intracellularly; these characteristics include resistance to the intracellular host defense mechanisms and no killing of the host cells (either by lysis or by inducing apoptosis). Once it has invaded host cells, *S. aureus* may be destroyed by the intracellular defense mechanisms, maintained as the normal-type in a relatively short time, or, for certain strains of *S. aureus*, switched to SCVs. For instance, upon infection with *S. aureus*, epithelial cells were found to contain mainly normal-type *S. aureus* in the beginning; however, the number of normal-type *S. aureus* decreased and the number of *S. aureus* SCVs increased with increasing postinfection time. *S. aureus* SCVs reached approximately 90% after 28 days [27].

S. aureus SCVs are phenotypically quite different from the normal-type strain and, clinically, their variations are mostly limited to types deficient in electron transport substrates (menadione and hemin) and those deficient in thiamine biosynthesis. As shown in Fig. 1, *S. aureus* SCVs were observed in at least one of the airway cultures from 24 pediatric patients with CF; 6 of these patients had menadione- and/or hemin-deficient SCVs [24]. The deficiencies of menadione, hemin, and thiamine caused the colonies to grow much more slowly when compared to the normal-type and kept them from being targeted by the host's intracellular defense mechanisms.

S. aureus SCVs are much smaller (10× difference in size), nonpigmented, and nonhemolytic colonies compared to the parent strain (normal-type or wild-type) (Fig. 2); their small size makes them frequently missed in hospital laboratories. Table 1 lists the characteristics of *S. aureus* SCVs. Their prominent features include decreased pigmentation and hemolysis, increased resistance to aminoglycosides, and an unstable colony phenotype. Unlike their normal-type, *S. aureus* SCVs are mostly non-virulent which allows them to be overlooked by host cell defenses. In the normal-type, *S. aureus* produces alpha-toxin which causes an intracellular signaling cascade that results in the lysis of host cells [28]. In *S. aureus* SCVs, the synthesis of alpha-toxin is downregulated and the host cells stay intact which provides these facultative bacteria a reservoir in which to persist [26]. Some SCV phenotypes could even survive the bacteriostatic environment of the lysosome; for instance, a menadione auxotroph SCV strain, obtained from an osteomyelitis patient, survived within the lysosome of endothelial cells for 48 h [29]. However, upon supplementing the deficient substrates (e.g., menadione, hemin, and thiamine), *S. aureus* SCVs may rapidly reverse to the normal-type and lyse the host cells [22]. It is believed that the ability of *S. aureus* SCVs to persist intracellularly is due in part to the ability of *S. aureus* to phenotypically switch from the parent strain to the SCVs.

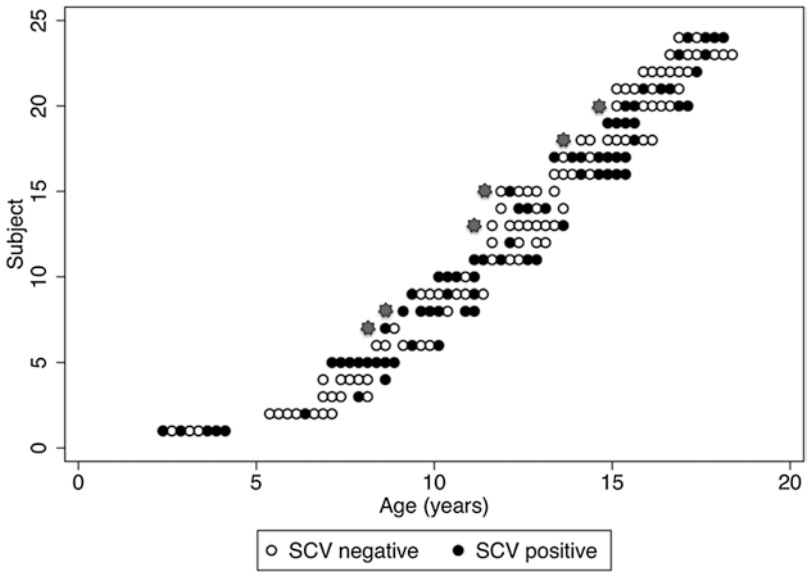


Fig. 1 Schematic of culture positivity for *S. aureus* SCVs among the 24 subjects from whom *S. aureus* SCV was isolated on one or more occasions. Each horizontal line of circles represents a series of culture results from one SCV-positive subject (each indicated by subject number on the *y*-axis) by age quarter during the study, with each culture plotted by subject age at the midpoint of each age quarter. Closed circles indicate cultures positive for *S. aureus* SCVs; open circles are negative. Asterisks indicate subjects who were culture positive for either menadione- or hemin-deficient *S. aureus* SCVs. (Reprinted with permission from Clinical Infectious Diseases 57:384–391 (2013) [24])

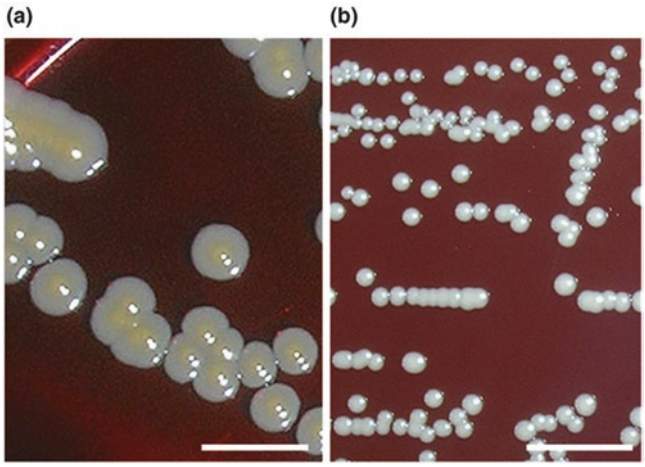


Fig. 2 Pictures of *S. aureus* of same clonal origin on sheep blood agar plates after 48 h incubation. Scale bar = 1 cm. (**a**) Normal-sized colonies, of 2–3 mm in size, show a typical golden pigmentation. (**b**) SCVs. (Reprinted with permission from Trends in Microbiology 17:54–58 (2009) [26])

Table 1 Characteristics of SCVs of *S. aureus*, as compared to parent strains

Phenotypic characteristics	
Colony size	10× smaller than normal-type colonies
Pigmentation	Weak
Hemolytic activity	Weak
Coagulase production	Weak
Resistance toward aminoglycosides	Increased
Auxotrophism	Present
Growth	Slow
Cell wall	Thick
Electrical potential across membrane	Low
Metabolism	
Tricarboxylic acid cycle	Reduced
Acetate catabolism	Reduced
Arginine deiminase pathway	Increased
Virulence determinants	
Toxin production	Weak or absent
Clumping factor	Increased levels
Fibronectin binding proteins	Increased levels
Polysaccharide intercellular adhesion	Increased
RNAIII	Very low levels
sigB	Upregulated
agr	Downregulated
hla	Downregulated

Reprinted with permission from Trends in Microbiology 17:54–58 (2009) [26]

Screening and Identification of *S. aureus* SCVs from Clinical Patient Samples

S. aureus SCVs have been isolated from a variety of clinical patient samples such as blood, bone, bronchial secretion, bronchoalveolar lavage, cerebrospinal fluid, joint aspiration, tissue aspirate, nares sample, oropharyngeal swab, pus, skin tissue, and sputum (Table 2). They have been characterized mainly by culturing for relatively long time periods and auxotrophy tests (Table 3). *S. aureus* SCVs are typically cultured, isolated, and identified as pinpoint, nonpigmented, nonhemolytic colonies after 24–72 h incubation on blood agar or Columbia agar. These characteristics set them apart from normal-type *S. aureus* which is ten times larger and golden. Normal-type *S. aureus* also grows much faster than SCVs; therefore, SCVs are overgrown by the normal-type and frequently overlooked. *S. aureus* SCVs can be further classified by testing them for thymidine, hemin, and menadione auxotrophy using agar disk diffusion tests; testing auxotrophy for hemin uses standard disks and testing auxotrophy for thymidine and menadione uses disks with thymidine and menadione, respectively. In addition, the samples must be confirmed as *S. aureus* by

Table 2 Types of clinical samples used for studies of *S. aureus* SCVs from human specimens

Disease		Sample studied	Reference
Cystic fibrosis		Bronchial secretion, sputum, bronchoalveolar lavage, or oropharyngeal swab	[6, 7, 18, 23, 24, 30, 31, 41–43]
Osteomyelitis		Bone, deep tissue aspirate	[4, 9, 17, 35]
Skin infection	Darier's disease	Skin tissue, anterior nare sample	[8]
	Hip abscess	Infected tissue	[44]
	Brain abscess	Tissue, pus	[30]
Implant/ device-related infections	Pacemaker	Blood	[9]
	Ventriculoperitoneal shunt infection	Cerebrospinal fluid	[36]
	Joint infection	Joint aspiration, tissue	[2, 47]

Table 3 Techniques applied to examine *S. aureus* SCVs in clinical samples

Technique	Procedure	Finding	Reference
Culture	Culture specimens on Columbia blood agar, brain-heart infusion agar, mannitol salt agar, and Schaedler agar. May subculture bacterial isolates representing each visible morphotype on blood agar plates	SCVs appeared as small, nonpigmented, and nonhemolytic colonies on Columbia agar while they grew normally on Schaedler agar; these SCVs were confirmed as *S. aureus* by coagulase tube test, or PCR for nuc and coa genes	[6, 7, 18, 23, 24, 30, 42]
Auxotrophy	Test auxotrophy for hemin using standard disks, and auxotrophy for thymidine and menadione using disks with thymidine and menadione, respectively	SCV demonstrated auxotrophy for hemin, menadione, or thymidine	[6, 18, 30, 31]

testing for the species specific genes *nuc* and *coa* via polymerase chain reaction (PCR) [18], 16S-rRNA-directed in situ hybridization [30], or genotypic analysis "Spa typing" [31].

Emergence and Prevalence of Clinical Cases of *S. aureus* SCVs

Due to their slow growth rate, atypical colony morphology, unusual biochemical reactions, and reduced coagulase activity, the isolation and identification of *S. aureus* SCVs have been challenging (especially in the early years). The roles of *S. aureus* SCVs in infections have been underestimated and likely have contributed to therapeutic failures in clinical settings. The first clinical observations of *S. aureus* SCVs were reported in the 1950s where pure growth of "dwarf-colony variants"

(now known as SCVs) of *S. aureus* was obtained in cultures from patients with abscesses, septic lesions, and whitlows [15, 32, 33]. Since the mid-1990s, *S. aureus* SCVs have been increasingly appreciated clinically after they were isolated from a variety of infected patients. *S. aureus* SCVs have been reported in clinical cases including CF, abscesses, bacteremia, pneumonia, septic arthritis, implant/device-related infection, and skin, soft tissue, and bone infections (Table 4). These clinical cases have shown the versatility of *S. aureus* SCVs and their presence in diverse clinical scenarios. *S. aureus* SCVs have been identified as the sole or predominant isolate in some cases [17] and have been increasingly seen in patients with recurrent, persistent infections, especially those that have been treated with antimicrobial therapy such as aminoglycosides and cell-wall-active antibiotics (Table 4).

Table 4 History of SCVs identified in diseases and their prevalence

Year	Disease	Finding	Refs.
1951	Abscess	Pure growth of "dwarf-colony variant" of *S. aureus* was obtained in primary culture of pus from an abscess and presented normal large-colony type of growth in the presence of 10% CO_2	[15]
1952	Closed septic lesion	*S. aureus* SCVs were isolated in pure culture from two independent closed septic lesions	[32]
1955	Recurrent whitlow	Pure growth of *S. aureus* SCVs were repeatedly obtained from a patient who had multiple whitlows treated with antibiotics in primary cultures of pus from two whitlows and a boil, and also from the patient's nose. There was strong evidence that these SCVs were the primary pathogens at least in the last two lesions	[33]
1969	Bacteremia, lymphatic leukemia, cadaver kidney recipient, etc.	*S. aureus* SCVs ("microcolonies") were recovered from the anterior nares of an asymptomatic adult, the blood of a patient being treated with Nafcillin for *Staphylococcal* bacteremia secondary to an infected aortic valve prosthesis, the blood of an anephric patient receiving Methicillin and chloromycetin, the blood of a patient with lymphatic leukemia receiving cortisone, methotrexate, and penicillin, and the blood of a cadaver kidney recipient receiving cortisone, penicillin, and polymyxin B treatment	[12]
1978	Pneumonia	*S. aureus* SCVs were revealed from a 29-year-old patient who developed right lower lobe pneumonia following chest trauma	[11]
1978	Infection (e.g., osteomyelitis)	*S. aureus* SCVs were identified as the sole or predominant isolate in eight human infection cases from osteomyelitis specimens, blood, subcutaneous abscess, and cerebrospinal fluid. These organisms were shown to be menadione- or thiamine-dependent	[17]
1987	Cystic fibrosis	Sputum, bronchial washings, pharyngeal swabs, tracheal aspirates, and Bartlett bronchial brushings were collected from 200 patients (0.5–37 years old), 95 patients harbored *S. aureus*, and 20/95 (21%) patients had thymidine-dependent *S. aureus* SCVs	[41]

(continued)

Table 4 (continued)

Year	Disease	Finding	Refs.
1995	Chronic osteomyelitis, septic arthritis	*S. aureus* SCVs were cultured from five patients with persistent and recurrent infections	[5]
1996	Sternoclavicular joint septic arthritis	Blood samples from an 11-year-old boy with shoulder discomfort were found to have *S. aureus* SCVs	[47]
1997	Chronic osteomyelitis	Bone specimens or deep tissue aspirates were acquired from 14 patients treated with gentamicin beads and 4/14 (29%) patients had *S. aureus* SCVs. One SCV was a menadione and three were hemin auxotrophs. There was infection recurrence in all four patients with SCVs but not in patients with normal-type *S. aureus* only	[4]
1998	Cystic fibrosis	Bronchial secretion samples were collected from 78 patients (6 months to 43 years). 53 patients harbored *S. aureus*, and among them, 26/53 (49%) patients had *S. aureus* SCVs	[7]
1998	Chronic osteomyelitis	Menadione auxotrophic *S. aureus* SCVs were recovered in multiple bone specimens from one patient with chronic osteomyelitis who had previously been treated with gentamicin beads	[34]
1999	Hip abscess	The first case of a fatal infection with *S. aureus* SCVs in an AIDS patient was reported and *S. aureus* methicillin-resistant SCVs were recovered from hip abscess	[9]
2000	Hip abscess	*S. aureus* SCVs were recovered from persistent wound infection in the right inguinal crural region after herniotomy	[44]
2001	Skin infection	*S. aureus* SCVs were detected from skin tissue and anterior nares in a 39-year-old male with Darier's disease	[8]
2002	Cystic fibrosis	63 *S. aureus* isolates were collected from sputum samples of children (1.5–19 years old); 20/63 (32%) contained *S. aureus* SCVs	[43]
2002	Chronic osteomyelitis	*S. aureus* SCVs were recovered from a patient treated with gentamicin beads	[35]
2003	Brain abscess	*S. aureus* SCVs were identified in a patient with brain abscess; the patient was treated with a combination of vancomycin and rifampin followed by prolonged treatment with teicoplanin, with no sign of infection at 9-month follow-up	[30]
2003	Cystic fibrosis	Sputum samples from 52 patients (21–72 years old) were found to present *S. aureus* and 24/52 (46%) patients had *S. aureus* SCVs	[23]
2003	Pacemaker-related infection	Blood samples from a recurrent pacemaker-related bloodstream infection contained *S. aureus* SCVs	[48]
2005	Ventriculoperitoneal shunt infection	*S. aureus* SCVs were recovered from cerebrospinal fluid in a 69-year-old woman with recurrent ventriculoperitoneal shunt-related meningitis	[36]
2006	Periprosthetic joint infection	Joint aspirations or intraoperative tissues from 5 (6%) out of 83 (66 total hip and 17 total knee arthroplasty) patients contained *S. aureus* SCVs. Intracellular cocci in fibroblasts were observed in periprosthetic tissue samples	[45]

(continued)

Table 4 (continued)

Year	Disease	Finding	Refs.
2007	Cystic fibrosis	Sputum samples and deep throat swabs were obtained from 252 patients (maximum of 61 years), and 17% (95% confidence interval, 10 to 25%) among *S. aureus* carriers had *S. aureus* SCVs	[6]
2008	Cystic fibrosis	*S. aureus* SCVs were identified in respiratory secretion samples from 8/40 (20%) patients harboring *S. aureus*, particularly those with advanced pulmonary disease and prolonged antibiotic exposures	[42]
2013	Cystic fibrosis	*S. aureus* SCVs were detected in 20 (16%) of 123 patients harboring *S. aureus*. SCVs and normal-type *S. aureus* strains showed identical genotypes in 14 patients, while 5 patients showed different genotypes	[18]
2013	Cystic fibrosis	Sputum, bronchoalveolar lavage, or oropharyngeal swabs were obtained from 100 pediatric patients (maximum of 16 years), and 24/100 (24%) patients had *S. aureus* SCVs	[24]
2015	Skin, soft tissue, and bone infections	Clinical samples were collected from skin, bone, and soft tissues, and 10 (15%) out of 66 samples with positive growth of *S. aureus* contained thymidine independent *S. aureus* SCVs	[46]
2016	Cystic fibrosis	9% of *S. aureus* positive patients were positive for *S. aureus* SCVs. 17 different SCV isolates and 12 corresponding normal-type isolates were obtained from 147 patients. 13 isolates were determined thymidine auxotroph, 2 isolates were auxotroph for hemin, and none of the tested isolates were auxotroph for both, respectively	[31]
2018	Cystic fibrosis	37 MRSA isolates from 28 patients were found to be SCVs, which presented higher rates of antibiotic resistance to moxifloxacin, erythromycin, and trimethoprim/sulfamethoxazole, compared to normal colony variant MRSA isolates. Moreover, patients with such SCVs had lower lung function, higher rates of persistent infection, compared to individuals with normal colony variant MRSA	[13]

Osteomyelitis

Osteomyelitis is an infection of the bone that occurs after trauma, surgery, presence of a foreign body such as a prosthesis or after hematogenous seeding. One of the major infecting organisms is *S. aureus* making *S. aureus* SCVs a suspect in patients that have persistent or recurrent infections. von Eiff et al. examined bone specimens and deep tissue aspirates from 14 patients with osteomyelitis [4]. Four (29%) out of the 14 patients had *S. aureus* SCVs. After antimicrobial (gentamicin) treatment for more than 4 weeks, strikingly, infection recurred in the four patients who had *S. aureus* SCVs whereas those without SCVs did not have recurrence [4].

Along with recurrent infection, patients were more likely to have *S. aureus* SCVs when they were treated with gentamicin beads; these beads release gentamicin over a period of weeks to months to provide a sustained local level of antibiotics and are commonly used to treat osteomyelitis. All four patients treated with gentamicin beads had *S. aureus* SCVs while the other patients who were not treated with gentamicin beads did not have *S. aureus* SCVs [4]. *S. aureus* SCVs were also recovered in two cases of chronic osteomyelitis where the patients were previously treated with gentamicin beads [34, 35]. These data may suggest that gentamicin beads might have selected for *S. aureus* SCVs (Table 5), which should alert physicians that gentamicin may select for *S. aureus* SCVs that could contribute to recurrent and persistent infections.

In 2006, Sendi et al. identified *S. aureus* SCVs in five periprosthetic joint infection patients [2]. These five patients had a mean age of 62, all experienced treatment failure prior to isolation of *S. aureus* SCVs despite as many as three surgical revisions and up to 22 months of antibiotics (e.g., intravenous flucloxacillin followed by a combination of rifampin and levofloxacin after a few days for methicillin-susceptible Staphylococcal infections). Even with antimicrobial therapies during early treatment consisting of various combinations of flucloxacillin,

Table 5 SCVs and normal-type of *S. aureus* in patients with or without previous local gentamicin treatment

Case no.	Colony type	Previous local gentamicin therapy	Other previous systemic antibiotics	Auxotroph	Cause of osteomyelitis	Recurrence of osteomyelitis[a]
1	n	No	PenG, Ctax, Cm, Cpfx	No	Postoperative	No
2	n	No	None	No	Hematogenous	No
3	n	No	Cm	No	Contiguous	No
4	n	No	None	No	Posttraumatic	No
5	n	No	None	No	Hematogenous	No
6	n	No	None	No	Hematogenous	No
7	n	No	Oxa	No	Posttraumatic	No
8	n	No	None	No	Hematogenous	No
9	n	No	Ctax	No	Postoperative	No
10	n	No	Amox/CA	No	Contiguous	No
11	SCV	Yes	Vm, Cfur	Hemin	Postoperative	Yes
12	SCV, n	Yes	Cm	Hemin	Postoperative	Yes
13	SCV, n	Yes	Vm	Hemin	Posttraumatic	Yes
14	SCV, n	Yes	Oxa, Ctax, Cm	Menadione	Postvaccination	Yes

Reprinted with permission from Clinical Infectious Diseases 25:1250–1251 (1997) [4]
Amox/CA amoxicillin/clavulanic acid, *Cfur* cefuroxime, *Cm* clindamycin, *Cpfx* ciprofloxacin, *Ctax* cefotaxime, *n* normal, *Oxa* oxacillin, *PenG* penicillin G, *SCV* small colony variant, *Vm* vancomycin
[a]Relapse of osteomyelitis occurring more than 1 year after primary diagnosis and treatment

vacomycin plus cefepime, ciprofloxacin, and rifampin, all patients had recurrent infections. The spacers were removed after detection of *S. aureus* SCVs and antimicrobial treatment was chosen based on susceptibility testing. A combination of levofloxacin and rifampin was administered for a course ranging between 5.5 and 7 weeks. Four patients proceeded to receive reimplantations, while one refused in fear of reinfection. Follow-up for these patients ranged between 12 and 48 months; three patients were cured while two were likely cured. Therefore, it seems that, in cases that involve persistent or recurrent infections, *S. aureus* SCVs should be examined and antibiotics that can eliminate SCVs may need to be considered in order to advance toward proper treatments and possibly avoid surgical revisions. Due to the stubborn nature of SCVs, removal of all implants and extensive debridement are recommended.

Implant/Device-Related Infection

S. aureus is one of the most common causes of infections associated with biomedical implants or devices. *S. aureus* SCVs have been isolated in cases of pacemaker and ventriculoperitoneal-shunt infections in 2003 and 2005, respectively [2, 36]. In these *S. aureus* SCV cases, the patients had poor clinical and microbiologic responses to prolonged antimicrobial therapies. Patients were treated unsuccessfully with antibiotics (e.g., cefuroxime) which led to multiple instances of recurrent fever, infection, hospitalization, and surgeries. These cases further emphasize the versatility and infectious nature of *S. aureus* SCVs. With the increasing use of invasive implants/devices, *S. aureus* SCVs are expected to become more common; implant/device-related infections that are persistent and resistant should be tested for *S. aureus* SCVs. Similar to the periprosthetic joint infection cases discussed above, the best way to treat *S. aureus* SCVs was to completely remove the foreign implant or device and administer appropriate antimicrobial treatment that is effective against *S. aureus* SCVs.

Cystic Fibrosis (CF)

CF is a progressive, genetic disease that causes persistent lung infections and may also affect the pancreas, liver, kidneys, and intestines. *S. aureus* SCVs have been frequently isolated in studies involving patients affected by CF [13]. *S. aureus* is one of the most common bacteria found in the respiratory tracts of children with CF [37] and continues to be one of the major pathogens along with *Pseudomonas aeruginosa* and *Haemophilus influenza* [38]. In order to combat these pathogens, long-term prophylactic oral antimicrobial agents such as tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole (SXT), and certain cephalosporins and penicillins [39, 40] are administered. Although normal-type *S. aureus* can be eliminated

from the airways, unfortunately, *S. aureus* SCVs can form and adapt to the hostile environment leading to chronic and recurrent infections. When looking at 9 studies involving a total of 1266 patients, *S. aureus* SCVs were identified in 9%, 16%, 17%, 20%, 21%, 24%, 32%, 46%, and 49%, respectively, among patients harboring *S. aureus* (Table 4) [6, 7, 18, 23, 24, 31, 41–43]. Alarmingly, carriers of *S. aureus* SCVs had been infected with *S. aureus* longer than those with the normal phenotype [42], showed significantly higher antimicrobial resistance rates than those with the normal phenotype [6], and the presence of SCVs was directly related to poor clinical outcomes [31]. Moreover, patients positive for SCVs were significantly older [6, 18, 42], more commonly co-colonized with *Pseudomonas aeruginosa* [6], and showed signs of more advanced disease, such as lower forced expiratory volume than patients who had only normal-type *S. aureus* [6, 42]. Lower weight, advanced age, and prior use of SXT were found to be independent risk factors for *S. aureus* SCV positivity [6].

Wolter et al. illustrated a unique pattern of culture positivity for *S. aureus* SCVs in 24 pediatric patients with CF [24]. The patients tended to have "alternating positive and negative culture positivity suggesting repeated selection and enrichment for *S. aureus* SCVs, incomplete detection, or both" (Fig. 1). Infection with *S. aureus* SCVs led to a greater drop in lung function and was independently associated with worse CF respiratory outcomes (Table 6). Patients treated with SXT for longer than 18 months or those receiving interventional aminoglycoside treatment were more likely to have SCVs [23, 24]. In fact, it was indicated that SXT was the strongest predictor of *S. aureus* SCV detection suggesting that SXT strongly selected for *S. aureus* SCVs. *S. aureus* SCVs should be a concern for all CF patients especially in those with reduced lung function and those treated with antibiotics for a long period of time. Screening and identification of these SCVs can help guide proper therapeutic treatments.

Abscess

S. aureus SCVs have been isolated from abscesses. One of the first clinical cases of *S. aureus* SCVs from an abscess patient showed pure tiny colonies (i.e., SCVs) in the cultures of pus samples. The smears of these tiny colonies presented typical Staphylococcal morphology and these tiny colonies reverted to typical large Staphylococcal colonies when cultured in the presence of carbon dioxide [15]. *S. aureus* SCVs were also identified from a patient with a persistent wound infection (abscess and fistula). A combination treatment of flucloxacillin and rifampicin for 4 weeks led to healing of the chronic wound infection [44].

In another report, methicillin-resistant *S. aureus* SCVs were identified in a patient with a brain abscess [30]. In this study, computed tomography (Fig. 3) showed a left temporal mass where, 10 years earlier, a neurosurgical intervention had been performed to treat a subarachnoid hemorrhage. The patient was treated with cefamandole for 2 weeks due to a febrile episode and *S. aureus* was confirmed via 16S

Table 6 SCV status as an independent predictor of change in lung function over the study period[a]

Predictor	Coefficient estimate (mean difference in change in FEV_1% predicted over study period[b])	95% Confidence interval	*P* value
Model 1			
Ever SCV positive on study	−11.00	−18.81, −3.18	0.007
Age at enrollment	−1.31	−2.43, −0.18	0.023
FEV_1% predicted at enrollment	−0.28	−0.48, −0.08	0.007
Model 2			
Ever SCV positive on study	−11.32	−19.10, −3.55	0.005
Age at enrollment	−1.51	−2.75, −0.26	0.019
FEV_1% predicted at enrollment	−0.28	−0.48, −0.08	0.007
Ever *Pseudomonas aeruginosa* positive on study	2.53	−4.75, 9.80	0.490
Model 3			
Ever SCV positive on study	−11.29	−19.61, −2.97	0.009
Age at enrollment	−1.29	−2.44, −0.14	0.028
FEV_1% predicted at enrollment	−0.28	−.48, −0.08	0.008
Ever MRSA positive on study	0.59	−5.92, 7.10	0.857
Model 4			
Ever SCV positive on study	−12.98	−21.55, −4.41	0.004
Age at enrollment	−1.34	−2.51, −0.17	0.026
FEV_1% predicted at enrollment	−0.26	−0.47, −0.06	0.013
Ever *Stenotrophomonas maltophilia* positive on study	4.41	−2.67, 11.49	0.218
Model 5			
Ever SCV positive on study	−10.91	−18.79, −3.03	0.008
Age at enrollment	−1.28	−2.41, −0.16	0.026
FEV_1% predicted at enrollment	−0.29	−0.49, −0.08	0.007
Any exacerbations on study	−1.04	−7.55, 5.46	0.749
Model 6[c]			
Ever SCV positive on study	−11.92	−20.18, −3.66	0.006
Age at enrollment	−1.29	−2.45, −0.12	0.031
FEV_1% predicted at enrollment	−0.28	−0.49, −0.08	0.007
Use of TMP–SMX during the study	2.19	−4.54, 8.92	0.517

Reprinted with permission from Clinical Infectious Diseases 57:384–391 (2013) [24]

FEV_1% percent predicted forced expiratory volume in 1 s, *MRSA* methicillin-resistant *S. aureus*, *SCV* small-colony variant, *TMP–SMX* trimethoprim–sulfamethoxazole

[a]Adjusting for sex did not alter the results (not shown). Each potential confounding variable was evaluated by adding it to the base model (model 1). Due to sample size constraints, we did not evaluate all potential confounding variables simultaneously

[b]For covariates coded as yes/no (such as culture positivity), this is the mean difference in change in FEV_1% predicted over the study period between subjects coded as yes versus those coded as no. For continuous covariates, this is the mean difference per 1 unit increase in covariate (e.g., for age, the mean difference per 1 year increase in age)

[c]Includes data for 59 participants who had antibiotic data reported. Similar results were obtained when adjusting for total quarters of TMP–SMX use

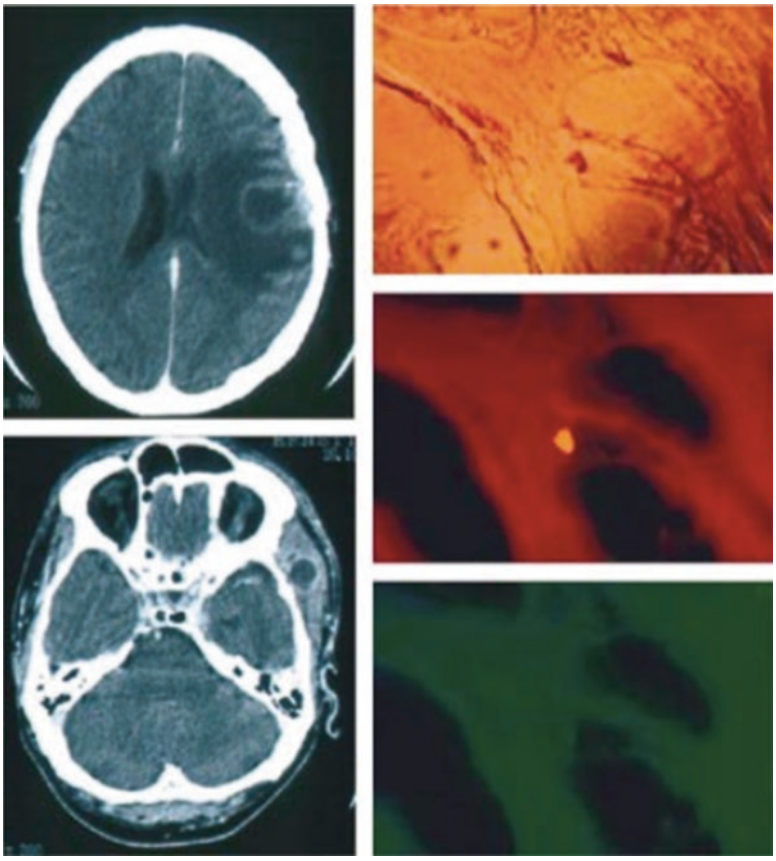


Fig. 3 Brain abscess caused by *S. aureus* SCVs. Left: cerebral computed tomography with contrast medium; (top) intracerebral abscess and (bottom) left temporal intramuscular abscess. Right: Detection of *S. aureus* cells by in situ hybridization of a tissue section obtained from brain abscess; (top) phase contrast microscopy, (middle) in situ hybridization using a Cy3-labeled *S. aureus* specific SA-P1 probe, and (bottom) control hybridization with a FLUOS-labeled *S. epidermidis* probe SEP1. (Reprinted with permission from Journal of Neurology, Neurosurgery, and Psychiatry 74:1000–1002 (2003) [30])

rRNA-directed in situ hybridization. *S. aureus* SCVs were subsequently identified after culturing tissue and pus samples from the abscess for a long time period (i.e., 72 h). This patient was treated with vancomycin, rifampin, and teicoplanin and no infection was observed at the 9-month follow-up. The medical history indicated that this patient had no signs of acute or recurrent infection between the two surgeries. The authors claimed that *S. aureus* SCVs were the causative microorganisms for the infection; to our understanding, this diagnosis was not conclusive, although it is possible that the surgery performed 10 years prior might be linked to the formation and later proliferation of *S. aureus* SCVs.

In 1999, the first case of a fatal infection with *S. aureus* SCVs was reported in an acquired immune deficiency syndrome (AIDS) patient (36-year-old man) who was under long-term treatment with trimethoprim/sulfamethoxazole for prophylaxis of *Pneumocystis carinii* pneumonia [9]. *S. aureus* methicillin-resistant SCVs were recovered from a hip abscess in the patient. Vancomycin treatment was administered but the patient's status deteriorated rapidly; the patient died of refractory septic shock 6 days after admission with fever and progressive pain (of 6 weeks duration) in the right hip [9].

Skin Infection

The first recorded case of a persistent and antimicrobial resistant skin infection due to *S. aureus* SCVs was reported in 2001 in a 39-year-old patient with Darier's disease [8]. The patient was hospitalized several times in previous years due to recurrent herpes virus infections and recurrent purulent infections. In 1999, the patient was hospitalized again. Methicillin-resistant *S. aureus* was isolated from skin and anterior nares leading to a 4-week intravenous course of antimicrobial therapy consisting of vancomycin, rifampicin, and clindamycin. A topical mupirocin ointment was also given for 2 weeks for the nasal mucosa. The skin condition did not significantly improve and topical treatments with steroids and antiseptics (povidone-iodine, chlorhexidine, and chlorquinaldol) were administered. Over a course of 28 months, 119 isolates were derived from 53 clinical specimens obtained from different areas of the affected skin and anterior nares. Hemin-auxotrophic SCVs along with various *S. aureus* strains were found in the skin infections. With this being said, *S. aureus* SCVs may be related to skin infections that persist for a long period of time and may be resistant to various therapeutic treatments.

Clinical Significance of *S. aureus* SCVs

From the clinical cases reported (Table 4), we can see that *S. aureus* SCVs have significant clinical implications because:

- *S. aureus* SCVs are found in a broad range of percentages among *S. aureus* positive patients such as 6% [45], 9% [31], 15% [46], 16% [18], 17% [6], 20% [42], 21% [41], 24% [24], 29% [4], 32% [43], 46% [23], 49% [7], and 100% [15, 32, 33]. *S. aureus* SCVs are responsible for [15, 32, 33], most likely responsible for [4, 17], or may contribute to [5–9, 11, 12, 18, 23, 24, 30, 31, 34–36, 41–48] the infections observed clinically.
- A variety of factors including lower weight, advanced age, and prior use of antibiotics may contribute to the development of *S. aureus* SCVs [6]. For instance, the history of antimicrobial treatments seems to contribute to increased occurrences

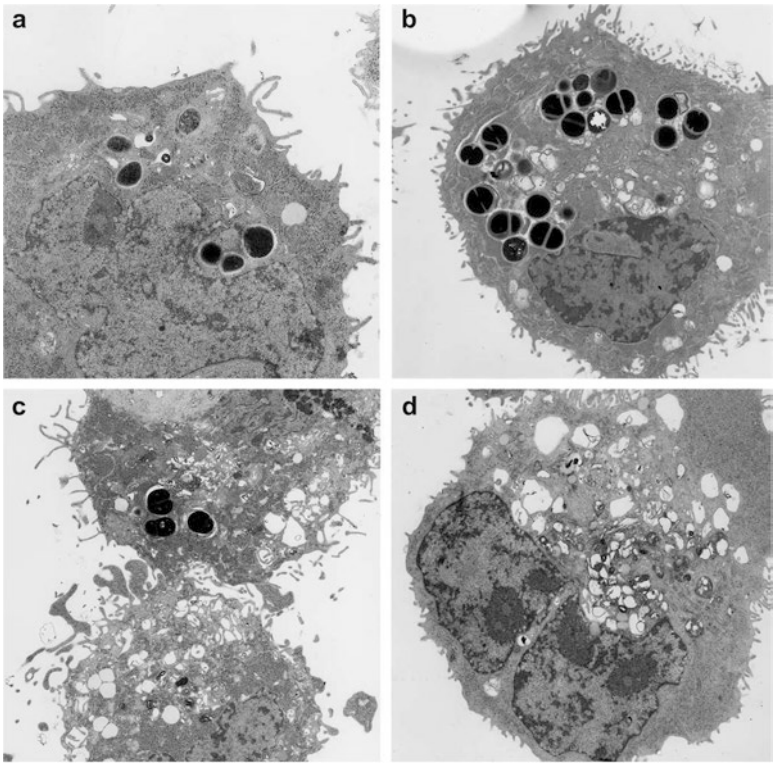


Fig. 4 Electron micrographs of keratinocyte HaCaT cells that were infected with isolates of clinical isogenic normal-type *S. aureus* and *S. aureus* SCVs. After incubation of infected HaCaT cells in the presence of lysostaphin for 30 min or 48 h (analogous to an intracellular persistence assay), cells were washed, dehydrated, and embedded in Epon. Ultrathin sections were counterstained and examined by electron microscopy. (**a**, **b**), Intracellular persistence of SCVs (SCV1) within viable HaCaT cells after (**a**) 30 min or (**b**) 48 h of incubation. Epithelial cells appear viable and show no signs of degeneration (original magnification, ×3400). (**c**, **d**) *S. aureus* of the normal phenotype (NP1) is incorporated (**c**) after 30 min by intact HaCaT cells; however, (**d**) after 48 h of incubation, most epithelial cells show severe lytic degeneration and release of bacteria (original magnification, ×3400). (Reprinted with permission from Clinical Infectious Diseases 32:1643–1647 (2001) [8])

of *S. aureus* SCVs, since *S. aureus* SCVs were more often obtained from patients who were treated with antibiotics (e.g., gentamicin) [4, 6, 9, 12, 33–35, 42, 45].

- *S. aureus* SCVs have the ability to persist longer within host cells compared to their wild-type strains (Fig. 4) [8], which may explain why *S. aureus* seems to be eliminated but infection may recur weeks or months later [33]. Because they reside intracellularly and have relatively low virulence, *S. aureus* SCVs can remain inside other cells and be protected from conventional antibiotic treatments as well as from the intracellular host defense mechanisms.
- *S. aureus* SCVs are often more resistant to antibiotics compared to their normal-type strain (Fig. 5) [6] and are difficult to eliminate. However, they can be treated but the optimal treatments still need to be identified and consequences of failure

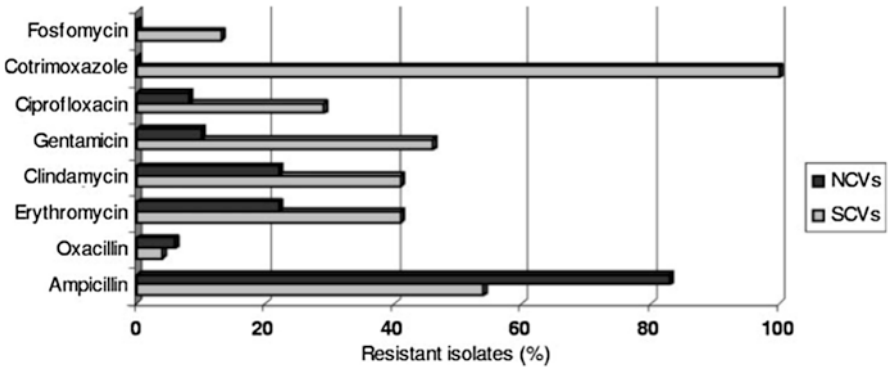


Fig. 5 Percent antimicrobial resistance of *S. aureus* isolates. Isolates with the SCV (n = 24) and normal colony variant or NCV (n = 110) phenotypes are compared. (Reprinted with permission from Journal of Clinical Microbiology 45:168–72 (2007) [6])

could be unexpected. In 2003, a patient with a brain abscess had *S. aureus* SCVs and was effectively treated with a combination of vancomycin and rifampin followed by prolonged treatment with teicoplanin; no signs of infection were observed at the subsequent 9-month follow-up [30]. However, various antibiotics have failed to treat *S. aureus* SCVs and led to recurrent infections [4] and might even have contributed to death [9].

- The presence of *S. aureus* SCVs most likely contributed to a poorer clinical outcome, since patients who had *S. aureus* SCVs were significantly more commonly co-colonized with other bacteria (e.g., *Pseudomonas aeruginosa*), infected with *S. aureus* longer, had chronic or persistent infections or infection recurrence, and presented signs of more advanced disease (e.g., lower forced expiratory volume) compared to patients who had only wild-type *S. aureus* [6, 18, 31, 42]. For instance, it was reported that four patients among 14 infected patients who had *S. aureus* SCVs all had infection recurrence while the remaining 10 patients with normal-type *S. aureus* showed no recurrence [4]. *S. aureus* SCVs were also more often observed among patients with chronic, persistent, or recurrent infections [5, 34, 36, 44, 48].

Summary and Perspectives

S. aureus SCVs have been observed in patients for more than half a century in a variety of infectious diseases including CF, sepsis, bacteremia, endocarditis, skin infections, rhinosinusitis, osteomyelitis, brain abscess, implant/device-related infections, etc. So far, clinical cases of *S. aureus* SCVs in CF patients have been documented relatively better compared to the other diseases, but this does not necessarily mean that *S. aureus* SCVs are less commonly found in the other infections. More clinical cases involving *S. aureus* infections should be examined for presence or absence of *S. aureus* SCVs. Similarly, SCVs of other bacteria should be carefully

Table 7 SCVs of non-*Staphylococcal* bacteria recovered from human specimens

Small colony variants of non-*Staphylococcal* bacteria recovered from human specimens	
Microorganism	Type or site of infection and/or specimen
Brucella melitensis	Subacute bacterial endocarditis (blood culture)
Burkholderia cepacia	Lung and other airway specimens from patients with cystic fibrosis
Burkholderia pseudomallei	Experimental melioidosis
Escherichia coli	Chronic prosthetic hip infection; urinary tract infection; human feces
Lactobacillus acidophilus	Human feces
Neisseria gonorrhoeae	Gonorrhoea (urethra, cervix, and vagina)
Pseudomonas aeruginosa	Lung and other airway specimens from patients with cystic fibrosis
Salmonella serovars	Typhoid fever

Reprinted with permission from Nature Reviews Microbiology 4:295–305 (2006) [28]

examined and treated; SCVs are not limited to *S. aureus* and have been reported for non-*Staphylococcus* bacteria that have been recovered from human specimens (Table 7) [28]. More details on diagnosis, pathogenesis, and treatment of *S. aureus* SCVs are discussed below.

Diagnosis *S. aureus* SCVs have been identified from various specimens including bronchial secretion, sputum, bronchoalveolar lavage, oropharyngeal swab, bone, tissue/joint aspirate, skin tissue, anterior nare sample, pus, cerebrospinal fluid, and blood. Currently, most hospital laboratories have the ability to isolate, characterize, and identify normal-type *S. aureus*, via targeting genes such as *nuc*, *clfA*, *eap*, and *coa*. In contrast to the normal-type, *S. aureus* SCVs are much smaller, nonhemolytic, and nonpigmented; these characteristics have made them difficult to recover and classify. Because of their slow growth and atypical morphology, *S. aureus* SCVs are often missed, misidentified, or misinterpreted by the automated systems routinely used in many clinical laboratories. For instance, *S. aureus* SCVs were misidentified as coagulase-negative *Staphylococcus* [9, 44, 48]. Therefore, special efforts should be taken to identify *S. aureus* SCVs when an infection is particularly resistant to treatment, persists for a long period, or fails to respond to adequate antimicrobial therapy. We recommend that *S. aureus* SCVs should be suspected whenever pinpoint colonies are noted on routine cultures (even with a small number), and such samples should be run for *S. aureus* SCVs using appropriate selective media and growth conditions.

Pathogenesis The development of *S. aureus* SCVs is likely when the presence of normal-type *S. aureus* lasts for hours, weeks, or longer; we have confirmed the formation of *S. aureus* SCVs in osteoblasts and macrophages [49], and that they may contribute to bone infections in vivo [50]. The presence and contribution of *S. aureus* SCVs in clinical infections most likely have been underestimated and underreported. It is likely that *S. aureus* SCVs have played an important role in infection persistence; however, complete pathogenesis for *S. aureus* SCVs has yet to be discovered. The ability of *S. aureus* SCVs to phenotypically switch back and forth between the nor-

mal and variant forms help the organism to evade both host defense and antimicrobial treatments thereby contributing to the persistence of their associated infections. Moreover, the menadione deficient strain of *S. aureus* SCVs can form more diverse and highly structured biofilms compared to the normal-type [51] and may contribute to their persistence as well.

Treatment *S. aureus* SCVs persist intracellularly within their host cells and they are often more resistant to antibiotics than the normal-type *S. aureus*. Their ability to "hide" intracellularly may protect them from intracellular host defense mechanisms and decrease their exposure to antibiotics. As a result, *S. aureus* SCVs are difficult to treat. Two approaches may be applied. One is to prevent the development of *S. aureus* SCVs by striking early in the acute infection stage before SCVs can develop by using effective local and systemic antimicrobial treatments; screening for *S. aureus* SCVs must be done if such treatments fail since certain antimicrobial treatments may be selective (Table 5) for the development of *S. aureus* SCVs. The other approach is to identify effective antimicrobial approaches to treat *S. aureus* SCV-associated infections. Currently, the optimal treatments for infections caused by *S. aureus* SCVs have not been identified. However, some treatments seem to be promising. A combination of levofloxacin and rifampin cured three patients with *S. aureus* SCVs [45], a combination of vancomycin and rifampin followed by prolonged treatment with teicoplanin presented no sign of infection at 9-month follow-up in a patient with an *S. aureus* SCV-associated brain abscess [30]. A combination of flucloxacillin and rifampicin led to healing of a persistent wound infection associated with *S. aureus* SCVs [44]. In preclinical studies, we have shown that antimicrobial peptides may be effective in eliminating *S. aureus* that are "hiding" in other cells [52], and tuning immune responses may be promising as well [53–55]. Nanomedicine, due to the unique characteristics of nanomaterials, is also emerging as improved or alternative therapies for intracellular pathogens like *S. aureus* SCVs [56]. Therefore, there are promising treatments and we recommend that antimicrobial agents (e.g., rifampin) which have potent intracellular activity should be used in treating infections caused by *S. aureus* SCVs.

Overall, *S. aureus* SCVs should be aggressively and accurately identified whenever infections induced by *S. aureus* fail apparently "adequate" antimicrobial therapy. The identification will help physicians end ineffective antimicrobial therapeutic treatments, which may inadvertently induce the development of *S. aureus* SCVs, and promptly initiate proper antimicrobial treatments. Failure to identity and treat *S. aureus* SCVs may lead to chronic, persistent, and recurrent infections, wound complications, and even death.

Acknowledgments We acknowledge financial support from the Office of the Assistant Secretary of Defense for Health Affairs, through the Peer Reviewed Medical Research Program, Discovery Award under Award No. W81XWH-17-1-0603. We also acknowledge financial support from AO Foundation, Osteosynthesis and Trauma Care Foundation, and the West Virginia National Aeronautics and Space Administration Experimental Program to Stimulate Competitive Research (WV NASA EPSCoR). Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the funding agencies. We thank Suzanne Danley for proofreading.

References

1. Styers D, Sheehan DJ, Hogan P, Sahm DF (2006) Laboratory-based surveillance of current antimicrobial resistance patterns and trends among Staphylococcus aureus: 2005 status in the United States. Ann Clin Microbiol Antimicrob 5:2. Epub 2006/02/14. PubMed PMID: 16469106; PMCID: Pmc1397857. https://doi.org/10.1186/1476-0711-5-2
2. Sendi P, Rohrbach M, Graber P, Frei R, Ochsner PE, Zimmerli W (2006) Staphylococcus aureus small colony variants in prosthetic joint infection. Clin Infect Dis 43(8):961–967. PubMed PMID: 16983605. https://doi.org/10.1086/507633
3. von Eiff C, Vaudaux P, Kahl BC, Lew D, Emler S, Schmidt A, Peters G, Proctor RA (1999) Bloodstream infections caused by small-colony variants of coagulase-negative staphylococci following pacemaker implantation. Clin Infect Dis 29(4):932–934. PubMed PMID: 10589914. https://doi.org/10.1086/520462
4. von Eiff C, Bettin D, Proctor RA, Rolauffs B, Lindner N, Winkelmann W, Peters G (1997) Recovery of small colony variants of Streptococcus aureus following gentamicin bead placement for osteomyelitis. Clin Infect Dis 25(5):1250–1251
5. Proctor RA, van Langevelde P, Kristjansson M, Maslow JN, Arbeit RD (1995) Persistent and relapsing infections associated with small-colony variants of Staphylococcus aureus. Clin Infect Dis 20(1):95–102. PubMed PMID: 7727677
6. Besier S, Smaczny C, von Mallinckrodt C, Krahl A, Ackermann H, Brade V, Wichelhaus TA (2007) Prevalence and clinical significance of Staphylococcus aureus small-colony variants in cystic fibrosis lung disease. J Clin Microbiol 45(1):168–172. PubMed PMID: 17108072; PMCID: PMC1828983. https://doi.org/10.1128/JCM.01510-06
7. Kahl B, Herrmann M, Everding AS, Koch HG, Becker K, Harms E, Proctor RA, Peters G (1998) Persistent infection with small colony variant strains of Staphylococcus aureus in patients with cystic fibrosis. J Infect Dis 177(4):1023–1029. PubMed PMID: 9534977
8. von Eiff C, Becker K, Metze D, Lubritz G, Hockmann J, Schwarz T, Peters G (2001) Intracellular persistence of Staphylococcus aureus small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with Darier's disease. Clin Infect Dis 32(11):1643–1647. PubMed PMID: WOS:000168588500020. https://doi.org/10.1086/320519
9. Seifert H, von Eiff C, Fatkenheuer G (1999) Fatal case due to methicillin-resistant Staphylococcus aureus small colony variants in an AIDS patient. Emerg Infect Dis 5(3):450–453. PubMed PMID: WOS:000080827600019
10. Wise RI (1956) Small colonies (G variants) of staphylococci: isolation from cultures and infections. Ann N Y Acad Sci 65(3):169–174. PubMed PMID: 13363212
11. Spagna VA, Fass RJ, Prior RB, Slama TG (1978) Report of a case of bacterial sepsis caused by a naturally occurring variant form of Staphylococcus aureus. J Infect Dis 138(2):277–278. Epub 1978/08/01. PubMed PMID: 681802
12. Quie PG (1969) Microcolonies (G-variants) of Staphylococcus aureus. Yale J Biol Med 41(5):394–403. PubMed PMID: 5780692; PMCID: PMC2591491
13. Suwantarat N, Rubin M, Bryan L, Tekle T, Boyle MP, Carroll KC, Jennings MT (2018) Frequency of small-colony variants and antimicrobial susceptibility of methicillin-resistant Staphylococcus aureus in cystic fibrosis patients. Diagn Microbiol Infect Dis 90(4):296–299. Epub 2018/01/19. PubMed PMID: 29343421. https://doi.org/10.1016/j.diagmicrobio.2017.11.012
14. Chen H, Wang Q, Yin Y, Li S, Niu DK, Wang H (2018) Genotypic variations between wild-type and small colony variant of Staphylococcus aureus in prosthetic valve infectious endocarditis: a comparative genomic and transcriptomic analysis. Int J Antimicrob Agents 51(4):655–658. Epub 2017/12/17. PubMed PMID: 29247687. https://doi.org/10.1016/j.ijantimicag.2017.12.006
15. Hale JH (1951) Studies on staphylococcus mutation: a naturally occurring "G" gonidial variant and its carbon dioxide requirements. Br J Exp Pathol 32(4):307–313. Epub 1951/08/01. PubMed PMID: 14886490; PMCID: Pmc2073169

16. Wise RI, Spink WW (1954) The influence of antibiotics on the origin of small colonies (G variants) of Micrococcus pyogenes var. aureus. J Clin Invest 33(12):1611–1622. PubMed PMID: 13211817; PMCID: PMC1072592. https://doi.org/10.1172/JCI103041
17. Acar JF, Goldstein FW, Lagrange P (1978) Human infections caused by thiamine- or menadione-requiring Staphylococcus aureus. J Clin Microbiol 8(2):142–147. PubMed PMID: 701460; PMCID: PMC275172
18. Yagci S, Hascelik G, Dogru D, Ozcelik U, Sener B (2013) Prevalence and genetic diversity of Staphylococcus aureus small-colony variants in cystic fibrosis patients. Clin Microbiol Infect 19(1):77–84. PubMed PMID: 22284387. https://doi.org/10.1111/j.1469-0691.2011.03742.x.
19. Tande AJ, Osmon DR, Greenwood-Quaintance KE, Mabry TM, Hanssen AD, Patel R (2014) Clinical characteristics and outcomes of prosthetic joint infection caused by small colony variant staphylococci. MBio 5(5):e01910–e01914. PubMed PMID: 25271290; PMCID: PMC4196237. https://doi.org/10.1128/mBio.01910-14
20. McNamara PJ, Proctor RA (2000) Staphylococcus aureus small colony variants, electron transport and persistent infections. Int J Antimicrob Agents 14(2):117–122. PubMed PMID: 10720801
21. Rollin G, Tan X, Tros F, Dupuis M, Nassif X, Charbit A, Coureuil M (2017) Intracellular survival of Staphylococcus aureus in endothelial cells: a matter of growth or persistence. Front Microbiol 8:1354. Epub 2017/08/05. PubMed PMID: 28769913; PMCID: Pmc5515828. https://doi.org/10.3389/fmicb.2017.01354
22. Balwit JM, van Langevelde P, Vann JM, Proctor RA (1994) Gentamicin-resistant menadione and hemin auxotrophic Staphylococcus aureus persist within cultured endothelial cells. J Infect Dis 170(4):1033–1037
23. Kahl BC, Duebbers A, Lubritz G, Haeberle J, Koch HG, Ritzerfeld B, Reilly M, Harms E, Proctor RA, Herrmann M, Peters G (2003) Population dynamics of persistent Staphylococcus aureus isolated from the airways of cystic fibrosis patients during a 6-year prospective study. J Clin Microbiol 41(9):4424–4427. PubMed PMID: WOS:000185246800066. https://doi.org/10.1128/Jcm.41.9.4424-4427.2003
24. Wolter DJ, Emerson JC, McNamara S, Buccat AM, Qin X, Cochrane E, Houston LS, Rogers GB, Marsh P, Prehar K, Pope CE, Blackledge M, Deziel E, Bruce KD, Ramsey BW, Gibson RL, Burns JL, Hoffman LR (2013) Staphylococcus aureus small-colony variants are independently associated with worse lung disease in children with cystic fibrosis. Clin Infect Dis 57(3):384–391. PubMed PMID: 23625938; PMCID: PMC3888146. https://doi.org/10.1093/cid/cit270
25. Garzoni C, Kelley WL (2009) Staphylococcus aureus: new evidence for intracellular persistence. Trends Microbiol 17(2):59–65. PubMed PMID: WOS:000264279200003. https://doi.org/10.1016/j.tim.2008.11.005
26. Sendi P, Proctor RA (2009) Staphylococcus aureus as an intracellular pathogen: the role of small colony variants. Trends Microbiol 17(2):54–58. PubMed PMID: WOS:000264279200002. https://doi.org/10.1016/j.tim.2008.11.004
27. Tuchscherr L, Medina E, Hussain M, Volker W, Heitmann V, Niemann S, Holzinger D, Roth J, Proctor RA, Becker K, Peters G, Loffler B (2011) Staphylococcus aureus phenotype switching: an effective bacterial strategy to escape host immune response and establish a chronic infection. EMBO Mol Med 3(3):129–141. PubMed PMID: WOS:000288727200003. https://doi.org/10.1002/emmm.201000115
28. Proctor RA, von Eiff C, Kahl BC, Becker K, McNamara P, Herrmann M, Peters G (2006) Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol 4(4):295–305. PubMed PMID: WOS:000236040200015. https://doi.org/10.1038/nrmicro1384
29. Schroder A, Kland R, Peschel A, von Eiff C, Aepfelbacher M (2006) Live cell imaging of phagosome maturation in Staphylococcus aureus infected human endothelial cells: small colony variants are able to survive in lysosomes. Med Microbiol Immunol 195(4):185–194. PubMed PMID: WOS:000241356100002. https://doi.org/10.1007/s00430-006-0015-0

30. Kipp F, Ziebuhr W, Becker K, Krimmer V, Hobeta N, Peters G, Von Eiff C (2003) Detection of Staphylococcus aureus by 16S rRNA directed in situ hybridisation in a patient with a brain abscess caused by small colony variants. J Neurol Neurosurg Psychiatry 74(7):1000–1002. PubMed PMID: 12810807; PMCID: 1738524
31. Masoud-Landgraf L, Zarfel G, Kaschnigg T, Friedl S, Feierl G, Wagner-Eibel U, Eber E, Grisold AJ, Kittinger C (2016) Analysis and characterization of Staphylococcus aureus small colony variants isolated from cystic fibrosis patients in Austria. Curr Microbiol 72(5):606–611. PubMed PMID: 26821237; PMCID: PMC4828482. https://doi.org/10.1007/s00284-016-0994-z
32. Sherris JC (1952) Two small colony variants of Staph. aureus isolated in pure culture from closed infected lesions and their carbon dioxide requirements. J Clin Pathol 5(4):354–355. Epub 1952/11/01. PubMed PMID: 13011225; PMCID: Pmc1023676
33. Goudie JG, Goudie RB (1955) Recurrent infections by a stable dwarf-colony variant of Staphylococcus aureus. J Clin Pathol 8(4):284–287. Epub 1955/11/01. PubMed PMID: 13271575; PMCID: Pmc1023871
34. von Eiff C, Lindner N, Proctor RA, Winkelmann W, Peters G (1998) Development of gentamicin-resistant small colony variants of S. aureus after implantation of gentamicin chains in osteomyelitis as a possible cause of recurrence. Zeitschrift fur Orthopadie und ihre Grenzgebiete 136(3):268–271. Epub 1998/09/16. PubMed PMID: 9736990
35. Rolauffs B, Bernhardt TM, von Eiff C, Hart ML, Bettin D (2002) Osteopetrosis, femoral fracture, and chronic osteomyelitis caused by Staphylococcus aureus small colony variants (SCV) treated by girdlestone resection--6-year follow-up. Arch Orthop Trauma Surg 122(9-10):547–550. PubMed PMID: 12483342. https://doi.org/10.1007/s00402-002-0435-2
36. Spanu T, Romano L, D'Inzeo T, Masucci L, Albanese A, Papacci F, Marchese E, Sanguinetti M, Fadda G (2005) Recurrent ventriculoperitoneal shunt infection caused by small-colony variants of Staphylococcus aureus. Clin Infect Dis 41(5):e48–e52. PubMed PMID: 16080075. https://doi.org/10.1086/432577
37. Cystic Fibrosis Foundation (2015) Cystic fibrosis foundation patient registry report. https://www.cff.org/Our-Research/CF-Patient-Registry/2015-Patient-Registry-Annual-Data-Report.pdf. Accessed 27 Apr 2017
38. Lyczak JB, Cannon CL, Pier GB (2002) Lung infections associated with cystic fibrosis. Clin Microbiol Rev 15(2):194–222. PubMed PMID: 11932230; PMCID: PMC118069
39. Kelly HW (1984) Antibiotic use in cystic-fibrosis. Drug Intell Clin Pharm 18(10):772–783. PubMed PMID: WOS:A1984TL05900001
40. Marks MI (1981) The pathogenesis and treatment of pulmonary infections in patients with cystic fibrosis. J Pediatr 98(2):173–179
41. Gilligan PH, Gage PA, Welch DF, Muszynski MJ, Wait KR (1987) Prevalence of thymidine-dependent Staphylococcus aureus in patients with cystic fibrosis. J Clin Microbiol 25(7):1258–1261. PubMed PMID: 3497170; PMCID: PMC269188
42. Schneider M, Muhlemann K, Droz S, Couzinet S, Casaulta C, Zimmerli S (2008) Clinical characteristics associated with isolation of small-colony variants of Staphylococcus aureus and Pseudomonas aeruginosa from respiratory secretions of patients with cystic fibrosis. J Clin Microbiol 46(5):1832–1834. PubMed PMID: 18322058; PMCID: PMC2395072. https://doi.org/10.1128/JCM.00361-08
43. Sadowska B, Bonar A, von Eiff C, Proctor RA, Chmiela M, Rudnicka W, Rozalska B (2002) Characteristics of Staphylococcus aureus, isolated from airways of cystic fibrosis patients, and their small colony variants. FEMS Immunol Med Microbiol 32(3):191–197. PubMed PMID: 11934563
44. Abele-Horn M, Schupfner B, Emmerling P, Waldner H, Goring H (2000) Persistent wound infection after herniotomy associated with small-colony variants of Staphylococcus aureus. Infection 28(1):53–54. PubMed PMID: WOS:000085370400014. https://doi.org/10.1007/s150100050014
45. Sendi P, Rohrbach M, Graber P, Frei R, Ochsner PE, Zimmerli W (2006) Staphylococcus aureus small colony variants in prosthetic joint infection. Clin Infect Dis 43(8):961–967. PubMed PMID: WOS:000240666200002. https://doi.org/10.1086/507633

46. Ansari S, Nepal HP, Gautam R, Shrestha S, Chhetri MR, Chapagain ML (2015) Staphylococcus aureus: methicillin resistance and small colony variants from pyogenic infections of skin, soft tissue and bone. J Nepal Health Res Counc 13(30):126–132. PubMed PMID: 26744197
47. Spearman P, Lakey D, Jotte S, Chernowitz A, Claycomb S, Stratton C (1996) Sternoclavicular joint septic arthritis with small-colony variant Staphylococcus aureus. Diagn Microbiol Infect Dis 26(1):13–15. PubMed PMID: 8950523
48. Seifert H, Wisplinghoff H, Schnabel P, von Eiff C (2003) Small colony variants of Staphylococcus aureus and pacemaker-related infection. Emerg Infect Dis 9(10):1316–1318. PubMed PMID: WOS:000185836000021
49. Hamza T, Li B (2014) Differential responses of osteoblasts and macrophages upon Staphylococcus aureus infection. BMC Microbiol 14:207
50. Hamza T, Dietz M, Pham D, Clovis N, Danley S, Li B (2013) Intra-cellular Staphylococcus aureus alone causes infection in vivo. Eur Cell Mater 25:341–350. discussion 50. Epub 2013/07/09. PubMed PMID: 23832687; PMCID: PMC3830899
51. Singh R, Ray P, Das A, Sharma M (2010) Enhanced production of exopolysaccharide matrix and biofilm by a menadione-auxotrophic Staphylococcus aureus small-colony variant. J Med Microbiol 59(Pt 5):521–527. PubMed PMID: 20110391. https://doi.org/10.1099/jmm.0.017046-0.
52. Noore J, Noore A, Li B (2013) Cationic antimicrobial peptide LL-37 is effective against both extra- and intracellular Staphylococcus aureus. Antimicrob Agents Chemother 57(3):1283–1290. Epub 2013/01/01; PubMed PMID: 23274662; PMCID: PMC3591932. https://doi.org/10.1128/aac.01650-12
53. Li B, Jiang B, Boyce BM, Lindsey BA (2009) Multilayer polypeptide nanoscale coatings incorporating IL-12 for the prevention of biomedical device-associated infections. Biomaterials 30(13):2552–2558. Epub 2009/02/14; PubMed PMID: 19215980; PMCID: PMC3699876. https://doi.org/10.1016/j.biomaterials.2009.01.042
54. Boyce BM, Lindsey BA, Clovis NB, Smith ES, Hobbs GR, Hubbard DF, Emery SE, Barnett JB, Li B (2012) Additive effects of exogenous IL-12 supplementation and antibiotic treatment in infection prophylaxis. J Orthop Res 30(2):196–202. Epub 2011/08/05. PubMed PMID: 21815205; PMCID: PMC3699881. https://doi.org/10.1002/jor.21520
55. Hamza T, Barnett JB, Li B (2010) Interleukin 12 a key immunoregulatory cytokine in infection applications. Int J Mol Sci 11(3):789–806. Epub 2010/05/19; PubMed PMID: 20479986; PMCID: 2869233. https://doi.org/10.3390/ijms11030789
56. Armstead AL, Li B (2011) Nanomedicine as an emerging approach against intracellular pathogens. Int J Nanomedicine 6:3281–3293. Epub 2012/01/10; PubMed PMID: 22228996; PMCID: PMC3252676. https://doi.org/10.2147/ijn.s27285

The Impact of Bacterial Biofilms in Transfusion Medicine

Sandra Ramirez-Arcos

Abstract Blood transfusion is a life-saving practice that started in the early 1800s. Blood components used for transfusion therapy for bleeding patients include platelet concentrates (PCs), red cell concentrates, and plasma. PCs are highly susceptible to bacterial contamination, due to their storage conditions in a nutrient-rich environment at ambient temperature, posing the most significant post-transfusion infectious risk. The predominant bacteria present in PCs are commensal inhabitants of the human skin such as *Staphylococcus epidermidis* (*S. epidermidis*) which are likely introduced at the time of blood collection. *S. epidermidis* and other common PC contaminants form surface-attached communities of matrix-embedded cells, known as biofilms, during PC storage. Biofilms are formed by bacteria adhering to either platelet cells or to the plastic of PC containers. Bacterial adhesion to PC containers is enhanced by the presence of plasma factors and can be reduced by physical or chemical modification of the PC storage bag. Biofilm formation can also be reduced by preventing platelet–bacteria interactions or by reducing the plasma content in PCs. The PC storage environment promotes biofilm formation by coagulase-negative staphylococci isolates traditionally considered to be biofilm negative, resulting in increased pathogenicity and missed detection during PC screening using automated culture systems. Recent studies have shown that the PC storage environment induces structural changes in the bacterial cell wall and biofilm matrix of *S. epidermidis* that could be responsible for resistance to immune clearance and persistent growth in this environment. Further studies are needed to deepen our understanding of the PC storage parameters responsible for triggering bacterial biofilm formation and to develop new strategies to improve PC safety for the benefit of transfusion patients.

Keywords Antimicrobial peptide · Bacterial adhesion · Bacterial contamination · Biofilm · Biofilm matrix · Bloodborne bacteria · Cell wall · Coagulase-negative staphylococci · Platelet–bacteria interaction · Septic transfusions · *Staphylococcus epidermidis*

S. Ramirez-Arcos (✉)
Centre for Innovation, Canadian Blood Services, Ottawa, ON, Canada

Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, ON, Canada
e-mail: sandra.ramirez@blood.ca

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_9

Introduction

The first human-to-human blood transfusion was reported by Dr. James Blundell in 1818; however, the use of blood transfusions for the treatment of bleeding patients was limited in the nineteenth century [1]. The main issue encountered at that time was blood clotting, which was overcome with the development of anticoagulant solutions [1–3]. Although transfusion of whole blood is still a routine practice in developing countries, blood components are usually manufactured for transfusion therapy in the industrialized world. A triage of blood components can be produced from whole blood: platelet concentrates (PCs), which are mainly used to treat or prevent thrombocytopenia (low platelet counts) in oncology or bleeding patients; red blood cell concentrates (RBCC) for the treatment of anemia and other RBC diseases; and plasma, to treat bleeding and trauma patients. RBCC are stored under refrigeration (1–6 °C or 2–6 °C, depending on the country) to preserve red blood cell functionality. Similarly, plasma units are frozen and stored at temperatures <18 °C to ensure stability of coagulation factors. PCs, however, are stored under agitation for up to 7 days in gas-permeable plastic containers, at temperatures of 20–24 °C, to avoid platelet aggregation and to maintain platelet function [4].

Good Manufacturing Practices ensure that blood operators maintain the safety, quality, identity, potency, and purity of blood components, criteria collectively covered by the acronym SQuIPP [5]. Significant advances have been made to increase the safety of blood products used for transfusion in recent years by reducing the occurrence of blood component units contaminated with viruses such as HIV, Hepatitis B, and Hepatitis C [6]. However, bacterial contamination of transfusable blood products remains the most significant post-transfusion infectious risk in developed countries. Bacteria are introduced into the donated blood during the venipuncture process and can be present in all blood components manufactured from whole blood. Within the three blood components, PCs are exquisitely susceptible to bacterial contamination due to their storage conditions. Measures implemented worldwide to mitigate the risk of transfusing bacterially contaminated PCs include donor screening with a questionnaire, donor skin disinfection, diverting the first aliquot of the donated blood, PC screening for the presence of bacteria, and pathogen inactivation technologies [6].

Bacterial contaminants of PCs include gram-positive and gram-negative bacteria with predominant species shown in Table 1. Gram-positive bacteria, such as coagulase-negative staphylococci and propionibacteria, are the predominant PC contaminants. Gram-negative bacteria can also be present in PCs originating from blood donor silent bacteremia or transient skin colonization, and can cause severe and often fatal septic shock in transfusion recipients. Frequently identified gram-negative PC contaminants include *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp., and *Serratia* spp. [6]. Both gram-positive and gram-negative bacteria can proliferate during PC storage, reaching high concentrations (Fig. 1, [7]).

Table 1 Predominant bacterial contaminants in platelet concentrates

Type	Species
Gram-positive bacteria	*Staphylococcus epidermidis*
	Cutibacterium acnes
	Corynebacterium spp.
	Staphylococcus aureus
	Streptococcus spp.
	Bacillus spp.
	Clostridium perfringens
Gram-negative bacteria	*Serratia marcescens*
	Escherichia coli
	Klebsiella pneumoniae
	Enterobacter spp.
	Pseudomonas spp.

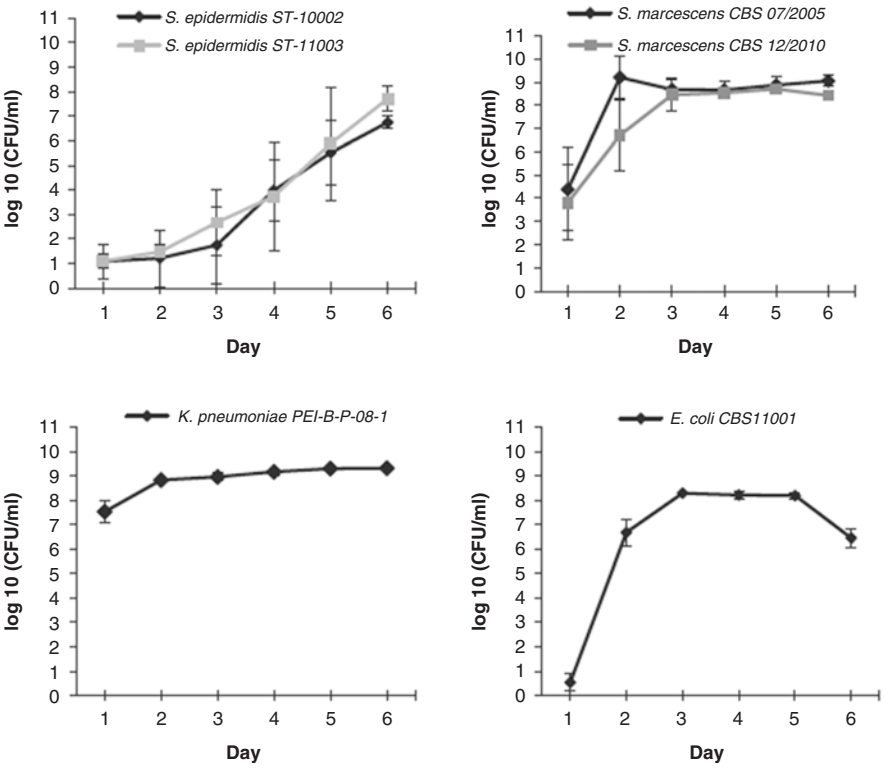


Fig. 1 Growth of gram-positive (*Staphylococcus epidermidis ST-10002 and ST*-11003) and gram-negative (*Serratia marcescens CBS 07/2005* and *CBS 12/2010, Klebsiella pneumoniae PEI-B-P-08-1*, *Escherichia coli CBS1101*) bacteria in PCs under standard storage conditions over 5 days. $N \geq 2 \pm$ SD. Reproduced with permission from Taha et al. [7]

Bacterial Biofilm Formation During Storage of Platelet Concentrates

Coagulase-negative staphylococci form surface-attached communities of matrix-embedded cells, known as biofilms, in the deeper layers of the skin [8]. Staphylococcal biofilms are resistant to the bactericidal action of the commonly used blood donor skin disinfectants chlorhexidine and isopropyl alcohol [9], and therefore can contaminate collected blood and derived blood components. Biofilm-positive coagulase-negative staphylococci have been isolated from contaminated PCs, indicating that these organisms are widely distributed within the healthy blood donor community [10, 11].

Bacterial adherence to blood transfusion sets, comprising transfusion bags and tubing, was first reported by Parment et al. [12]. Isolates of *Serratia marcescens* (*S. marcescens*), *Serratia liquefaciens* (*S. liquefaciens*), *Pseudomonas aeruginosa*, and *S. epidermidis* were used in adherence studies in relation to connecting tubes (polyvinyl chloride) and PC bags. *S. marcescens* isolated from contaminated blood bags showed greater adherence to the tubing of the transfusion sets compared to isolates from other sources [12].

More recently, the dynamics of bacterial growth and biofilm formation during PC storage have been extensively studied using the predominant aerobic PC contaminant *S. epidermidis* as a model organism. *S. epidermidis* is part of the normal skin flora and forms biofilms in PCs by adhering to the inner surface of PC storage containers or by a direct interaction of bacterial cells with activated platelets (Fig. 2, [13]). Using a bioimaging system, Motoyama et al. [14] showed formation of floating microcolonies of *S. epidermidis* during initial stages of PC storage.

Importantly, the PC storage environment promotes biofilm formation by *S. epidermidis* and other coagulase-negative staphylococcal isolates traditionally considered to be biofilm negative [13, 15, 16]. Of note, biofilms formed in stored PCs have increased pathogenicity as demonstrated using a nematode killing assay [17].

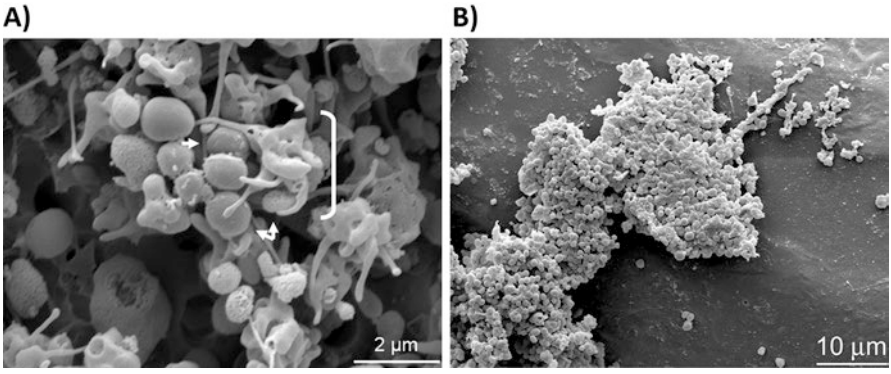


Fig. 2 Biofilm formation of *Staphylococcus epidermidis* in platelet concentrates. (**A**) Platelet–staphylococcal cells attachment. (**B**) Staphylococcal biofilm adhered to the inner surface of platelet storage bags. Reproduced with permission from Greco et al. [13]

Furthermore, missed detection of *S. epidermidis*, *Staphylococcus capitis, Staphylococcus aureus* (*S. aureus*), and *S. marcescens* during PC screening with automated culture methods has been attributed to slow growth and/or biofilm formation ability of these organisms [13, 15, 16, 18, 19]. Reports of septic transfusion reactions involving PCs with false negative screening results which were contaminated with biofilm-forming *S. epidermidis* [20] and *S. aureus* [19] highlight the harmful impact that bacterial biofilm formation during PC storage may have on the safety of transfusion patients.

Bacterial Adhesion to PC Storage Containers

Bacterial adherence to the inner surface of PC storage bags likely contributes to missed detection, as fewer bacteria would be available in the supernatant samples taken for PC screening. Staphylococcal adhesion to PC containers is enhanced by the presence of plasma proteins such as fibrinogen, which likely mediate bacterial adherence to the containers [21]. Hadjesfandiari et al. [22] demonstrated higher bacterial adhesion and bacterial biofilm formation on rough compared to smooth surfaces of PC containers. Strategies to prevent bacterial adherence to PC containers have been recently developed. In a study conducted by Wilson-Nieuwenhuis et al. [23], PC containers were physically flattened resulting in altered roughness and reduced hydrophobicity of the PC bag surface. The authors demonstrated that biofilm formation by *S. marcescens* and *S. epidermidis* was reduced in PCs stored for up to 5 days in the flattened containers. A different approach has been presented by Hadjesfandiari et al. [24] with the application of an antifouling polymer to coat PC containers. The coating process decreased fibrinogen adsorption, platelet adhesion, and bacterial biofilm formation. Similarly, when approximately 65% of plasma content of PCs is replaced with a platelet additive solution, biofilm formation by *S. epidermidis* and *S. liquefaciens* is significantly decreased supporting the role of plasma proteins in bacterial adhesion during PC storage (Fig. 3, [25]).

Bacteria–Platelet Interaction During PC Storage

Bacteria–platelet interaction is crucial for the development of infections such as endocarditis. The interaction follows a sequence of events starting with bacterial cells bound to a platelet receptor, either directly or through bridging proteins, triggering a signal-transduction cascade. The result of this interaction is platelet activation and aggregation [26]. Platelet binding proteins differ within bacterial species; some examples include the clumping factor A in *S. aureus* [27] and the fibrinogen binding protein SdrG in *S. epidermidis* [28]. Similar platelet–bacteria interactions likely occur in contaminated PCs. When PCs are mixed with methoxypoly(ethylene glycol), the platelet surface is covalently modified (PEGylated PCs) resulting in a significant reduction in platelet–bacteria interaction

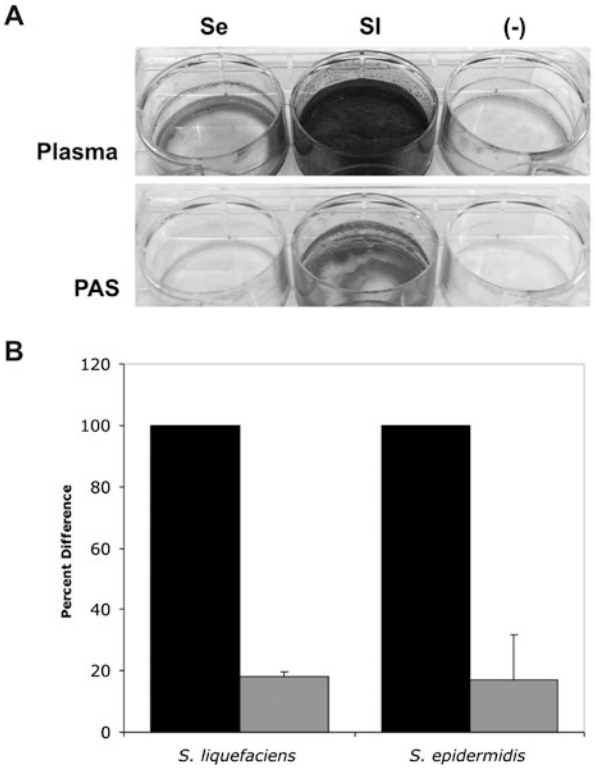


Fig. 3 Biofilm formation by *Staphylococcus epidermidis* (Se) and *Serratia liquefaciens* (Sl) in platelet concentrates (PCs) suspended in either plasma or platelet additive solution (PAS). (**A**) Day 5 biofilms were stained with 0.3% crystal violet. (**B**) Eluted stain intensity at λ = 492 nm was measured for biofilms in PAS-PCs ($n = 4$, grey) and normalized to biofilms grown in plasma-PCs ($n = 6$, black). Reproduced with permission from Greco et al. [25]

and biofilm formation by *S. epidermidis* [29]. Electron microscopy studies have shown bacterial cells "embraced" by activated platelets (mixed biofilms) in PC units contaminated with *S. aureus* or *S. epidermidis* (Fig. 2a) [13, 19]. The molecular mechanisms of these interactions have yet to be elucidated; however, it has been shown that the platelet content plays a key role in bacterial biofilm formation during PC storage. When PCs are filtered, and platelet concentration is reduced by approximately 1000-fold, biofilm formation by *S. epidermidis* is significantly reduced [13].

Biofilm Resistance to Immune Clearance During PC Storage

Recent studies have demonstrated that mature *S. epidermidis* biofilms grown in PCs are resistant to the bactericidal action of a combination of three synthetic antimicrobial peptides, the platelet-derived peptide (PD4) and two arginine–tryptophan repeats (RW3 and RW4) [30]. The mechanisms of resistance against killing by antimicrobial

peptides are diverse including peptide degradation by proteases, repulsion by the biofilm matrix, efflux pumps and chemical modification of the bacterial cell wall and cell membrane [31]. It is presently unknown which of these mechanisms is responsible for the bacterial resistance to antimicrobial peptides observed in PCs. Investigations aimed at understanding the physiological changes that bacteria undergo when growing in PCs as biofilms have revealed that the chemical composition and structure of the cell wall and biofilm matrix of *S. epidermidis* change in this milieu [32]. The peptidoglycan, a major component of the cell wall, of *S. epidermidis* biofilms grown in PCs has a simpler chemical composition and reduced content of serine in comparison to the peptidoglycan of the biofilms grown in laboratory media. Additionally, the protein content of the biofilm matrix of *S. epidermidis* is prominently increased in cells grown in PCs compared to cells grown in media [32]. The role that these molecular modifications might play in *S. epidermidis* resistance to the action of antimicrobial peptides released by platelets during PC storage merits further investigation.

Safety Implications for Transfusion Patients

Showing a direct link between the transfusion of PC units contaminated with biofilms and clinical outcomes is very difficult. It would require a follow up of PC transfusion recipients beyond the first 24 h post-transfusion. Skin flora bacteria such as *S. epidermidis* do not cause a typical septic transfusion reaction (septic shock) as these organisms do not produce pyrogens [6]. However, biofilm-positive skin flora organisms could colonize biomedical devices implanted in the transfusion patients and cause infections days later that would not be linked to the transfusion event. The relationship between the organism isolated from a biomedical device and a PC contaminant could only be discerned by molecular testing, ideally by whole-genome sequencing of the two organisms.

Future Approaches

Data summarized in this chapter have provided insights of the impact of bacterial biofilms on PC safety. However, several questions remain unanswered and warrant further studies. It is important to understand the regulatory mechanisms involved in the physiological changes that bacterial biofilms undergo when growing in PCs and their mechanism of resistance to immune clearance. It is also vital to deepen biofilm studies in pathogenic gram-positive bacteria such as *S. aureus* and streptococci, as these organisms pose a major safety risk to PC transfusion recipients despite improved PC screening algorithms [33–35]. Furthermore, changes in PC production by the adoption of platelet additive solutions, modification of PC storage conditions (i.e., PC storage under refrigeration), or implementation of pathogen inactivation

technologies should be validated considering differences in bacterial growth dynamics and biofilm formation. Finally, consideration should be given to differences in bacterial growth in PCs of isolates of the same species. The path to improve patient transfusion safety cannot advance without understanding the complex interactions between bacteria and platelets and resistance to immune factors present in PCs and other blood components.

Concluding Remarks

This review chapter highlights the role that bacterial biofilm formation plays during transfusion of blood from donor to recipient (vein to vein). Normal skin flora form biofilms in the skin, which can be transferred to the donated blood during venipuncture. Furthermore, biofilm-negative bacteria convert to a biofilm-positive phenotype during PC storage. Structural modifications of the bacterial cell surface may be responsible for resistance to immune factors present in PCs and enhanced pathogenicity. Development and implementation of measures to prevent bacterial biofilm formation during PC storage should become a priority in transfusion medicine to prevent serious transfusion events in the highly susceptible population of PC transfusion patients.

References

1. Greenwalt TJ (1997) The short history of transfusion medicine. Transfusion 37:550–563
2. Lewisohn R (1924) The citrate method of blood transfusion after ten years. Boston Med Surg J 190:733
3. Weil R (1915) Sodium citrate in the transfusion of blood. JAMA 64:425
4. Canadian Blood Services. Circular of information. https://blood.ca/en/hospital-services/products/component-types/circular-information
5. Ramirez-Arcos S, Marks DC, Acker JP, Sheffield WP (2016) Quality and safety of blood products (Editorial). J Blood Transfus 2016:2482157
6. Ramirez-Arcos S, Goldman M (2012) Bacterial contamination. In: Popovsky M (ed) Transfusion reactions, 4th edn. American Association of Blood Banks Press, Bethesda, MD, pp 53–181
7. Taha M, Kalab M, Yi QL, Maurer E, Jenkins C, Schubert P, Ramirez-Arcos S (2016) Bacterial survival and distribution during buffy coat platelet production. Vox Sang 111:333–340
8. Brandwein M, Steinberg D, Meshner S (2016) Microbial biofilms and the human skin microbiome. NPJ Biofilms Microbio 2:3
9. Taha M, Kala M, Yi Q, Landry C, Greco-Stewart V, Braassinga A, Sifri CD, Ramirez-Arcos S (2014) Biofilm-forming skin microflora bacteria are resistant to the bactericidal action of disinfectants used during blood donation. Transfusion 54:2974–2982
10. Greco C, Mastronardi C, Pagotto F, Mack D, Ramirez-Arcos S (2008) Assessment of biofilm-forming ability of coagulase-negative staphylococci isolated from contaminated platelet preparations in Canada. Transfusion 48(5):969–977
11. Martini R, Hörner R, Rampelotto RF, Garzon LR, Nunes MS, Teixeira MD, Graichen DÂ (2016) Investigation of biofilm formation in coagulase-negative staphylococci isolated from platelet concentrate bags. Rev Inst Med Trop Sao Paulo 58:1

12. Parment PA, Gabriel M, Bruse GW, Stegali S, Ahearn DG (1993) Adherence of *Serratia marcescens, Serratia liquefaciens, Pseudomonas aeruginosa* and *Staphylococcus epidermidis* to blood transfusion bags (CPD-SAGMAN sets). Scand J Infecti Dis 26:721–724
13. Greco C, Martincic I, Gusinjac A, Kalab M, Yang AF, Ramirez-Arcos S (2007) *Staphylococcus epidermidis* forms biofilms under simulated platelet storage conditions. Transfusion 47:1143–1153
14. Motoyama Y, Yamaguchi N, Matsumoto M, Ichijo T, Nagumo H, Kagami N, Tani Y, Satake M, Nasu M (2009) *Staphylococcus epidermidis* forms floating micro-colonies in platelet concentrates at the early stage of contamination. J Health Sci 55:726–731
15. Ali H, Greco-Stewart VS, Jacobs MR, Yomtovian RA, Rood IG, de Korte D, Ramírez-Arcos SM (2014) Characterization of the growth dynamics and biofilm formation of Staphylococcus epidermidis strains isolated from contaminated platelet units. J Med Microbiol 63:884–891
16. Greco-Stewart VS, Ali H, Kumaran D, Kalab M, Rood IG, De Korte D, Ramirez-Arcos S (2013) Biofilm formation by *Staphylococcus capitis* strains isolated from contaminated platelet concentrates. J Med Microbiol 62:1051–1059
17. Hodgson SD, Greco-Stewart V, Jimenez CS, Sifri CD, Brassinga AK, Ramirez-Arcos S (2014) Enhanced pathogenicity of biofilm-negative *Staphylococcus epidermidis* isolated from platelet preparations. Transfusion 54:461–470
18. Greco-Stewart VS, Brown EE, Parr C, Kalab M, Jacobs MR, Yomtovian RA, Ramirez-Arcos SM (2012) *Serratia marcescens* strains implicated in adverse transfusion reactions form biofilms in platelet concentrates and demonstrate reduced detection by automated culture. Vox Sang 102:212–220
19. Loza-Correa M, Kou Y, Taha M, Kalab M, Ronholm J, Schlievert PM, Cahill MP, Skeate R, Cserti-Gazdewich C, Ramirez-Arcos S (2017a) Septic transfusion case caused by a platelet pool with visible clotting due to contamination with *Staphylococcus aureus*. Transfusion 57:1299–1303
20. Kou Y, Pagotto F, Hannach B, Ramirez-Arcos S (2015) Fatal false-negative transfusion infection involving a buffy coat platelet pool contaminated with biofilm-positive Staphylococcus epidermidis: a case report. Transfusion 55:2384–2389
21. Loza-Correa M, Kalab M, Yi QL, Eltringham-Smith LJ, Sheffield WP, Ramirez-Arcos S (2017b) Comparison of bacterial attachment to platelet bags with and without preconditioning with plasma. Vox Sang 112:401–407
22. Hadjesfandiari N, Schubert P, Fallah Toosi S, Chen Z, Culibrk B, Ramirez-Arcos S, Devine DV, Brooks DE (2016) Effect of texture of platelet bags on bacterial and platelet adhesion. Transfusion 56:2808–2818
23. Wilson-Nieuwenhuis JST, Dempsey-Hibbert N, Liauw CM, Whitehead KA (2017) Surface modification of platelet concentrate bags to reduce biofilm formation and transfusion sepsis. Colloids Surf B Biointerfaces 160:126–135
24. Hadjesfandiari N, Weinhart M, Kizhakkedathu JN, Haag R, Brooks DE (2018) Development of antifouling and bactericidal coatings for platelet storage bags using dopamine chemistry. Adv Healthc Mater 7(5). https://doi.org/10.1002/adhm.201700839
25. Greco CA, Zhang JG, Kalab M, Yi QL, Ramirez-Arcos SM, Gyongyossy-Issa MI (2010) Effect of platelet additive solution on bacterial dynamics and their influence on platelet quality in stored platelet concentrates. Transfusion 50:2344–2352
26. Fitzgerald JR, Timothy J, Foster TJ, Cox D (2006) The interaction of bacterial pathogens with platelets. Nat Rev 4:445–457
27. Siboo IR, Cheung AL, Bayer AS, Sullam PM (2001) Clumping factor A mediates binding of *Staphylococcus aureus* to human platelets. Infect Immun 69:3120–3127
28. Brennan MP, Loughman A, Devocelle M, Arasu S, Chubb AJ, Foster TJ, Cox D (2009) Elucidating the role of *Staphylococcus epidermidis* serine-aspartate repeat protein G in platelet activation. J Thromb Haemost 7:1364–1372
29. Greco CA, Maurer-Spurej E, Scott MD, Kalab M, Nakane N, Ramírez-Arcos SM (2011) PEGylation prevents bacteria-induced platelet activation and biofilm formation in platelet concentrates. Vox Sang 100:336–339

30. Alabdullatif M, Atreya CD, Ramirez-Arcos S (2018) Antimicrobial peptides: an effective approach to prevent bacterial biofilm formation in platelet concentrates. Transfusion 58:2013–2021
31. Joo H-S, C-l F, Otto M (2016) Bacterial strategies of resistance to antimicrobial peptides. Philos Trans R Soc B 371:20150292
32. Loza-Correa M, Ayala JA, Perelman I, Hubbard K, Kalab M, Yi QL, Taha M, de Pedro MA, Ramirez-Arcos S (2019) The peptidoglycan and biofilm matrix of Staphylococcus epidermidis undergo structural changes when exposed to human platelets. PLoS One 14:e0211132
33. Abela MA, Fenning S, Maguire KA, Morris KG (2018) Bacterial contamination of platelet components not detected by BacT/ALERT. Transf Med 28:65–70
34. Brailsford SR, Tossell J, Morrison R, McDonald CP, Pitt TL (2018) Failure of bacterial screening to detect *Staphylococcus aureus:* the English experience of donor follow-up. Vox Sang. https://doi.org/10.1111/vox.12670
35. Haass KA, Sapian MRP, Savinkina A, Kuehnert MJ, Basavaraju SV (2019) Transfusion-transmitted infections reported to the National Healthcare Safety Network Hemovigilance Module. Transf Med Rev 33(2):84–91. https://doi.org/10.1016/j.tmrv.2019.01.001

Part III
Advanced Antimicrobial Strategies to Treat Infection

Antimicrobial Materials in Arthroplasty

Julie Shaner, Noreen Hickock, and Antonia F. Chen

Abstract With an increase in the number of total joint arthroplasty procedures being performed, the number of surgical site infections (SSI) and peri-prosthetic joint infections (PJI) are also expected to increase. In addition to portending significant morbidity and mortality, the growing number of prosthetic associated infections also presents a significant social and economic burden. There are current antimicrobial resistance strategies available for clinical use and more are being developed and are in the laboratory development and testing phases. However, resistance to treatment include limited implant host interface vascularity that contributes to the inability of systemically administered antibiotics to effectively reach and exert a full effect where most needed. Recognition of the limitation of systemic antibiotics and the growing problem presented by PJI have led to more recent efforts focused on local antimicrobial control at or around surgically implanted materials. Current and developing methods of achieving prophylactic local antimicrobial control in arthroplasty include using antibiotic loaded bone cement, intrawound antibiotic powders, antiseptic lavages, biocompatible antimicrobial delivery devices and coatings, and modified implants.

Keywords Prosthetic · Joint · Infection · Biofilm · Antibiotics · Antimicrobial · Antiseptic · Implants · Delivery devices · Chitosan · Hydrogel · Surface · Metal · Coatings

J. Shaner
Temple University Hospital, Philadelphia, PA, USA
e-mail: julie.shaner@TUHS.Temple.edu

N. Hickock
Thomas Jefferson University Hospital, Philadelphia, PA, USA
e-mail: noreen.hickok@jefferson.edu

A. F. Chen (✉)
Brigham and Women's Hospital, Boston, MA, USA
e-mail: afchen@bwh.harvard.edu

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_10

Introduction

Hip and knee arthroplasty are proven to be successful in clinical practice. They have led to high survivorship and resulted in reduced pain, function, and improved quality of life with low morbidity and mortality [1–3]. For these reasons, the number of joint replacement procedures continues to rise, with the number of total hip arthroplasty (THA) procedures slated to increase 71% and total knee arthroplasty (TKA) 85% by 2030 [4]. Despite reduced rates of revision performed for aseptic loosening and wear due to advances in components design and improved surgical technique, the rate of peri-prosthetic joint infection (PJI) remains unchanged, making it a very common mode of failure in total joint arthroplasty (TJA) [5, 6]. Revision for PJI is performed in less than 2% of primary TJAs [7] and up to 20% of revision arthroplasties, including limb salvage surgery [8]. With anticipated continued growth of total joint procedures performed, so too will the numbers of PJI [9]. PJI is associated with significant morbidity, increased rates of mortality, and costs associated with PJI are projected to exceed $1.6 billion by 2020 [10, 11]. The estimated PJI cost for sensitive organism PJI is over $60,000, while resistant organisms (e.g., methicillin-resistant organisms) is greater than $100,000 for per case [12, 13]. For these reasons, current and future efforts focused on preventing and/or eradicating PJI are paramount.

Current Methods of PJI Prevention

The first step in reducing PJI is prevention. Current methods have focused primarily on reducing risk through control of the operative environment and patient factors. In the operating room, foot traffic control, laminar flow, air filtration systems, hooded surgical gowns, good sterile techniques, and surgical efficiency have been adopted to minimize the opportunity for microbial contamination of the surgical field [14, 15]. Patient focused factors include administration of systemic perioperative antibiotics, presurgical skin cleansing, nasal methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* decolonization, and selecting patients who have undergone modifiable risk factor optimization [16, 17]. Likewise, despite efforts to minimize these patient-related risk factors, host disparities leading to increased PJI susceptibility are not always identifiable or modifiable. In fact, only the use of perioperative systemic antibiotics is supported by consensus recommendation and is considered standard of care [18]. Unfortunately, regardless of efforts to maintain a sterile operating room (OR) condition, bacterial and fungal bioaerosols cannot be completely eliminated from the surgical environment. Various pathogens have been found on inanimate OR surfaces, as one study demonstrated that 16.6% of 283 objects sampled from 35 operating rooms of teaching hospitals in the USA were positive for pathogens [19].

Biofilm and Limitations of Systemic Preventative Strategies

Device-associated infections are generally assumed to occur due to a low numbers of contaminating bacteria that occurs during the operative procedure. Implanted material has been shown to allow establishment of infection with an inoculum (10–100 bacterial colony forming units (CFU)) that is ~10,000 times lower than that required for its establishment in the absence of an implant [20], suggesting that the host response to the hardware to defend is compromised. Specifically, upon placement, implants are rapidly coated with serum proteins such as albumin, fibronectin, and fibrin[ogen], proteins that are critical for osseointegration but unfortunately provide an ideal surface for bacterial adhesion. The presence of the implant further complicates the situation as this foreign body causes activation of the immune system and an inflammatory response, neither of which can adequately eradicate the adherent bacteria [21]. Finally, the bacteria use the proteinaceous matrix as well as secretion of its own biofilm proteins [22, 23] to encase the adherent bacteria within a bacterial biofilm that further protects microorganisms from surveillance by host immune cells and antibiotics [24].

Bacterial colonization is the process from microbial adhesion to establishing a mature biofilm layer that takes only a few hours [25]. Biofilm bacteria tend to be metabolically indolent and are comprised of a high percentage of persisters [26, 27]. This suppressed metabolic state decreases the consequences of antibiotic treatment as antibiotics are targeted at rapidly growing cells, including functions such as cell wall synthesis, protein synthesis, or DNA replication. Thus, bacteria contained within a biofilm have 10 to 1000-fold less antibiotic susceptibility than free floating planktonic bacteria in culture [23, 28]. Importantly, due to avascularity of implanted material and subsequent impaired blood circulation in the bone environment, only low drug concentrations are delivered to the bone-implant interface with the result that systemic antibiotic treatments are usually ineffective at eliminating bacterial biofilms [29]. To date, there is no systemic treatment capable of rapid and complete biofilm destruction, which leaves local control and contaminated implant extraction as some of the few viable options for the treatment of PJI [30]. Infection prevention is key, as treatment is difficult due to pathogen colonization of implants, pathogen recalcitrance to antibiotic treatment when adhered to implants, and pathogen persistence in tissue despite removal of the implant.

Focus on Local Control

Microbial colonization of implanted material furnished the theory of a proposed "race for the surface," in which bacteria and host cells compete for implant survivorship [31]. While this concept is not entirely accurate, as host peri-implant osseointegration or fibrous tissue encapsulation does not eliminate survivorship of bacterial micro-colonies, it has focused efforts on providing local antimicrobial control. The goal of local prophylactic control is to keep microbial infections from occurring at

or around the site of implantation. Local drug delivery can reduce bacteria concentration around implants and potentially prevent bacterial adherence. Compared to intravenous antibiotics, local antibiotic delivery offers higher drug concentration in relevant tissue and reduced systemic toxicity. Potential methods of achieving local peri-implant microbial control in TJA include use of antibiotic loaded bone cement (ABLC), antibiotic powders, antiseptic irrigation, biocompatible delivery devices and coatings, and modified implants.

Antibiotic Bone Cement

Discovery of antibiotic elution from polymethylmethacrylate (PMMA) bone cement into the tissue surrounding implants resulted in the use of antibiotic-loaded bone cement (ALBC) for infection prophylaxis in TJA [32]. Elution from ABLC shows an initial sharp peak of antibiotic release followed by decreased but constant release observed over the following days to week; a retrieval study demonstrated that gentamicin and vancomycin loaded hip spacers continued to release a reduced but constant concentration of local antibiotic 3–6 months after implantation [33]. While a multitude of antibacterial and antifungal pharmacologic agents can be added to bone cement, preferred characteristics include: thermal stability, powder form, antimicrobial efficacy over a wide spectrum, antimicrobial effect at low concentrations, high PMMA elution, minimal disruption of bone cement mechanical properties, and low risk of delayed hypersensitivity or allergy [34] (Table 1). Due to their wide spectrum coverage, including most organisms associated with PJI, vancomycin and gentamicin

Table 1 Antibacterial and antifungal pharmacologic agents that can be added to bone cement

Type of antibiotic	Activity against	g/40g PMMA
Vancomycin	Gram-positive bacteria, including methicillin-resistant organisms	0.5–4
Cefazolin	Gram-positive infections, limited gram-negative coverage	1–2
Erythromycin	Aerobic gram-positive *cocci* and bacilli	0.5–1
Linezolid	Multidrug-resistant gram-positive *cocci* such as MRSA	1.2
Meropenem	Gram-positive and gram-negative bacteria, anaerobes, and *Pseudomonas*	0.5–4
Tobramycin	Gram-negative bacteria (*Pseudomonas*)	1–4.8
Gentamicin	Gram-negative bacteria (*E. coli*, *Klebsiella*, and *Pseudomonas aeroginosa*). Aerobic bacteria	0.25–4.8
Ceftazidime	Gram-negative bacteria (*Pseudomonas*)	2
Cefotaxime	Gram-negative bacteria, not against *Pseudomonas*	2
Ceftaroline	Gram-negative bacteria, not against *Pseudomonas*	2–4
Ciprofloxacin	Gram-negative organisms (*Enterobacteriaceae*)	0.2–3
Colistin	Gram-negative bacteria	0.24
Aztreonam	Gram-negative bacteria	4
Amphotericin deoxycholate	Fungus	200
Voriconazole	Fungus	300–600

have broad clinical application in orthopedics. The literature surrounding the practice of prophylactic ALBC to prevent infection is controversial, as some studies support this practice while others have suggested that this strategy is not ideal as a prophylactic measure [35, 36]. Prolonged exposure to antibiotics does not provide any additional benefit and may lead to systemic toxicity, reduced mechanical properties of cement, and can contribute to microbial antibiotic resistance [37, 38].

Antibiotic Powder

The increase in drug-resistant organisms is due to the overutilization of antibiotics, which highlights the importance of reducing antibiotic exposure and minimizing unnecessary antibiotic prescriptions. Guidelines support systemic antibiotic perioperative prophylaxis administration within 60 min before surgical incision to prevent SSI (ASHP guidelines). However, there are no guidelines for administration of local antibiotics. The purpose of using topical antibiotics is achieving a high antibiotic concentration at the surgical site while minimizing the adverse effects associated with systemic exposure [39]. Systemic antibiotics show decreased surgical wound infection when administered within 1–2 h before incision; locally applied antibiotic powder requires less time for activity onset and achievement of high local concentrations at the desired site [40, 41]. A potential disadvantage of topical antibiotics is that the typical application occurs prior to closure to prevent dilution or removal with irrigation of the surgical bed. This limits their use in isolation, as without coadministration of preoperative systemic antibiotics, the late timing may provide inadequate prevention although isolated administration has not been conducted in any clinical studies to date.

Despite limited systemic bioavailability and diminished risk for adverse events, documented complications of local antibiotics in powder form including culture negative seromas, ototoxicity and transient hearing loss, nephropathy, and anaphylactic circulatory collapse have been reported with use in spine surgery [42, 43]. Other concerns regarding high concentrations of locally administered antibiotics include the effect on osteoblast physiology and the potential for accelerated bearing surface wear [44]. High local vancomycin concentrations <1000 mg/L have minimal effect on osteoblast-like cells, with osteoblast cell death at concentrations >10,000 mg/L of vancomycin [45, 46]. Tobramycin or cefazolin concentrations <200 mg/L do not affect osteoblast cells, where 200–400 mg/L alters cell replication, and >10,000 mg/L causes cell death [47]. Combined systemic cefazolin and local gentamicin has shown the greatest efficacy in in vivo animal model studies when compared to topical antibiotic options alone or with other combinations of systemic and topical antibiotics [48]. Few studies have evaluated the use of topical intrawound antibiotics for TJA infection prophylaxis, since it is not a commonly adopted practice. However, a retrospective study reporting on 125 consecutive patients who underwent THA compared intravenous antibiotics alone to intravenous antibiotics and 2 g of locally applied topical vancomycin. The placement of the powdered vancomycin resulted in markedly fewer infections in THA patients [49]. The growing antibiotic resistance and possible formation of culture negative seromas accentuate the need to develop alternative local antimicrobial strategies.

Antiseptic Irrigation

There are currently no set standards for wound irrigation for SSI prevention at the time of primary TJA, as there is a lack of evidence and minimal high-level studies [50]. Currently used solutions include 0.9% saline, antiseptics (e.g., povidone–iodine complex, chlorhexidine, or hydrogen peroxide), antibiotic solutions (e.g., bacitracin/polymyxin), and castile soap. Antiseptics are favored over antibiotics, as they have less likelihood of resistance due to the fact that they target various aspects of microbial cell biology with different mechanisms of action [51].

The commonly used povidone–iodine complex, formed by association of iodine with povidone (a synthetic carrier polymer), has no microbicidal activity [52]. In an aqueous medium, the povidone–iodine complex releases free iodine (the antimicrobial component) to reach an equilibrium; as the iodine-consuming germicidal activity proceeds, the povidone–iodine reservoir releases more free iodine [53, 54]. Iodine exhibits microbicidal activity by oxidizing nucleotides, respiratory chain cytosolic enzymes, and bacterial cell membrane fatty/amino acids. This oxidation causes their denaturation [55]. In terms of cytotoxicity, a recent study showed that povidone–iodine complex enhanced wound healing via tumor necrosis factor beta (TGF β) with increased granulation and enhanced neovascularization [56]. Thus, povidone–iodine complex offers favorable efficacy due to its ability for biofilm penetration, activity across a broad spectrum of bacteria, fungi, protozoa, and viruses, and decreased resistance development. Its lack of cytotoxicity as evidenced by its lack of adverse effects on wound healing and its anti-inflammatory properties are an added benefit as prolonged inflammation contributes to extracellular matrix defective remodeling and can cause failure of reepithelialization and development of chronic wounds [57]. Intraoperative flushing of the surgical site with povidone–iodine complex (0.35% dilution) has resulted in reduced TJA infection rates [58].

Chlorhexidine (CHD) is being used in multiple healthcare applications, since it has a broad-spectrum of antimicrobial activity and a fast onset of action. Applications including hand and oral hygiene, skin preparation, and impregnation into surgical meshes, catheter sites, and wound dressings at various concentrations [59]. CHD has a faster onset of action compared to povidone–iodine complex against more microorganisms and has been shown to be less cytotoxic when applied to healthy tissue [60]. CHD is a frequently used bactericidal antiseptic that acts through disruption of microbial cellular membranes [61]. Several in vitro and in vivo animal studies have sought to investigate the safety of CHD-based irrigants, evaluating the effects of its exposure on different anatomic structures including arteries, veins, and collagen. These studies have not found any toxicity at low concentrations and have demonstrated no effects on mechanical properties of collagen-based structures such as tendons [62, 63]. There are no known reports of CHD resistance despite long-term use. Mounting evidence may suggest that antiseptics should be used preferentially instead of systemic and topical antibiotics, however further investigation is needed to make that determination.

Modified Implants

While antibiotic powders and antiseptic irrigations offer options for treating the peri-implant local environment, emerging technologies involving implant surface modification facilitate both peri-implant and direct implant surface antimicrobial activity. The overall goals of these modifications are prevention of bacterial adhesion and formation of biofilm while avoiding conditions that may foster acquisition of antibiotic resistance. These implant surface modifications permit different strategies to display active molecules and/or prevent bacterial adhesion.

Two main strategies are implemented to produce activated implants. A selected drug or biomolecule can be mixed with the substrate of the bulk device, or it can be grafted onto the surface to produce biomolecular loaded coatings. Surface coatings require the apposition of a certain substance onto a desired object, adding layers to the existing surface. Some surface coatings consist of a biodegradable delivery from which bioactive agents are released. Examples of such bioactive delivery devices are bioactive glasses, hydrogels and chitosan. A variety of techniques including direct chemical coupling, dip coating, layer by layer (LBL), and electrophoretic deposition (EPD) are used to produce implant surface coatings. The aim of implant mediated antimicrobial activity is to prevent primary microbial adhesion by repelling or killing planktonic microbial cells. Coating activity is active or passive depending on whether agents are locally delivered to surrounding tissue or prevent adhesion or function by contact killing [64, 65].

Topographies: Perhaps the most straightforward surfaces are those created by topographically modifying the surface on the nanoscale to prevent bacterial adhesion. These surfaces are inspired by naturally antimicrobial surfaces, such as shark skin that has 3D riblet microstructure, lotus leaves that have micro-size bulge shape, and gecko skin that has hair-like nanostructure [66–68]. The patterning has been explored for uses in catheters, and on metal surfaces where micro and nanotopographic modulations affect the ability of bacterium to adhere to the surface. While numerous designs exist, and even at least one company, these topographic surfaces are predominantly characterized ex vivo; at least one rat skin test found that the surfaces retained activity in the presence of at least wound fluid. In general, these surfaces are reported to decrease bacterial colonization by up to three logs [69]. In addition, surfaces have altered charge, roughness, porosity, and hydrophobicity to affect bacterial adhesion [70].

Antimicrobial Implant Surfaces

Example of active antimicrobial molecules that have been used to modify implant surfaces include a nitric oxide (NO) releasing material, antimicrobial peptides, antibiotics, antibacterial polymers, and inorganic antibacterial metal elements [24, 71]. Currently available and developing modified implants for orthopedic use have primarily involved applied strategies to titanium (Ti) substrates, and to a lesser extent

cobalt chrome (Co-Cr) and allograft bone [72]. Titanium and its alloys (Ti-6Al-7Nb, Ti-5Al-2·5Fe, and Ti-6Al-4V) are often used in orthopedics due to biocompatibility, corrosion resistance, and chemical and mechanical properties, and are therefore the focus of most research involving surface treatment. Due to the frequent use of press fit techniques in THA and growing use in TKA, with ultimate stability reliant on successful host implant in-growth or on-growth, modified implant strategies developed for use in the trauma setting may not be appropriately applied to the realm of arthroplasty. Therefore, the ideal antimicrobial arthroplasty implant should maintain its biomechanical properties, remain biocompatible, promote or be non-inhibitory toward osteoblast activity, and provides effective anti-infectivity [73–75]. We and others have explored the efficacy of using direct grafting to permanently render the biomaterial surface antimicrobial. Unlike controlled-release systems, antibiotics are not eluted from these surfaces and thus have the potential to remain antimicrobial during the osseointegration period. Direct grafting of vancomycin [76–78] on titanium, Ti-6Al-4V, and on allograft bone reduced adhesion by *S. aureus* and *S. epidermidis* [79, 80], with direct efficacy in small [45] and large [81] animal models of osteomyelitis. Similarly, antimicrobial peptides have been tethered to surfaces with retention of antimicrobial activity [82–85]. Perhaps of greatest interest is a report in which tissue plasminogen activator (tPA) was coated on polystyrene and examined in vitro and in vivo. The induction of fibrinolysis by tPA significantly reduced bacterial colonization [86].

More recently, vancomycin and complementary antibiotics was immobilized in the matrix of UHMWPE to render the bearing surfaces antimicrobial. With an eccentric clustering of the antimicrobial, elution of vancomycin occurred at bactericidal levels for >3 weeks. Importantly, gamma radiation of the implant for sterilization resulted in permanent immobilization of some of the antibiotics, leading to permanently antimicrobial UHMWPE components to prevent infection. It is noteworthy that these surfaces were also used to treat infection, as it had continued activity in a rabbit model of osteomyelitis, outperforming antibiotic-loaded bone cement [87]. These surfaces, however, remain in the development phase.

Hydrogels

Cross-linked polymers and hydrogels are often used for various biomedical purposes due to their biocompatibility, ability for local pharmacological agent delivery and capacity to produce specific elution patterns [88]. The broad structure of these polymers and hydrogels encourages cell survival and proliferation, has compositional similarities to extracellular matrices, and is readily resorbable. Poly-electrolyte hydrogels bearing amino acid residues approximates biologic tissue by permitting bioactivity, while also forming a physical barrier to bacterial adhesion. Ionic functional groups permit complex formation with drug molecules and/or metal ions. Aside from the primary role as an ion and drug delivery system, hydrogels ionic interactions also control the release kinetics into the environment. The kinetics of release is determined by the strength of the interaction between the hydrogel car-

boxyl group and drug amine group. Hydrogels are therefore utilized as an antibacterial coating that provides fast resorption and local protection in the short-term.

A current clinical use includes a hydrogel coating referred to as defensive antibacterial coating (DAC, Novagenit SRL, Mezzolombardo, Italy). Novagenit SRL, Mezzolombardo, Italy). The composition consists of hyaluronan that is covalently linked and poly-D,L-lactide, and undergoes hydrolytic degradation within 72 h in vivo. During the dissolution phase, the hydrogel completely releases a variety of antibacterials impregnated within the gel. In a prospective observational multicenter study, 380 patients were in the treatment group that received antibiotic loaded DAC coating applied intraoperatively to the surfaces of total hip or total knee prosthesis, or a control group. Although only short-term results were available, it demonstrated good safety and efficacy without local or systemic side effects, and there was a ten-fold reduction in early SSIs [89].

Chitosan

Chitosan (CTS) is a biocompatible, biodegradable polymer developed from renewable resources that are natural. It is derived from the deacetylation of chitin, which is a naturally occurring biopolymer that comprises the exoskeleton of crustaceans, can be found in fungal cells walls, and is found in abundance in other biological materials. The antibacterial and antifungal properties of chitosan are hypothesized to derive from the polycationic characteristics and are mediated by electrostatic forces between negative residues at cell surfaces and protonated amino groups (NH_3^+) in chitosan. The antimicrobial activity of CTS is influenced by the numbers of these protonated amines present in chitosan where these numbers increase with greater degrees of deacetylation, as well as its film-forming properties and cationicity. When formed as a film, CTS has selective permeability to CO_2 and O_2 gases, strong mechanical properties, and exhibits high permeability to water. This biopolymer is susceptible to accelerated angiogenesis, enzymatic degradation, limited fibrous encapsulation, increased cellular adhesion, and innate ability to deliver and link to growth factors [90].

The miscibility of the substance with which chitosan is blended can influence both the mechanical properties and surface morphology of the biologic film. In addition, chitosan-based films can be tuned when combining with other hydrocolloids or proteins, where antibiotics are often combined with CTS as a drug delivery system. Using chitosan–gelatin composites, ampicillin release could be rate-controllable by changing the polymer ratio within deposited films in an in vitro model [91]. CTS also functions well as a delivery device for other bioactive agents. For example, chitosan has been combined with gentamicin-loaded bioactive glass (CS/BG/GS), forming a composite coating that transforms a brittle glass coating to a more compliant structure [92]. Similar to ALBC, release kinetics show an initial burst followed by slower release. Within 5 days, the CS/BG/GS composite released 40% gentamicin but maintained sustained release over a period of 8 weeks. This inhibited in vitro bacterial growth for 2 days, led to cellular proliferation up to

10 days. Finally, CTS has been combined with the poly anionic polymer hyaluronic acid (HA) and applied to Ti; this coating showed decreased adherence of *S. aureus* and *E. coli* in vitro. It is thought that coatings consisting of hydrophilic CTS and HA inhibit bacterial adhesion, which is typically greater on hydrophobic materials [93].

Metal Ion Coating

Zinc, copper, silver, gold, and magnesium nanoparticles (NPs) are clusters of atoms that range from 1 to 100 nm. These NPs exhibit antimicrobial activity by an ion release mechanism that has intrinsic antimicrobial properties. These can serve as agents for antimicrobial implants [94]. Metal ions are bactericidal, especially silver and copper ions, which is secondary to the oligodynamic effect, which is the noxious effect that these metal ions have against living cells [95]. Copper exposure to microorganisms can permeate membrane integrity and can lead to cell death. Furthermore, copper can cause hydrolysis and displace cell organelles. Copper also contributes to viral inactivation or cell death by altering protein structure to change their function or forming complexes with proteins. Due to affinity for DNA, copper can break hydrogen bonds within DNA, which leads to cross-linking within the strands and opens the double helix resulting in DNA destruction [96]. An in vivo animal study simulated an *S. aureus* PJI to evaluate the antibacterial effect of a spacer (Ti6A14V) coated with 4× Cu-TiO2. This coating used a sol–gel substrate to integrate and deliver copper ions. In the presence of copper ions, there was a significant reduction in bacterial growth rate, with the highest reductions (4×) found in the copper TiO2-coating group. In addition to desirable antibacterial activity, coatings integrated with the implant coating were also found to have good durability. In particular, it was noted that this antibacterial Cu-TiO2 coating had good efficacy against MRSA, a particularly problematic microorganism responsible for a growing number of PJI [97]. However, some bacteria expressed copper tolerance genes, minimizing its potential efficacy [98].

Silver (Ag) is the most prevalent antimicrobial metal used in applications within biomedical science, and its activity has been known for many years. Antibacterial activity is attributed to the solvated ionic or nanoparticle form as opposed to bulk material [99]. The benefit of elemental NPs is the large surface area to volume ratio, thus amplifying release of ions and the consequent antimicrobial effect. In addition, the shape of the silver nanoparticle appears to be important [100]. As these ions are gradually released from surface coatings into the surrounding tissue, they become hydroxylated to form highly reactive components, including reactive oxygen species [101]. These cause bacterial cell membrane oxidation and result in greater cell permeability and death. Despite their antimicrobial activity, silver ions are not routinely applied to implants due to concerns of cytotoxicity with resultant decreased biocompatibility [102]. The use of silver NP loaded polymers show a burst of silver release for the first 3 days and decreased release over the subsequent 2 days [103]. However, due to high shear forces between the implant and bone surfaces in arthroplasty, polymer coatings do not adequately meet mechanical requirements given the force of load bearing

implants. An alternative is incorporation of silver into inorganic coatings like glass or ceramic, which demonstrate antibacterial activities against gram-positive and -negative bacteria in vitro with no remarkable cytotoxicity [104, 105]. Recently, HA coatings doped with silver NPs implanted in an animal model showed osseointegration similar to conventional HA implants, indicating good osseointegrative properties [106]. Enhanced silver loaded Ti showed successful in vitro inhibition of *S. aureus* growth with maintenance of good cellular activity [107]. Selected delivery devices along with layering techniques have been used to control the release of silver ions while maintaining cyto-compatible concentrations.

Silver Clinical Use-Case Series

More importantly, there are several reports on in vivo clinical application of silver coatings with respect to arthroplasty-related implants. Silver coating of Modular Universal Tumor and Revision System (MUTARS) megendoprosthesis (implantcast, Buxtehude, Germany) is accomplished by galvanic deposition of elementary silver on the surface of the titanium–vanadium prostheses. The first prospective case series included 20 patients with bone tumors (humerus, tibia, and femur) that were treated with an implant with this specific coating. There were no local or systemic toxic side effects of the silver coating. Blood silver levels never exceeded 56.4 (0.056 μg/mL) parts per billion (ppb), which is considered non-toxic, and there were no aberrant liver and kidney laboratory parameters. There were no signs of foreign body reaction or chronic inflammation in histological analysis [108]. A separate 51 patient case series that received a proximal tibia or proximal femur replacement using a tumor endoprosthesis with a similar silver coating found an infection rate of 5.9% (3 of 51 patients) in the silver group after 5 year follow-up compared to a historical control of uncoated implants in the same hospital with a 17.6% (13 of 74 patients) infection rate [109]. Another case series of 32 patients reported on the use of silver coated megendoprostheses in those undergoing soft tissue or bone resection surgery (26 patients) or revision arthroplasty (6 patients), of which 7 patients (23%) developed local argyria, which is a local reaction to silver often manifest in the skin. Silver levels were similar between patients with and without argyria with regards to serum levels and aspirated postoperative seroma. There was no association with the length of prosthesis, which was an indicator of how much silver was present. There were no elevated liver or kidney serum levels, and no significant difference in hemoglobin and leukocytes with or without argyria. Four out of seven patients with local argyria had peripheral neurological deficit, with two present prior to surgery, and the remaining two with no details on potential cause given [110].

Further clinically used silver coatings are produced by anodization of Ti alloy substrate with absorption of low amounts of silver within an aqueous solution; these are used in the *Agluna* tumor prostheses (Accentus Medical Ltd., Oxfordshire, United Kingdom). In contrast to the galvanized silver implants, a retrospective review of 394 consecutive patients that underwent resection and endoprosthesis

placement for bone tumors showed 12.4% PJI in the anodized-silver treated group compared to 7.5% in the non-silver group; however, the patients that received silver had a higher baseline risk of infection [111]. However, in this study, patients who received the anodized silver prosthesis were assigned to this treatment group based on elevated preoperative risk for infection. This may reflect different local silver concentrations thus different antibacterial effect, as it may relate to the method of silver-Ti substrate incorporation.

Custom made endoprostheses (Stanmore Implants Worldwide Ltd., Elstree, United Kingdom) are made with on ionic silver "stitched" into the titanium alloy surface by titanium alloy anodization with silver absorption from an aqueous solution [112]. The surface modification is directly integrated into the substrate, then silver is added by an ion exchange reaction where 5 μm circular features are formed. The maximum amount of silver allowed on a typical endoprostheses is 5 mg.

A retrospective case-control study compared 85 patients that received a silver-coated tumor prosthesis (2006–2011) to 85 patients that received the same prosthesis without a silver coating (2001–2011) with a 12-month minimum follow-up. The indications for tumor prosthesis implantation included 50 primary reconstructions, and 120 revisions for infection (79 one-stage revisions and 41 two-stage revisions). There were significantly less post-operative infections in the silver group (11.8%) compared to the non-silver group (22.4%, $p = 0.03$). For those that developed subsequent infection, debridement, antibiotic treatment with implant retention (DAIR) was successful in the seven infected patients who received a silver implant, whereas only 6 of 19 patients (31.6%) in the non-silver group ($p = 0.048$) were successfully treated with DAIR. When performing two-stage revision for infection, the silver group had an overall success rate of 86% versus 57% in the matched control group ($p = 0.05$). There were no implant specific adverse events, including argyria [112].

Non-metal Element Coating

Non-metal elements, such as chlorine, hydrogen, oxygen, or iodine, are commonly used in medicine given their antimicrobial properties. However, they are rarely used as antibacterial coating technologies in orthopedics due to their inadequate mechanical properties. An in vitro study of selenium covalently bound onto a Ti surface prevented *S. aureus* and *S. epidermidis* adhesion without impact on osteoblast activity [113].

Iodine is an ideal bioactive molecule, as it rapidly kills bacteria, fungi, mycobacteria, viruses, and spores. While the exact mechanism is unknown, it is known that iodine can penetrate into microorganisms and leads to cell death by attacking key groups of nucleotides, proteins, and fatty acids [114]. Aqueous solutions are often unstable, as there are at least seven iodine species that exist in a complex equilibrium; of those different species, molecular iodine (I_2) is mostly responsible for antimicrobial efficacy [115]. The problems with aqueous solutions were overcome when iodophors were developed with "iodine carriers" such as povidone–iodine complex. In addition, an electrolyte-based process has been used for iodine coating

of implants for limb salvage and megaendoprostheses. A prospective case series that followed 222 patients that received iodine coated implants were evaluated for post-operative infections, compromised status (bone tumor cases), degenerative disease, limb deformity, fractures, or non-unions with an average follow-up of 18.4 months. This series reported on a variety of implants, but included 10 hips and 4 knee prostheses. The author distinguished between "preventative" and "therapeutic" cases. One patient had a suspected iodine allergy, although all patients underwent preoperative patch testing for potential iodine allergy. Thyroid serum levels and thyroid function were evaluated and found to be unaffected. Mechanical implant failure occurred in two cases without further specification, and overall no implant loosening and good radiographical bone integration were reported. Of the 158 patients who received iodine coated implants preventatively for an immune compromised state in the setting of tumor resection, only three cases of acute infection (1.9%) were noted, of which all three were reportedly treated with DAIR without recurrence of infection at latest follow-up [116].

Synthetic Peptide Coatings

Antimicrobial peptides are an alternative strategy for infection prevention, as they do not rely on bacteria metabolic activity for efficacy. However, the native antimicrobial peptides suffer from problems of suboptimal efficacy and systemic toxicity which have been largely circumvented through the design of synthetic peptides. These engineered cationic amphipathic peptides (eCAPs) bind to bacteria then create pores in the bacterial membranes of gram-positive and -negative organisms. One eCAP WLBU2 synthetic peptide maximizes antimicrobial activity while causing minimal toxicity in mammalian cells, and decreases biofilm mass compared untreated implants in a surgical implant infection animal model. It has been shown to have in vivo efficacy in a murine model against *S. aureus*, and in vivo efficacy against clinical strains of *S. aureus*. Unlike antibiotics, the property of antimicrobial peptides cell lysis is independent of metabolism. However, concern remains for maintenance of bactericidal action of antimicrobial peptides in vivo with exposure to protease activity. However, there is optimism that this can be overcome with carefully designed D-enantiomers such as WLBU2 [117], but these materials are not yet ready for clinical use.

Barriers to Development/Implementation

Due to the low prevalence of PJI, most studies evaluating effective treatments to prevent PJI cannot achieve statistical significance without requiring prohibitively large clinical studies. Because of this, most proposed therapies will have to be tested in cases of established infection such as revision for established PJI. Studies such as these will allow insight into the effects of the treatments on colonization of the

implant surface and subsequent reestablishment of infection. However, as mouse models suggest that bacteria colonize the bone matrix during infection [118], it is possible that the bar may be much higher for prevention of reinfection in these cases.

There are a plethora of suggested coatings for antimicrobial implants, indwelling catheters, and other readily infected materials, such as the ventilator tubes associated with assisted respiration. Many of the materials/composites lack the mechanical robustness required for orthopedic applications. Nevertheless, mechanical considerations aside, the progress of these surfaces into small and then large animal models has been slow. We would suggest that several factors figure into this. Firstly, it is well-accepted that implant-associated infections are due to formation of biofilms [119] and biofilms formed in vitro may lack important components of those formed in a particular tissue environment, further hampered by the fact that there is no "accepted" model for a physiological biofilm. Secondly, antimicrobial efficacy is severely attenuated against biofilm bacteria so that antimicrobial activity needs to be determined against biofilm bacteria—either those forming on the antimicrobial surface or on adjacent material. In this context, it is not clear the degree of inhibition that needs to be attained to have a surface that is antimicrobial, in vivo. In our studies, our vancomycin-modified surfaces achieved between 1 and 3 logs (94–99.9%) inhibition [79, 80] and this inhibition was sufficient to markedly reduce infection in a large animal model [81]. However, with 10^{7-8} bacteria in a biofilm, these reductions only bring the numbers of ~10^5 bacteria, more than enough bacteria to propagate the infection. More animal studies should be performed to determine reasonable reductions in bacterial colonization by antimicrobial surfaces. Furthermore, animal models may not mimic human chronic wound care, as human patients have various underlying medical conditions that cannot be replicated in the animal model that complicate healing [120].

Safety and efficacy properties of developed antimicrobial surface modifications are often first tested in vitro which is limited in its translation to in vivo animal and human environments. For example, cytotoxicity data from isolated cells may be more pronounced than an in vivo system that contains three-dimensional matrices and vascular systems [121, 122]. Certainly, the financial costs from research and development of modified implants and the many stages of testing, is not insignificant. While the biomaterial market is worth over $300 billion US Dollars and is increasing 20% per year [123], it remains important to balance the clinical need with the cost of development.

Conclusion/Summary

Implant-associated infections remain a problem that is increasing due to the growing number of prostheses being implanted, and efforts toward prevention are a continued area of interest. Implant modification strategies may play a future role in both preventing bacterial adhesion and biofilm formation, and eradicating implant associated infections. Despite the current challenges facing translational medicine

development of antimicrobial surface technology, with mounting worldwide pressure to diminish the incidence of PJI, continued efforts will be made. It is not unrealistic to expect to see multifunctional smart surfaces in the field of orthopedics in the foreseeable future. Implant modification remains a growing area of research with limited clinical implementation, which highlights the need for further translational science in this field.

References

1. Berry DJ, Harmsen WS, Cabanela ME, Morrey BF (2002) Twenty-five-year survivorship of two thousand consecutive primary Charnley total hip replacements. J Bone Joint Surg Am 84:171–177
2. Ranawat CS, Flynn WF Jr, Saddler S, Hansraj KK, Maynard MJ (1993) Long-term results of the total condylar knee arthroplasty: a 15-year survivorship study. Clin Orthop Relat Res 286:94-102
3. Verra W, Kernkamp W, Van Hilten J (2016) Patient satisfaction and quality of life at least 10 years after total hip or knee arthroplasty. Int J Orthop 2:5–9
4. Sloan M, Premkumar A, Sheth NP (2018) Projected volume of primary total joint arthroplasty in the U.S., 2014 to 2030. J Bone Joint Surg Am 17:1455–1460. https://doi.org/10.2106/JBJS.17.01617
5. Bozic KJ, Kurtz SM, Lau E (2009) The epidemiology of revision total hip arthroplasty in the United States. J Bone Joint Surg Am 91:128–133. https://doi.org/10.2106/JBJS.H.00155
6. Bozic KJ, Kurtz SM, Lau E (2010) The epidemiology of revision total knee arthroplasty in the United States. Clin Orthop Relat Res 468:45–51. https://doi.org/10.1007/s11999-009-0945-0
7. Kurtz SM, Lau EC, Son MS, Chang ET, Zimmerli W, Parvizi J (2018) Are we winning or losing the battle with periprosthetic joint infection: trends in periprosthetic joint infection and mortality risk for the medicare population. J Arthroplasty 33:3238–3245
8. Lentino JR (2003) Prosthetic joint infections: bane of orthopedists, challenge for infectious disease specialists. Clin Infect Dis 36:1157–1161
9. Kamath AF, Ong KL, Lau E, Chan V, Vail TP, Rubash HE et al (2015) Quantifying the burden of revision total joint arthroplasty for periprosthetic infection. J Arthrop 30:1492–1497. https://doi.org/10.1016/j.arth.2015.03.035
10. Kurtz SM, Lau E, Watson H, Schmier JK, Parvizi J (2012) Economic burden of peri-prosthetic joint infection in the United States. J Arthroplast 18:394–400. https://doi.org/10.1089/sur.2017.068.
11. Zmistowski B, Karam JA, Durinka JB, Casper DS, Parvizi J (2013) Periprosthetic joint infection increases the risk of one-year mortality. J Bone Joint Surg Am 124:2177–2184. https://doi.org/10.2106/JBJS.L.00789.
12. Bozic KJ, Ries MD (2005) The impact of infection after total hip arthroplasty on hospital and surgeon resource utilization. J Bone Joint Surg Am 87(8):1746–1751
13. Parvizi J, Pawasarat IM, Azzam KA, Joshi A, Hansen EN, Bozic KJ (2010) Periprosthetic joint infection: the economic impact of methicillin-resistant infections. J Arthroplasty 25:103–107. https://doi.org/10.1016/j.arth.2010.04.011
14. Anis HK, Sodhi N, Klika AK, Mont MA, Barsoum WK, Higuera CA, Molloy RM (2018) Is operative time a predictor for post-operative infection in primary total knee arthroplasty? J Arthroplast 18:31150–31151. https://doi.org/10.1016/j.arth.2018.11.022.
15. Illingworth KD, Mihalko WM, Parvizi J, Sculco T, McArthur B, El Bitar Y, Saleh KJ (2013) How to minimize infection and thereby maximize patient outcomes in total joint arthroplasty: a multicenter approach: AAOS exhibit selection. J Bone Joint Surg Am 95:e50. https://doi.org/10.2106/JBJS.L.00596

16. Kunutsor SK, Whitehouse MR, Blom AW, Beswick AD, INFORM Team (2016) Patient-related risk factors for periprosthetic joint infection after total joint arthroplasty: a systematic review and meta-analysis. PLoS One 11(3):e0150866. https://doi.org/10.1371/journal.pone.0150866
17. Pruzansky JS, Bronson MJ, Grelsamer RP, Strauss E, Moucha CS (2014) Prevalence of modifiable surgical site infection risk factors in hip and knee joint arthroplasty patients at an urban academic hospital. J Arthroplast 29:272–276. https://doi.org/10.1016/j.arth.2013.06.019
18. Aboltins CA, Berdal JE, Casas F, Corona PS, Cuellar D, Ferrari MC, Hendershot E, Huang W, Kuo FC, Malkani A, Reyes F, Rudelli S, Safir O, Seyler T, Tan TL, Townsend R, Tuncay I, Turner D, Winkler H, Wouthuyzen-Bakker M, Yates AJ, Zahar A (2019) Hip and knee section, prevention, antimicrobials (systemic): proceedings of international consensus on orthopedic infections. J Arthroplasty 34(2S):S279–S288
19. Munoz-Price LS, Birnbach DJ, Lubarsky DA et al (2012) Decreasing operating room environmental pathogen contamination through improved cleaning practice. Infect Control Hosp Epidemiol 33:897–904. https://doi.org/10.1086/667381
20. Elek SD, Conen PE (1957) The virulence of Staphylococcus pyogenes for man; a study of the problems of wound infection. Br J Exp Pathol 38:573–586
21. Moriarty TF, Harris LG, Mooney RA, Wenke JC, Riool M, Zaat SAJ, Moter A, Schaer TP, Khanna N, Kuehl R, Alt V, Montali A, Liu J, Zeiter S, Busscher HJ, Grainger DW, Richards RG (2019) Recommendations for design and conduct of preclinical in vivo studies of orthopedic device-related infection. J Orthop Res 37:271–287
22. Dastgheyb S, Hammoud S, Ketonis C, Liu AY, Fitzgerald K, Parvizi J, Purtill J, Ciccotti M, Otto M, Hickok NJ (2015a) Staphylococcal persistence due to biofilm formation in synovial fluid containing prophylactic cefazolin. Antimicrob Agents Chemother 59:2122–2128. https://doi.org/10.1128/AAC.04579-14
23. Delaney LJ, MacDonald D, Leung J, Fitzgerald K, Sevit AM, Eisenbrey JR, Patel N, Forsberg F, Kepler CK, Fang T, Kurtz SM, Hickok NJ (2019) Ultrasound-triggered antibiotic release from PEEK clips to prevent spinal fusion infection: initial evaluations. Acta Biomater 93:12–24. https://doi.org/10.1016/j.actbio.2019.02.041
24. Hickok NJ, Shapiro IM (2012) Immobilized antibiotics to prevent orthopaedic implant infections. Adv Drug Deliv Rev 64:1165–1176. https://doi.org/10.1016/j.addr.2012.03.015
25. Hola V, Ruzicka F, Votava M (2006) The dynamics of Staphylococcus epidermis biofilm formation in relation to nutrition, temperature and time. Scripta Medica 79:169–174
26. Urish KL, DeMuth PW, Kwan BW, Craft DW, Ma D, Haider H, Tuan RS, Wood TK, Davis CM (2016) Antibiotic-tolerant Staphylococcus aureus biofilm persists on arthroplasty materials. Clin Orthop Relat Res 474:1649–1656. https://doi.org/10.1007/s11999-016-4720-8
27. Van den Bergh B, Fauvart M, Michiels J (2017) Formation, physiology, ecology, evolution and clinical importance of bacterial persisters. FEMS Microbiol Rev 41:219–251. https://doi.org/10.1093/femsre/fux001
28. Dastgheyb S, Shapiro IM, Hickok NJ, Otto M (2015b) Biofilms cause recalcitrance of staphylococcal joint infection to antibiotic treatment. J Infect Dis 211:641–650. https://doi.org/10.1093/infdis/jiu514.
29. Goodman SB, Yao Z, Keeney M, Yang F (2013) The future of biologic coatings for orthopaedic implants. Biomaterials 34:3174–3183
30. Hendricks SK, Kwok C, Shen M, Horbett TA, Ratner BD, Bryers J (2000) Plasma-deposited membranes for controlled release of antibiotic to prevent bacterial adhesion and biofilm formation. J Biomed Mater Res 50:160–170
31. Gristina AG, Naylor P, Myrvik Q (1988) Infections from biomaterials and implants: a race for the surface. Med Prog Technol 14:205–224
32. Bucholz HW, Engelbrecht H (1970) Uber die Depotwirkung einiger antibiotika bei vermischung mit dem kunstharz palacos. Chirurg 41:511–515
33. Bertazzoni Minelli E, Benini A, Magnan B, Bartolozzi P (2014) Release of gentamicin and vancomycin from temporary human hip spacer in tow stage revision of infected arthroplasty. J Antimicrobe Chemother 52:329–334

34. Murray WR (1984) Use of antibiotic-containing bone cement. Clin Orthop 190:89–95
35. Bourne RB (2004) Prophylactic use of antibiotic bone cement: an emerging standard-in the affirmative. J Arthroplast 19:69–72
36. Jiranek W (2005) Antibiotic-loaded cement in total hip replacement: current indications, efficacy, and complications. Orthopedics 28:873–877
37. Huiras P, Logan JK, Papadopoulos S, Whitney D (2012) Local antimicrobial administration for prophylaxis of surgical site infections. Pharmacotherapy 32:1006–1019
38. Walenkamp GHIM, Vree TB, Van Rens TJ (1986) Gentamicin-PMMA beads: pharmacokinetic and nephrotoxicological study. Clin Orthop Relat Res 205:171–183
39. Chen AF, Fleischman A, Austin MS (2018) Use of intrawound antibiotics in orthopaedic surgery. JAAOS 26:371–378
40. Classen DC, Evans RS, Pestotnik SL, Horn SD, Menlove RL, Burke JP (1992) The timing of prophylactic administration of antibiotics and the risk of surgical-wound infection. N Engl J Med 326:281–286
41. Hawn MT, Richman JS, Vick CC, Deierhoi RJ, Graham LA, Henderson WG, Itani KM (2013) Timing of surgical antibiotic prophylaxis and the risk of surgical site infection. JAMA Surg 148:649–657
42. Ghobrial GM, Cadotte DW, Williams K, Fehlings MG, Harrop JS (2015) Complications from the use of intrawound vancomycin in lumbar spinal surgery: a systematic review. Neurosurg Focus 39:E11. https://doi.org/10.3171/2015.7.FOCUS15258
43. Mariappan R, Manninen P, Massicotte EM, Bhatia A (2013) Circulatory collapse after topical application of vancomycin powder during spine surgery: case report. J Neurosurg Spine 19:381–383
44. Qadir R, Ochsner JL, Chimento GF, Meyer MS, Waddell B, Zavatsky JM (2014) Establishing a role for vancomycin powder application for prosthetic joint infection prevention—results of a wear simulation study. J Arthroplast 29:1449–1456. https://doi.org/10.1016/j.arth.2014.02.012
45. Antoci V, Adams CS, Hickok NJ, Shapiro IM, Parvizi J (2007) Antibiotics for local delivery systems cause skeletal cell toxicity in vitro. Clin Orthop Relat Res 462:200–206
46. Edin ML, Miclau T, Lester GE, Lindsey RW, Dahners LE (1996) Effect of cefazolin and vancomycin on osteoblasts in vitro. Clin Orthop Relat Res 333:245–251
47. Miclau T, Edin ML, Lester GE, Lindsey RW, Dahners LE (1995) Bone toxicity of locally applied aminoglycosides. J Orthop Trauma 9:401–406
48. Cavanaugh DL, Berry J, Yarboro SR, Dahners LE (2009) Better prophylaxis against surgical site infection with local as well as systemic antibiotics: an in vivo study. J Bone Joint Surg Am 91:1907–1912
49. Omrani FA, Emami M, Sarzaeem M, Zarei R, Yeganeh A (2015) The effect of intra-wound vancomycin powder application in reducing surgical site infections after total hip arthroplasty. Biosci Biotechnol Res Asia 12:2383–2386
50. Blom AW, Brown J, Taylor AH, Pattision G, Whitehouse S, Bannister C (2004) Infection after total knee arthroplasty. Bone Joint J 86:688–691
51. Leaper DJ, Schultz G, Carville K, Fletcher J, Swanson T, Drake R (2012) Extending the TIME concept: what have we learned in the past 10 years. Int Wound J 9:1–19
52. Ripa S, Bruno R, Reder R (2002) Clinical applications of Povidone-iodine as a topical antimicrobial. Handbook of topical antimicrobials industrial applications, industrial applications in consumer products and pharmaceuticals. CRC Press
53. Fleischer W, Reimer K (1997) Povidone-iodine in antisepsis–state of the art. Dermatology 195:3–9
54. Rackur H (1985) New aspects of mechanism of action of povidone-iodine. J Hosp Infect 6:13–23
55. Kanagalingam J, Feliciano R, Hah JH, Labib H, Le TA, Lin JC (2015) Practical use of povidone-iodine antiseptic in the maintenance of oral health and in the prevention and treatment of common oropharyngeal infections. Int J Clin Pract 69:1247–1256

56. Wang L, Qin W, Zhou Y, Chen B, Zhao X, Zhao H, Mi E, Wang Q, Ning J (2017) Transforming growth factor β plays an important role in enhancing wound healing by topical application of Povidone-iodine. Sci Rep 20:991. https://doi.org/10.1038/s41598-017-01116-5
57. Schultz GS, Sibbald RG, Falanga V, Ayello EA, Dowsett C, Harding K, Romanelli M, Stacey MC, Teot L, Vanscheidt W (2003) Wound bed preparation: a systematic approach to wound management. Wound Repair Regen 11:S1–S28
58. Brown NM, Cipriano CA, Moric M, Sporer SM, Della Valle CJ (2012) Dilute betadine lavage before closure for the prevention of acute postoperative deep periprosthetic joint infection. J Arthroplast 27:27–30. https://doi.org/10.1016/j.arth.2011.03.034
59. Mohammadi Z, Abbott PV (2009) The properties and applications of chlorhexidine in endodontics. Int Endod J 42:288–302. https://doi.org/10.1111/j.1365-2591.2008.01540.x
60. Lim KS, Kam PC (2008) Chlorhexidine-pharmacology and clinical applications. Anesth Intensive Care 36:502–512
61. Kuyyakanond T, Quesnel LB (1992) The mechanism of action of chlorhexidine FEMS. Microbiol Lett 100(1–3):211
62. Brennan SS, Foster ME, Leaper DJ (1986) Antiseptic toxicity in wounds healing by secondary intention. J Hosp Infect 8:263–267
63. Han Y, Giannitsios D, Duke K, Steffen T, Burman M (2011) Biomechanical analysis of chlorhexidine power irrigation to disinfect contaminated anterior cruciate ligament grafts. Am J Sports Med 39:1528–1533. https://doi.org/10.1177/0363546511401175
64. Hetrick EM, Schoenfisch MH (2006) Reducing implant-related infections: active release strategies. Chem Soc Rev 35:780–789
65. Zaborowska M, Welch K, Branemark R, Khalilpour P, Engqvist H, Thomsen P, Trobos M (2015) Bacteria-material surface interactions: methodological development for the assessment of implant surface induced antibacterial effects. J Biomed Mater Res 103:179–187. https://doi.org/10.1002/jbm.b.33179
66. Bixler GD, Theiss A, Bhushan B, Le SC (2014) Anti-fouling properties of microstructured surfaces bio-inspired by rice leaves and butterfly wings. J Colloid Interface Sci 419:114–133
67. Jaggessar A, Shahali H, Mathew A, Yarlagadda PKDV (2017) Bio-mimicking nano and micro-structured surface fabrication for antibacterial properties in medical implants. J Nanobiotechnol 15:64. https://doi.org/10.1186/s12951-017-0306-1
68. Mann EE, Manna D, Mettetal MR, May RM, Dannemiller EM, Chung KK, Brennan AB, Reddy ST (2014) Surface micropattern limits bacterial contamination. Antimicrob Resist Infect Control 3:28
69. Xu B, Wei Q, Mettetal MR, Han J, Rau L, Tie J, May RM, Pathe ET, Reddy ST, Sullivan L, Parker AE, Maul DH, Brennan AB, Mann EE (2017) Surface micropattern reduces colonization and medical device-associated infections. J Med Microbiol 66:1692–1698
70. Hasan J, Chatterjee K (2015) Recent advances in engineering topography mediated antibacterial surfaces. Nanoscale 7:15568–15575
71. Deupree SM, Schoenfisch MH (2009) Morphological analysis of the antimicrobial action of nitric oxide on Gram-negative pathogens using atomic force microscopy. Acta Biomater 5:1405–1415
72. Hickok NJ, Ketonis C, Adams CS (2017) Tethered antibiotics. In: Ducheyne P, Healy KE, Hutmacher DW, Grainger DW, Kirkpatrick CJ (eds) Comprehensive biomaterials, 2nd edn. Elsevier
73. Bernthal NM, Stavrakis AI, Billi F, Cho JS, Kremen TJ, Simon SI, Cheung AL, Finerman GA, Lieberman JR, Adams JS, Miller LS (2010) A mouse model of post-arthroplasty Staphylococcus aureus joint infection to evaluate in vivo the efficacy of antimicrobial implant coatings. PLoS One 5(9):e12580. https://doi.org/10.1371/journal.pone.0012580.
74. Corvec S, Portillo ME, Pasticci BM, Borens O, Trampuz A (2012) Epidemiology and new developments in the diagnosis of prosthetic joint infection. Int J Artif Organs 35:923–934
75. Ratner BD, Schoen FJ (2013) The concept and assessment of biocompatibility. In: Ratner BD, Hoffman AS, Schoen FJ, Lemons JE (eds) Biomaterials science: an introduction to materials in medicine, 3rd edn. Elsevier, Amsterdam, The Netherlands

76. Jose B, Antoci V, Zeiger AR, Wickstro E, Hickok NJ (2005) Vancomycin covalently bonded to titanium beads kills *Staphylococcus aureus*. Chem Biol 12:1041–1048
77. Lawson MC, Bowman CN, Anseth KS (2007) Vancomycin derivative photopolymerized to titanium kills S. epidermidis. Clin Orthop Relat Res 461:96–105
78. Parvizi J, Wickstrom E, Zeiger AR, Adams CS, Shapiro IM, Purtill JJ, Sharkey PF, Hozack WJ, Rothman RH, Hickok NJ (2004) Frank Stinchfield Award. Titanium surface with biologic activity against infection. Clin Orthop Relat Res 429:33–38
79. Antoci V, Adams CS, Parvizi J, Davidson HM, Composto RJ, Freeman TA, Wickstrom E, Zeiger AR, Ducheyne P, Jungkind D, Shapiro IM, Hickok NJ (2008) Vancomycin-modified Ti alloy inhibits S. epidermidis biofilm formation: implications for treatment of periprosthetic infection. Biomaterials 29:4684–4690
80. Ketonis C, Barr S, Adams CS, Shapiro IM, Parvizi J, Hickok NJ (2011) Vancomycin bonded to bone grafts prevents bacterial colonization. Antimicrob Agents Chemother 55:487–494
81. Stewart S, Barr S, Engiles J, Hickok N, Shapiro IM, Richardson DW, Parvizi J, Schaer TP (2012) Vancomycin-modified implant surface inhibits biofilm formation and supports bone healing in an infected osteotomy model in sheep—a proof-of concept-study. J Bone Joint Surg Am 94:1406–1141
82. de la Fuente-Núñez C, Cardoso MH, de Souza Cândido E, Franco OL, Hancock RE (2016) Synthetic antibiofilm peptides. Biochim Biophys Acta 1858(5):1061–1069
83. Gabriel M, Nazmi K, Veerman EC, Nieuw Amerongen AV, Zentner A (2006) Preparation of LL-37-grafted titanium surfaces with bactericidal activity. Bioconjug Chem 17:548–550
84. Humblot V, Yala JF, Thebault P, Boukerma K, Hequet A, Berjeaud JM, Pradier CM (2009) The antibacterial activity of Magainin I immobilized onto mixed thiols self-assembled monolayers. Biomaterials 30:3503–3512
85. Khoo X, Hamilton P, O'Toole GA, Snyder BD, Kenan DJ, Grinstaff MW (2009) Directed assembly of PEGylated-peptide coatings for infection-resistant titanium metal. J Am Chem Soc 131:10992–10997
86. Kwiecinski J, Na M, Jarneborn A, Jacobsson G, Peetermans M, Verhamme P, Jin T (2015) Tissue plasminogen activator coating on implant surfaces reduces Staphylococcus aureus biofilm formation. Appl Environ Microbiol 82:394–401. https://doi.org/10.1128/AEM.02803-15
87. Suhardi VJ, Bichara DA, Kwok SJJ, Freiberg AA, Rubash H, Malchau H, Yun SH, Muratoglu OK, Oral E (2017) A fully functional drug-eluting joint implant. Nat Biomed Eng 1:80. https://doi.org/10.1038/s41551-017-0080
88. Overstreet D, McLaren A, Calara F, Vernon B, McLemore R (2015) Local gentamicin delivery from resorbable viscous hydrogels is therapeutically effective. Clin Orthop Relat Res 473:337–347
89. Romano CL, Malizos K, Capuano C, Mezzoprete R, D'Arienzo M, Van Der Straeten C, Scarponi S, Drago L (2016) Does an antibiotic-loaded hydrogel coating reduce early post-surgical infection after joint arthroplasty? J Bone Joint Infect 19:34–41. https://doi.org/10.7150/jbji.15986
90. Di Martino A, Sittinger M, Risbud MV (2005) A versatile biopolymer for orthopaedic tissue-engineering. Biomaterials 26:5983–5990
91. Patel KD, Singh RK, Lee EJ, Han CM, Won JE (2014) Tailoring solubility and drug release from electrophoretic deposited chitosan-gelatin films on titanium. Surf Coat Technol 242:232–236
92. Pishbin F, Mourino V, Flor S, Kreppel S, Salih V, Ryan MP, Boccaccini AR (2014) Electrophoretic deposition of gentamicin-loaded glass/chitosan composite coatings for orthopaedic implants. ACS Appl Mater Interfaces 11:8796–8806. https://doi.org/10.1021/am5014166.
93. Chua PH, Neoh KG, Shi Z, Kang ET (2008) Structural stability and bio-applicability assessment of hyaluronic acid-chitosan polyelectrolyte multilayers on titanium substrates. J Biomed Mater Res 87:1061–1074
94. Slavin YN, Asnis J, Häfeli UO, Bach H (2017) Metal nanoparticles: understanding the mechanisms behind antibacterial activity. J Nanobiotechnology 15(1):65

95. Ricketts CR, Lowbury EJ, Lawrence JC, Hall M, Wilkins MD (1970) Mechanism of prophylaxis by silver compounds against infection of burns. Br Med J 23:444–446
96. Thurman RB, Gerba CP (1989) The molecular mechanisms of copper and silver ion disinfection of bacteria and viruses. Crit Rev Environ Control 18:259–315
97. Mauerer A, Stenglein S, Schulz-Drost S, Schorner C, Taylor D, Krinner S, Heidenau F, Adler W, Forst R (2017) Antibacterial effect of a 4x Cu-TiO2 coating simulating acute periprosthetic infection—an animal model. Molecules 22(7):1042. https://doi.org/10.3390/molecules22071042
98. Elguindi J, Wagner J, Rensing C (2009) Genes involved in copper resistance influence survival of Pseudomonas aeruginosa on copper surfaces. J Appl Microbiol 106:1448–1455
99. Lemire JA, Harrison JJ, Turner RJ (2013) Antimicrobial activity of metals: mechanisms, molecular targets and applications. Nat Rev Microbiol 11:371–384
100. Kumari M, Pandey S, Giri VP, Bhattacharya A, Shukla R, Mishra A, Nautiyal CS (2017) Tailoring shape and size of biogenic silver nanoparticles to enhance antimicrobial efficacy against MDR bacteria. Microb Pathog 105:346–355
101. Chernousova S, Epple M (2013) Silver as antibacterial agent: ion, nanoparticle, and metal. Angew Chem Int Ed Engl 52:1636–1653
102. Moseke C, Gbureck U, Elter P, Drechsler P, Zoll A, Thull R, Ewald A (2011) Hard implant coatings with antimicrobial properties. J Mater Sci Mater Med 22:2711–2720
103. Furno F, Morley KS, Wong B, Sharp BL, Arnold PL, Howdle SM, Bayston R, Brown PD, winship PD, Reid HJ (2004) Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection? J Antimicrob Chemoth 54:1019–1024
104. Chen W, Liu Y, Courtney HS, Bettenga M, Agrawal CM, Bumgardner JD, Ong JL (2006) In vitro anti-bacterial and biological properties of magnetron co-sputtered silver-containing hydroxyapatite coating. Biomaterials 27:5512–5517
105. Feng QL, Kim TN, Wu J, Park ES, Kim JO, Lim DY, Cui FZ (1998) Antibacterial effects of Ag-Hap thin films on alumina substrates. Thin Solid Films 335:214–219
106. Łapaj Ł, Woźniak W, Markuszewski J (2018) Osseointegration of hydroxyapatite coatings doped with silver nanoparticles: scanning electron microscopy studies on a rabbit model. Folia Morphol (Warsz) 78(1):107–113. https://doi.org/10.5603/FM.a2018.0055
107. Zhang Y, Dong C, Yang S, Chiu TW, Wu J, Xiao K, Huang Y, Li X (2018) Enhanced silver loaded antibacterial titanium implant coating with novel hierarchical effect. J Biomater Appl 32:1289–1299. https://doi.org/10.1177/0885328218755538
108. Hardes J, Ahrens H, Gebert C, Streitbuerger A, Buerger H, Erren M, Gunsel A, Wedemeyer C, Saxler G, Winkelmann W, Gosheger G (2007) Lack of toxicological side-ffects in silver-coated megaprostheses in humans. Biomaterials 28:2869–2875
109. Hardes J, von Eiff C, Streitbuerger A, Balke M, Budny T, Henrichs MP, Hauschild G, Ahrens H (2010) Reduction of periprosthetic infection with silver-coated megaprostheses in patients with bone sarcoma. J Surg Oncol 101:389–395
110. Glehr M, Leithner A, Friesenbichler J, Goessler W, Avian A, Andreou D, Et a (2013) Argyria following the use of silver-coated megaprostheses: no association between the development of local argyria and elevated silver levels. Bone Joint J 95:988–992
111. Parry MC, Laitinen MK, Albergo JI, Gaston CL, Stevenson JD, Grimer RJ, Jeys LM (2018) Silver-coated (Agluna®) tumour prostheses can be a protective factor against infection in high risk failure patients. Eur J Surg Oncol 18:32031–32036. https://doi.org/10.1016/j.ejso.2018.12.009.
112. Wafa H, Grimer RJ, Reddy K, Jeys L, Abudu A, Carter SR et al (2015) Retrospective evaluation of the incidence of early periprosthetic infection with silver-treated endoprostheses in high-risk patients: case-control study. Bone Joint J 97:252–257
113. Holinka J, Pilz M, Kubista B, Presterl E, Windhager R (2013) Effects of selenium coating of orthopaedic implant surfaces on bacterial adherence and osteoblastic cell growth. Bone Joint J 95:678–682
114. Oduwole KO, Glynn AA, Molony DC et al (2010) Anti-biofilm activity of sub inhibitiory povidone-iodine concentrations against staphylococcus epidermidis and Staphylococcus aureus. J Orthop Res 28:1252–1256

115. Haley CE, Marling-Carson M, Smith JW, Luby JP, Mackowiak PA (1985) Bactericidal activity of antiseptics against methicillin-resistant Staphylococcus aureus. J Clin Microbiol 21:991
116. Tsuchiya H, Shirai T, Nishida H, Murakami H, Kabata T, Yamamoto N, Watanabe K, Nakase J (2012) Innovative antimicrobial coating of titanium implants with iodine. J Orthop Sci 17:595–604
117. Mandell JB, Deslouches B, Montelaro RC, Shanks RMQ, Doi Y, Urish KL (2017) Elimination of antibiotic resistant surgical implant biofilms using and engineered cationic amphipathic peptide WLBU2. Sci Rep 22:18098. https://doi.org/10.1038/s41598-017-17780-6
118. Shi M, de Mesy Bentley KL, Palui G, Mattoussi H, Elder A, Yang H (2017) The roles of surface chemistry, dissolution rate, and delivered dose in the cytotoxicity of copper nanoparticles. Nanoscale 9(14):4739–4750
119. Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science 284:1318–1322
120. Lipsky BA, Hoey C (2009) Topical antimicrobial therapy for treating chronic wounds. Clin Infect Dis 49:1541–1549
121. Leaper DJ, Durani D (2008) Topical antimicrobial therapy of chronic wounds healing by secondary intention using iodine products. Int Wound J 5:361–368
122. van Meurs SJ, Gawlitta D, Heemstra KA, Poolman RW, Vogely HC, Kruyt MC (2014) Selection of an optimal antiseptic solution for intraoperative irrigation: an in vitro study. J Bone Joint Surg Am 96:285–291
123. Chu PK, Liu X (2008) Biomaterials fabrication and processing handbook. CRC Press, Taylor & Francis Group, Boca Raton, FL, p ix

Antimicrobial Endodontic Materials

Xiaogang Cheng and Xiaohua Liu

Abstract Bacteria and their by-products are the primary cause of pulpal and periapical diseases that are one of the most common oral diseases. Root canal treatment (RCT) is the most effective procedure to treat pulpal and periapical diseases with severe infection. RCT aims to eliminate the infection from root canals and place filling materials to seal the space to prevent reinfection. Because of the complicated anatomical structure of the tooth root canal, complete elimination of the bacteria that reside in a root canal and the dentinal tubules via mechanical preparation is extremely difficult, if not impossible. Therefore, antimicrobial endodontic materials are indispensable for infection elimination during RCT. Based on the procedure of RCT, different antimicrobial endodontic materials have been developed for root canal irrigation, medication, and obturation (sealing). This chapter discusses the antimicrobial endodontic biomaterials that are used during the three steps of the RCT. Advantages and limitations of each material are emphasized. In addition, recent developments and future research directions of antimicrobial endodontic biomaterials are presented.

Keywords Antimicrobial · Endodontic · Dental materials · Root canal treatment · Pulpal and periapical diseases · Tooth

X. Cheng
Biomedical Sciences Department, Texas A&M University College of Dentistry, Dallas, TX, USA

Department of Operative Dentistry and Endodontics, School of Stomatology, The Fourth Military Medical University, Xi'an, Shaanxi, PR China

X. Liu (✉)
Biomedical Sciences Department, Texas A&M University College of Dentistry, Dallas, TX, USA
e-mail: xliu@tamhsc.edu

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_11

Introduction

Pulpal and periapical diseases, which are one of the most common oral diseases, can cause considerable discomfort, orofacial pain, and ultimately loss of teeth [1]. The diseases give rise to physical and mental suffering, and as a result compromise a patient's quality of life. In addition, the pulpal and periapical diseases act as a reservoir for infection, and are associated with the occurrence of systemic diseases (such as bacterial endocarditis and cardiovascular diseases) [2, 3]. A variety of evidence has confirmed that bacteria and their by-products are the primary cause of pulpal and periapical diseases [4, 5]. There are over 700 bacteria species that are the source of infectious pulpal and periapical diseases, and among them approximately 150 bacteria species have been identified from the infected root canals [6, 7]. An infected root canal usually contains 2–10 bacteria species and approximately 10^3–10^7 bacterial cells [8].

While Gram-negative obligate anaerobes are the main pathogens in infected root canals, Gram-positive facultative anaerobes also contribute to root canal infections. The majority of the bacteria isolated from the canal with necrotic pulp are *black-pigmented bacteria*, including *Fusobacterium nudeatum* and *Peptostreptococcus micros* [9, 10]. The bacteria isolated from the symptomatic canals with infected pulp include *Prevotella intermedia*, *Porphyromonas endodontalis*, *Porphyromonas gingivalis*, and *Prevotella nigrescens* [11–13]. In addition, *Actinomyces* [14, 15], *Spirochetes*, *Candida* [16, 17], *Lactobacillus*, and *Streptococcus* were also found in infected canals [7, 16, 18, 19]. However, the bacterium that is most frequently isolated from endodontically treated root canals is *Enterococcus* spp. [20].

Bacterial biofilms play an important role in the occurrence and development of pulpal and periapical diseases [21]. It was reported that the antibiotic resistance of a bacterial biofilm is 10–1000 times higher than its planktonic counterpart [22]. A bacterial biofilm inside or outside of the root canal usually consists of multi-strains [23, 24], except that the *Enterococcus faecalis* (*E. faecalis*) can specifically form a mono-species biofilm [25]. Due to the complicated anatomical structure of the root canal system, conventional techniques cannot completely eliminate the bacteria or the biofilms [26, 27], which are sources of persistent infection of the pulpal and periapical diseases.

Root canal treatment (RCT) is the most widely used and the most effective procedure to treat pulpal and periapical diseases with severe infection. The fundamental goal of the RCT is to eliminate the infection from root canals and place filling materials to seal the space to prevent reinfection [28]. The RCT procedure consists of root canal preparation and shaping, disinfection, and obturation [29, 30]. Each step of the RCT procedure plays an important role in infection elimination. As described above, due to the complicated anatomical structure of the root canal, complete elimination of the bacteria that reside in a root canal and the dentinal tubules via mechanical preparation is extremely difficult, if not impossible. Accordingly, antimicrobial endodontic materials that are used for root canal irrigation, medication,

and obturation (sealing) are indispensable for infection elimination [26–28]. This chapter discusses the antimicrobial endodontic materials that are used during the three steps of the RCT.

Antimicrobial Irrigants and Irrigation Techniques

Antimicrobial Irrigants

Generally, an excellent root canal irrigant should have: (1) an effective antibacterial effect that directly kills the bacteria residing in infected canals; (2) lubrication function that indirectly enhances the disinfection effect of root canal mechanical preparation; (3) a tissue dissolution effect that dissolves the infected tissues; and (4) good biocompatibility with the surrounding tissues. Clinically, the commonly used root canal irrigants are sodium hypochlorite (NaOCl), chlorhexidine (CHX), ethylene diamine tetraacetic acid (EDTA), strong acid electrolyzed water (SAEW), and mixture tetracycline citric acid and detergent (MTAD) [31].

NaOCl is the most frequently used and most effective irrigant [32], and it possesses a broad spectrum of antibacterial effects on bacteria, phage, spore, saccharomycetes, and viruses [31]. The bactericidal effect of NaOCl is caused by the hypertonicity and the ability to perform protein oxidation and hydrolysis that destroys bacterial cell membranes. Its hypertonicity increases the permeability of bacterial cell membranes, which results in osmotic extraction of intracellular fluids, shrinking and lysis of bacterial cells, and finally cell death [33]. The pH value of NaOCl is approximately 11–12. When NaOCl directly contacts proteins, nitrogen, formaldehyde, and acetaldehyde are generated in a short period of time, which dissolves the proteins. In addition, chloramine is generated when the hydrogen atoms in the amino group are replaced by chlorine atoms, which provides a strong antibacterial effect. The antibacterial effect of NaOCl increases with concentration [34]. However, the toxicity of NaOCl also increases with concentration. At low concentrations, NaOCl induces an inflammatory reaction. However, a high concentration of NaOCl causes a strong tissue reaction, especially when the NaOCl reaches periapical tissues [35, 36]. It was reported that 5.25% NaOCl completely killed *Candida albicans*, *E. faecalis*, and *Bacillus* [37]. However, there were no significant differences of the bactericidal effects when the NaOCl concentrations were 1.0%, 2.5%, and 5.25% [38]. Clinical investigations further indicated that the bacteria were still culturable in 1/3 to 1/2 of the canals that were treated with mechanical preparation followed by 5% NaOCl [39]. While the recommended NaOCl concentration is 0.5–1% [40], there is no consensus on the standard concentration of NaOCl applicable for RCT. It is generally accepted that the use of a low concentration of NaOCl for a relatively long period of time can achieve both disinfection and low toxicity [38]. It is noticeable that although NaOCl is the most effective irrigant for root canal disinfection, it is incapable of removing the smear layer.

CHX is a cationic antimicrobial that can adhere to the cell wall of bacteria, destroy the integrity of the cell membrane, cause drainage of cell contents, and finally kill the bacteria. CHX has a bacteriostatic effect at a concentration of 0.2%, while 2% CHX presents a bactericidal effect [41]. It was reported that 0.2% CHX effectively reduces the number of bacteria inside infected canals [42]. In fact, it was confirmed that 0.2% CHX eliminated bacteria that invaded dentinal tubules at a depth of 500 μm [43]. CHX gel or solution at a concentration of 2% effectively reduced or eliminated *E. faecalis* in the root canal system [44]. One study showed that after treatment with 2% CHX for two minutes, the bacteria in both the root canal wall and dentinal tubules were completely eliminated [45]. Comparison of the bactericidal effects of CHX to NaOCl is complex and often has confusing results. For example, an in vitro study found that the bactericidal effect of 1% and 2% CHX was as effective as that of 5.25% NaOCl [46]. However, there were studies showing that the bactericidal effect of 4% NaOCl was higher than that of 0.2% CHX when they were used to treat four types of specific black-pigmented bacteria and facultative anaerobes [47]. Also, there were studies indicating that the bactericidal effect of CHX was stronger than that of NaOCl [48, 49]. CHX does not dissolve tissues and has less toxicity compared to NaOCl. The combination of CHX and NaOCl achieved a better bactericidal and tissue dissolution effect than the use of CHX and NaOCl alone [50]. However, the combination of CHX and NaOCl could not remove the smear layer inside the infected canals.

Bacteria can infect the smear layer inside root canals, and the smear layer helps bacteria become resistant to antimicrobials [51]. EDTA is a chelator that can effectively remove the smear layer while it possesses a limited bactericidal effect. Therefore, EDTA has to be combined with other irrigants to enhance a bactericidal effect. Because NaOCl is the most effective irrigant with a minimal capability to remove the smear layer, the combination of EDTA and NaOCl is an ideal protocol for root canal irrigation [52].

SAEW is prepared by electrolysis of a sodium chloride solution. Fresh SAEW has a pH value of 2.3 to 2.7 [53, 54], and has a bactericidal effect for 48 h. Because of its antimicrobial activity against bacteria and viruses, SAEW has been widely used in food safety and surgical site disinfection [54–57]. In recent years, SAEW has been used as a potential root canal irrigant [58, 59]. The bactericidal effect of SAEW depends mainly on its low pH value, high oxidation-reduction potential (ORP), and the synergies of HClO, Cl_2, H_2O_2, and OH^-. Generally, a low pH value influences the permeability of cell membranes and results in the inability of bacteria to reproduce. A high ORP affects metabolic compounds within bacteria and causes cell death. OH^- and H_2O_2 destroy bacteria by damaging the cell lipid membrane, denaturing proteins, as well as preventing enzyme activation by severing DNA [57, 58, 60]. It was reported that SAEW had a similar antibacterial effect to that of 5.25% NaOCl against both flow and static *E. faecalis* biofilms [61] (Fig. 1). In addition to the antibacterial effect, SAEW is also effective for smear layer removal without decreasing the hardness of dentin [62].

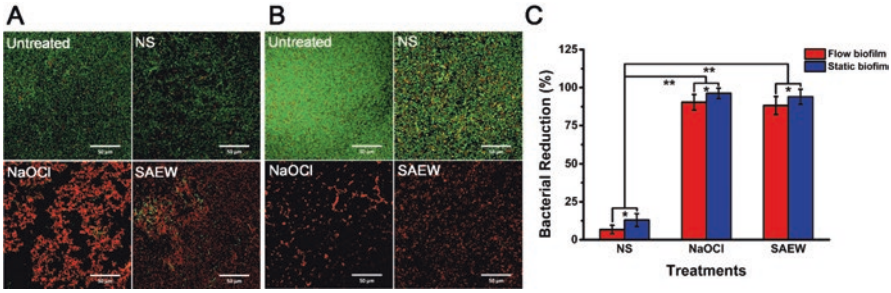


Fig. 1 (**A**, **B**) CLSM images of *E. faecalis* biofilms, and (**C**) the relative bacterial reductions after treatment. (**A**) and (**B**) are the flow and static *E. faecalis* biofilms, respectively; (**C**) is relative bacterial reductions in the flow and static *E. faecalis* biofilms. For the untreated group, *E. faecalis* biofilms formed after 72 h incubation with no treatment. Green fluorescence represented viable bacterial cells while red fluorescence represented dead cells. For both the flow and static *E. faecalis* biofilms, bacterial reductions in the SAEW and 5.25% NaOCl groups were significantly greater than that in the NS group. There was significant difference in bacterial reductions between the flow and static biofilms in both the SAEW and 5.25% NaOCl groups. NS, NaOCl, and SAEW represented *E. faecalis* biofilms treated with normal saline, 5.25% NaOCl, and SAEW, respectively. (From Cheng et al. [61] copyright 2016 American Association of Endodontists. Reprinted with permission $* p < 0.05$, $** p < 0.001$)

MTAD is a mixture of 3% doxycycline, 4.25% citric acid, and detergent (Tween 80), and was developed in 2003 [63]. MTAD is effective for smear layer removal, and its erosion to dentin is much less severe than that of EDTA [64]. Several studies showed that the bactericidal effect of MTAD was more effective than that of conventional irrigants, such as NaOCl and EDTA [65]. One study showed that MTAD combined with 0.2% CHX or 1.3% NaOCl effectively killed *E. faecalis* [66, 67]. Another study found that the bactericidal effect of MTAD combined with 1.3% NaOCl on *E. faecalis* located in the apical third of the root canal was as effective as that of 5.25% NaOCl combined with 15% EDTA [68]. However, the bactericidal effect of NaOCl (6% and 1%) on *E. faecalis* was more effective than that of 2% CHX and MTAD [69]. MTAD had less cytotoxicity than that of 5.25% NaOCl and EDTA, but was more than that of 2.63%, 1.31%, and 0.66% NaOCl [70]. However, the antifungal effect of MTAD was much less than that of 6% NaOCl or 2% CHX [71]. In addition, MTAD could not eliminate bacterial biofilms inside infected canals [72]. Overall, the antibacterial effectiveness of MTAD needs further investigation.

Besides NaOCl, CHX, EDTA, SAEW, and MTAD, hydrogen peroxide, ozone, and stannous fluoride can also be used as root canal irrigants. It was reported that the bactericidal effect of ozone on *E. faecalis* was as effective as that of 2.5% NaOCl [73]. As described above, all of the irrigants have a certain level of bactericidal effect and possess both pros and cons. Currently, a better root canal irrigation procedure can be the combination of two or more irrigants.

Irrigation Techniques

While irrigants have bactericidal capability, they have limited infiltration capacity inside the root canal system. This is because the canal is a closed channel that causes gas entrainment and produces a vapor lock effect during irrigant delivery. The apical vapor lock effect has an adverse effect on bactericidal action [74]. Therefore, clinicians usually adopt several "mechanical forces" to enhance the infiltration capacity of the irrigants to achieve better bactericidal effect during RCT; currently, the widely used "mechanical forces" include ultrasound and lasers. Passive ultrasonic irrigation (PUI) was first introduced to RCT in 1980, and it can effectively remove a smear layer, organic tissues, and bacterial biofilms [75]. Studies showed that PUI plus NaOCl not only effectively removed the smear layer inside infected root canals but also significantly improved the clean condition of the biofilm-infected dentin [76–78]. Prior to the use of PUI, however, the apical third of a root canal has to be enlarged to at least an international standardization organization (ISO) size of 35–40 to allow needle placement to within 1–2 mm of the apical seat [79, 80], which limits PUI application. Laser systems used for laser-assisted irrigation (LAI) mainly include the Nd:YAG laser, Er:YAG laser, and the Er,Cr:YSGG laser, and antimicrobial photodynamic therapy (aPDT) [81, 82]. Among them, the Nd:YAG laser and Er:YAG laser have the most effective bactericidal effect. The Nd:YAG laser (1064 nm) could present an effective bactericidal effect up to 1 mm into dentin and reduce more than 99% of *E. faecalis* in a number of inoculated root canals [83, 84]. However, it was also reported that Nd:YAG irradiation was not effective against nonpigmented bacteria or bacterial biofilms because its irradiation was well absorbed in melanin and dark pigmented tissues [85]. Therefore, higher energy densities are required, which may induce a lethal thermal effect [86]. In fact, it was reported that Nd:YAG irradiation caused thermal damage such as structural changes, carbonization, and cracks to dentin when it is activated at higher power ($\geq$ 3W) [87]. The bactericidal potential of the Er:YAG laser is related to the evaporation effect of cellular water, which expands quickly during the laser pulse and leads to the disintegration of the bacterial cell wall [88]. However, carbonization, cracks, and craters were found in the dentin treated with laser radiations without a coolant [89, 90]. It was reported that the Er:YAG laser significantly enhanced the disinfection efficacy of NaOCl for endodontic treatment [91–93]. The mechanism of the Er:YAG laser for the root canal treatment is attributed to vapor bubble expansion and the implosion with secondary cavitation effects that induce high-speed fluid motion in and out of the canal [94, 95]. The collapsed shock waves and acoustic streaming of the fluid produced during the process of laser-assisted irrigation exert a large shear stress on the root canal wall [96, 97]. The shear stress facilitates the penetration of NaOCl into deep dentin layers to perform a bactericidal effect. Studies have confirmed that Er:YAG laser irradiation at 0.5 W for 30 s combined with NaOCl irrigation was the optimal protocol and might be considered as a new alternative to conventional root canal disinfection [91, 92] (Table 1). Although PUI and LAI have presented encouraging bactericidal effects, they are incapable of completely eliminating infections from infected canals.

Table 1 Bacterial counts (CFU/mL, Mean ± SD) before and after treatments and bacterial reductions (BR, Mean ± SD) in different groups

	On the surface of the root canal wall			100 μm inside the dentinal tubule			200 μm inside the dentinal tubule			300 μm inside the dentinal tubule		
Groups	Before ($\times10^5$)	After	BR (%)	Before ($\times10^5$)	After	BR (%)	Before ($\times10^4$)	After	BR (%)	Before ($\times10^3$)	After	BR (%)
Normal saline (NS)	6.60 ± 0.80	1.79 ± 0.11 ($\times10^5$)	72.74 ± 1.78	1.26 ± 0.13	3.43 ± 0.20 ($\times10^4$)	72.56 ± 1.67	1.69 ± 0.15	5.48 ± 0.28 ($\times10^3$)	67.49 ± 1.32	4.81 ± 0.20	2.45 ± 0.05 ($\times10^3$)	49.02 ± 1.08
5.25% NaClO	8.11 ± 0.47	10.00 ± 16.16 (×1)	99.99 ± 0.00	1.54 ± 0.12	1.92 ± 3.10 (×1)	99.99 ± 0.00	1.49 ± 0.27	4.00 ± 6.25 (×1)	99.98 ± 0.03	3.11 ± 0.72	16 ± 3.46 (×1)	99.52 ± 0.09
Nd:YAG	6.28 ± 0.45	5.63 ± 0.41 ($\times10^4$)	91.04 ± 0.22	1.18 ± 0.14	1.10 ± 0.10 ($\times10^4$)	90.62 ± 0.52	1.69 ± 0.10	2.90 ± 0.45	82.92 ± 1.72 ($\times10^3$)	2.85 ± 0.13	6.60 ± 0.09 ($\times10^2$)	76.81 ± 0.81
Er:YAG/ NaClO/NS/ DW	7.25 ± 0.45	0.00 ± 0.00 (×1)	100.00 ± 0.00	1.36 ± 0.08	0.00 ± 0.00 (×1)	100.00 ± 0.00	1.61 ± 0.09	0.00 ± 0.00 (×1)	100.00 ± 0.00	3.94 ± 0.18	5.00 ± 1.83 (×1)	99.87 ± 0.04
Er:YAG/NS/ DW	7.54 ± 0.34	2.33 ± 0.13 ($\times10^2$)	99.97 ± 0.00	1.42 ± 0.06	45.92 ± 0.80 (×1)	99.97 ± 0.00	1.46 ± 0.10	9.20 ± 0.44 ($\times10^2$)	93.69 ± 0.13	3.22 ± 0.15	3.78 ± 0.21 ($\times10^2$)	88.25 ± 0.64
Er,Cr:YSGG	6.03 ± 0.28	4.04 ± 0.15 ($\times10^4$)	93.29 ± 0.08	1.13 ± 0.11	7.98 ± 0.32 ($\times10^3$)	92.91 ± 0.45	1.33 ± 0.10	1.59 ± 0.24 ($\times10^3$)	88.11 ± 1.02	3.58 ± 0.17	5.60 ± 0.08 ($\times10^2$)	84.34 ± 0.53
aPDT	9.28 ± 0.26	1.74 ± 0.29 ($\times10^4$)	98.13 ± 0.26	1.76 ± 0.22	3.36 ± 0.23 ($\times10^3$)	98.08 ± 0.13	1.08 ± 0.38	3.00 ± 0.53 ($\times10^2$)	97.08 ± 0.46	2.20 ± 0.14	66.70 ± 3.08 (×1)	96.96 ± 0.08

From Cheng et al. [92] copyright 2016 Journal of Medical Microbiology

Antimicrobial Drugs for Root Canal Medication

Following root canal preparation and irrigation, root canal medication is applied to place disinfectants inside the canal space during the interval between the two appointments. The aims of root canal medication are to kill the residual bacteria inside the canals, to reduce the periapical inflammatory reaction, to relieve periapical pain, and to promote the repair of periapical tissues [98]. Based on chemical characteristics, the disinfectants for root canal medication include: (1) phenolic compounds (e.g., eugenol and camphorated single chlorophenol); (2) aldehydes (e.g., formaldehyde cresol formocresol); (3) calcium hydroxide ($Ca(OH)_2$); (4) halide (e.g., iodine potassium iodide (IKI)); and (5) antibiotics [98]. Considering the concern of the toxicity of phenolic compounds and aldehydes, $Ca(OH)_2$, IKI, and antibiotics are more widely used in the clinic and are described below.

$Ca(OH)_2$ is a base with a pH value of approximately 12.5, and was first introduced to endodontics more than a century ago. $Ca(OH)_2$ possesses antibacterial effects and the ability of promoting the repair and mineralization of hard tissues [99]. The antibacterial effect of $Ca(OH)_2$ is associated with hydroxide ions released under a humid environment. The hydroxide ion is a strong oxyradical that can destroy the bacterial cell membrane, denature the proteins, destroy the bacterial DNA, and as a result kill the bacteria [98]. To perform its antibacterial effect, $Ca(OH)_2$ needs to be in direct contact with bacteria under water which is not always satisfied in the clinic. In addition, the antibacterial effect of $Ca(OH)_2$ has a positive correlation to the local concentration of hydroxide ions [100]. Studies showed that $Ca(OH)_2$ killed bacteria located inside dentinal tubules, owing to the diffusion of the hydroxide ions to deep dentinal tubules. However, more research indicated that $Ca(OH)_2$ could not completely eliminate the bacteria inside the canal system, especially the *E. faecalis* [101, 102]. In addition, dentin acts as a buffer and consequently weakens the antibacterial effect of $Ca(OH)_2$ [103].

IKI can penetrate into dentinal tubules to present a bactericidal effect that lasts only for a short period of time [104]. It was shown that the bactericidal effect of IKI combined with $Ca(OH)_2$ was much stronger than $Ca(OH)_2$ alone [105]. IKI was also used to enhance the elimination of *E. faecalis* [106]. Meanwhile, the addition of IKI to $Ca(OH)_2$ did not change the alkalinity or cytotoxicity of the $Ca(OH)_2$ [107].

When antibiotic preformulations are used for root canal medication, they might also induce antibiotic resistance in bacteria and drug allergies [98]. The mixture of metronidazole, ciprofloxacin, and dimethylamine tetracycline was found to improve the repair of periapical tissues [108]. Corticosteroids have been used for endodontic therapy for many years. They are very effective for relieving tooth pain with pulp vitality, but are less effective to teeth with necrotic pulp tissue [109]. In addition, Gram-positive bacteria are more sensitive to tetracycline of low concentration than Gram-negative bacteria [110].

Antimicrobial Endodontic Sealers

As the final step of the RCT, a root canal filling is performed by obturating the canal system using bio-inert materials, such as gutta percha and endodontic sealers. Gutta percha is mainly used for the obturation of the canal space. Endodontic sealers are used to fill the gap between the gutta percha and the root canal wall, to seal the canal system (e.g., the irregular region), and to entomb the residual bacteria inside the canals [111]. An ideal endodontic sealer should possess the following properties: (1) it can be easily transported into the canal; (2) it can seal the lateral canal and apical foramen; (3) it does not shrink after transporting into the canal; (4) it is stable and not affected by the environment humidity; (5) it has a bacteriostasis function or at least does not promote the growth of bacteria; (6) it is radiopaque; (7) it does not stain the tooth structure; (8) it does not irritate the periapical tissues; (9) it is sterile or can be sterilized before transporting into the canal; and (10) it can be removed easily from the canal when needed [112].

Most of the commercial endodontic sealers have a certain degree of short-term antibacterial activity, due to the release of antibacterial ingredients in the sealer prior to the fixation. For example, zinc oxide eugenol-based (ZnOE-based) sealers release free eugenol, and epoxy amine resin-based sealers release formaldehyde and bisphenol-A diglycidyl ether during the curing process [113, 114]. However, it was reported that those sealers no longer had an antibacterial effect hours or days after fixation because of the loss of the antibacterial components [114, 115]. While studies also showed that the ZnOE-based and epoxy amine resin-based sealers presented a certain antibacterial effect up to 30 days and even 60 days [116–118], their cytotoxicity should be taken into consideration [119]. The released antibacterial components (e.g., eugenol and formaldehyde) could trigger moderate to severe cytotoxicity to cells and periapical tissues [120, 121]. In addition, the loss of the antibacterial components led to shrinkage of the sealer, which compromised the sealing effect [122, 123].

Commercial endodontic sealers include zinc oxide eugenol (ZnOE)-based sealers (e.g., Endomethasone C), epoxy amine resin-based sealers (e.g., AH Plus), calcium hydroxide-based sealers (e.g., Apexit Plus) and mineral trioxide aggregate (MTA)-based sealers (e.g., MTA Fillapex) [29]. Studies showed that different root canal sealers presented different antibacterial effects on bacteria associated with infected root canals [124–126]. ZnOE-based and epoxy amine resin-based root canal sealers were found to have the largest antibacterial spectrum and the most effective antibacterial effect [116, 125, 127, 128]. They were able to present an effective antibacterial effect on *Fusobacterium nucleatum*, *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli*, and *Candida albicans* [125, 127, 129]. However, they displayed a weak antibacterial effect on *E. faecalis* which is one of the most persistent microorganisms associated with refractory periapical periodontitis [130, 131]. In addition, the freshly mixed ZnOE-based and epoxy amine resin-based root canal sealers had the most effective antibacterial effect, which, however, lasted only for a short period of time [131–134]. Many attempts were made to

improve the antibacterial effect of the commonly used root canal sealers. Root canal sealers such as Kerr pulp canal sealer EWT, AH Plus, and RealSeal SE, which were simply mixed with antibiotics (such as amoxicillin, metronidazole, and doxycycline), showed a better antibacterial effect than their unmodified counterparts when they were freshly mixed. However, they had no benefit of a long-term antibacterial effect [135–137]. AH Plus, Apexit Plus, and Canals mixed with hinokitiol improved the antibacterial effect, but they also exhibited increased cytotoxicity [138]. Similar to $Ca(OH)_2$-based sealers, silicon-based endodontic sealers showed a weak antibacterial effect [130] [134]. In addition, root canal sealers mixed or coated with cationic nanoparticles and silver ions were developed and showed an improved short-term antibacterial effect [115, 139, 140].

Quaternary ammonium salts (QASs) are cationic antimicrobials and have excellent antibacterial properties [141–147]. QASs kill a wide range of both Gram-positive and Gram-negative bacteria through electrostatic interactions with multiple anionic targets on bacterial surfaces [121, 148]. Since the 1970s, QASs have been widely used in the synthesis of antibiotics, disinfection of the environment, and water sterilization [143, 149]. In oral medicine, QASs are mainly used in the development of antibacterial composite resins, primers, and adhesives [150–152], and the application of QASs for root canal sealers is limited. Root canal sealers incorporated with QASs showed significantly more effective short-term antibacterial effects than unmodified counterparts. However, they showed no apparently improved long-term antibacterial effect [148, 153, 154]. Silica bioactive glass has very good biocompatibility and can promote the proliferation, mineralization, and differentiation of stem cells [120]. Silicon-containing root canal sealers have been developed due to their favorable biocompatibility. But they achieved a similar weak antibacterial effect to that of $Ca(OH)_2$-based sealers [130, 133, 134, 155, 156].

During the process of RCT, endodontic sealers can easily extrude the apical foramen and reach the periapical region and even the maxillary sinus [157, 158]. Direct contact of the endodontic sealers or their by-products with the periapical tissues or cells may induce a chronic inflammatory response, which decreases the success rate of RCT. Therefore, the endodontic sealer should have good biocompatibility to the periapical tissues and cells. Endodontic sealers show the highest toxicity when they are freshly prepared. ZnOE-based sealers released free eugenol and zinc oxide during the curing process, which presented a high level of cytotoxicity to human gingival fibroblasts, human periodontal ligament stem cells, and osteoblasts [159]. In addition, they also induced an inflammatory response to soft and bone tissues [160]. Epoxy amine resin-based sealers released formaldehyde and bisphenol-A diglycidyl ether during the curing process and presented moderate to severe cytotoxic effects both in vitro and in vivo [161]. MTA-based sealers had good cytocompatibility, which was probably due to the release of Ca^{2+} when contacting directly with water [162].

As mentioned above, an ideal endodontic sealer should be water insoluble to ensure a good sealing effect and to reduce the occurrence of micro-leakage, therefore enhancing the long-term therapeutic effect [163]. However, the majority of the endodontic sealers have shown some water solubility [164]. For example, there was

about 1–7% of weight loss for ZnOE-based sealers when they were incubated in an environment of 95% humidity and 37°C [165]. It was also shown that the AH 26, an epoxy amine resin-based sealer, lost about 2.6% of weight when stored in water for 28 days [164]. MTA-based sealers had a water solubility similar to that of epoxy amine resin-based sealers [166].

Recently, our group synthesized a unique type of substrate for long-term antibacterial endodontic sealers via grafting a series of novel quaternary ammonium polymethacrylate salts (QAPMs) on the surface of mono-dispersed silica-based bioactive glass nanospheres (SBG-NS) (Fig. 2) [167]. The silica-based bioactive glass was selected as the core sealing material because of its excellent biocompatibility. QAPM is a type of quaternary ammonium cationic antimicrobial and can kill a broad spectra of both Gram-positive and Gram-negative bacteria. During the synthesis process, a sol–gel process was first used to prepare mono-dispersed SBG-NS. Next, a series of quaternary ammonium methacrylate salts (QAMs) were synthesized and grafted onto the surface of the SBG-NS via a coupling reaction and a free radical polymerization process. The QAPM-containing SBG-NS (SBG-QAPM) presented both a long-term antibacterial effect and excellent cytocompatibility and biocompatibility (Fig. 3). In addition, the nano-sized SBG-NS readily penetrated into dentinal tubules and entombed any residual bacteria within the tubules, therefore providing another layer of protection from reinfection. Therefore, the SBG-QAPMs are promising substrates for the development of long-term antibacterial endodontic sealers [157].

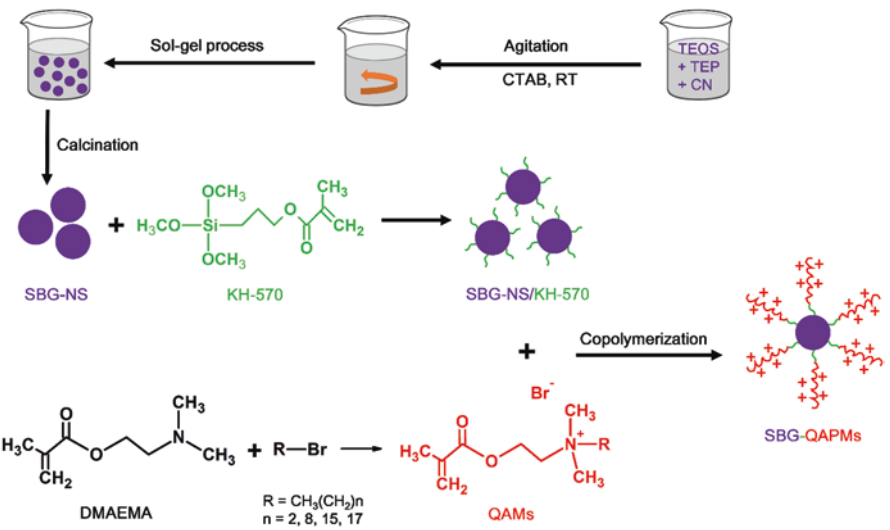


Fig. 2 Schematic diagram of synthesizing antimicrobial endodontic materials SBG-QAPMs. (From Cheng et al. [167] copyright 2017 The Royal Society of Chemistry. Reprinted with permission)

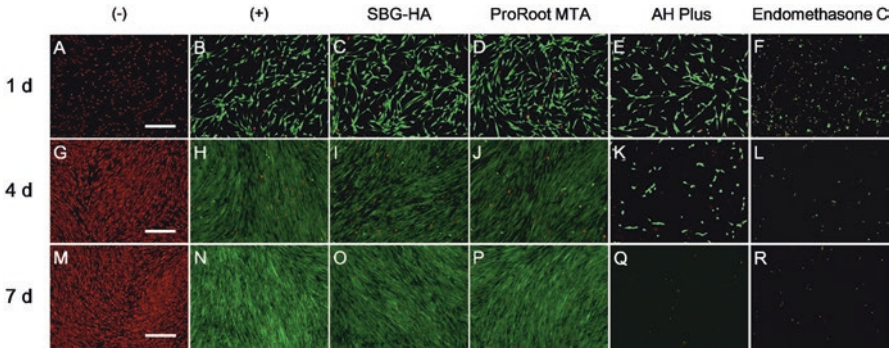


Fig. 3 In vitro cytocompatibility (LIVE/DEAD staining) of the antimicrobial endodontic material SBG-HA, commercial products Endomethasone C, AH Plus, and ProRoot MTA to periodontal ligament stem cells. The green fluorescence represents viable cells while the red fluorescence represents dead cells. Scale bar = 500 μm. (From Cheng et al. [167] copyright 2017 The Royal Society of Chemistry. Reprinted with permission)

Conclusions

The elimination of infection within the root canal is crucial for the success of RCT, which is currently carried out through a variety of chemo-mechanical techniques. Due to the complicated anatomical structure of the tooth root, it is well known that complete eradication of the bacteria in the canal and dentin tubules is virtually impossible, regardless of instrumentation and irrigation procedures. The residual bacteria can cause reinfection, leading to failure of the endodontic treatment. Therefore, antimicrobial endodontic materials are widely used during RCT. Each canal irrigation, medication, and sealing material has its advantages and limitations, and none of them can meet all the requirements, especially in terms of the efficacy and toxicity. The development of new antimicrobial endodontic materials with long-term efficacy and high biocompatibility are expected to enhance the disinfection effect, to improve the success rate of RCT, and as a result, to promote and maintain the long-term outcome of endodontic therapy.

References

1. Kureishi A, Chow A (1988) The tender tooth. Dentoalveolar, pericoronal, and periodontal infections. Infectious disease clinics of North America 2(1):163–182
2. Tao L, Herzberg MC (1998) Identifying in vivo expressed streptococcal genes in endocarditis. Methods in enzymology 310:109–116
3. Genco R, Offenbacher S, Beck J (2002) Periodontal disease and cardiovascular disease: epidemiology and possible mechanisms. The Journal of the American Dental Association 133:14S–22S
4. Kalowski M (1976) Result of bacteriological studies in cases of difficulties encountered during endodontic treatment of non-vital teeth with periapical lesions. Czasopismo stomatologiczne 29(11):989–995

5. Sundqvist GK, Eckerbom M, Larsson A, Sjögren U (1979) Capacity of anaerobic bacteria from necrotic dental pulps to induce purulent infections. Infection and immunity 25(2):685–693
6. Paster BJ, Boches SK, Galvin JL, Ericson RE, Lau CN, Levanos VA et al (2001) Bacterial diversity in human subgingival plaque. J Bacteriol 183(12):3770–3783
7. Bergenholtz G (1974) Micro-organisms from necrotic pulp of traumatized teeth. Odontologisk revy 25(4):347–358
8. Bystrom A, Sundqvist G (1981) Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scandinavian journal of dental research 89(4):321–328
9. Fouad AF, Barry J, Caimano M, Clawson M, Zhu Q, Carver R et al (2002) PCR-based identification of bacteria associated with endodontic infections. J Clin Microbiol 40(9):3223–3231
10. Sundqvist G (1994) Taxonomy, ecology, and pathogenicity of the root canal flora. Oral surgery, oral medicine, and oral pathology 78(4):522–530
11. Gomes BP, Drucker DB, Lilley JD (1994) Associations of specific bacteria with some endodontic signs and symptoms. International endodontic journal 27(6):291–298
12. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr (1998) Microbial complexes in subgingival plaque. J Clin Periodontol 25(2):134–144
13. Baumgartner JC, Falkler WA Jr (1991) Bacteria in the apical 5 mm of infected root canals. Journal of endodontics 17(8):380–383
14. Gomes BP, Pinheiro ET, Gade-Neto CR, Sousa EL, Ferraz CC, Zaia AA et al (2004) Microbiological examination of infected dental root canals. Oral Microbiol Immunol 19(2):71–76
15. Kalfas S, Figdor D, Sundqvist G (2001) A new bacterial species associated with failed endodontic treatment: identification and description of Actinomyces radicidentis. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics 92(2):208–214
16. Molander A, Reit C, Dahlen G, Kvist T (1998) Microbiological status of root-filled teeth with apical periodontitis. International endodontic journal 31(1):1–7
17. Baumgartner JC, Watts CM, Xia T (2000) Occurrence of Candida albicans in infections of endodontic origin. Journal of endodontics 26(12):695–698
18. Chavez De Paz LE, Dahlen G, Molander A, Moller A, Bergenholtz G (2003) Bacteria recovered from teeth with apical periodontitis after antimicrobial endodontic treatment. International endodontic journal 36(7):500–508
19. Foschi F, Cavrini F, Montebugnoli L, Stashenko P, Sambri V, Prati C (2005) Detection of bacteria in endodontic samples by polymerase chain reaction assays and association with defined clinical signs in Italian patients. Oral Microbiol Immunol 20(5):289–295
20. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB (2006) Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. Journal of endodontics 32(2):93–98
21. Takahashi K (1998) Microbiological, pathological, inflammatory, immunological and molecular biological aspects of periradicular disease. International endodontic journal 31(5):311–325
22. Hoyle BD, Costerton JW (1991) Bacterial resistance to antibiotics: the role of biofilms. Progress in drug research. Fortschritte der Arzneimittelforschung. Progres des recherches pharmaceutiques 37:91–105
23. Su L, Gao Y, Yu C, Wang H, Yu Q (2010) Surgical endodontic treatment of refractory periapical periodontitis with extraradicular biofilm. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 110(1):e40–e44
24. Sundqvist G (1992) Ecology of the root canal flora. Journal of endodontics 18(9):427–430
25. Noguchi N, Noiri Y, Narimatsu M, Ebisu S (2005) Identification and localization of extraradicular biofilm-forming bacteria associated with refractory endodontic pathogens. Applied and environmental microbiology 71(12):8738–8743
26. Nair PN, Henry S, Cano V, Vera J (2005) Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after “one-visit” endodontic treatment. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics 99(2):231–252

27. Sjogren U, Figdor D, Persson S, Sundqvist G (1997) Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. International endodontic journal 30(5):297–306
28. Kreisler M, Kohnen W, Beck M, Al Haj H, Christoffers AB, Gotz H et al (2003) Efficacy of NaOCl/H2O2 irrigation and GaAlAs laser in decontamination of root canals in vitro. Lasers in surgery and medicine 32(3):189–196
29. Tyagi S, Mishra P, Tyagi P (2013) Evolution of root canal sealers: an insight story. European Journal of General Dentistry 2(3):199
30. Zehnder M (2006) Root canal irrigants. Journal of endodontics 32(5):389–398
31. Becker TD, Woollard GW (2001) Endodontic irrigation. General dentistry 49(3):272–276
32. Siqueira JF Jr, Machado AG, Silveira RM, Lopes HP, de Uzeda M (1997) Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of Enterococcus faecalis from the root canal, in vitro. International endodontic journal 30(4):279–282
33. Pashley EL, Birdsong NL, Bowman K, Pashley DH (1985) Cytotoxic effects of NaOCl on vital tissue. Journal of endodontics 11(12):525–528
34. Shih M, Marshall FJ, Rosen S (1970) The bactericidal efficiency of sodium hypochlorite as an endodontic irrigant. Oral surgery, oral medicine, and oral pathology 29(4):613–619
35. Rutberg M, Spangberg E, Spangberg L (1977) Evaluation of enhanced vascular permeability of endodontic medicaments in vivo. Journal of endodontics 3(9):347–351
36. Hulsmann M, Hahn W (2000) Complications during root canal irrigation—literature review and case reports. International endodontic journal 33(3):186–193
37. Smith JJ, Wayman BE (1986) An evaluation of the antimicrobial effectiveness of citric acid as a root canal irrigant. Journal of endodontics 12(2):54–58
38. Siqueira JF Jr, Rocas IN, Favieri A, Lima KC (2000) Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite. Journal of endodontics 26(6):331–334
39. Cvek M, Nord CE, Hollender L (1976) Antimicrobial effect of root canal debridement in teeth with immature root. A clinical and microbiologic study. Odontologisk revy 27(1):1–10
40. Bergenholtz G, Spangberg L (2004) Controversies in endodontics. Critical reviews in oral biology and medicine 15(2):99–114
41. White RR, Hays GL, Janer LR (1997) Residual antimicrobial activity after canal irrigation with chlorhexidine. Journal of endodontics 23(4):229–231
42. Delany GM, Patterson SS, Miller CH, Newton CW (1982) The effect of chlorhexidine gluconate irrigation on the root canal flora of freshly extracted necrotic teeth. Oral surgery, oral medicine, and oral pathology 53(5):518–523
43. Heling I, Sommer M, Steinberg D, Friedman M, Sela MN (1992) Microbiological evaluation of the efficacy of chlorhexidine in a sustained-release device for dentine sterilization. International endodontic journal 25(1):15–19
44. Gomes BP, Souza SF, Ferraz CC, Teixeira FB, Zaia AA, Valdrighi L et al (2003) Effectiveness of 2% chlorhexidine gel and calcium hydroxide against Enterococcus faecalis in bovine root dentine in vitro. International endodontic journal 36(4):267–275
45. Vahdaty A, Pitt Ford TR, Wilson RF (1993) Efficacy of chlorhexidine in disinfecting dentinal tubules in vitro. Endodontics & dental traumatology 9(6):243–248
46. Vianna ME, Gomes BP, Berber VB, Zaia AA, Ferraz CC, de Souza-Filho FJ (2004) In vitro evaluation of the antimicrobial activity of chlorhexidine and sodium hypochlorite. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics 97(1):79–84
47. Buck RA, Eleazer PD, Staat RH, Scheetz JP (2001) Effectiveness of three endodontic irrigants at various tubular depths in human dentin. Journal of endodontics 27(3):206–208
48. Jeansonne MJ, White RR (1994) A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. Journal of endodontics 20(6):276–278
49. Oncag O, Hosgor M, Hilmioglu S, Zekioglu O, Eronat C, Burhanoglu D (2003) Comparison of antibacterial and toxic effects of various root canal irrigants. International endodontic journal 36(6):423–432

50. Kuruvilla JR, Kamath MP (1998) Antimicrobial activity of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate separately and combined, as endodontic irrigants. Journal of endodontics 24(7):472–476
51. Torabinejad M, Handysides R, Khademi AA, Bakland LK (2002) Clinical implications of the smear layer in endodontics: a review. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics 94(6):658–666
52. Yamada RS, Armas A, Goldman M, Lin PS (1983) A scanning electron microscopic comparison of a high volume final flush with several irrigating solutions: part 3. Journal of endodontics 9(4):137–142
53. Nagamatsu Y, Chen K-K, Tajima K, Kakigawa H, Kozono Y (2002) Durability of bactericidal activity in electrolyzed neutral water by storage. Dental materials journal 21(2):93–104
54. Kubota A, Goda T, Tsuru T, Yonekura T, Yagi M, Kawahara H et al (2015) Efficacy and safety of strong acid electrolyzed water for peritoneal lavage to prevent surgical site infection in patients with perforated appendicitis. Surgery today 45:876–879
55. Rahman S, Ding T, Oh D-H (2010) Effectiveness of low concentration electrolyzed water to inactivate foodborne pathogens under different environmental conditions. Int J Food Microbiol 139(3):147–153
56. Ozaki M, Ohshima T, Mukumoto M, Konishi H, Hirashita A, Maeda N et al (2012) A study for biofilm removing and antimicrobial effects by microbubbled tap water and other functional water, electrolyzed hypochlorite water and ozonated water. Dental materials journal 31(4):662–668
57. Park H, Hung Y-C, Chung D (2004) Effects of chlorine and pH on efficacy of electrolyzed water for inactivating Escherichia coli O157: H7 and Listeria monocytogenes. Int J Food Microbiol 91(1):13–18
58. Chen X, Li P, Wang X, Gu M, Zhao C, Sloan A et al (2013) Ex vivo antimicrobial efficacy of strong acid electrolytic water against Enterococcus faecalis biofilm. Int Endod J 46(10):938–946
59. G-i H, Uemura M, Weine FS, Toda T (1996) Removal of smear layer in the root canal using oxidative potential water. J Endod 22(12):643–645
60. Kiura H, Sano K, Morimatsu S, Nakano T, Morita C, Yamaguchi M et al (2002) Bactericidal activity of electrolyzed acid water from solution containing sodium chloride at low concentration, in comparison with that at high concentration. Journal of Microbiological Methods 49(3):285–293
61. Cheng X, Tian Y, Zhao C, Qu T, Ma C, Liu X et al (2016) Bactericidal effect of strong acid electrolyzed water against flow enterococcus faecalis biofilms. Journal of endodontics 42(7):1120–1125
62. Qing Y, Akita Y, Kawano S, Kawazu S, Yoshida T, Sekine I (2006) Cleaning efficacy and dentin micro-hardness after root canal irrigation with a strong acid electrolytic water. J Endod 32(11):1102–1106
63. Shabahang S, Pouresmail M, Torabinejad M (2003) In vitro antimicrobial efficacy of MTAD and sodium hypochlorite. Journal of endodontics 29(7):450–452
64. Mancini M, Armellin E, Casaglia A, Cerroni L, Cianconi L (2009) A comparative study of smear layer removal and erosion in apical intraradicular dentine with three irrigating solutions: a scanning electron microscopy evaluation. Journal of endodontics 35(6):900–903
65. Torabinejad M, Shabahang S, Aprecio RM, Kettering JD (2003) The antimicrobial effect of MTAD: an in vitro investigation. Journal of endodontics 29(6):400–403
66. Shabahang S, Torabinejad M (2003) Effect of MTAD on Enterococcus faecalis-contaminated root canals of extracted human teeth. Journal of endodontics 29(9):576–579
67. Portenier I, Waltimo T, Orstavik D, Haapasalo M (2006) Killing of Enterococcus faecalis by MTAD and chlorhexidine digluconate with or without cetrimide in the presence or absence of dentine powder or BSA. Journal of endodontics 32(2):138–141
68. Kho P, Baumgartner JC (2006) A comparison of the antimicrobial efficacy of NaOCl/Biopure MTAD versus NaOCl/EDTA against Enterococcus faecalis. Journal of endodontics 32(7):652–655

69. Dunavant TR, Regan JD, Glickman GN, Solomon ES, Honeyman AL (2006) Comparative evaluation of endodontic irrigants against Enterococcus faecalis biofilms. Journal of endodontics 32(6):527–531
70. Zhang W, Torabinejad M, Li Y (2003) Evaluation of cytotoxicity of MTAD using the MTT-tetrazolium method. Journal of endodontics 29(10):654–657
71. Ruff ML, McClanahan SB, Babel BS (2006) In vitro antifungal efficacy of four irrigants as a final rinse. Journal of endodontics 32(4):331–333
72. Clegg MS, Vertucci FJ, Walker C, Belanger M, Britto LR (2006) The effect of exposure to irrigant solutions on apical dentin biofilms in vitro. Journal of endodontics 32(5):434–437
73. Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M (2004) Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. Journal of endodontics 30(11):778–781
74. Tay FR, Gu LS, Schoeffel GJ, Wimmer C, Susin L, Zhang K et al (2010) Effect of vapor lock on root canal debridement by using a side-vented needle for positive-pressure irrigant delivery. Journal of endodontics 36(4):745–750
75. Plotino G, Pameijer CH, Grande NM, Somma F (2007) Ultrasonics in endodontics: a review of the literature. J Endod 33(2):81–95
76. George R, Meyers IA, Walsh LJ (2008) Laser activation of endodontic irrigants with improved conical laser fiber tips for removing smear layer in the apical third of the root canal. Journal of endodontics 34(12):1524–1527
77. De Moor RJ, Meire M, Goharkhay K, Moritz A, Vanobbergen J (2010) Efficacy of ultrasonic versus laser-activated irrigation to remove artificially placed dentin debris plugs. Journal of endodontics 36(9):1580–1583
78. Ordinola-Zapata R, Bramante CM, Aprecio RM, Handysides R, Jaramillo DE (2014) Biofilm removal by 6% sodium hypochlorite activated by different irrigation techniques. International endodontic journal 47(7):659–666
79. Huang TY, Gulabivala K, Ng YL (2008) A bio-molecular film ex-vivo model to evaluate the influence of canal dimensions and irrigation variables on the efficacy of irrigation. International endodontic journal 41(1):60–71
80. Peeters HH, Gutknecht N (2014) Efficacy of laser-driven irrigation versus ultrasonic in removing an airlock from the apical third of a narrow root canal. Australian endodontic journal 40(2):47–53
81. Meire MA, Coenye T, Nelis HJ, De Moor RJ (2012) Evaluation of Nd:YAG and Er:YAG irradiation, antibacterial photodynamic therapy and sodium hypochlorite treatment on Enterococcus faecalis biofilms. International endodontic journal 45(5):482–491
82. Gordon W, Atabakhsh VA, Meza F, Doms A, Nissan R, Rizoiu I et al (2007) The antimicrobial efficacy of the erbium, chromium:yttrium-scandium-gallium-garnet laser with radial emitting tips on root canal dentin walls infected with Enterococcus faecalis. Journal of the American Dental Association 138(7):992–1002
83. Klinke T, Klimm W, Gutknecht N (1997) Antibacterial effects of Nd:YAG laser irradiation within root canal dentin. Journal of clinical laser medicine & surgery 15(1):29–31
84. Moritz A, Schoop U, Goharkhay K, Jakolitsch S, Kluger W, Wernisch J et al (1999) The bactericidal effect of Nd:YAG, Ho:YAG, and Er:YAG laser irradiation in the root canal: an in vitro comparison. Journal of clinical laser medicine & surgery 17(4):161–164
85. Hellingwerf KJ, Hoff WD, Crielaard W (1996) Photobiology of microorganisms: how photosensors catch a photon to initialize signalling. Molecular microbiology 21(4):683–693
86. Camargo SE, Valera MC, Camargo CH, Fonseca MB, Menezes MM (2005) Effects of Nd:YAG laser irradiation on root canal dentin wall: a scanning electron microscopic study. Photomedicine and laser surgery 23(4):399–404
87. Spencer P, Cobb CM, McCollum MH, Wieliczka DM (1996) The effects of CO2 laser and Nd:YAG with and without water/air surface cooling on tooth root structure: correlation between FTIR spectroscopy and histology. Journal of periodontal research 31(7):453–462
88. Aoki A, Ishikawa I, Yamada T, Otsuki M, Watanabe H, Tagami J et al (1998) Comparison between Er:YAG laser and conventional technique for root caries treatment in vitro. Journal of dental research 77(6):1404–1414

89. Yamazaki R, Goya C, Yu DG, Kimura Y, Matsumoto K (2001) Effects of erbium,chromium:YSGG laser irradiation on root canal walls: a scanning electron microscopic and thermographic study. Journal of endodontics 27(1):9–12
90. Bolhari B, Ehsani S, Etemadi A, Shafaq M, Nosrat A (2014) Efficacy of Er,Cr:YSGG laser in removing smear layer and debris with two different output powers. Photomedicine and laser surgery 32(10):527–532
91. Cheng X, Guan S, Lu H, Zhao C, Chen X, Li N et al (2012) Evaluation of the bactericidal effect of Nd: YAG, Er: YAG, Er, Cr: YSGG laser radiation, and antimicrobial photodynamic therapy (aPDT) in experimentally infected root canals. Lasers in surgery and medicine 44(10):824–831
92. Cheng X, Chen B, Qiu J, He W, Lv H, Qu T et al (2016) Bactericidal effect of Er:YAG laser combined with sodium hypochlorite irrigation against Enterococcus faecalis deep inside dentinal tubules in experimentally infected root canals. J Med Microbiol 65(2):176–187
93. Macedo RG, Wesselink PR, Zaccheo F, Fanali D, Van Der Sluis LW (2010) Reaction rate of NaOCl in contact with bovine dentine: effect of activation, exposure time, concentration and pH. International endodontic journal 43(12):1108–1115
94. Blanken J, De Moor RJ, Meire M, Verdaasdonk R (2009) Laser induced explosive vapor and cavitation resulting in effective irrigation of the root canal. Part 1: a visualization study. Lasers in surgery and medicine 41(7):514–519
95. Koch JD, Jaramillo DE, DiVito E, Peters OA (2016) Irrigant flow during photon-induced photoacoustic streaming (PIPS) using Particle Image Velocimetry (PIV). Clin Oral Investig 20(2):381–386
96. Matsumoto H, Yoshimine Y, Akamine A (2011) Visualization of irrigant flow and cavitation induced by Er: YAG laser within a root canal model. J Endod 37(6):839–843
97. Levy G, Rizoiu I, Friedman S, Lam H (1996) Pressure waves in root canals induced by Nd: YAG laser. Journal of endodontics 22(2):81–84
98. El Karim I, Kennedy J, Hussey D (2007) The antimicrobial effects of root canal irrigation and medication. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics 103(4):560–569
99. Foreman PC, Barnes IE (1990) Review of calcium hydroxide. International endodontic journal 23(6):283–297
100. Nerwich A, Figdor D, Messer HH (1993) pH changes in root dentin over a 4-week period following root canal dressing with calcium hydroxide. Journal of endodontics 19(6):302–306
101. Peters LB, van Winkelhoff AJ, Buijs JF, Wesselink PR (2002) Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. International endodontic journal 35(1):13–21
102. Siqueira JF Jr, de Uzeda M (1996) Disinfection by calcium hydroxide pastes of dentinal tubules infected with two obligate and one facultative anaerobic bacteria. Journal of endodontics 22(12):674–676
103. Haapasalo HK, Siren EK, Waltimo TM, Orstavik D, Haapasalo MP (2000) Inactivation of local root canal medicaments by dentine: an in vitro study. International endodontic journal 33(2):126–131
104. Fuss Z, Mizrahi A, Lin S, Cherniak O, Weiss EI (2002) A laboratory study of the effect of calcium hydroxide mixed with iodine or electrophoretically activated copper on bacterial viability in dentinal tubules. International endodontic journal 35(6):522–526
105. Molander A, Reit C, Dahlen G (1999) The antimicrobial effect of calcium hydroxide in root canals pretreated with 5% iodine potassium iodide. Endodontics & dental traumatology 15(5):205–209
106. Siren EK, Haapasalo MP, Waltimo TM, Orstavik D (2004) In vitro antibacterial effect of calcium hydroxide combined with chlorhexidine or iodine potassium iodide on Enterococcus faecalis. Eur J Oral Sci 112(4):326–331
107. Torneck CD, Smith JS, Grindall P (1973) Biologic effects of endodontic procedures on developing incisor teeth. IV. Effect of debridement procedures and calcium hydroxide-

camphorated parachlorophenol paste in the treatment of experimentally induced pulp and periapical disease. Oral surgery, oral medicine, and oral pathology 35(4):541–554
108. Hoshino E, Kurihara-Ando N, Sato I, Uematsu H, Sato M, Kota K et al (1996) In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. International endodontic journal 29(2):125–130
109. Moskow A, Morse DR, Krasner P, Furst ML (1984) Intracanal use of a corticosteroid solution as an endodontic anodyne. Oral surgery, oral medicine, and oral pathology 58(5):600–604
110. Heling I, Pecht M (1991) Efficacy of Ledermix paste in eliminating Staphylococcus aureus from infected dentinal tubules in vitro. Endodontics & dental traumatology 7(6):251–254
111. Ørstavik D (2005) Materials used for root canal obturation: technical, biological and clinical testing. Endodontic topics 12(1):25–38
112. Grossman LLI, Oliet S, Del Río CE (1988) Endodontic practice. Lea & Febiger, New York
113. Hume WR (1986) The pharmacologic and toxicological properties of zinc oxide-eugenol. The Journal of the American Dental Association 113(5):789–791
114. Leonardo MR, da Silva LAB, Tanomaru Filho M, da Silva RS (1999) Release of formaldehyde by 4 endodontic sealers. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 88(2):221–225
115. Kreth J, Kim D, Nguyen M, Hsiao G, Mito R, Kang M et al (2008) The antimicrobial effect of silver ion impregnation into endodontic sealer against Streptococcus mutans. Open Dentistry Journal 2:18–23
116. Wang Z, Shen Y, Haapasalo M (2014) Dentin extends the antibacterial effect of endodontic sealers against Enterococcus faecalis biofilms. Journal of endodontics 40(4):505–508
117. Du T, Wang Z, Shen Y, Ma J, Cao Y, Haapasalo M (2015) Combined antibacterial effect of sodium hypochlorite and root canal sealers against enterococcus faecalis biofilms in dentin canals. Journal of endodontics 41(8):1294–1298
118. Sagsen B, Er O, Esel D, Yagmur G, Altintop Y (2009) In vitro pharmacodynamic activities of root canal sealers on Enterococcus faecalis. J Contemp Dent Pract 10:35–42
119. Zhou HM, Du TF, Shen Y, Wang ZJ, Zheng YF, Haapasalo M (2015) In vitro cytotoxicity of calcium silicate-containing endodontic sealers. Journal of endodontics 41(1):56–61
120. Qu T, Liu X (2013) Nano-structured gelatin/bioactive glass hybrid scaffolds for the enhancement of odontogenic differentiation of human dental pulp stem cells. Journal of Materials Chemistry B 1(37):4764–4772
121. Buffet-Bataillon S, Tattevin P, Bonnaure-Mallet M, Jolivet-Gougeon A (2012) Emergence of resistance to antibacterial agents: the role of quaternary ammonium compounds—a critical review. International journal of antimicrobial agents 39(5):381–389
122. Gorduysus M, Avcu N, Gorduysus O, Pekel A, Baran Y, Avcu F et al (2007) Cytotoxic effects of four different endodontic materials in human periodontal ligament fibroblasts. J Endod 33(12):1450–1454
123. Jones JR (2013) Review of bioactive glass: from hench to hybrids. Acta Biomater 9(1):4457–4486
124. Faria-Júnior N, Tanomaru-Filho M, Berbert FLCV, Guerreiro-Tanomaru J (2013) Antibiofilm activity, pH and solubility of endodontic sealers. Int Endod J 46(8):755–762
125. Poggio C, Lombardini M, Colombo M, Dagna A, Saino E, Arciola CR et al (2011) Antibacterial effects of six endodontic sealers. International Journal of Artificial Organs 34(9):908–913
126. Saha S, Samadi F, Jaiswal J, Ghoshal U (2010) Antimicrobial activity of different endodontic sealers: an in vitro evaluation. Journal of Indian Society of Pedodontics and Preventive Dentistry 28(4):251
127. Heyder M, Kranz S, Völpel A, Pfister W, Watts DC, Jandt KD et al (2013) Antibacterial effect of different root canal sealers on three bacterial species. Dental Materials 29(5):542–549
128. Mozini ACA, Vansan LP, Sousa Neto MD, Pietro R (2009) Influence of the length of remaining root canal filling and post space preparation on the coronal leakage of Enterococcus faecalis. Brazilian Journal of Microbiology 40(1):174–179

129. Miyagak DC, de Carvalho EM, Robazza CR, Chavasco JK, Levorato GL (2006) In vitro evaluation of the antimicrobial activity of endodontic sealers. Brazilian oral research 20(4):303–306
130. Willershausen I, Callaway A, Briseño B, Willershausen B (2011) In vitro analysis of the cytotoxicity and the antimicrobial effect of four endodontic sealers. Head Face Med 7:15
131. Smadi L, Khraisat A, Al-Tarawneh S, Mahafzah A, Salem A (2008) In vitro evaluation of the antimicrobial activity of nine root canal sealers: direct contact test. Tropical dental journal 31(124):11–18
132. Shantiaee Y, Dianat O, Janani A, Ahari GK (2010) In vitro evaluation of the antibacterial activity of three root canal sealers. Iranian endodontic journal 5(1):1–5
133. Farmakis ETR, Kontakiotis EG, Tseleni-Kotsovili A, Tsatsas VG (2012) Comparative in vitro antibacterial activity of six root canal sealers against Enterococcus faecalis and Proteus vulgaris. Journal of investigative and clinical dentistry 3(4):271–275
134. Prestegaard H, Portenier I, Ørstavik D, Kayaoglu G, Haapasalo M, Endal U (2014) Antibacterial activity of various root canal sealers and root-end filling materials in dentin blocks infected ex vivo with Enterococcus faecalis. Acta Odontologica Scandinavica 72(8):970–976
135. Sharma D, Grover R, Pinnameneni PS, Dey S, Raju PR (2014) Evaluation of efficacy of combinations of five endodontic sealers with five antibiotics against Enterococcus Faecalis–An in-vitro study. Journal of international oral health: JIOH 6(2):90
136. Baer J, Maki JS (2010) In vitro evaluation of the antimicrobial effect of three endodontic sealers mixed with amoxicillin. J Endod 36(7):1170–1173
137. Hoelscher AA, Bahcall JK, Maki JS (2006) In vitro evaluation of the antimicrobial effects of a root canal sealer-antibiotic combination against Enterococcus faecalis. J Endod 32(2):145–147
138. Shih Y-H, Lin D-J, Chang K-W, Hsia S-M, Ko S-Y, Lee S-Y et al (2014) Evaluation physical characteristics and comparison antimicrobial and anti-inflammation potentials of dental root canal sealers containing hinokitiol in vitro. PloS one 9(6):e94941
139. Kishen A, Shi Z, Shrestha A, Neoh KG (2008) An investigation on the antibacterial and antibiofilm efficacy of cationic nanoparticulates for root canal disinfection. J Endod 34(12):1515–1520
140. Kreth J, Kim D, Nguyen M, Hsiao G, Mito R, Kang M et al (2008) The antimicrobial effect of silver ion impregnation into endodontic sealer against Streptococcus mutans. The open dentistry journal 2:18
141. Zhang H, Oyanedel-Craver V (2013) Comparison of the bacterial removal performance of silver nanoparticles and a polymer based quaternary amine functiaonalized silsesquioxane coated point-of-use ceramic water filters. Journal of hazardous materials 260:272–277
142. Li L, Pu T, Zhanel G, Zhao N, Ens W, Liu S (2012) New biocide with both N-chloramine and quaternary ammonium moieties exerts enhanced bactericidal activity. Advanced healthcare materials 1(5):609–620
143. Kochan J, Scheidle M, van Erkel J, Bikel M, Büchs J, Wong JE et al (2012) Characterization of antibacterial polyethersulfone membranes using the respiration activity monitoring system (RAMOS). Water research 46(16):5401–5409
144. Sajomsang W, Gonil P, Tantayanon S (2009) Antibacterial activity of quaternary ammonium chitosan containing mono or disaccharide moieties: preparation and characterization. International journal of biological macromolecules 44(5):419–427
145. Liu WS, Wang CH, Sun JF, Hou GG, Wang YP, Qu RJ (2015) Synthesis, characterization and antibacterial properties of dihydroxy quaternary ammonium salts with long chain alkyl bromides. Chemical biology & drug design 85(1):91–97
146. Yang Z, Degorce-Dumas J-R, Yang H, Guibal E, Li A, Cheng R (2014) Flocculation of Escherichia coli using a quaternary ammonium salt grafted carboxymethyl chitosan flocculant. Environmental science & technology 48(12):6867–6873

147. Jennings MC, Ator LE, Paniak TJ, Minbiole KP, Wuest WM (2014) Biofilm-eradicating properties of quaternary ammonium amphiphiles: simple mimics of antimicrobial peptides. ChemBioChem 15(15):2211–2215
148. Beyth N, Shvero DK, Zaltsman N, Houri-Haddad Y, Abramovitz I, Davidi MP et al (2013) Rapid kill—novel endodontic sealer and Enterococcus faecalis. PloS one 8(11):e78586
149. Calvani M, Critelli L, Gallo G, Giorgi F, Gramiccioli G, Santaniello M et al (1998) L-Carnitine esters as "soft", broad-spectrum antimicrobial amphiphiles. Journal of medicinal chemistry 41(13):2227–2233
150. Beyth N, Yudovin-Farber I, Perez-Davidi M, Domb AJ, Weiss EI (2010) Polyethyleneimine nanoparticles incorporated into resin composite cause cell death and trigger biofilm stress in vivo. Proceedings of the National Academy of Sciences 107(51):22038–22043
151. Cheng L, Weir MD, Zhang K, Arola DD, Zhou X, Xu HH (2013) Dental primer and adhesive containing a new antibacterial quaternary ammonium monomer dimethylaminododecyl methacrylate. Journal of dentistry 41(4):345–355
152. Melo MA, Wu J, Weir MD, Xu HH (2014) Novel antibacterial orthodontic cement containing quaternary ammonium monomer dimethylaminododecyl methacrylate. Journal of dentistry 42(9):1193–1201
153. Kesler Shvero D, Abramovitz I, Zaltsman N, Perez Davidi M, Weiss E, Beyth N (2013) Towards antibacterial endodontic sealers using quaternary ammonium nanoparticles. International endodontic journal 46(8):747–754
154. Barros J, Silva M, Rodrigues M, Alves F, Lopes M, Pina-Vaz I et al (2014) Antibacterial, physicochemical and mechanical properties of endodontic sealers containing quaternary ammonium polyethylenimine nanoparticles. Int Endod J 47(8):725–734
155. Mohammadi Z, Yazdizadeh M (2006) Evaluation of the antibacterial activity of new root canal sealers. The Journal of clinical dentistry 18(3):70–72
156. Wang X, Chang J, Hu S (2012) A study on the sealing ability and antibacterial activity of Ca 3 SiO 5/CaCl 2 composite cement for dental applications. Dental materials journal 31(4):617–622
157. Camps J, About I (2003) Cytotoxicity testing of endodontic sealers: a new method. J Endod 29(9):583–586
158. Batur YB, Ersev H (2008) Five-year follow-up of a root canal filling material in the maxillary sinus: a case report. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology 106(4):e54–e56
159. Schwarze T, Fiedler I, Leyhausen G, Geurtsen W (2002) The cellular compatibility of five endodontic sealers during the setting period. J Endod 28(11):784–786
160. Yesilsoy C, Koren LZ, Morse DR, Kobayashi C (1988) A comparative tissue toxicity evaluation of established and newer root canal sealers. Oral Surgery, Oral Medicine, Oral Pathology 65(4):459–467
161. Silva EJ, Santos CC, Zaia AA (2013) Long-term cytotoxic effects of contemporary root canal sealers. Journal of Applied Oral Science 21(1):43–47
162. Swierenga S, MacManus J, Whitfield J (1976) Regulation by calcium of the proliferation of heart cells from young adult rats. In Vitro 12(1):31–36
163. Grossman LI (1958) An improved root canal cement. The Journal of the American Dental Association 56(3):381–385
164. Schafer E, Zandbiglari T (2003) Solubility of root-canal sealers in water and artificial saliva. International endodontic journal 36(10):660–669
165. Camps J, Pommel L, Bukiet F, About I (2004) Influence of the powder/liquid ratio on the properties of zinc oxide-eugenol-based root canal sealers. Dental materials 20(10):915–923
166. Gomes-Filho JE, Watanabe S, Bernabe PF, de Moraes Costa MT (2009) A mineral trioxide aggregate sealer stimulated mineralization. Journal of endodontics 35(2):256–260
167. Cheng X, Qu T, Ma C, Xiang D, Yu Q, Liu X (2017) Bioactive mono-dispersed nanospheres with long-term antibacterial effects for endodontic sealing. J Mater Chem B 5(6):1195–1204

Advances in Polysaccharide-Based Antimicrobial Delivery Vehicles

Vaishali Pawar, M. C. Bavya, K. Vimal Rohan, and Rohit Srivastava

Abstract Antimicrobial resistance is one of the major causes for morbidity and mortality in sepsis patients. Trying to circumvent the challenge with newer antibiotics has led to the drug misuse and bacterial recalcitrance. Recently, polysaccharides have proffered inexplicable contributions in the field of antimicrobial drug delivery. Structural hierarchy and tunability in biochemical and mechanical properties make polysaccharides unique. Some of the polysaccharides in the naïve state itself pose antimicrobial properties in inhibiting bacterial colonization via blocking carbohydrate receptor associated with host–bacterial responses. While, rest of the saccharides upon modification delivers antibacterial drugs onto targeted sites with sustained or burst release depending upon the need. Ongoing research keeps pace in promoting polysaccharides for local as well as systemic therapy due to its attractive features, mainly biocompatibility, mechanical strength, stimuli responsiveness, protein affinity and reduced toxicity. This chapter presents the updates of prominent polysaccharides involved in the field of antimicrobial drug delivery.

Keywords Polysaccharide · Antimicrobial · Drug delivery · Antibiotic resistance · Biocompatibility · Biomaterial

Introduction

Recent developments in the field of biomaterial science and regenerative medicine have led to innovations in the development of "bioactive materials" capable of producing biological responses, especially in the area of antimicrobial applications. Of

Vaishali Pawar, M. C. Bavya, K. Vimal Rohan and Rohit Srivastava contributed equally to this work.

V. Pawar · M. C. Bavya · R. Srivastava (✉)
Indian Institute of Technology, Mumbai, Maharashtra, India
e-mail: rsrivasta@iitb.ac.in

K. V. Rohan
Pariyaram Medical College, Pariyaram, Kerala, India

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_12

particular significance, the materials meant for orthopedic and other implant-related infections should have the ability to defend against microbial invasions and produce a favorable environment for bone regeneration.

Typically, after orthopedic procedures or implant fixation, cells grow onto an implant and further upon the appropriate conditions, the proteins and cells envelop the implant. The major challenge here is material acceptance and a plausible risk of microbial growth, which may further elicit negative responses and pave the way for rejection of the implant or give rise to infections. Therefore, it is of the foremost importance to treat orthopedic implant-associated disorders with biocompatible materials for minimizing such complications [1].

The etiology behind purulent bacterial infections is due to the recalcitrant behavior of bacteria upon drug exposure, developing resistance toward antibiotics [2]. A recent report suggests that implant-associated infections and the subsequent risk of causing morbidity and mortality are on the rise and have to be addressed quickly [3]. Generally, infections are treated using systemic antibiotics, debridement therapy, implant removal, and complicated surgeries, which may require long-term rehabilitation procedures [4, 5]. In such cases, there is a considerable rise in capital, without guaranteeing a successful clinical outcome [6, 7]. However, the persistent growth of microorganisms and their genetic mutation has led to newer alternative research on the modification of existing drug delivery vehicles [8]. The selection of biomaterials is based on their innate antimicrobial activity or having the capability to imbibe antibacterial activity upon tuning their chemistry. Also, the ability to mimic the extracellular matrix and cause minimal harm to tissues may be considered for their use as carrier vehicles in medical sciences to treat antimicrobial infections related to orthopedic applications [9].

Solution for these life-intimidating complications is in developing biopolymeric antimicrobial drug delivery carriers or coatings, which could promote adequate bone tissue linkage. A novel approach amongst biopolymers is the use of polysaccharide carriers, which have equipped the ability to mimic the extracellular matrix, capability of tailoring their properties for improving antimicrobial properties, and ample biocompatibility with an ease of tuning surface functionalities to serve as an antimicrobial aid for bone and implant applications.

To define polysaccharides, it is important to know that they are biomaterials belonging to the class of simple sugars, derived from monosaccharides via glycosidic linkages, which are of significant research interest globally [10]. To put it into simpler terms, polysaccharides are hefty molecules originated from the Greek word “Poly” meaning many and “Saccharide” meaning sweet [11]. Polysaccharides can be chemically modified based on their reactive groups and structural diversity into functional and structural components of cells (glycoproteins, glycolipids); they are also capable of serving as a storage depot (glycogen) (Table 1) [12].

These characteristics of polysaccharides have led them to establish the field of drug delivery. Polysaccharides also serve as excellent antimicrobial agents due to the presence of functional groups (amine, aldehyde, carboxyl, and hydroxyl). The ease of tailoring them for more specific microbial targeting has made them suitable for use as antimicrobial agents [13]. Polysaccharides (like chitosan and alginate) exhibit inherent bioactivity with good cytocompatibility, degradability, miscibility,

Table 1 Widely used polysaccharides, their classification, composition, availability, and functions

Polysaccharides	Classifications	Components	Availability	Function
Glucose	Homo	Primary monosaccharide	Plants, algae	Energy
Heparin	Hetero	D-glucoronic acid, N-sulpho D-glucosamine, L-iduronic acid	Blood, mast cells	Anticoagulant
Glycogen	Homo	Glucose	Liver, muscles	Storage depot
Starch	Homo	Glucose	Grains, vegetables	Paper manufacturing, textiles
Hyaluroic acid	Hetero	D-glucoronic acid, N-acetyl D-glucosamine	Skin, connective tissue	Shock absorber, lubricant
Alginate	Homo	D-mannuronate	Algae	Wound dressings
Chitosan	Homo	L-glucoronate	Crustacean, mushrooms	Wound dressings, hemostatic
Dextran	Homo	ȁ-D-glucopyronosyl of sucrose	Bacteria	Nutrition, fermentation
Cellulose	Homo	Glucose	Basic structure in plant, vegetables, cells, wood,	Paper, plastics, explosives, photographic films
Pectin	Hetero	Homogalactoronan, Rhamnogalactoronun	Fruit extract, component of cell wall of many plants	Preparation of jam, jelly and flavoring agent
Chrondoitin sulfate	Hetero	D-glucoronic acid, N-acetyl D-galactosamine-O-Sulfate	Cartilage	Bone-cartilage formation, cartilage accumulation

antioxidant, antitumor, antiviral, and antimicrobial activity [14, 15]. Additionally, other polysaccharides upon surface modification have proved to be excellent antibacterial carriers for delivering drugs [15, 16].

This chapter discusses how polysaccharides are tuned for antimicrobial drug delivery applications, and provides in-depth knowledge about widely used polymers in surgical site wounds and orthopedic implant-associated applications (such as alginate, chitosan, carrageenan, dextran, guar gum, hyaluronic acid, cellulose, and pectin).

Overview of Polysaccharides as Biological Macromolecules

Carbohydrate-based polysaccharides are of paramount importance and have been eye-catching due to their contribution as drug delivery vehicles and their pivotal role in biomolecular recognition. The structure of polysaccharides can be linear or highly branched, having a general formula of $C_x(H_2O)_y$ wherein x can vary from 200

to 2500 [17]. Physicochemical properties of polysaccharides are manipulated using intermolecular H-bonding associations and chain conformations. Owing to the presence of abundant hydroxyl groups in the repeating units of polysaccharides, inter- and intramolecular H-bonding occur easily, which imparts insolubility after drying, one of the required properties for gel and film formation. Polysaccharides are mostly present in helical conformations in solution form, and their stability depends upon the ionic concentration and temperature of the solution [18].

Polysaccharides are considered to be the most abundant biological macromolecules present in nature. They are distributed widely in plants, algae, fungi, microorganisms, and animals [19]. These biological macromolecules play an important role in various physiological functions of life. Several decades ago, polysaccharides found their use in pharmaceuticals, foodstuff, biomaterials and biofuels, and now due to the growing interest and deeper investigations, it is being proved that the value of polysaccharides in several novel bio applications is vast [20]. Of their important medical applications (Fig. 1), antimicrobial, anticancer, antiaging, and antiviral, as well as their role in immunomodulator, antioxidant, and being hypoglycemic are some of the indispensable applications proven by polysaccharides in biomedical science [17, 21]. Among the class of polysaccharides, β-glucans have been clinically tested for antitumor activity [22], which is a polysaccharide extracted from *Ganoderma lucidum* [23] and lentinans [24]. Many naturally occurring polysaccharides have been reported to possess antiviral, particularly anti-herpes, anti-influenza, and anti-HIV activity [22]. Cellulose, hemicellulose, and lignin can stimulate bowel movement, aid in preventing diverticulosis and hemorrhoids.

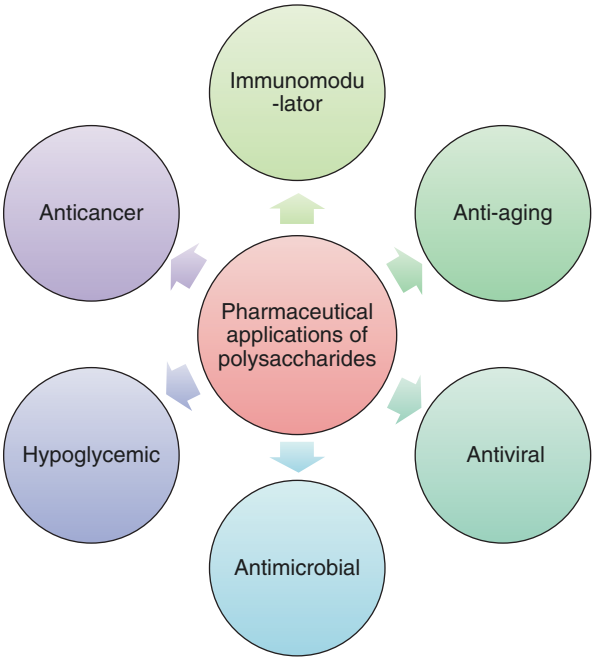


Fig. 1 Important medical applications of polysaccharides

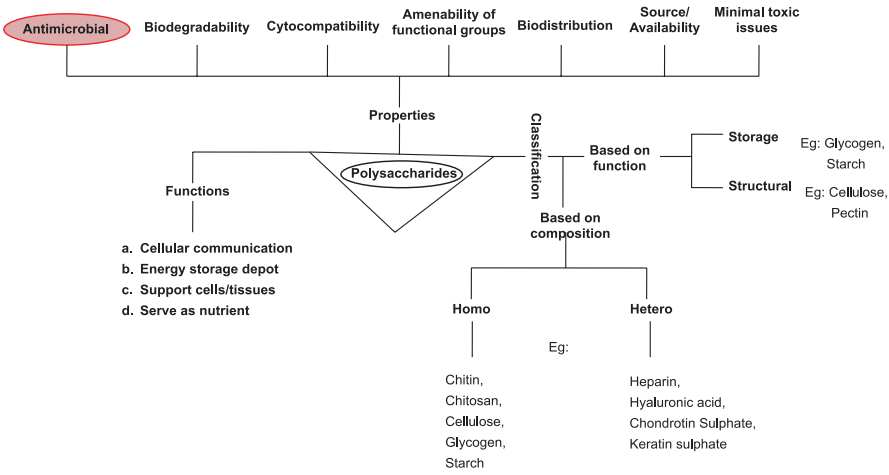


Fig. 2 Overview of polysaccharides as biological macromolecules

However, the use of cellulose materials is restricted due to the absence of enzymes in the human body for its degradation. Few amongst the most commonly used polysaccharides are chitosan, alginate, starch, gelatin, cellulose, pectin, and dextran. According to study reports, it could be a combination of polysaccharides, that produces a desirable impact in biological applications rather than a single polysaccharide [18]. Hence, a blend of two or more polysaccharides has been prepared to develop biomaterials having necessary properties. Likewise, polysaccharides are also manipulated to blend with synthetic polymers. The optimization/tailoring of synthetic polymers is often completed to improve its suitability to find uses in biomedical applications. An overview of the use of polysaccharides as novel biological macromolecules is represented in Fig. 2.

Polysaccharides as Drug Delivery Vehicles

Drug delivery or delivering drugs is defined as the distribution of therapeutically active molecules with certain approaches, formulations, and technologies inside the body to achieve a required therapeutic response with improved safety profiles. Fast changing trends in the global market scenario has contributed toward challenges in product development and technology, for the potential growth of pharmaceutical industries. However, in the current practice, bio-based materials have gained tremendous attention to be engineered as modified drug delivery vehicles [25]. Polysaccharides are of special interest, due to their unique properties such as stability, easy availability, non-toxicity, etc. and the ease of tailoring their end functional groups has allowed them to be a suitable candidate for drug delivery [26]. Moreover, the merit is in the customization or modification of polysaccharides chemically and

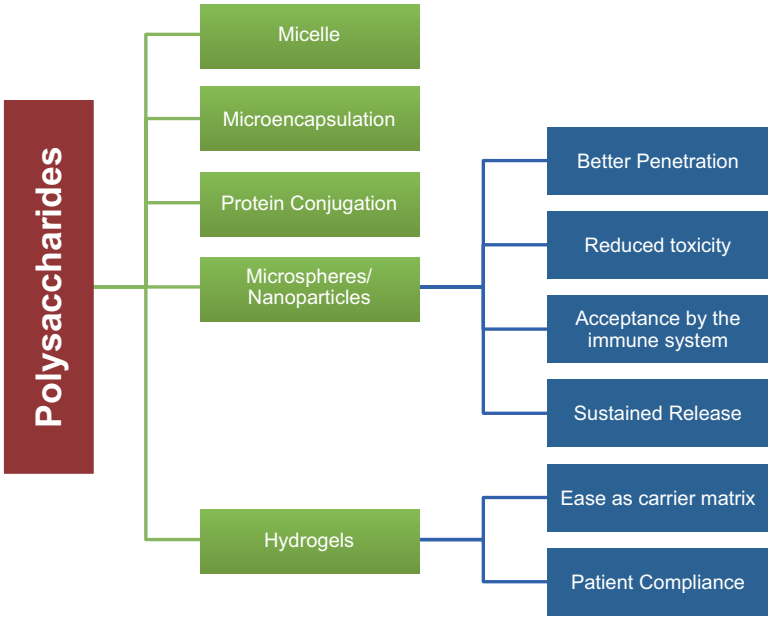


Fig. 3 The way in which polysaccharides can be modified to find use in biomedical applications

biochemically marking them as an appropriate carrier in the field of drug delivery. The advantageous properties of polysaccharides allow their use in drug delivery especially to target organs/tissues with different delivery routes and with variable release profiles [27–29].

Figure 3 explains the way in which polysaccharides have been modified to find multiple uses in biomedical applications. Polysaccharides can be formulated as nanoparticles, microparticles, monoliths, hydrogels, sponges, and beads to incorporate drugs. In drug delivery systems, drug loading is an important parameter when concerned with pharmaceutical formulations, wherein they are largely correlated with a matrix structure, surface area, and porosity of the polysaccharides [30]. Tuning the surface modification of polysaccharides also plays a pivotal role in the extent of bioavailability and the release profile of the entrapped drug. This chapter briefly explains the possible ways polysaccharides are used in the field of drug delivery mainly as antimicrobials.

Polysaccharides as Antimicrobial Agents

The consistent growth of polysaccharides into different branches of science has been thoroughly established due to their unique properties as discussed in the introduction. In general, factors like biodegradability, cytocompatibility, biodistribution, modification of functional groups, and minimal side effects prove the effectiveness of polysaccharides (natural as well as synthetic proficient) in drug delivery [31, 32].

According to the Structural Activity Relationship (SAR), functional groups present in some polysaccharides have an innate antibacterial ability capable of being used in biomedical applications. Khemakhem et al. reported the antibacterial activity of polysaccharides that were extracted from olives [33]. A study conducted by Anitha et al. on leaf extracts of *Citrus grandis* provided strong evidence that the composition of the plant was polysaccharide and had reactive functional groups like amine, amide, aromatic alcohol, alkane, alcohol, esters, phenol, and nitro compounds primarily responsible for its antimicrobial activity. The work led by Sehei et al. clearly pointed out the role of the carboxylic groups in polysaccharides showing antibacterial activity. However, the extent of killing bacteria depended upon the virulent strains and further redecorating the group to strengthen antibacterial activity. The study also detailed out the modification of carboxylic groups into amides and esters to increase antibacterial activity [34]. Therefore, in short, the major functional groups responsible for antimicrobial activity are C-O, C=C, -C-H, N-O, C-H, O-H, N-H, =C-H, and C=O [35]. Tuning the activity of functional groups may result in much more potential antimicrobial activity. Amongst all natural polysaccharides, chitosan has an inherent broad-spectrum antimicrobial activity and, thus, it has also been widely used as an antimicrobial delivery vehicle.

Polysaccharide-Based Antimicrobial Delivery Vehicles

Table 2 summarizes the key findings of each polysaccharide-based antimicrobial drug delivery vehicles.

Table 2 Key findings of polysaccharides

Polysaccharides	Type	Antimicrobial delivery vehicles	Most targeted bacteria	Key findings
Chitosan	Cationic polysaccharide	Nanoparticle Microparticle Coatings Films Sponges Hydrogels	*Staphylococcus epidermidis, Staphylococcus aureus, Salmonella* sp.	Inherent antibacterial property. Inhibits Gram-positive bacteria selectively inhibiting *Staphylococcus aureus* via inhibiting RNA and protein synthesis
Alginate	Hydrophilic linear polysaccharide	Sponges Hydrogels Nanofibers Nanoparticle Microparticle Beads	*Staphylococcus aureus, Micrococcus, Escherichia coli, Bacillus subtillis, Propionibacterium acne, Escherichia faecalis, Escherichia cloaceae*	Excellent antibacterial carrier and drug delivery vehicle. Strong gelling property helps in achieving uniform bonding with antibacterial molecules

(continued)

Table 2 (continued)

Polysaccharides	Type	Antimicrobial delivery vehicles	Most targeted bacteria	Key findings
Carrageenan	High molecular weight polysaccharide	Nanoparticle Microparticle	*Staphylococcus aureus Bacillus cereus Escherichia colistrains*	Available in three fractions. Among which Kappa carrageenan is widely used as antimicrobial drug delivery vehicle due to its ideal properties in combining with antimicrobial formulations
Pectin	Highly branched polysaccharide	Nanoparticle Microparticle	*Escherichia coli, Staphylococcus epidermidis, Helicobacter pylori*	Pectin is easily amenable to form three-dimensional network in enhancing antibacterial activity with other carrier molecules
Dextran	Complex polysaccharide of glucan	Nanoparticle Microparticle Hydrogel	*Staphylococcus aureus, Bacillus cereus, Listeria monocytogene, Bacillus luteus, Klebsiela pnemoniae, Pseudomonas aeruginosa Escherichia coli*	Dextran serves to be a good organic carrier matrix in combination with organic and inorganic materials
Guar gum	Hydrocolloid	Nanoparticle Microparticle Hydrogel	*Staphylococcus aureus Escherichia coli*	Gel formation property with minimal toxicity makes guar gum suitable to get mixed with antimicrobial compounds
Hyaluronic acid	Non-sulfated glycosamino glycan	Nanoparticle Microparticle Hydrogel Scaffold	*Staphylococcus epidermidis, Staphylococcus aureus Pseudomonas aeruginosa*	No direct influence in inhibiting bacteria. But provokes responses for promoting wound healing and helps in reducing prolonged inflammation cascade and matrix stabilization
Cellulose	Linear polysaccharide	Nanoparticle Microparticle Hydrogel	*Staphylococcus aureus Escherichia coli Salmonella cholerasuis*	Widely distributed polysaccharide. Applications of cellulose are never limited for antimicrobial carrier, since it also serves as an excellent molecule in enhancing bone responses and mineralization

Chitosan

Chitosan is a naturally occurring polysaccharide obtained by the alkaline deacetylation of chitin, which is present in the exoskeleton of insects, crustaceans, and fungal cell walls. Chitosan is regarded as the second most abundant polysaccharide present after cellulose. It is a copolymer of 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-N-acetyl-D-glucopyranose units linked via a β-1,4-linkage. Chitosan is not soluble in neutral or alkaline pH and hence is strictly insoluble in water. However, in acidic conditions (pH < 6), free amino groups present in the chitosan molecules get protonated to dissolve chitosan. The solubility of chitosan depends upon N-acetyl groups and distribution of the free amino groups present [36]. The polymer is completely soluble in dilute acids like acetic acid, malic acid, lactic acid, and formic acid [37]. Generally, the viscosity of chitosan increases with increases in chitosan concentration. Owing to its polycationic nature, chitosan is very active and can easily react with an anionic polysaccharide, proteins, fatty acids, and phospholipids. For many years, chitosan has been extensively used as a biomaterial for various applications in the biomedical field due to its unique properties like biocompatibility and biodegradability. It has also exhibited excellent hemostasis and tissue regeneration properties to find use as a wound dressing material. HemCon™, Axiostat®, Tegaderm®, etc. are chitosan-based wound dressings, are the FDA approved, and are commercially available in the market [38].

Chitosan has been extensively used as a drug delivery carrier and numerous articles have been published since the 1990s on its use highlighting that interest is still high in chitosan as a biomaterial [39]. The main merits of this polysaccharide are properties like (a) non-toxicity, (b) cost-effectiveness, (c) organic solvents not required for solubilization, (d) polycationic nature for ease of chemical tailoring and, finally, (e) carrier matrix ability for delivery systems such as films, sponges, hydrogels, etc. Chitosan has limited applications for the delivery of hydrophobic drugs as a result of its insoluble nature in organic solvents, which gave rise to various derivatives of chitosan [40]. Drugs can be directly mixed into viscous solutions of chitosan or can be conjugated via a hydrolyzable bond to a chitosan backbone and further formulated into different delivery vehicles [41]. Chitosan binds easily to proteins, DNA, and RNA that can be useful for the prevention and treatment of infections using vaccines or gene therapy.

Chitosan Nanoparticles

Chitosan nanoparticles (CNPs) demonstrate better antibacterial potential than chitosan, which is attributed to the polycationic nature of CNPs having a greater surface area to interact with bacterial cell walls compared to pure chitosan [42]. In the previous report, CNPs loaded with different antibiotics have been developed as a delivery carrier. Results demonstrated that the antibiotic-loaded CNPs inhibit and destroy the growth of both Gram-positive and Gram-negative bacteria [43]. A study performed by Madureira et al. found that the bare CNPs prepared by an ionic gelation method possessed antimicrobial activity [44].

Elbi et al. developed fucoidan-coated ciprofloxacin-loaded CNPs for the treatment of intracellular and biofilm infections of *Salmonella*. It was observed that the fucoidan-coated CNPs exhibited anti-*Salmonella* activity twofolds higher than CNPs and sixfolds higher than ciprofloxacin alone [45]. Piras et al. developed antimicrobial peptide temporin B loaded CNPs for long-term antibacterial activity against clinical isolates of *Staphylococcus epidermidis* [46]. Recently, CNPs have been explored as effective inhibitors of multidrug-resistant skin microorganisms with an average minimum inhibitory concentration (MIC) of 1.5 mg/mL [47]. It was observed that the integration of lysozymes into the CNPs improved antibacterial performance possibly due to the ability of nanoparticles to penetrate the cell membrane, enzyme activities and interference with bacterial metabolism [48].

Chitosan Microparticles

Chitosan microparticles prepared by an ionic crosslinking method employ strong antibacterial activity against various microorganisms via binding to the bacteria outer membrane protein A and lipopolysaccharide [49]. Chitosan microparticles exerted broad-spectrum antimicrobial activity against antibiotic-resistant microorganisms [50]. Jeon et al. reported the application of chitosan microparticles for the treatment of metritis and provided promising evidence for the use of chitosan microparticles as an antimicrobial agent for controlling the growth of pathogens [51]. Shen et al. developed carboxylated chitosan/silver hydroxyapatite hybrid microparticles prepared via a simple gas diffusion method. Excellent antimicrobial activity of hybrid microspheres against *Staphylococcus aureus* could be attributed to the synergistic effect of silver ions and carboxylate chitosan [52]. Cefepime loaded O-carboxymethyl chitosan microspheres with sustained bactericidal activity and enhanced biocompatibility was previously reported [53]. The dual delivery of growth factors and antibiotics from chitosan microparticles was reported for antibacterial activity against *Staphylococcus aureus* and promoting osteoblast proliferation. Significant antibacterial activity was observed along with remarkable proliferation of osteoblasts in the presence of cefazolin (50–100 μg/mL) and BMP 7 as compared to BMP 7 alone, which indicated that the cefazolin might play a role in the proliferation of osteoblasts [54]. Curcumin-conjugated chitosan microparticles showed good anti-inflammatory, antioxidant, and antibacterial activity [55]. Chitosan–alginate microspheres prepared by Ca^{2+} ionic crosslinking method demonstrated greater antibacterial and antibiofilm activity against multidrug-resistant microbial pathogens [56].

Chitosan Coatings

A viscous solution of chitosan was obtained by dissolving chitosan in an acidic solution. Using ionic or polyelectrolyte complexes can enhance the bioadhesive property of chitosan. Due to the polycationic nature of chitosan, it can readily react

with negatively charged mucins, which are present on/in mucosal tissues. Thus, a drug-loaded chitosan solution or chitosan-coated implant enhances the in vivo residence time in the target tissues and ultimately helps to increase bioavailability. Abdelbary et al. prepared chitosan-coated liposomes loaded with ciprofloxacin hydrochloride via a thin film hydration method for ocular delivery. Mucoadhesive chitosan-coated liposomes demonstrated improved antibiotic retention, in vitro/in vivo antibiotic elution and physicochemical stability [57]. Norowski et al. proved the efficacy of tetracycline-loaded chitosan-coated titanium implants against pathogenic bacteria responsible for implant-associated infections for almost 7 days. Additionally, coated implants demonstrated a slight inflammatory response similar to uncoated implants, when tested using a rodent muscle pouch model. However, the coated implant did not exhibit any cytotoxic effects on human fibroblasts and osteoblast cells [58].

Chitosan Films

Chitosan films have also provided to be a good platform for drug delivery because they can be easily applied over the surgical sites or wound surface due to its flexible nature. Noel et al. demonstrated that amikacin- and daptomycin-loaded chitosan films have shown excellent antibacterial activity against *Staphylococcus aureus* for almost 72 h [59]. Further, Smith et al. evaluated the ability of daptomycin/vancomycin-loaded chitosan films prepared using chitosan with 61, 71, and 80% degree of deacetylation (DDA) to prevent or lessen musculoskeletal fixation device-related infections. The results indicated that the chitosan films with 80% DDA had a great potential to prevent *Staphylococcus aureus*-mediated musculoskeletal infections [60].

Chitosan Sponges

Chitosan sponges possess an excellent ability to provide a higher release of antibiotics above the MIC for a longer period of time and increased loading efficiency owing to their porous network. Chitosan sponges can be easily loaded with antibiotic drugs simply by dissolving them in a chitosan solution. Previously, antibiotic-loaded chitosan sponges have been employed as a sustained release system for wound healing in dental surgery [61]. Gentamycin containing chitosan bars have been developed for the treatment of bone infections [62]. Noel et al. investigated the drug releasing chitosan sponge for the prevention of orthopedic and musculoskeletal infections. Chitosan sponges prepared by lyophilization were dipped into a 10 mL of an antibiotic solution containing 5 mg/mL of vancomycin and amikacin each. The release of vancomycin (40 μg/mL) and amikacin (13 μg/mL) showed that these sponges have potential clinical applications for the prevention of early-stage infections in small surgeries [63]. Chitosan acetate sponges commercialized as HemCon™ burn dressings incorporated with silver

nanoparticles (AgNPs) [64] and a bilayer chitosan wound dressing loaded with silver sulfadiazine [65] showed synergistic bacterial inhibition activity in burns and wound infections. *Phaechamud* and *Charoenteeraboon* developed doxycycline-loaded glutaraldehyde cross-linked/non-cross-linked chitosan sponges and evaluated their antibacterial activity [66]. They observed that the non-cross-linked sponges showed a slower release of drugs as compared to cross-linked sponges because the former could form a gel network, which might have prevented drug diffusion. Pawar et al. developed cefuroxime- and ciprofloxacin-loaded chitosan sponges for the prophylaxis and treatment of orthopedic implant-associated infections. Results showed that the cefuroxime and ciprofloxacin chitosan sponges provided sustainable antibacterial activity against *Staphylococcus aureus* for 25 and 13 days, respectively [67].

Chitosan Hydrogels

Hydrogels are physical or chemical cross-linked polymer networks that contain high hydrophilic groups or domains. Hydrogels can be formulated into different shapes and sizes so that they can be easily applied into any irregular shape wounds and defects. The release of drugs from the hydrogel matrix as a function of time is categorized as swelling-controlled, diffusion-controlled, and chemically controlled mechanisms. However, the primary mechanism for regulating therapeutic drug release is the diffusion of the drug from the hydrogel matrix [68]. Injectability, rapid clearance, and degradation behavior of chitosan hydrogels makes them an excellent local delivery carrier for various biomedical applications [69]. Wu et al. have developed a gentamycin-loaded chitosan/carboxy methylcellulose (CMC) hydrogel cross-linked with genipin. It was observed that genipin concentrations played an important role in the release profile and provided adequate antibacterial efficacy with good osteoblastic cell responses [70]. In the previous report published by Chen et al., the hydrogel was formed by mixing chitosan and hydroxyl propyl methyl cellulose (HPMC) for the targeted delivery of photodynamic inactivator toluidine blue into *Staphylococcus aureus* biofilms [71]. A composite complex containing chitosan, CMC, and magnetic iron oxide showed the controlled release of different antibiotics with minimum cell toxicity [72].

Alginate

Alginate is a hydrophilic linear polysaccharide, which is widely used in the biomedical field for various applications. Alginate is a salt of alginic acid commonly seen in brown algae. Alginate is produced on a large scale by two methods, the alginic acid method and calcium alginate method. The presence of phycocolloids in the thalus serves as the integral material in providing strength and resilience to the algal component. This causes the accumulation of divalent ions aiding in gel

formation [73]. Properties like solubility, degradability, stability and sterilization, and biological parameters like immunogenicity, compatibility, and non-toxicity has allowed alginate to find uses in numerous biomedical applications [74]. It has strong gelling properties in the presence of Ca^{2+} ions, thus, widely being used in drug delivery and controlled release applications. Alginate-based antibacterial formulations and studies that have been conducted are discussed below.

Alginate Sponges/Hydrogels

Alginate dressings constitute cellulose fibers obtained from seaweed. An ideal wound dressing material should have high absorbance with minimum adhesion and be nonadherent [75]. It should also be readily available with hydrophilicity properties, causing no hypersensitive reactions. Alginate has the aforementioned properties and is widely used as a wound dressing materials [76, 77]. Moreover, alginate being bacteriostatic, nonallergenic, hemostatic, hydrophilic, highly absorbable, and biocompatible contributes significantly to biomaterials used as dressings to resist bacterial infections [78]. The ability to absorb liquid exudates and transform them into viscous gum makes alginate an appropriate candidate to be used in wound dressings and as an immobilizing vehicle for drug delivery, improving antibacterial properties. Research has unequivocally proven that alginate-based formulations help reduce the wound bed bioburden by reducing microbial invasion [79]. A review article by Stephan et al. elucidated that the combination of alginate–silver was clinically relevant from older times and was effective for the treatment of "at risk" wound infections. The author explained that once a lesion was created on the skin surface, the microbes could easily gain entry and remain in a quiescent stage and upon sensing a favorable environment, they could start to multiply and cause life intimidating sepsis, paving the way to fatalities. Therefore, a combination of alginate–silver on the skin impeded the indocile behavior of microorganisms and destroyed them from causing infections and associated complications [80]. The review performed in the year 2018 by Deborah et al. demonstrated that the alginate-based hydrogels meant for wound applications could hinder bacterial colony formation and enhance faster healing [81]. A general comparison study conducted by Weigand et al. using alginate wound dressing, pure alginate, and alginate containing silver revealed the improved binding of pure alginate to elastase, minimizing free radical production and pro-inflammatory cytokines. The results also suggested that alginate was useful as a basic wound dressing material in the management of exudating wounds capable of hindering microbial growth and in being clinically relevant [82].

Alginate Nanofibers

A recent study conducted by Rafiq et al. developed nanofibers containing sodium alginate-poly(vinyl alcohol) (SA-PVA) and encapsulated essential oils via electro spinning for desirable antibacterial properties [83]. The main intention of the study

was to replace antibiotics with essential oils (cinnamon, clove, and lavender: 0.5, 1, 1.5 %). Essential oils are known to possess excellent antibacterial properties, where limitations are faced in its validation. As the study proved, cinnamon oil was the best combination with SA-PVA nanofibers for antibacterial applications. A study performed by Kokkarachedu et al. reported that the synthesis of nano zinc oxide alginate antibacterial cellulose fibers had the potential to destroy *Escherichia coli*. The study revealed that sodium alginate was an excellent carrier for biomedical applications, and also the findings stated that there was a significant influence on varying the concentration of sodium alginate during fiber synthesis on inhibiting bacteria multiplication [84].

Alginate Micro/Nanoparticles

Alginate is the most commonly used polymer for preparing microparticles. Alginate nanoparticles are not common due to the formation of aggregates or difficulty in tailoring them to the nano level [85]. Alginate is combined with silver or chitosan to serve as antibacterial nanoparticles [86]. A study conducted by Trandafilovic et al. described the use of alginate for providing a controlled platform for synthesizing zinc oxide nanoparticles (ZnO NP) against *Escherichia coli* and *Staphylococcus aureus*. The authors substantiated the importance of alginate in the field of drug delivery, tissue engineering, and other biomedical applications as a carrier by narrating properties like an affinity toward divalent metal ions and the reaction of alginate toward metals [87]. The study conducted in 2016 developed a sodium alginate stabilized silver/mesoporous silica nanocomposite system to destroy Gram-positive and Gram-negative bacteria. Here, the author implemented a green way for nanocomposite preparation. Mesoporous silica was capped onto AgNPs. Sodium alginate was used in the study to stabilize and enhance the biocompatibility of the composite system [88]. The author Adam et al. developed chitosan–alginate nanoparticles against the treatment of bacteria *Propionibacterium acnes*. After benzoyl peroxide encapsulation in the chitosan–alginate nanoparticles, *Propionibacterium acnes* was inhibited and the nanoparticles exhibited anti-inflammatory property causing reduced toxic effects to eukaryotic cells [89]. A study conducted by Jianhua et al. described the synthesis of Ɛ-polylysine encapsulated chitosan–alginate nanoparticles for antibacterial activity. The formulation demonstrated enhanced inhibition of bacteria when tested against *Staphylococcus aureus, Micrococcus, Escherichia coli, and Bacillus subtillis*. Alginate in the formulation extensively absorbed moisture from the environment and proved to serve as a barrier for bacterial entry. Interestingly, the study concluded that Ɛ-polylysine nanoparticles resulted in a threefold bacterial inhibition over the free drug [90]. The study conducted by Joana et al. included ocular delivery of daptomycin-utilized chitosan-coated alginate nanoparticles. The study was conducted to inhibit methicillin-resistant *Staphylococcus aureus* (MRSA). The formulation was prepared by an ionotropic pre-gelation method in alginate followed by polyelectrolyte chitosan complex formation reactions. The formulation was effective in treating endophthalmitis where the alginate core provided a moist ocular bed and the antibiotic was powerful in destroying the bacteria [91].

Alginate Beads

In recent studies, it was seen that alginate beads served as an inert nonallergic carrier for tetracycline delivery. The studies promise the use of alginate beads in biomedical applications due to its enhanced compatibility and human compliance [92]. Here, the author developed alginate beads by dropping calcium chloride and immobilizing tetracycline into the beads for sustained antibacterial activity. The results provided evidence of the active disintegration of Gram-positive and Gram-negative bacteria by inhibiting their protein synthesis. Therefore, the authors suggested future prospects for beads to be used in open wounds, hospital room premises, and surgical drapes for enhanced patient compliance. In another study conducted by Selda et al. amoxicillin was covalently immobilized to alginate. The results proved that the amoxicillin-immobilized alginate actively inhibited cell wall synthesis in *Staphylococcus aureus* and *Escherichia coli* species [93]. Another study conducted by Hebeish et al. emphasized surface modification using nanocomposite coatings incorporated with silver composites, and proved that alginate had minimal toxicity and could be an excellent carrier for sustained drug delivery avoiding dosing frequency, whereas silver actively disintegrated and killed bacteria to a great extent [94]. The recent study reported by Deepathomas et al. employed zinc/alginate beads as a carrier matrix for the controlled delivery of rifampicin and it was found that encapsulation efficiency improved as the polymer quantity for bead preparation improved, and the beads exhibited good antibacterial properties with good compatibility toward eukaryotic cells [95].

Alginate Composite Gel System

Alginate has found uses in tissue engineering hydrogels exhibiting antibacterial properties owing to its smooth and moist bed properties improving cell loading efficiency [96]. A study conducted for *anti-staphylococcal* activity in 2017 revealed the use of alginate as an immobilizing matrix as it promoted quicker wound re-epithelization and helped absorbing wound exudates and preventing cross infection [97]. The authors prepared sodium alginate-polyvinyl alcohol (SA-PVA) hydrogels encapsulating vancomycin coated with polyelectrolytes and vitamin C. The formulation was found to exhibit extended antibiotic release over time, and effectively disrupted *Staphylococcus aureus.* The study was performed in 2010 for assessing the in vitro antibacterial efficacy of sodium alginate and Na-CMC as a carrier hydrogel matrix for gatifloxacin. The study proved that, with an increase in sodium alginate concentration, more encapsulation and greater antibacterial effects were seen against *Staphylococcus aureus* and *Escherichia coli*. The alginate used in the study was used to enhance the mucoadhesive force, and the antibiotic effectively reduced the total bacterial count [98]. In our lab, we developed CNPs and povidone iodine loaded in in situ alginate composite hydrogels for prophylaxis and the treatment of orthopedic implant-associated infections, which was found to be a promising candidate for preclinical and clinical applications [28]. A study conducted by Shilpa et al. developed AgNPs through an ecofriendly approach involving sodium alginate and chitosan composite films.

The author described the use of natural polymer alginates to serve as a stabilizer and reducing agent for metallic nanoparticles and their suitability for antibacterial applications [99].

Carrageenan

Carrageenans are high molecular weight polysaccharides, which have found immense applications in the biomedicine and food industry [100]. Carrageenan is mainly available in three fractions out of which kappa carrageenan is widely used in antimicrobial studies due to its cytocompatibility, degradability, mechanical strength, hydrophilicity, and gelling properties. Another study conducted by Swarup et al. prepared carrageenan-based ZnO NP for improved antibacterial properties. The results confirmed that the combination of carrageenan/ZnO NP showed a good antibacterial effect with ample thermal stability, good mechanical strength, and water vapor barrier properties [101]. A study conducted by Shojaee et al. confirmed the antibacterial film forming ability of kappa carrageenan incorporated with zataria multiflora boiss (ZEO) and metha pulegium (MEO) essential oil [102]. Though both essential oils have the capacity to destroy bacteria, ZEO-incorporated carrageenan films were found to be more potent antibacterial films. The formulation was found to disrupt *Staphylococcus aureus* followed by *Bacillus cereus* and later *Escherichia coli* strains. A study conducted by Annabella et al. revealed the optimal elasticity, smooth morphology to absorb exudates, adhesiveness, and excellent mechanical strength of carrageenan. Multilayer assemblies of polyethyleneimine and carrageenan exhibited a synergistic effect against pathogenic bacteria. The results exhibited contact killing of *Staphylococcus aureus, Escherichia cloacae,* and *Escherichia faecalis* [103]. A study conducted by Fawal et al. also proved the contact killing effect of bacteria. Carrageenan films were plasticized with glycerol and encapsulated with citric acid. Upon contact with citric acid, more bacteria died. The reason might be due to the unfavorable acidic content. The results proved the inhibition of *Staphylococcus aureus, Proteus mirabilus, Pseudomonas aeruginosa, Escherichia coli,* and *Dickeya chrysanthami* strains indicating the antibacterial potency of the formulation [104].

Pectin

Pectin is a natural polysaccharide obtained from various fruit extracts using enzymatic or catalytic methods. Pectins are widely present as a constituent of the cell wall of many plants. Pectin is a highly branched polysaccharide macromolecule, consisting of at least three domains: (a) Homogalactoronan, (b) Rhamnogalactoronun I, and (c) Rhamnogalactoronun II. Homogalactoronan is the major component of the pectin polysaccharide, which is basically composed of chains of D-galacturonic acid units linked by α (1-4) glycosidic linkages that can be methyl esterified (some extent <10%) and in some cases partially acetyl esterified. A highly concentrated

solution of pectin can be easily formulated into a flexible, three-dimensional hydrogel network, which is widely used for biomedical applications. A water-insoluble pectin gel could be obtained by using divalent or trivalent cations that can swell in an aqueous medium but do not dissolve.

Ciprofloxacin hydrochloride-loaded pectin microspheres have been developed for the treatment of osteomyelitis. The microspheres were prepared using the spray drying method, which exhibited a release of ciprofloxacin for 48 h. In vivo results demonstrated that the biodegradable pectin microspheres were able to maintain aseptic conditions at the site without impeding new bone formation [105]. Pallavicini et al. prepared AgNPs with pectin (P-AgNPs) wherein pectin acted as a reductant and coating agent. It was observed that P-AgNPs demonstrated excellent antibacterial and antibiofilm action at a lower Ag^+ ion release rate against *Escherichia coli* and *Staphylococcus epidermidis*, as compared to ionic silver. In addition, P-AgNPs were able to promote fibroblast proliferation and, thus, it could be a potential medication for wound healing as well as for effective prophylaxis of implant-associated surgical site infections [106]. In a previous report published by Martinez et al., pectin-polyvinyl alcohol (P-PVA) cryogel patches were developed as a controlled release system for enrofloxacin and keratinase enzyme for antimicrobial treatment in wounds and scars. In this report, pectin with a different degree of esterification (71%, 62%, 55%, and 33%) and three concentrations (0.50%, 0.75% and 1% w/v) were tested to optimize enrofloxacin and keratinase release. Results suggested that the PVA cryogel containing pectin at a 0.50% w/v concentration and 55% degree of esterification exhibited the highest release of keratinase [107]. Finally, it was observed that the controlled release of enrofloxacin and keratinase could be modified by tailoring the amount and concentration of pectin with different degrees of acetylation in the PVA cryogel patches developed for antimicrobial treatment. The levofloxacin-loaded silver phosphate (Ag_3PO_4)-pectin microspheres could be used as an effective antimicrobial agent for medical applications against *Escherichia coli* and *Staphylococcus aureus* [108]. Silva et al. developed amoxicillin-loaded covalent TiO_2-co-pectin microspheres containing Fe_3O_4 nanoparticles for the treatment of *Helicobacter pylori* associated ulcers. The nanostructured pectin microspheres showed great pharmacological potential [109]. In one of the previous report, a novel bioactive zinc cross-linked pectin–sodium alginate based film was prepared for antimicrobial activity particularly for disinfection of medical devices [110]. Therefore, it could be concluded that the biopolymer pectin played a significant role as an active antibacterial carrier molecule in vivid formulations for hindering and destroying bacteria colonization.

Dextran

Dextran is a complex glucan synthesized via polymerization of α-D–glucopyronosyl of sucrose catalyzed by the dextransucrase enzyme. Dextran generally shows a negative effect on thrombocyte aggregation and coagulation factors [111]. Therefore, dextran is commonly used as an adjuvant and not used in higher concentrations for

formulations. Though dextran does not have any direct influence on antibacterial and osteoinductive properties, it serves as an excellent carrier matrix in combination with other organic and inorganic materials [112]. Research conducted by Yang et al. revealed the use of dextran as a capping agent in the preparation of antibacterial AgNPs. Results confirmed the equal distribution of the size and shape of AgNPs due to dextran capping. The formed nanoparticles were highly potent and destroyed Gram-positive and Gram-negative bacteria (*Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*), with minimal toxic reactions when tested on mouse fibrosarcoma cells [113]. Studies also reported the use of dextran as an immobilizing matrix hydrogel for enhancing antibacterial activity. The study performed by Jiaul et al. synthesized formulations to destroy biofilms, which are the reason for the most debilitating disorders. The study performed utilized biocides incorporated with dextran methacrylate hydrogels. The results suggested that the direct loading of a biocide in the hydrogel formulation had >99.99% annihilating effect on *Staphylococcus aureus, Escherichia coli,* and MRSA biofilm formation. The study suggested the formulation as a potential candidate especially for topical infections [114].

Felicetta et al. developed a dextran hydrogel loaded with gentamicin. The result showed that the high antibacterial efficacy lasted, up to 24 days maintained the ideal hydrogel properties, and was capable of disrupting the already formed bacterial biofilm. The result also proved that the formulation was more potent than the pure gentamicin sulfate [115]. The study performed by Hogue et al. was for the development of a dextran methacrylate hydrogel with biocide loading to destroy biofilm formation. Apart from biofilms, the formulation was 100% efficacious to MRSA, and its activity was maintained for up to 5 days. Besides antibacterial applications dextran-based hydrogels also proved suitability for use in enhanced cellular growth, differentiation, and proliferation [116]. A study conducted by Nina et al. illustrated the application of dextran in wound dressings and skin tissue engineering applications. The nanofibers were synthesized using an electrospinning technique involving three polymers: polycaprolactone, cellulose acetate, and dextran incorporating tetracycline hydrochloride into the fibers. The author highlighted that the prepared fibers improved adhesion and proliferation of cells and exhibited sustained antibacterial drug release with good antimicrobial activity against Gram-positive and Gram-negative bacteria [117]. Another interesting study conducted by Maggie et al. described the use of dextran aldehyde in the form of a hydrogel in preventing bacterial adhesion and further limiting their growth after surgical procedures. The study aimed to compare antibacterial activity, biocompatibility and wound healing capacity of the hydrogel. The findings of the study concluded that the wound closure after a period of 72 h upon the application of the formulation had ample biocompatibility and good antibacterial properties [118].

Author Milorad et al. have developed AgNPs stabilized with dextran sulfate involving a chemical reduction green synthetic method. The approach was put forth to utilize nontoxic, biodegradable polysaccharides for the reduction and stabilization of the prepared nanoparticles. The results obtained for the study were extremely convincing in that the dextran sulfate stabilized AgNPs exhibited strong antibacterial properties against *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes,*

Bacillus luteus, Klebsiela pnemoniae, Pseudomonas aeruginosa, and *Escherichia coli* [119]. A study conducted by Afeesh et al. reported the synthesis of scaffolds using polyurethane and dextran incorporated with ciprofloxacin. The results illustrated that the antibacterial drug ciprofloxacin was released in a controlled manner with the aid of dextran as a nanocarrier, where the cells were unaffected, and the scaffold possessed good bacterial inhibition properties [120].

Guar gum

Guar gum is a hydrocolloid, which has tremendous applications in medical science. The ability to form a thick paste without gel formation makes it unique for antibacterial applications with improved patient compliance [121]. A study conducted by Balbir et al. prepared guar gum/polyaniline/polyacrylic acid based interpenetrating hydrogels by a two-step polymerization process. The resulting gel was confirmed to be electrically conductive with antibacterial properties [122]. The study conducted by Reema et al. proved the antibacterial applications of guar gum in combination with acrylic acid incorporated polyaniline. The results confirmed the antibacterial properties of the hydrogel on *Staphylococcus* and *Escherichia coli* species [123]. A study conducted by Runa et al. modified guar gum intrinsically to a novel biopolymer for wound healing applications thereby obstructing bacterial entry. This evidence proved the promotion of wound closure with no trace of antibacterial entry. Further, it induced the proliferation and migration of cells at the scar tissue [124].

Hyaluronic Acid (HA)

Hyaluronic acid (HA) is a biopolysaccharide belonging to the class of non-sulfated glycosaminoglycans, with constant disaccharide seen mainly in connective and epithelial tissues [125]. HA is non-immunogenic, cytocompatible, biodegradable, angiogenic, and osteoconductive [126]. HA plays a pivotal role in the wound healing cascade [127]. HA enhances inflammation essential for promoting wound healing and later minimizes long-term inflammation and aids in stabilization of the matrix and therefore is regarded as a good carrier for antibacterial applications [128]. Though HA does not contribute directly to biocidal activity, it serves as an excellent carrier matrix for antibacterial activity [129]. One such study performed by Leyre et al. elucidated the development of layer-by-layer assembly of HA with chitosan onto poly(ethylene terephthalate). The findings concluded that the coating resulted in inhibiting bacterial adhesion. The selective layer approach enabled the long-term release of antibacterial components making it suitable for implementing in implant substrates [130]. The study reported by Andrea et al. explained in detail the influence of HA in annihilating bacteria. The study conducted on 15 ATCC strains revealed that HA was found to exhibit dose-dependent growth inhibition of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*,

Candida glabrata, *Candida parapsilosis*, and *Enterococci* [131]. The fast resorbable HA-based hydrogel when tested preclinically and clinically was found to be effective and safe for intraoperative use and can be easily spread onto direct implant sites for bacterial adhesion prevention [132]. A study conducted by Isbelle et al. modified HA to exhibit antibacterial characteristics by grafting with antimicrobial peptide (nisin) and formulating it in the form of hydrogels. The prepared antibacterial hydrogels when tested on *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* revealed antibacterial activity suggesting the effectiveness of the formulation for use as wound dressing materials, contact lenses, cosmetics and for other biomedical formulations [126].

Cellulose

Cellulose is a linear polysaccharide consisting of repeated glucose subunits and one of the most abundant polysaccharides on earth [133]. The major structural components in plants are made up of cellulose and are very suitable candidates for biomedical applications [134]. Some of the exciting characteristic features include bioavailability, degradability, low density, ease of reproducibility, enhanced chemical persistence, and thermal constancy making it suitable for several other multifarious applications too [135, 136]. Though cellulose has no role in possessing antibacterial activity, a facile approach in producing antimicrobial cellulose components is of much research interest [137, 138]. Considering these aspects, a study conducted by Kamyar et al. was successful and has concluded that cellulose could contribute to the area of drug delivery especially transdermal or wound dressing patches [139]. A study conducted by Afeesh et al. revealed the use of cellulose acetate together with zein and polyurethane for wound dressing applications. The author produced substantiating evidence for the use of a cellulose biopolymer in a study due to its hydrophilicity and good adsorption characteristics, which are considered to be the essential requisites of wound dressing preventing antimicrobial attack [140]. Incorporating a minimal amount of antibiotic streptomycin sulfate to the wound mat improved bactericidal activity with controlled release of the formulation improving patient compliance. Applications of cellulose are never limited for antimicrobial, since it also serves as an excellent molecule in enhancing bone responses and mineralization. A study conducted by Sa Liu et al. reported the importance of cellulose as a carrier material for destroying bacteria. The authors developed a bacterial cellulose/collagen and hydroxylpropyltrimethyl ammonium chloride chitosan mesh composite. The finding suggested growth impairment of *Staphylococcus aureus* and *Escherichia coli* proving mesh biocompatibility and antimicrobial ability [141]. A study performed by Susan et al. involved noncovalently combined cellulose to aid as a stabilizer in synthesizing ZnO-silver heterostructure nanoparticles. Antibacterial studies were evaluated using *Salmonella cholerasuis* and *Staphylococcus aureus* exhibited significant inhibition of bacterial growth [142]. A study performed by Mazhar et al. prepared a nanocomposite film of regenerated bacterial cellulose embedding ZnO NP into it. The results showed an

excellent bactericidal effect with reduced toxic reactions upon testing in vitro and favored cell adhesion [143]. A further study developed nanocellulose films consisting of phytogenic nano-bactericides of silver and found that cellulose, when fine-tuned and non-covalently bonded with metallic particles, could effectively target bacteria and the same was proved with the exotic species compendium of activities to protect the ecosystem (ESCAPE) communities who are at the largest risk of threats due the economical crisis.

Conclusion

Implant materials should be biocompatible, provide a favorable environment for bone tissue regeneration and should have the ability to prevent the adhesion and growth of microorganisms. Despite tremendous advances in the prophylaxis and the treatment of implant-associated infections, it remains a most devastating problem in orthopedics. Various antimicrobial delivery vehicles have been developed to encounter the bacteria present at the implant site. Polysaccharide-based antimicrobial delivery carriers are amongst the most novel approaches employed for the management of implant related infections. They are widely used in the biomedical field for drug delivery applications due to their advantageous properties such as non-toxicity, easy availability, biocompatibility, capability of tailoring their functionalities for improving antimicrobial properties, and biodegradability. They can be fine-tuned via chemical modifications, blending of two or more polymers, surface modification and conjugation with other polymers or the drug itself to develop controlled and sustained release antimicrobial delivery systems. Chitosan has inherent antibacterial activity. Thus, it is a promising candidate amongst polysaccharides for antimicrobial delivery due to potential synergistic activity with other antimicrobial agents. Considering the advantages of polysaccharide-based antimicrobial delivery vehicles, we should dedicate our research to develop a novel commercially available sustained release, effective and nontoxic delivery system for infection prophylaxis. In the next 10 years, we hope that new polysaccharide-based formulations will emerge to eradicate the bacteria present at various implant sites for a prolonged period and reduce chances of infection.

References

1. Suryavanshi AV, Borse V, Pawar V, Sindhu KR, Srivastava R (2016) Material advancements in bone-soft tissue fixation devices. Sci Adv Today 2:25236
2. Liu Y, Zheng Z, Zara JN, Hsu C, Soofer DE, Lee KS et al (2012) The antimicrobial and osteoinductive properties of silver nanoparticle/poly (dl-lactic-co-glycolic acid)-coated stainless steel. Biomaterials 33:8745–8756. https://doi.org/10.1016/j.biomaterials.2012.08.010
3. Riool M, De Boer L, Jaspers V, Loos CMVD, Wamel WB, Wu G et al (2014) Staphylococcus epidermidis originating from titanium implants infects surrounding tissue and immune cells. Acta Biomater 10:5202–5212. https://doi.org/10.1016/j.actbio.2014.08.012

4. Lu H, Liu Y, Guo J, Wu H, Wang J, Wu G (2016) Biomaterials with antibacterial and osteoinductive properties to repair infected bone defects. Int J Mol Sci 17:334–352. https://doi.org/10.3390/ijms17030334
5. Borse V, Pawar V, Shetty G, Mullaji A, Srivastava R (2016) Nanobiotechnology perspectives on prevention and treatment of ortho-paedic implant associated infection. Curr Drug Deliv 13:175–185. https://doi.org/10.2174/1567201812666150812141849
6. Biedenbach DJ, Moet GJ, Jones RN (2004) Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program (1997-2002). Diagn Microbiol Infect Dis 50:59–69. https://doi.org/10.1016/j.diagmicrobio.2004.05.003
7. Brusselaers N, Vogelaers D, Blot S (2011) The rising problem of antimicrobial resistance in the intensive care unit. Ann Intensive Care 1:47–54. https://doi.org/10.1186/2110-5820-1-47
8. Campoccia D, Montanaro L, Arciola CR (2013) A review of the clinical implications of anti-infective biomaterials and infection-resistant surfaces. Biomaterials 34:8018–8029. https://doi.org/10.1016/j.biomaterials.2013.07.048
9. Mohammadi M, Mousavi Shaegh SA, Alibolandi M, Ebrahimzadeh MH, Tamayol A, Jaafari MR et al (2018) Micro and nanotechnologies for bone regeneration: recent advances and emerging designs. J Control Release 274:35–55. https://doi.org/10.1016/j.jconrel.2018.01.032
10. Engelking L (2008) Polysaccharides and carbohydrate structure. In: Textbook of veterinary physiological chemistry, 3rd edn. Elsevier, Amsterdam, pp 270–279. https://doi.org/10.1111/j.1939-165x.2005.tb00053.x
11. Liu Z, Jiao Y, Wang Y, Zhou C, Zhang Z (2008) Polysaccharides-based nanoparticles as drug delivery systems. Adv Drug Deliv Rev 60:1650–1662. https://doi.org/10.1016/j.addr.2008.09.001
12. Bacic A, Fincher GB, Stone AB (2009) Chemistry, biochemistry, and biology of 1-3 Beta glucans and related polysaccharides, 1st edn. Elsevier, Amsterdam, pp 1–350. https://doi.org/10.1016/B978-0-12-373971-1.X0001-5
13. Gupta BS, Edwards JV (2009) Textile materials and structures for wound care products. In: Rajendran S (ed) Advanced textiles for wound care. Woodhead Publishing Series in Textiles, Boca Raton, New York, London, pp 48–96. https://doi.org/10.1533/9781845696306.1.48
14. Sebaaly C, Kassem S, Grishina E, Kanaan H, Sweidan A, Chmit MS et al (2014) Anticoagulant and antibacterial activities of polysaccharides of red algae Corallina collected from lebanese coast. J Appl Pharm Sci 4:30–37. https://doi.org/10.7324/JAPS.2014.40406
15. Thunyakipisal P, Saladyanant T, Hongprasong N, Pongsamart S, Apinhasmit W (2010) Antibacterial activity of polysaccharide gel extract from fruit rinds of durio zibethinus murr. against oral pathogenic bacteria. J Investig Clin Dent 89:74–75. https://doi.org/10.1111/j.2041-1626.2010.00017.x
16. Zhang N, Wardwell PR, Bader RA (2013) Polysaccharide-based micelles for drug delivery. Pharmaceutics 5:329–352. https://doi.org/10.3390/pharmaceutics5020329
17. Zheng Y, Bai L, Zhou Y, Tong R, Zeng M, Li X et al (2019) Polysaccharides from Chinese herbal medicine for anti-diabetes recent advances. Int J Biol Macromol 121:1240–1253. https://doi.org/10.1016/j.ijbiomac.2018.10.072
18. Wasupalli GK, Verma D (2018) Polysaccharides as biomaterials. In: Thomas S, Balakrishnan P, Sreekala MS (eds) Fundamental biomaterials: polymers. Woodhead Publishing Series in Biomaterials, London, pp 37–70. https://doi.org/10.1016/B978-0-08-102194-1.00003-7
19. Liu L, Li M, Yu M, Shen M, Wang Q, Yu Y et al (2019) Natural polysaccharides exhibit anti-tumor activity by targeting gut microbiota. Int J Biol Macromol 121:743–751. https://doi.org/10.1016/j.ijbiomac.2018.10.083
20. Liu J, Willför S, Xu C (2015) A review of bioactive plant polysaccharides: biological activities, functionalization, and biomedical applications. Bioact Carbohydr Diet Fibre 5:31–61. https://doi.org/10.1016/j.bcdf.2014.12.001
21. Han G, Wang F, Chen Q, Liu F, Shao X, Ling P (2017) Recent advances in polysaccharides for osteoarthritis therapy. Eur J Med Chem 139:926–935. https://doi.org/10.1016/j.ejmech.2017.08.048

22. Shi L (2016) Bioactivities, isolation and purification methods of polysaccharides from natural products: a review. Int J Biol Macromol 92:37–48. https://doi.org/10.1016/j.ijbiomac.2016.06.100
23. Lin Z, Zhang H (2004) Anti-tumor and immunoregulatory activities of Ganoderma lucidum and its possible mechanisms. Acta Pharmacol Sin 25:1387–1395
24. Zheng R, Jie S, Hanchuan D, Moucheng W (2005) Characterization and immunomodulating activities of polysaccharide from Lentinus edodes. Int Immunopharmacol 5:811–820. https://doi.org/10.1016/j.intimp.2004.11.011
25. García-González CA, Alnaief M, Smirnova I (2011) Polysaccharide-based aerogels—promising biodegradable carriers for drug delivery systems. Carbohydr Polym 86:1425–1438. https://doi.org/10.1016/j.carbpol.2011.06.066
26. Malafaya PB, Silva GA, Reis RL (2007) Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. Adv Drug Deliv Rev 59:207–233. https://doi.org/10.1016/j.addr.2007.03.012
27. Pawar V, Srivastava R (2016) Layered assembly of chitosan nanoparticles and alginate gel for management of post-surgical pain and infection. In: 16th Int. Conf. Nanotechnol. - IEEE NANO 2016, pp 241–244. https://doi.org/10.1109/NANO.2016.7751388
28. Pawar V, Topkar H, Srivastava R (2018) Chitosan nanoparticles and povidone iodine containing alginate gel for prevention and treatment of orthopedic implant associated infections. Int J Biol Macromol 115:1131–1141. https://doi.org/10.1016/j.ijbiomac.2018.04.166
29. Pawar V, Borse V, Thakkar R, Srivastava R (2018) Dual-purpose injectable doxorubicin conjugated alginate gel containing polycaprolactone microparticles for anti-cancer and anti-inflammatory therapy. Curr Drug Deliv 15:716–726. https://doi.org/10.2174/1567201814666171013151750
30. Mehling T, Smirnova I, Guenther U, Neubert RHH (2009) Polysaccharide-based aerogels as drug carriers. J Non Cryst Solids 355:2472–2479. https://doi.org/10.1016/j.jnoncrysol.2009.08.038
31. Dang JM, Leong KW (2006) Natural polymers for gene delivery and tissue engineering. Adv Drug Deliv Rev 58:487–499. https://doi.org/10.1016/j.addr.2006.03.001
32. Gaber M, Mabrouk MT, Freag MS, Khiste SK, Fang JY, Elkhodairy KA et al (2018) Protein-polysaccharide nanohybrids: hybridization techniques and drug delivery applications. Eur J Pharm Biopharm 133:42–62. https://doi.org/10.1016/j.ejpb.2018.10.001
33. Khemakhem I, Abdelhedi O, Trigui I, Ayadi MA, Bouaziz M (2018) Structural, antioxidant and antibacterial activities of polysaccharides extracted from olive leaves. Int J Biol Macromol 106:425–432. https://doi.org/10.1016/j.ijbiomac.2017.08.037
34. Hirosawa S, Takahashi Y, Hashizume H, Miyake T, Akamatsu Y (2014) Synthesis and antibacterial activity of tripropeptin C derivatives modified at the carboxyl groups. J Antibiot (Tokyo) 67:265–268. https://doi.org/10.1038/ja.2013.128
35. Ma YL, Zhu DY, Thakur K, Wang CH, Wang H, Ren YF et al (2018) Antioxidant and antibacterial evaluation of polysaccharides sequentially extracted from onion (Allium cepa L.). Int J Biol Macromol 111:92–101. https://doi.org/10.1016/j.ijbiomac.2017.12.154
36. Agnihotri SA, Mallikarjuna NN, Aminabhavi TM (2004) Recent advances on chitosan-based micro- and nanoparticles in drug delivery. J Control Release 100:5–28. https://doi.org/10.1016/j.jconrel.2004.08.010
37. Ahmed S, Ikram S (2016) Chitosan based scaffolds and their applications in wound healing. Achiev Life Sci 10:27–37. https://doi.org/10.1016/j.als.2016.04.001
38. Niekraszewicz A (2005) Chitosan medical dressings. Fibres Text East Eur 13:16–18. https://doi.org/10.1111/1468-2427.00255
39. Harkins AL, Duri S, Kloth LC, Tran CD (2014) Chitosan-cellulose composite for wound dressing material. Part 2. Antimicrobial activity, blood absorption ability, and biocompatibility. J Biomed Mater Res Pt B Appl Biomater 102:1199–1206. https://doi.org/10.1002/jbm.b.33103
40. You J, Li W, Yu C, Zhao C, Jin L, Zhou Y et al (2013) Amphiphilically modified chitosan cationic nanoparticles for drug delivery. J Nanopart Res 15:1–10. https://doi.org/10.1007/s11051-013-2123-2

41. Pawar V, Dhanka M, Srivastava R (2018) Cefuroxime conjugated chitosan hydrogel for treatment of wound infections. Colloids Surf B Biointerfaces 173:776–787. https://doi.org/10.1016/j.colsurfb.2018.10.034
42. Qi L, Xu Z, Jiang X, Hu C, Zou X (2004) Preparation and antibacterial activity of chitosan nanoparticles. Carbohydr Res 339:2693–2700. https://doi.org/10.1016/j.carres.2004.09.007
43. Ibrahim HM, El-Bisi MK, Taha GM, El-Alfy EA (2015) Chitosan nanoparticles loaded antibiotics as drug delivery biomaterial. J Appl Pharm Sci 5:85–90. https://doi.org/10.7324/JAPS.2015.501015
44. Madureira AR, Pereira A, Castro PM, Pintado M (2015) Production of antimicrobial chitosan nanoparticles against food pathogens. J Food Eng 167:210–216. https://doi.org/10.1016/j.jfoodeng.2015.06.010
45. Elbi S, Biswas R, Baranwal G, Sathianarayanan S, Rajan VK, Jayakumar R et al (2017) Fucoidan coated ciprofloxacin loaded chitosan nanoparticles for the treatment of intracellular and biofilm infections of Salmonella. Colloids Surf B Biointerfaces 160:40–47. https://doi.org/10.1016/j.colsurfb.2017.09.003
46. Piras AM, Maisetta G, Sandreschi S, Gazzarri M, Bartoli C, Grassi L et al (2015) Chitosan nanoparticles loaded with the antimicrobial peptide temporin B exert a long-term antibacterial activity in vitro against clinical isolates of *Staphylococcus epidermidis*. Front Microbiol 6:1–10. https://doi.org/10.3389/fmicb.2015.00372
47. Pintado MM, Tavaria FK, Silva S, Costa EM, Veiga M (2018) Exploring chitosan nanoparticles as effective inhibitors of antibiotic resistant skin microorganisms—from in vitro to ex vitro testing. Carbohydr Polym 201:340–346. https://doi.org/10.1016/j.carbpol.2018.08.083
48. Wu T, Wu C, Fu S, Wang L, Yuan C, Chen S et al (2017) Integration of lysozyme into chitosan nanoparticles for improving antibacterial activity. Carbohydr Polym 155:192–200. https://doi.org/10.1016/j.carbpol.2016.08.076
49. Jeon SJ, Oh M, Yeo WS, Galvão KN, Jeong KC (2014) Underlying mechanism of antimicrobial activity of chitosan microparticles and implications for the treatment of infectious diseases. PLoS One 9:e92723. https://doi.org/10.1371/journal.pone.0092723
50. Ma Z, Kim D, Adesogan AT, Ko S, Galvao K, Jeong KC (2016) Chitosan microparticles exert broad-spectrum antimicrobial activity against antibiotic-resistant micro-organisms without increasing resistance. ACS Appl Mater Interfaces 8:10700–10709. https://doi.org/10.1021/acsami.6b00894
51. Jeon SJ, Ma Z, Kang M, Galvão KN, Jeong KC (2016) Application of chitosan microparticles for treatment of metritis and in vivo evaluation of broad spectrum antimicrobial activity in cow uteri. Biomaterials 110:71–80. https://doi.org/10.1016/j.biomaterials.2016.09.016
52. Shen J, Jin B, Qi YC, Jiang Q, Gao XF (2017) Carboxylated chitosan/silver-hydroxyapatite hybrid microspheres with improved antibacterial activity and cytocompatibility. Mater Sci Eng C 78:589–597. https://doi.org/10.1016/j.msec.2017.03.100
53. Liu Z, Wang C, Liu Y, Peng D (2017) Cefepime loaded O-carboxymethyl chitosan microspheres with sustained bactericidal activity and enhanced biocompatibility. J Biomater Sci Polym Ed 28:79–92. https://doi.org/10.1080/09205063.2016.1244372
54. Mantripragada VP, Jayasuriya AC (2016) Effect of dual delivery of antibiotics (vancomycin and cefazolin) and BMP-7 from chitosan microparticles on *Staphylococcus epidermidis* and pre-osteoblasts in vitro. Mater Sci Eng C 67:409–417. https://doi.org/10.1016/j.msec.2016.05.033
55. Saranya TS, Rajan VK, Biswas R, Jayakumar R, Sathianarayanan S (2018) Synthesis, characterisation and biomedical applications of curcumin conjugated chitosan microspheres. Int J Biol Macromol 110:227–233. https://doi.org/10.1016/j.ijbiomac.2017.12.044
56. Thaya R, Vaseeharan B, Sivakamavalli J, Iswarya A, Govindarajan M, Alharbi NS et al (2018) Synthesis of chitosan-alginate microspheres with high antimicrobial and antibiofilm activity against multi-drug resistant microbial pathogens. Microb Pathog 114:17–24. https://doi.org/10.1016/j.micpath.2017.11.011
57. Abdelbary G (2011) Ocular ciprofloxacin hydrochloride mucoadhesive chitosan-coated liposomes. Pharm Dev Technol 16:44–56. https://doi.org/10.3109/10837450903479988

58. Norowski PA, Courtney HS, Babu J, Haggard WO, Bumgardner JD (2011) Chitosan coatings deliver antimicrobials from titanium implants: a preliminary study. Implant Dent 20:56–67. https://doi.org/10.1097/ID.0b013e3182087ac4
59. Noel SP, Courtney H, Bumgardner JD, Haggard WO (2008) Chitosan films: a potential local drug delivery system for antibiotics. Clin Orthop Relat Res 466:1377–1382. https://doi.org/10.1007/s11999-008-0228-1
60. Smith JK, Bumgardner JD, Courtney HS, Smeltzer MS, Haggard O (2015) Antibiotic-loaded chitosan film for infection prevention: a preliminary in vitro characterization. J Biomed Mater Res Pt B Appl Biomater 94:203–211. https://doi.org/10.1002/jbm.b.31642.Antibiotic-loaded
61. Oungbho K, Müller BW (1997) Chitosan sponges as sustained release drug carriers. Int J Pharm 156:229–237. https://doi.org/10.1016/S0378-5173(97)00201-9
62. Chen Aimin DZ, Chunlin H, Juliang B, Tinyin Z (1999) Antibiotic loaded chitosan bar—an in vitro, in vivo study of a possible treatment for osteomyelitis. Clin Orthop Relat Res 366:239–247. PMID: 10627741
63. Noel SP, Courtney HS, Bumgardner JD, Haggard WO (2010) Chitosan sponges to locally deliver amikacin and vancomycin: a pilot in vitro evaluation. Clin Orthop Relat Res:2074–2080. https://doi.org/10.1007/s11999-010-1324-6
64. Huang L, Dai T, Xuan Y, Tegos GP, Hamblin MR (2011) Synergistic combination of chitosan acetate with nanoparticle silver as a topical antimicrobial: efficacy against bacterial burn infections. Antimicrob Agents Chemother 55:3432–3438. https://doi.org/10.1128/AAC.01803-10
65. Hao JY, Mi FL, Shyu SS, Wu YB, Schoung JY, Tsai YH et al (2002) Control of wound infections using a bilayer chitosan wound dressing with sustainable antibiotic delivery. J Biomed Mater Res 59:438–449. https://doi.org/10.1002/jbm.1260
66. Phaechamud T, Charoenteeraboon J (2008) Antibacterial activity and drug release of chitosan sponge containing doxycycline hyclate. AAPS PharmSciTech 9:829–835. https://doi.org/10.1208/s12249-008-9117-x
67. Pawar V, Bulbake U, Khan W, Srivastava R (2019) Chitosan sponges as a sustained release carrier system for theprophylaxis of orthopedic implant-associated infections. Int J Biol Macromol 134:100–112. https://doi.org/10.1016/j.ijbiomac.2019.04.190
68. Bhattarai N, Gunn J, Zhang M (2010) Chitosan-based hydrogels for controlled, localized drug delivery. Adv Drug Deliv Rev 62:83–99. https://doi.org/10.1016/j.addr.2009.07.019
69. Harris M, Alexander C, Wells CM, Bumgardner JD, Carpenter DP, Jennings JA (2017) Chitosan for the delivery of antibiotics. In: Jessica Jennings JB (ed) Chitosan based biomater, 1st edn. Elsevier Publishing, Amsterdam, pp 147–173. https://doi.org/10.1016/B978-0-08-100228-5.00006-7
70. Meng G, He J, Wu Y, Wu F, Gu Z (2014) Antibiotic-loaded chitosan hydrogel with superior dual functions: antibacterial efficacy and osteoblastic cell responses. ACS Appl Mater Interfaces 6:10005–10013. https://doi.org/10.1021/am502537k
71. Chen CP, Hsieh CM, Tsai T, Yang JC, Chen CT (2015) Optimization and evaluation of a chitosan/hydroxypropyl methylcellulose hydrogel containing toluidine blue O for antimicrobial photodynamic inactivation. Int J Mol Sci 16:20859–20872. https://doi.org/10.3390/ijms160920859
72. Grumezescu AM, Andronescu E, Ficai A, Bleotu C, Mihaiescu DE, Chifiriuc MC (2012) Synthesis, characterization and in vitro assessment of the magnetic chitosan-carboxymethylcellulose biocomposite interactions with the prokaryotic and eukaryotic cells. Int J Pharm 436:771–777. https://doi.org/10.1016/j.ijpharm.2012.07.063
73. Skjk-Braek G, Grasdalen H, Larsen B (1986) Monomer sequence and acetylation pattern in some bacterial alginates. Carbohydr Res 154:239–250. https://doi.org/10.1016/S0008-6215(00)90036-3
74. Sachan NK, Pushkar S, Jha A, Bhattcharya A (2009) Sodium alginate: the wonder polymer for controlled drug delivery. J Pharm Res 2:1191–1199
75. Dawn Hunt S (2016) Self-care and postoperative dressing management. Br J Nurs 25:1–6. https://doi.org/10.12968/bjon.2016.25.15.s34

76. Cooper C (2013) Fundamentals of hand therapy: clinical reasoning and treatment guidelines for common diagnoses of the upper extremity. In: Cooper C (ed) Wound care, 2nd edn. Elsevier, Amsterdam, pp 206–218. https://doi.org/10.1016/C2011-0-05791-5
77. Leveriza-Oh M, Phillips TJ (2012) Dressings and postoperative care. In: Dockery GD, Crawford ME (eds) Lower extremtremity soft tissue cutaneous plastic surgery, 2nd edn. Elsevier, Amsterdam, pp 478–488. https://doi.org/10.1016/B978-0-323-02752-6.50013-4
78. Rinaudo M (2014) Biomaterials based on a natural polysaccharide: alginate. TIP 17:92–96. https://doi.org/10.1016/s1405-888x(14)70322-5
79. Koehler J, Brandl FP, Goepferich AM (2018) Hydrogel wound dressings for bioactive treatment of acute and chronic wounds. Eur Polym J 100:1–11. https://doi.org/10.1016/j.eurpolymj.2017.12.046
80. Percival SL, McCarty SM (2014) Silver and alginates: role in wound healing and biofilm control. Adv Wound Care 4:407–414. https://doi.org/10.1089/wound.2014.0541
81. Simoes D, Miguel SP, Ribeiro MP, Coutinho P, Mendonça AG, Correia IJ (2018) Recent advances on antimicrobial wound dressing: a review. Eur J Pharm Biopharm 127:130–141. https://doi.org/10.1016/j.ejpb.2018.02.022
82. Wiegand C, Heinze T, Hipler UC (2009) Comparative in vitro study on cytotoxicity, antimicrobial activity, and binding capacity for pathophysiological factors in chronic wounds of alginate and silver-containing alginate. Wound Repair Regen 17:511–521. https://doi.org/10.1111/j.1524-475X.2009.00503.x
83. Rafiq M, Hussain T, Abid S, Nazir A, Masood R (2018) Development of sodium alginate/PVA antibacterial nanofibers by the incorporation of essential oils. Mater Res Express 5:035007. https://doi.org/10.1088/2053-1591/aab0b4
84. Varaprasad K, Raghavendra GM, Jayaramudu T, Seo J (2016) Nano zinc oxide-sodium alginate antibacterial cellulose fibres. Carbohydr Polym 135:349–355. https://doi.org/10.1016/j.carbpol.2015.08.078
85. Paques JP, Van Der Linden E, Van Rijn CJM, Sagis LMC (2014) Preparation methods of alginate nanoparticles. Adv Colloid Interface Sci 209:163–171. https://doi.org/10.1016/j.cis.2014.03.009
86. Li P, Dai YN, Zhang JP, Wang AQ, Wei Q (2008) Chitosan-alginate nanoparticles as a novel drug delivery system for nifedipine. Int J Biomed Sci 4:221–228. PMID: 23675094
87. Trandafilović LV, Božanić DK, Dimitrijević-Branković S, Luyt AS, Djoković V (2012) Fabrication and antibacterial properties of ZnO-alginate nanocomposites. Carbohydr Polym 88:263–269. https://doi.org/10.1016/j.carbpol.2011.12.005
88. Pandey S, Ramontja J (2016) Sodium alginate stabilized silver nanoparticles–silica nanohybrid and their antibacterial characteristics. Int J Biol Macromol 93:712–723. https://doi.org/10.1016/j.ijbiomac.2016.09.033
89. Friedman AJ, Phan J, Schairer DO, Champer J, Qin M, Pirouz A et al (2013) Antimicrobial and anti-inflammatory activity of chitosan-alginate nanoparticles: a targeted therapy for cutaneous pathogens. J Invest Dermatol 133:1231–1239. https://doi.org/10.1038/jid.2012.399
90. Liu J, Xiao J, Li F, Shi Y, Li D, Huang Q (2018) Chitosan-sodium alginate nanoparticle as a delivery system for ε-polylysine: preparation, characterization and antimicrobial activity. Food Control 91:302–310. https://doi.org/10.1016/j.foodcont.2018.04.020
91. Costa JR, Silva NC, Sarmento B, Pintado M (2015) Potential chitosan-coated alginate nanoparticles for ocular delivery of daptomycin. Eur J Clin Microbiol Infect Dis 34:1255–1262. https://doi.org/10.1007/s10096-015-2344-7
92. Ozseker EE, Akkaya A (2016) Development of a new antibacterial biomaterial by tetracycline immobilization on calcium-alginate beads. Carbohydr Polym 151:441–451. https://doi.org/10.1016/j.carbpol.2016.05.073
93. Guler S, Ozseker EE, Akkaya A (2016) Developing an antibacterial biomaterial. Eur Polym J 84:326–337. https://doi.org/10.1016/j.eurpolymj.2016.09.031
94. Hebeish A, Ramadan M, Montaser A, Krupa I, Farag A (2015) Molecular characteristics and antibacterial activity of alginate beads coated chitosan polyacrylonitrile copolymer loaded silver nanocomposite. J Sci Res Rep 5:479–488. https://doi.org/10.9734/jsrr/2015/14775

95. George M, Abraham TE (2006) Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan—a review. J Control Release 114:1–14. https://doi.org/10.1016/j.jconrel.2006.04.017
96. Nam SY, Nho YC, Hong SH, Chae GT, Jang HS, Suh TS et al (2004) Evaluations of poly(vinyl alcohol)/alginate hydrogels cross-linked by γ-ray irradiation technique. Macromol Res 12:219–224. https://doi.org/10.1007/BF03218391
97. George L, Bavya MC, Rohan KV, Srivastava R (2017) A therapeutic polyelectrolyte–vitamin C nanoparticulate system in polyvinyl alcohol–alginate hydrogel: an approach to treat skin and soft tissue infections caused by *Staphylococcus aureus*. Colloids Surf B Biointerfaces 160:315–324. https://doi.org/10.1016/j.colsurfb.2017.09.030
98. Kesavan K, Nath G, Pandit JK (2010) Sodium alginate based mucoadhesive system for gatifloxacin and its in vitro antibacterial activity. Sci Pharm 78:941–957. https://doi.org/10.3797/scipharm.1004-24
99. Sharma S, Sanpui P, Chattopadhyay A, Ghosh SS (2012) Fabrication of antibacterial silver nanoparticle—sodium alginate-chitosan composite films. RSC Adv 2:5837–5843. https://doi.org/10.1039/c2ra00006g
100. Whistler RL, BeMiller JN (2012) Industrial gums: polysaccharides and their derivatives, 3rd edn. Wiley Academic Press, San Diego, New York, Boston, pp 234–251. https://doi.org/10.1016/C2009-0-03188-2
101. Roy S, Rhim JW (2019) Carrageenan-based antimicrobial bionanocomposite films incorporated with ZnO nanoparticles stabilized by melanin. Food Hydrocoll 90:500–507. https://doi.org/10.1016/j.foodhyd.2018.12.056
102. Shojaee-Aliabadi S, Hosseini H, Mohammadifar MA, Mohammadi A, Ghasemlou M, Hosseini SM et al (2014) Characterization of κ-carrageenan films incorporated plant essential oils with improved antimicrobial activity. Carbohydr Polym 101:582–591. https://doi.org/10.1016/j.carbpol.2013.09.070
103. Briones AV, Sato T, Bigol UG (2014) Antibacterial activity of polyethylenimine/carrageenan multilayer against pathogenic bacteria. Adv Chem Eng Sci 4:233–241. https://doi.org/10.4236/aces.2014.42026
104. El-Fawal G (2014) Preparation, characterization and antibacterial activity of biodegradable films prepared from carrageenan. J Food Sci Technol 51:2234–2239. https://doi.org/10.1007/s13197-013-1255-9
105. Cevher E, Mülazimoglu L, Gürcan D, Alper M, Araman A, Özsoy Y (2006) The preparation of ciprofloxacin hydrochloride-loaded chitosan and pectin microspheres their evaluation in an animal osteomyelitis model. J Bone Joint Surg Br 88:270–275. https://doi.org/10.1302/0301-620X.88B2.16328
106. Dacarro G, Curtosi S, Milanese C, D'Agostino A, Bertoglio F, Taglietti A et al (2017) Silver nanoparticles synthesized and coated with pectin: an ideal compromise for anti-bacterial and anti-biofilm action combined with wound-healing properties. J Colloid Interface Sci 498:271–281. https://doi.org/10.1016/j.jcis.2017.03.062
107. Martínez YN, Cavello I, Hours R, Cavalitto S, Castro GR (2013) Immobilized keratinase and enrofloxacin loaded on pectin PVA cryogel patches for antimicrobial treatment. Bioresour Technol 145:280–284. https://doi.org/10.1016/j.biortech.2013.02.063
108. Bayón B, Bucalá V, Castro GR (2016) Development of antimicrobial hybrid mesoporous silver phosphate-pectin microspheres for control release of levofloxacin. Micropor Mesopor Mater 226:71–78. https://doi.org/10.1016/j.micromeso.2015.12.041
109. da Silva EP, Sitta DA, Fragal VH, Cellet TP, Mauricio MR, Garcia FP et al (2014) Covalent TiO2/pectin microspheres with Fe3O4 nanoparticles for magnetic field-modulated drug delivery. Int J Biol Macromol 67:43–52. https://doi.org/10.1016/j.ijbiomac.2014.02.035
110. Nešić A, Onjia A, Davidović S, Dimitrijević S, Errico ME, Santagata G et al (2017) Design of pectin-sodium alginate based films for potential healthcare application: study of chemico-physical interactions between the components of films and assessment of their antimicrobial activity. Carbohydr Polym 157:981–990. https://doi.org/10.1016/j.carbpol.2016.10.054

111. Polifka JE, Habermann J (2014) Anticoagulants, thrombocyte aggregation inhibitors, fibrinolytics and volume replacement agents. In: Schaefer RKMC, Peters P (eds) Drugs during pregnancy and lactation: treatment options and risk assessment, 3rd edn. Elsevier, Amsterdam, pp 225–249. https://doi.org/10.1016/B978-0-12-408078-2.00010-X
112. Sagitha P, Reshmi CR, Sundaran SP, Binoy A, Mishra N, Sujith A (2019) In-vitro evaluation on drug release kinetics and antibacterial activity of dextran modified polyurethane fibrous membrane. Int J Biol Macromol 126:717–730. https://doi.org/10.1016/j.ijbiomac.2018.12.155
113. Yang G, Lin Q, Wang C, Li J, Wang J, Zhou J et al (2012) Synthesis and characterization of dextran-capped silver nanoparticles with enhanced antibacterial activity. J Nanosci Nanotechnol 12:3766–3774. https://doi.org/10.1166/jnn.2012.5865
114. Hoque J, Haldar J (2017) Direct synthesis of dextran-based antibacterial hydrogels for extended release of biocides and eradication of topical biofilms. ACS Appl Mater Interfaces 9:15975–15985. https://doi.org/10.1021/acsami.7b03208
115. De Cicco F, Reverchon E, Adami R, Auriemma G, Russo P, Calabrese EC et al (2014) In situ forming antibacterial dextran blend hydrogel for wound dressing: SAA technology vs. spray drying. Carbohydr Polym 101:1216–1224. https://doi.org/10.1016/j.carbpol.2013.10.067
116. Ritz U, Kögler P, Höfer I, Frank P, Klees S, Gebhard S et al (2016) Photocrosslinkable polysaccharide hydrogel composites based on dextran or pullulan-amylose blends with cytokines for a human co-culture model of human osteoblasts and endothelial cells. J Mater Chem B 4:6552–6564. https://doi.org/10.1039/c6tb00654j
117. Liao N, Unnithan AR, Joshi MK, Tiwari AP, Hong ST, Park CH et al (2015) Electrospun bioactive poly (e{open}-caprolactone)-cellulose acetate-dextran antibacterial composite mats for wound dressing applications. Colloids Surfaces A Physicochem Eng Asp 469:194–201. https://doi.org/10.1016/j.colsurfa.2015.01.022
118. Tiyaboonchai W, Rodleang I, Ounaroon A (2015) Mucoadhesive polyethylenimine-dextran sulfate nanoparticles containing Punica granatum peel extract as a novel sustained-release antimicrobial. Pharm Dev Technol 20:426–432. https://doi.org/10.3109/10837450.2013.879884
119. Bankura KP, Maity D, Mollick MR, Mondal D, Bhowmick B, Bain MK et al (2012) Synthesis, characterization and antimicrobial activity of dextran stabilized silver nanoparticles in aqueous medium. Carbohydr Polym 1110:156–161. https://doi.org/10.1016/j.carbpol.2012.03.089
120. Unnithan AR, Barakat NM, Tirupathi Pichiah PB, Gnanasekaran G, Nirmala R, Cha YS et al (2012) Wound-dressing materials with antibacterial activity from electrospun polyurethane-dextran nanofiber mats containing ciprofloxacin HCl. Carbohydr Polym 90:1786–1793. https://doi.org/10.1016/j.carbpol.2012.07.071
121. Cano AI, Cháfer M, Chiralt A, González-Martínez C (2015) Physical and microstructural properties of biodegradable films based on pea starch and PVA. J Food Eng 167:59–64. https://doi.org/10.1016/j.jfoodeng.2015.06.003
122. Kaith BS, Sharma R, Kalia S (2015) Guar gum based biodegradable, antibacterial and electrically conductive hydrogels. Int J Biol Macromol 75:266–275. https://doi.org/10.1016/j.ijbiomac.2015.01.046
123. Sharma R, Kaith BS, Kalia S, Pathania D, Kumar A, Sharma N et al (2015) Biodegradable and conducting hydrogels based on Guar gum polysaccharide for antibacterial and dye removal applications. J Environ Manage 162:37–45. https://doi.org/10.1016/j.jenvman.2015.07.044
124. Auddy RG, Abdullah MF, Das S, Roy P, Datta S, Mukherjee A (2013) New guar biopolymer silver nanocomposites for wound healing applications. Biomed Res Int 2013:912458. https://doi.org/10.1155/2013/912458
125. Necas J, Bartosikova L, Brauner P, Kolar J (2008) Hyaluronic acid (hyaluronan): a review. Vet Med (Praha) 53:397–411. https://doi.org/10.17221/1930-VETMED
126. Lequeux I, Ducasse E, Jouenne T, Thebault P (2014) Addition of antimicrobial properties to hyaluronic acid by grafting of antimicrobial peptide. Eur Polym J 51:182–190. https://doi.org/10.1016/j.eurpolymj.2013.11.012
127. Saranraj P, Naidu MA (2013) Hyaluronic acid production and its applications—a review. Int J Pharm Biol Arch 4:853–859. ISSN 0976-3333

128. Suzuki K, Anada T, Miyazaki T, Miyatake N, Honda Y, Kishimoto KN et al (2014) Effect of addition of hyaluronic acids on the osteoconductivity and biodegradability of synthetic octacalcium phosphate. Acta Biomater 10:531–543. https://doi.org/10.1016/j.actbio.2013.09.005
129. Rodan GA, Martin TJ (1981) Role of osteoblasts in hormonal control of bone resorption—a hypothesis. Calcif Tissue Int 33:349–351. https://doi.org/10.1007/BF02409454
130. Pérez-Álvarez L, Ruiz-Rubio L, Azua I, Benito V, Bilbao A, Vilas-Vilela JL (2019) Development of multiactive antibacterial multilayers of hyaluronic acid and chitosan onto poly(ethylene terephthalate). Eur Polym J 112:31–37. https://doi.org/10.1016/j.eurpolymj.2018.12.038
131. Ardizzoni A, Neglia RG, Baschieri MC, Cermelli C, Caratozzolo M, Righi E et al (2011) Influence of hyaluronic acid on bacterial and fungal species, including clinically relevant opportunistic pathogens. J Mater Sci Mater Med 22:2329–2338. https://doi.org/10.1007/s10856-011-4408-2
132. Gaetano G, Giuseppe P, Salvatore PF, Susanna M, Sara S, Luca RC (2018) Hyaluronic-based antibacterial hydrogel coating for implantable biomaterials in orthopedics and trauma: from basic research to clinical applications. In: Haider AH (ed) Hydrogels, 1st edn. IntechOpen, London, pp 179–200. https://doi.org/10.5772/intechopen.73203
133. Petrauskaite O, Gomes PS, Fernandes MH, Juodzbalys G, Stumbras A, Maminskas J et al (2013) Biomimetic mineralization on a macroporous cellulose-based matrix for bone regeneration. Biomed Res Int 2013:1–9. https://doi.org/10.1155/2013/452750
134. Cheng H, Yang X, Che X, Yang M, Zhai G (2018) Biomedical application and controlled drug release of electrospun fibrous materials. Mater Sci Eng C 90:750–763. https://doi.org/10.1016/j.msec.2018.05.007
135. Khattak S, Wahid F, Liu LP, Jia SR, Chu LQ, Xie YY et al (2019) Applications of cellulose and chitin/chitosan derivatives and composites as antibacterial materials: current state and perspectives. Appl Microbiol Biotechnol 103:1989–2006. https://doi.org/10.1007/s00253-018-09602-0
136. Konwarh R, Karak N, Misra M (2013) Electrospun cellulose acetate nanofibers: the present status and gamut of biotechnological applications. Biotechnol Adv 31:421–437. https://doi.org/10.1016/j.biotechadv.2013.01.002
137. Jia B, Mei Y, Cheng L, Zhou J, Zhang L (2012) Preparation of copper nanoparticles coated cellulose films with antibacterial properties through one-step reduction. ACS Appl Mater Interfaces 4:2897–2902. https://doi.org/10.1021/am3007609
138. Foresti ML, Vázquez A, Boury B (2017) Applications of bacterial cellulose as precursor of carbon and composites with metal oxide, metal sulfide and metal nanoparticles: a review of recent advances. Carbohydr Polym 157:447–467. https://doi.org/10.1016/j.carbpol.2016.09.008
139. Khoshnevisan K, Maleki H, Samadian H, Shahsavari S, Sarrafzadeh MH, Larijani B et al (2018) Cellulose acetate electrospun nanofibers for drug delivery systems: applications and recent advances. Carbohydr Polym 198:131–141. https://doi.org/10.1016/j.carbpol.2018.06.072
140. Unnithan AR, Gnanasekaran G, Sathishkumar Y, Lee YS, Kim CS (2014) Electrospun antibacterial polyurethane-cellulose acetate-zein composite mats for wound dressing. Carbohydr Polym 102:884–892. https://doi.org/10.1016/j.carbpol.2013.10.070
141. Liu S, Chu M, Zhu Y, Li L, Wang L, Gao H et al (2017) A novel antibacterial cellulose based biomaterial for hernia mesh applications. RSC Adv 7:11601–11607. https://doi.org/10.1039/c6ra26216c
142. Azizi S, Ahmad MB, Hussein MZ, Ibrahim NA (2013) Synthesis, antibacterial and thermal studies of cellulose nanocrystal stabilized ZnO-Ag heterostructure nanoparticles. Molecules 18:6269–6280. https://doi.org/10.3390/molecules18066269
143. Baker S, Volova T, Prudnikova SV, Shumilova AA, Perianova OV, Zharkov SM et al (2018) Bio-hybridization of nanobactericides with cellulose films for effective treatment against members of ESKAPE multi-drug-resistant pathogens. Appl Nanosci 8:1101–1110. https://doi.org/10.1007/s13204-018-0717-9

Mechanisms of Action and Chemical Origins of Biologically Active Antimicrobial Polymers

Jeff Shen, Geoffrey C. Gurtner, Lynette Cegelski, and Yunzhi Peter Yang

Abstract The creation of synthetic tissues for patients with traumatic or debilitating injuries and diseases has proven to be a rapidly growing field. Scaffold design plays a crucial role in determining the biocompatibility, function and longevity of these engineered tissues. Biodegradable polymers with high levels of biocompatibility and functional flexibility are currently the primary choice for scaffold construction. Due to the fiscal and healthcare-related costs of replacing scaffolds during the healing process, manufacturing transplants with the ability to withstand foreign infection is tantamount to the success of the field. Antimicrobial polymers (AMPs) can serve as materials for such synthetic transplants. A variety of AMPs bearing different chemical motifs and biological effects have been studied with regard to their viability as biocompatible engineering materials. This review discusses the merits and faults of AMPs in their potential applications toward tissue scaffold design.

Keywords Antimicrobial · Polymer · Scaffold · Tissue engineering · Synthetic · Biocompatible · Transplant · Chitosan · Quaternized ammonium

J. Shen
Department of Chemistry, Stanford University, Stanford, CA, USA

Genentech, San Francisco, CA, USA
e-mail: shenj45@gene.com

G. C. Gurtner
Departments of Surgery and Materials Science and Engineering, Stanford University School of Medicine, Stanford, CA, USA
e-mail: ggurtner@stanford.edu

L. Cegelski
Department of Chemistry, Stanford University, Stanford, CA, USA
e-mail: cegelski@stanford.edu

Y. P. Yang (✉)
Departments of Orthopaedic Surgery, Materials Science and Engineering, and Bioengineering, Stanford University School of Medicine, Stanford, CA, USA
e-mail: ypyang@stanford.edu

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_13

Introduction

In recent years, the engineering of synthetic tissues, including tendon and bone transplants, has reached stages where it is now feasible to create highly biomimetic and biocompatible synthetic implants. However, successful clinical translation of these developing technologies hinges upon infection-free incorporation of the implants, as well as maintaining an antimicrobial environment in the absence of a traditional immune response, as is often the case with these transplants.

Due to the circumstances surrounding the cases where transplants are required, such as in trauma or disease-induced organ and tissue loss, the immune system is incapable of handling microbial infections at the site of the transplants. Microorganism-related infections provide the most serious complications in the healthcare industry, particularly when medical devices and hospital equipment are involved [1]. For example, contamination of catheters can lead to high levels of discomfort and illness within medical patients. Furthermore, treatment of these infections often requires complete removal of the implants, which prove to be costly and inconvenient procedures for both the patient and hospital. More serious infections can occur when the microbes migrate from the infection site to the spinal fluid or the brain, as traditional antibiotics cannot treat infections in those locations. Therefore, prevention of these microbial infections is a high priority and mandatory step to ensure safe, long-lasting transplants, especially when using synthetic materials.

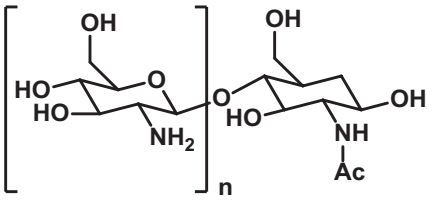
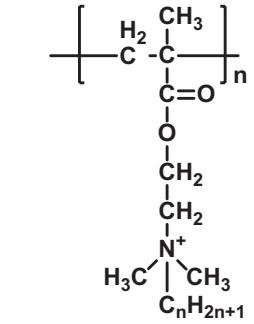
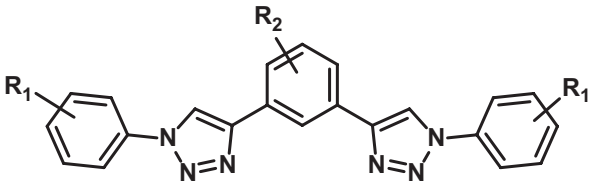
Many research groups have dedicated their efforts toward synthesizing biocompatible materials that provide their host with antibiotic activity. One strategy has been to employ traditional antibiotics in a sustained release fashion, generally through encapsulation of the antibiotics in materials that slowly degrade over a long time scale [2]. By incorporating this into synthetic transplants, the transplant itself will gradually release antibiotics into the surrounding area, keeping the microbial infections at bay. The major disadvantage to this is that most antibiotics bind to their targets with very high affinity, but a single point mutation in the gene that encodes for the antibiotics' target could lead to resistance against the drug [3]. This is exacerbated when there are large and diverse populations of microbes incubating over a long period of time, as is the case when transplants are involved.

Another antibacterial design approach relies on selecting and tuning the chemical and biochemical properties of the materials used to construct or coat a transplant, as certain materials exhibit bactericidal properties due to electronics and sterics [4]. Because the mechanisms of action of such antimicrobial polymers rely on general properties, particularly with regard to the microbial cell surface and cell membrane, resistance is often avoided, even after long exposure to the same antimicrobial materials [5]. This is attributed to the difficulty inherent in microbes enacting large scale changes in biochemical properties to alter a target such as the cell wall or cell membrane, as single mutations would not be sufficient to generate resistance. This chapter will focus primarily on polymers of this type with inherent antimicrobial properties and will include a discussion of their proposed mechanisms of action against microorganisms and activity with human cells, a perspective of current limitations in the field, and future opportunities.

Overview of Different Types of Antimicrobial Polymers

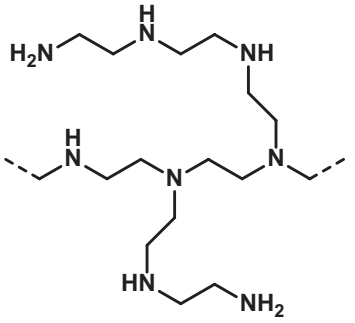
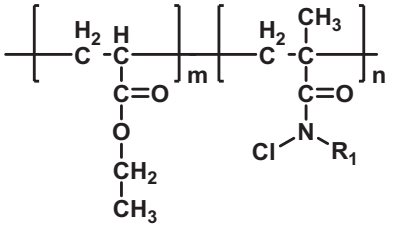
Currently, there are a wide variety of antimicrobial polymers (AMPs) that have demonstrated high selectivity and strong fungicidal, bactericidal, and antiviral capabilities. They are derived from diverse chemical origins and rely on particular chemical functionality for their mechanisms of action. Table 1 provides a summary of

Table 1 Chemical structures and general mechanisms of AMPs

Polymer	Mechanism of action
 Chitosan	Interaction between positively charged chitosan and negatively charged microbial membranes leads to perforations in the membrane. Activity is pH dependent
 Quaternary Ammonium-containing Compounds	Integration of hydrophobic tail into microbial membrane impairs membrane integrity. Leads to leakage and cell lysis
 Synthetic Protein Mimics	Mimicry of antimicrobial peptides, often designed to exhibit enhanced stability and microbial targeting, serving to penetrate or impair cell barriers

(continued)

Table 1 (continued)

Polymer	Mechanism of action
 Polyethylenimines	Inhibits attachment of viral and bacterial organisms to host cells, thus preventing microbial biofilm formation on host cells and tissue. Additionally, electrostatic interactions impair bacterial membrane integrity
 N-Halamines	Oxidative halogens can target specific thiols or amino groups on proteins, leading to inactivation and eventual cell death

representative polymers, their notable chemical moieties that contribute to their biological activity, and the generally understood basis for their antimicrobial properties.

Overall, it can be noted that the majority of the AMPs rely on charge interactions between the AMPs and their target microbes. Generally, positively charged AMPs are attracted toward negatively charged microbes and this interaction is the basis of many antimicrobial activities. However, there are certain types of AMPs that derive their activity from biomimetic properties, such as the synthetic mimics of antimicrobial peptides (SMAMPs), and halogen-based polymers, which serve to transfer halogens such as chlorine through direct contact to microbial membranes or proteins at the cell surface.

Chitosan-Based Polymers

Chitosan is a well-known natural polymer that consists of acetylated and non-acetylated glucosamines that are attached linearly with an ether bond. It is a hetero polysaccharide with polycationic character and derives its chemical flexibility mostly from its amine groups, which can be functionalized to provide further versatility and utility to this polymer. It can be found naturally in the cell walls of fungi,

green algae, or in insect cuticles [6]. It is generally produced through the deacetylation of chitin, which is the fully acetylated form of chitosan that is found commonly in many living organisms. For the production of chitosan, chitin is generally submerged in a basic solution and the degree of deacetylation (DDA) is determined via UV spectroscopy [7, 8].

Chitosan's properties are heavily dependent on the degree of acetylation of the polymer, as this affects the viscosity, charge distribution and ultimately bactericidal abilities of chitosan [9]. The currently accepted DDA for chitosan requires at least a 40% deacetylation for the polymer to be considered chitosan. A key property of chitosan is its solubility in aqueous solutions. It is insoluble in water or alkaline media, but becomes soluble in solutions at and below pH 6.5 due to the pKa of the amines. Protonation of the amino groups enables the production of a cationic polysaccharide, while the overall balance of charge of the polymer is also dependent on the acetylation density. Thus, the fewer the acetyl groups on the polymer, the more cationic properties the chitosan will have. The bactericidal properties of chitosan are ascribed to its cationic character, enabling it to bind to negatively charged membranes and biomolecules such as phosphate-rich teichoic acids [10]. Chitosan is also an attractive polymer for its highly biodegradable and biocompatible properties. It is easily hydrolyzed by enzymes, such as lysozymes or cellulases. There are also enzymes specific to chitosan but not chitin called chitosanases that hydrolyze the glycosidic bonds between the deacetylated glucosamines [11, 12].

In terms of its antimicrobial activity, chitosan has been shown to be effective against yeast, bacteria, fungi, with more activity against gram-positive rather than gram-negative bacteria. It has been noted that chitosan primarily prevents bacterial growth rather than directly killing bacteria [13]. After being separated from the chitosan through membrane filtration, bacteria can continue to grow, demonstrating that chitosan does not permanently damage bacteria [14]. Furthermore, this suggests that chitosan is associated outside of bacteria and needs this association for its antimicrobial efficacy. Therefore, chitosan is mostly a bacteriostatic, although it exhibits bactericidal properties when it accesses the bacterial membrane, suffocating bacteria by physically blocking access to nutrients [15]. One curious observation is that the potency of chitosan is not dependent on molecular weight, as the minimum inhibitory concentrations (toward bacteria) of chitosan polymers ranging from 80 to 1500 kDa were all within one order of magnitude [16].

The mechanism of action of chitosan-based polymers is generally assumed to require interactions with the cell envelope, which alters cell surface properties, leading to disruption of cellular function and cell leakage. This is thought to be due to the protonated amino groups allowing chitosan to form strong electrostatic interactions with the negatively charged surface of most microorganisms. It has been shown that chitosan loses its antimicrobial activity above pH 7 and that its antimicrobial activity is dependent on acetylation. These factors determine how positively charged the chitosan polymer is, and both support the idea that the protonated amino groups are essential for chitosan to function as an antibacterial molecule [17].

Chitosan has been functionalized by many different research groups at its amino site in order to modify its biochemical properties. For example, a modified chitosan

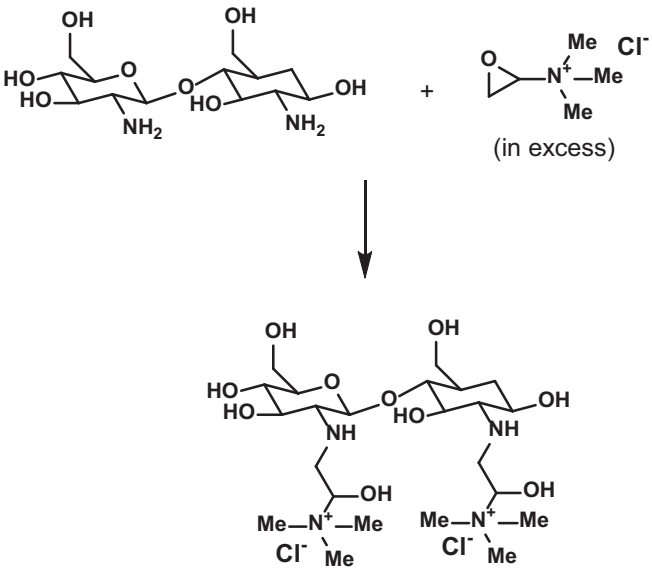


Fig. 1 The reaction scheme for synthesis of the modified chitosan, a one-step process. (Modified from [14])

was formed through the reaction of glycidyltrimethylammonium chloride with chitosan to generate an alkylated version of chitosan with extra quaternary ammonium groups, as seen in Fig. 1 [1]. This chitosan derivative exhibited enhanced antimicrobial activity over regular chitosan, due to the inclusion of the quaternary ammonium groups. Other modifications include the addition of a vinyl sulfonic acid sodium salt to the amino group to generate a zwitterionic structure that has optimal antibacterial properties at pH 5.75 but dropped significantly when the pH was increased to 6.2 [18]. It can be seen that chitosan's chemical structure lends itself readily to modifications that allow its antimicrobial properties to be hybridized with other chemical motifs. Chitosan also has the ability to chelate metal ions such as Cu^{2+}, which can also contribute to additional antimicrobial properties [19]. These options for modification lead to interesting combinations of antimicrobial activities that can have broad spectrum applications due to the diverse chemical groups that can be added to chitosan.

Polymers Containing Quaternized Ammonium

Compounds that contain quaternized ammonium salts (QAS) are another widely studied set of polymers due to their strong antimicrobial properties. Unlike chitosan, these are generally not found naturally and are synthetic polymers that have biocompatible backbones with the quaternized salt attached as a pendant group. The

Environmental Protection Agency (EPA) has revealed that QAS polymers are the most popular when it comes to household disinfectants, as they are found in over 50% of commercially available products [20]. They are also found in cosmetic products, mouthwash, and surface finishings.

In making these polymers, backbones such as polyethylene glycol (PEG), polynorbornene, and poly(ε-caprolactone) (PCL), are used to render the material properties of the compounds. The QAS chains are then added to provide chemical and antimicrobial properties. Generally, the QAS chains that contain 8–18 carbons have the best antimicrobial properties, with shorter lengths being better against gram-positive bacteria and longer lengths better against gram-negative bacteria [21]. The QAS chains also protect polymers like PEG by imparting resistance to redox and acid–base reactions as it protects vulnerable groups on the base polymer. Lastly, due to the diverse structures that QAS polymers can take, they can be made water soluble or water insoluble, meaning that they can be used in a wide variety of medical devices and synthetic transplants.

The mechanism of action of QAS polymers is proposed to be through penetration of the cell membrane and cell wall, leading to eventual cell death [22]. Higher weight polymers have been shown to have higher positive charge densities, which strengthens the adsorption of the polymers onto the surface of microorganisms. Adsorption then facilitates the ability of the polymers to enter the cell membrane. The hole-boring mechanism of action for QAS polymers has been verified through atomic force microscopy and fluorescence correlation spectroscopy. Another factor affecting the activity of these polymers is the structure of the counter anions, with Cl^- being the most effective toward antimicrobial performance. It has been postulated that using the correct counter anion facilitates dissociation of the quaternary salts [23].

The synthesis of these QAS polymers varies greatly, due to the different backbones that can be used. Examples in recent literature include biodegradable versions of PCL that have been grafted with alkyne chains containing QAS motifs or poly(ethylene glycol) methacrylate (PEGMA) with pyridine groups. The addition of the QAS polymers greatly enhances the ability of the polymer to inhibit and kill bacteria, particularly gram-positive bacteria. Naturally occurring primary ammonium modified cellulose has also been discovered in certain bacterial cells as part of a phosphoethanolamine modification, although these polymers have the inverse effect of promoting adhesion to other bacterial fibers and do not impact viability of the producing organisms [24, 25]. However, this does open the door for considerations of generating biosynthetically modified cellulose as a feasible alternative to chemically produced polymers.

Values of minimum inhibitory concentrations (MICs) of different antimicrobial polymers enable quantitative comparisons of the ability of these polymers to prevent bacterial growth. Table 2 shows a comparison between alkyl group functionalized polymers with quaternized nitrogen and commonly used sources of antibacterial potential, such as silver and streptomycin. The MICs for QAS polymers are comparable to traditional antibiotics and silver, although cell lysis and undesired toxicity can occur, as will be discussed in section “Cytotoxicity of Polymers.”

Table 2 Comparison of minimum inhibitory concentrations (MICs) between antibacterial compounds and alkylated QAS polymers

Antibacterial material	MIC in *E. coli* (μg/mL)	MIC in *B. subtillis* (μg/mL)
Silver nanoparticles	12.5	>25
Streptomycin	12.5	25
Ethyl-QAS polymers	200	200
Butyl-QAS polymers	200	200
Hexyl-QAS polymers	12.5	4
Octyl-QAS polymers	4	4
Decyl-QAS polymers	12.5	6
Phenylethyl-QAS polymers	12.5	12.5

Synthetic Protein Mimics

Currently, antimicrobial peptides are being studied as a class of antibiotics that can be used in place of traditional drugs. The major advantage that peptides confer over traditional antibiotics is that they demonstrate broad-spectrum activity against pathogens instead of targeting specific epitopes or enzymes in the microorganisms. There are currently hundreds of antimicrobial peptides that have been studied and databases exist to keep track of them. However, these peptides have had little to no success in being developed as FDA-approved antibiotics. The major obstacle is currently the synthesis of these peptides, as protein synthesis methods are too costly to create industrial amounts of these macromolecules [26].

A new strategy toward simplifying synthesis is to create chemically similar mimics that have the same functional groups as the peptides, whilst being readily accessible via straightforward chemistry like click chemistry. Figure 2 showcases a relatively short process used to create a synthetic mimic of an antimicrobial peptide, which was shown to have strong selectivity against *Staphylococcus aureus* and *Escherichia coli*, while requiring a 10–50 fold increase in concentration before lysing human red blood cells.

The mechanism of action of SMAMPs follows the trend of membrane interaction as seen with most antimicrobial polymers. Currently, there are a few different models that attempt to describe how SMAMPs interact with their target membranes. In the toroidal pore model, the SMAMPs bend the membrane of the target microorganism in order to form toroidal pores that lead to leakage of macromolecules. In the carpet model, the SMAMPs act as a detergent by covering the surface of the membranes, eventually dissolving the membrane and leading to large lesions on the cell surface [27]. Furthermore, there is evidence that SMAMPs target intracellular DNA and RNA, and that they inhibit cell-wall synthesis and nucleic acid synthesis.

SMAMPs have low frequency in selecting for resistant strains while maintaining high target selectivity and fast acting permeabilization of bacterial membranes, making them naturally potent against biofilms [28]. Biofilms derive antibiotic resistance, in part, from their low growth and metabolic rates, which are overcome by SMAMPs. Toward this, it has been shown by Barron et al. that antimicrobial peptoids have strong activities against Pseudomonas biofilms and *Mycobacterium tuberculosis* [29].

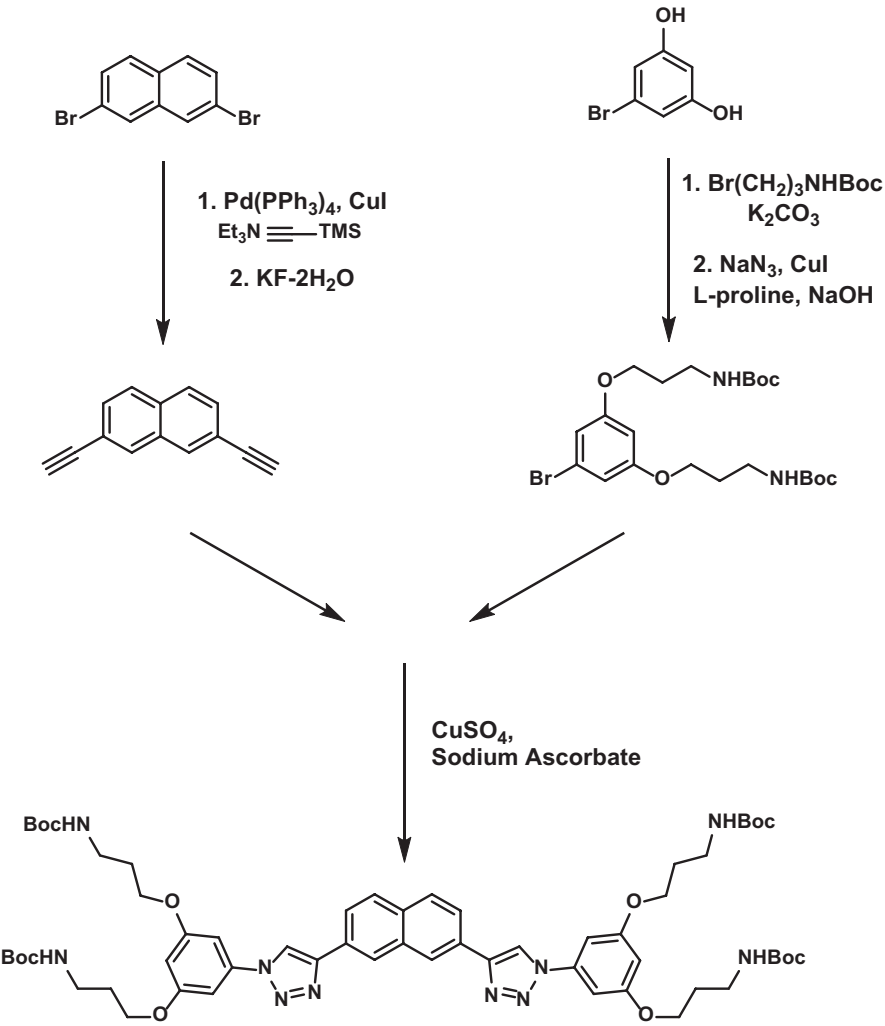


Fig. 2 Standard representative scheme of click chemistry being used to synthesize SMAMPs containing triazoles. (Modified from [18])

Polyethylenimines

Polyethylenimine (PEI) is a synthetic, cationic polymer that is not biodegradable but contains multiple, differently functionalized nitrogens. This allows for a wide variety of chemical modifications toward these amino groups, as the variable substitution levels on the amines of this polymer have different reactivity profiles. For example, alkylation of these polymers was shown to greatly increase the bactericidal activity against *S. aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *E. coli* by over 30% [30]. This strong bactericidal activity is due to the polycationic nature of PEI, as well as the ability of the alkyl groups to greatly

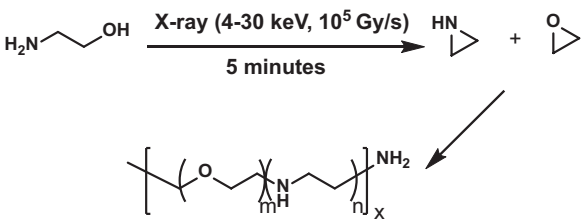


Fig. 3 Synthesis of linear-like PEI-co-PEG via X-ray irradiation. (Modified from [23])

increase association with bacterial membranes. Another property of the polymer is that it can be synthesized as either a branched or linear form, again allowing for flexibility in its functionalization and material properties.

Functionalized PEI polymers have been shown to be potent transfection agents in addition to exhibiting antibacterial properties [31]. This is likely due to its ability to bind to DNA, thus helping gene transfection as PEI-DNA complexes help open up the DNA to gene therapy agents. This property has been translated toward antiviral properties, as it has been shown that PEI can be used to inhibit the activities of papillomaviruses and cytomegaloviruses. Incubation of cells with PEI caused the virus to be unable to bind to the cells, and PEI was also shown to lack cytotoxic effects at the relevant concentrations required for viral inhibition [32]. It is thought that PEI inhibits the viral ability to bind to heparin sulfate proteoglycans that most strains of human papillomavirus rely on.

Synthesis of PEI polymers is relatively straightforward, as it generally involves acid-catalyzed polymerization of aziridines, followed by hydrolysis. Furthermore, it can also be synthesized in conjunction with other polymers, such as PEG, to form copolymers in an efficient and quick manner, as shown in Fig. 3 [33]. This method yields the copolymer with a mono-dispersive molecular weight and can be performed in an aqueous solution, ideal for translation into biological purposes.

Halamines

There are two types of halogen containing polymers that are being studied with respect to antimicrobial properties. The first category is halamines, which are molecules that contain nitrogen-halogen covalent bonds. These are the more common type of halogenated polymers, and the second type refers to polymers with halogens attached to other atoms. Halamines are highly stable in both aqueous and dry conditions, environmentally friendly, and have shown stability over long periods of time. These polymers provide a source of slowly released, active halogen species that inhibit the activity of many types of microbial organisms. They are commonly used as coatings made via electrogeneration or polymerization on the surfaces of textiles and healthcare products [34].

Halamines have been shown to have broad-spectrum activities against microorganisms, and are considered safe for human health [35]. They are also used for their unique ability to recharge halogens, as they can be reacted with halogen donors such as sodium hypochlorite. This gives halamines their renewable nature, meaning that

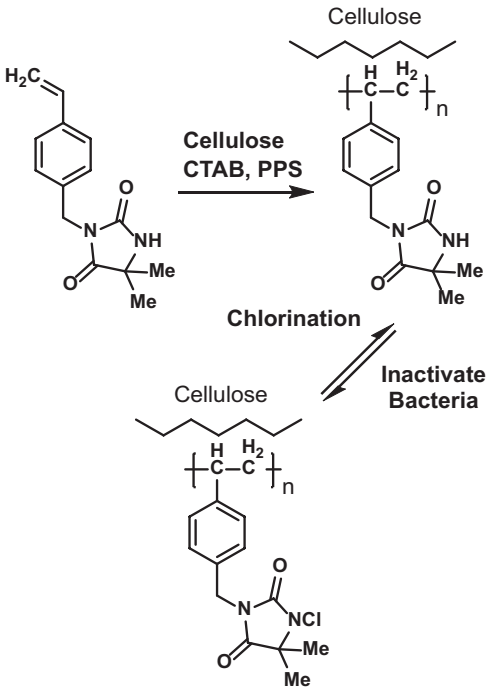


Fig. 4 Synthesis of *N*-halamine biocidal cellulose. Cetrimonium bromide (CTAB), polyphenylene sulfide (PPS)

they can retain their antimicrobial properties indefinitely, even after their initial dose of active halogens has been released [36]. These polymers have been shown to be effective against *S. aureus* and *E. coli* in cotton swatch tests and could regenerate up to 70% of the chlorine lost after washing [37]. The activity of halamines is attributed to the released halogens being active radicals that inhibit or inactivate microbes. This is attributed to the radicals' ability to rapidly penetrate membranes of microorganisms and attack key proteins, as well as DNA. This is seen in iodine, chlorine and bromine, and they are all strongly active against fungi, bacteria, spores and viruses.

The synthesis of halamines generally involves the formation of a covalent bond of an *N*-halamine precursor with the target polymer. For example, cellulose is chlorinated and converted into biocidal cellulose in Fig. 4 [38]. Another common precursor for N-halamines is hydantoin, as it contains two secondary amines that are readily available for reaction, which can then be halogenated with ease. N-halamines can also be copolymerized with monomers such as siloxane, which can then be coated onto cotton fabrics and are highly potent against both gram-negative and gram-positive bacteria [39].

Cytotoxicity of Polymers

As previously mentioned, an important drawback to using antimicrobial polymers often lies in the mechanism of their unique potency, which serves as a double-edged sword. Although these polymers can provide nearly indefinite resistance toward

Table 3 Comparison of DOBAB activity against different human and bacterial cells

Cell type	Cell count	DOBAB conc. at 50% survival (μg/mL)
Kidney epithelial cells	10^5	3400
3T3(cloneA31) fibroblasts	10^4	631
SV40-SVT2 fibroblasts	10^4	631
E. coli	2×10^7	17.7
S. typhimurium	2×10^7	6.3
P. aeruginosa	3×10^7	3.2

growth of microorganisms on materials used for transplants and tissue growth, they also act indiscriminately, thereby targeting and lysing human cells as well. Therefore, when evaluating the effectiveness of certain polymeric materials, selectivity of potency toward human and foreign cells and organisms is a crucial component of determining the usefulness of a polymer.

In terms of selectivity, a key value to look at is the hemolytic capability of the polymers. Due to the negatively charged surface of red blood cells (RBCs), care must be taken when designing polymers that have polycationic charges. It is often seen that the polymers not only target bacterial cell surfaces, but RBCs as well. An example of this can be seen in a brominated ammonium compound, dimethyldioctadecylammonium bromide (DODAB), as shown in Table 3 [40]. As expected, the fibroblasts required roughly five times less material to fall below a 50% survival in comparison to kidney epithelial cells, but the material still shows promising selectivity against multiple strains of bacteria.

Furthermore, it is fairly common to see alkyl chain lengths and molecular weight strongly affect the hemolytic capabilities of the polymers. Figure 5 shows a study conducted on QAS polymers of different alkyl chain lengths and different molecular weights [41]. The general trend is that the more hydrophobic the molecules are, the more hemolytic activity they show, but increasing the molecular weight of the polymer offsets this to a degree.

Future Directions

Tissue engineering is an emerging interdisciplinary field that combines various disciplines, including chemistry, biology, and material science [42]. Although the current approach toward scaffold design principally utilizes polyhydroxyl acids due to their degradation profile for controlled drug release, the combination of drugs and scaffold material is a promising direction for the field [43]. The long-term goal would be to create scaffolds that have minimal infection risks without relying on the degradation of the scaffold itself for timed release. Toward this endeavor, the field has yet to design polymers with sufficient material and antibiotic properties that pose no significant threat to the surrounding cells and tissues.

At the present, a viable subset of AMPs is quaternary ammonium salts due to their broad spectrum antimicrobial activity and long-term biocidal efficiency [44].

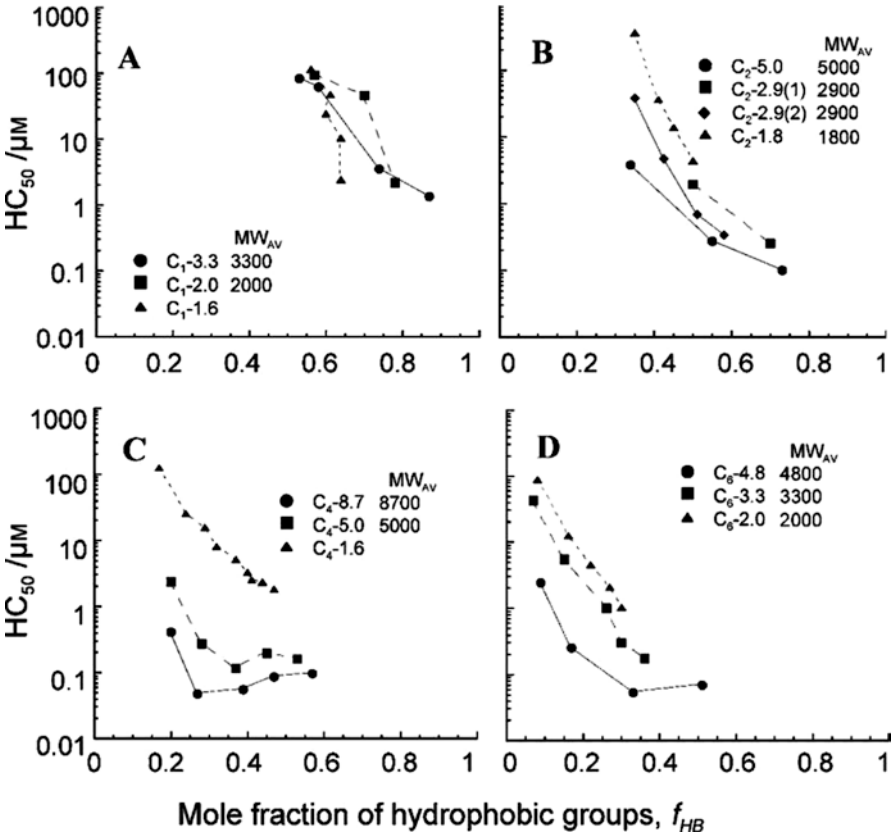


Fig. 5 Comparison of alkyl chain lengths and molecular weights to hemolytic ability (HC_{50}). (Modified from [29])

Much effort has been directed toward improving the biocompatibility of such AMPs, as they have generally proven toxic at high concentrations to nearby erythrocytes. Chitosan derivatives containing QAS moieties have been explored for their relatively low toxicity and flexible material properties [45]. Although these polymers have been tested in vitro, many of the synthesized materials have not been implemented or characterized in vivo. In addition, the long-term viability of these materials as scaffolds has been largely untouched, despite their long-lived antimicrobial properties.

Another subset of antimicrobial materials involves the application of peptides or peptoids due to their antibiofilm properties. These molecules exert substantial effects toward biofilm prevention and dispersal, as well as direct killing of biofilm cells [46–48]. Their biocompatibility is also not of concern as these peptides often have human origins [49]. However, these molecules are not suitable toward scaffold construction and instead could serve as material coatings, for example, which have a propensity to require maintenance over time. Lastly, the cost-effectiveness of bulk manufacturing of these peptides is of concern, causing these coatings to remain elusive in practical applications [50].

Conclusion

There are a wide variety of polymers and molecules that have been synthesized over recent years to exhibit strongly antimicrobial properties. The biggest setbacks toward application of these polymers are cost of production (generally for protein mimics), renewability and degradation rate, and cytotoxicity. However, there are promising polymers that have been functionalized to ameliorate these concerns, and both engineering and chemical advances have pushed our ability to generate these molecules with both financial and temporal expediency.

The direction of the AMP field seems to be trending toward protein mimics or antimicrobial peptides, as well as a hybrid of multiple subsets of AMPs. These types of polymers are naturally biocompatible and biodegradable, and generally avoid the cytotoxic pitfall that other types of polymers fall into. The major issue for protein-inspired antimicrobial molecules is their production costs. However, due to the rapid rate of advancement in biological sciences, it is only a matter of time before affordable synthetic paths or large-scale bio-production of these molecules becomes feasible, thus facilitating the route toward commercial application of these polymers. At the same time, the need is great and many opportunities exist for the entry of alternative and creative solutions to identify and develop antimicrobial treatment and prevention strategies.

Acknowledgments This work was partially supported by NIH grants R01AR057837, R01AR074458, R01AR072613, R01GM117278, and U01AR069395.

References

1. Kenawy E-R, Worley SD, Broughton R (2007) The chemistry and applications of antimicrobial polymers: a state-of-the-art review. Biomacromolecules 8(5):1359–1384
2. Gao P, Nie X, Zou M, Shi Y, Cheng G (2011) Recent advances in materials for extended-release antibiotic delivery system. J Antibiot 64(9):625–634
3. Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJV (2014) Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 13(1):42–51
4. Kim CH, Choi JW, Chun HJ, Choi KS (1997) Synthesis of chitosan derivatives with quaternary ammonium salt and their antibacterial activity. Polym Bull 38:387–393
5. Ilker MF, Nüsslein K, Tew GN, Coughlin EB (2004) Tuning the hemolytic and antibacterial activities of amphiphilic polynorbornene derivatives. J Am Chem Soc 126(48):15870–15875
6. Raafat D, Sahl H-G (2009) Chitosan and its antimicrobial potential—a critical literature survey. Microb Biotechnol 2(2):186–201
7. Tan S (1998) The degree of deacetylation of chitosan: advocating the first derivative UV-spectrophotometry method of determination. Talanta 45(4):713–719
8. Yuan Y, Chesnutt BM, Haggard WO, Bumgardner JD (2011) Deacetylation of chitosan: material characterization and in vitro evaluation via albumin adsorption and pre-osteoblastic cell cultures. Materials 4(12):1399–1416
9. Singla AK, Chawla M (2001) Chitosan: some pharmaceutical and biological aspects—an update. J Pharm Pharmacol 53(8):1047–1067

10. Foster LRA, Ho S, Hook J, Basuki M, Marçal H (2015) Chitosan as a biomaterial: influence of degree of deacetyla-tion on its physiochemical, material and biological proper-ties. PLoS One 10(8):e0135153
11. Kimoto H (2002) Biochemical and genetic properties of Paenibacillus glycosyl hydrolase having chitosanase activity and discoidin domain. J Biol Chem 277(17):14695–14702
12. Rivas LA, Parro V, Moreno-Paz M, Mellado RP (2000) The Bacillus subtilis 168 csn gene encodes a chitosanase with similar properties to a streptomyces enzyme. Microbiology 146(11):2929–2936
13. Raafat D, Bargen KV, Haas A, Sahl H-G (2008) Insights into the mode of action of chitosan as an antibacterial compound. Appl Environ Microbiol 74(12):3764–3773
14. Jarry C, Chaput C, Chenite A, Renaud M-A, Buschmann M, Leroux J-C (2001) Effects of steam sterilization on thermogelling chitosan-based gels. J Biomed Mater Res 58(1):127–135
15. Benhabiles M, Salah R, Lounici H, Drouiche N, Goosen M, Mameri N (2012) Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste. Food Hydrocoll 29(1):48–56
16. Tamara F, Lin C, Mi F-L, Ho Y-C (2018) Antibacterial effects of chitosan/cationic peptide nanoparticles. Nanomaterials 8(2):88
17. Liu XF, Guan YL, Yang DZ, Li Z, Yao KD (2000) Antibacterial action of chitosan and carboxymethylated chitosan. J Appl Polym Sci 79(7):1324–1335
18. Jung B-O, Kim C-H, Choi K-S, Lee YM, Kim J-J (1999) Preparation of amphiphilic chitosan and their antimicrobial activities. J Appl Polym Sci 72(13):1713–1719
19. Miyoshi H, Shimura K, Watanabe K, Onodera K (1992) Characterization of some fungal chitosans. Biosci Biotechnol Biochem 56(12):1901–1905
20. Liang Z, Zhu M, Yang Y-W, Gao H (2013) Antimicrobial activities of polymeric quaternary ammonium salts from poly (glycidyl methacrylate)s. Polym Adv Technol 25(1):117–122
21. Gilbert P, Moore L (2005) Cationic antiseptics: diversity of action under a common epithet. J Appl Microbiol 99(4):703–715
22. Rawlinson L-AB, Ryan SM, Mantovani G, Syrett JA, Haddleton DM, Brayden DJ (2010) Antibacterial effects of poly(2-(dimethylamino ethyl)methacrylate) against selected gram-positive and gram-negative bacteria. Biomacromolecules 11(2):443–453
23. Kanazawa A, Ikeda T, Endo T (1993) Polymeric phosphonium salts as a novel class of cationic biocides. II. Effects of counter anion and molecular weight on antibacterial activity of polymeric phosphonium salts. J Polym Sci A Polym Chem 31(6):1441–1447
24. Thongsomboon W, Serra DO, Possling A, Hadjineophytou C, Hengge R, Cegelski L (2018) Phosphoethanolamine cellulose: a naturally produced chemically modified cellulose. Science 359(6373):334
25. Hollenbeck EC, Antonoplis A, Chai C, Thongsomboon W, Fuller GG, Cegelski L (2018) Phosphoethanolamine cellulose enhances curli-mediated adhesion of uropathogenic Escherichia coli to bladder epithelial cells. Proc Natl Acad Sci 115(40):10106
26. Fu T-H, Li Y, Thaker HD, Scott RW, Tew GN (2013) Expedient synthesis of SMAMPs via click chemistry. ACS Med Chem Lett 4(9):841–845
27. Li Y, Xiang Q, Zhang Q, Huang Y, Su Z (2012) Overview on the recent study of antimicrobial peptides: origins, functions, relative mechanisms and application. Peptides 37(2):207–215
28. Batoni G, Maisetta G, Brancatisano FL, Esin S, Campa M (2011) Use of antimicrobial peptides against microbial biofilms: advantages and limits. Curr Med Chem 18(2):256–279
29. Kapoor R, Wadman MW, Dohm MT, Czyzewski AM, Spormann AM, Barron AE (2011) Antimicrobial peptoids are effective against Pseudomonas aeruginosa biofilms. Antimicrob Agents Chemother 55(6):3054–3057
30. Lin J, Qiu S, Lewis K, Klibanov A (2002) Bactericidal properties of flat surfaces and nanoparticles derivatized with alkylated polyethylenimines. Biotechnol Prog 18(5):1082–1086
31. Coll J-L, Chollet P, Brambilla E, Desplanques D, Behr J-P, Favrot M (1999) In vivo delivery to tumors of DNA complexed with linear polyethylenimine. Hum Gene Ther 10(10):1659–1666

32. Spoden GA, Besold K, Krauter S, Plachter B, Hanik N, Kilbinger AFM, Lambert C, Florin L (2011) Polyethylenimine is a strong inhibitor of human papillomavirus and cytomegalovirus infection. Antimicrob Agents Chemother 56(1):75–82
33. Samal SK, Dash M, Vlierberghe SV, Kaplan DL, Chiellini E, Blitterswijk CV, Moroni L, Dubruel P (2012) Cationic polymers and their therapeutic potential. Chem Soc Rev 41(21):7147
34. Hui F, Debiemme-Chouvy C (2013) Antimicrobial N-halamine polymers and coatings: a review of their synthesis, characterization, and applications. Biomacromolecules 14(3):585–601
35. Chen Y, Wang L, Yu H, Shi Q, Dong X (2007) Synthesis, characterization, and antibacterial activities of novel N-halamine copolymers. J Mater Sci 42(11):4018–4024
36. Denyer SP, Stewart G (1998) Mechanisms of action of disinfectants. Int Biodeterioration Biodegradation 41:261
37. Ren X, Kocer HB, Worley S, Broughton R, Huang T (2009) Rechargeable biocidal cellulose: Synthesis and application of 3-(2,3-dihydroxypropyl)-5,5-dimethylimidazolidine-2,4-dione. Carbohydr Polym 75(4):683
38. Ulman KN, Shukla SR (2015) Admicellar polymerization and its application in textiles. Adv Polym Technol 35(3):307–325
39. Kocer HB, Worley S, Broughton R, Huang T (2011) A novel N-halamine acrylamide monomer and its copolymers for antimicrobial coatings. React Funct Polym 71(5):561
40. Carmona-Ribeiro A, Carrasco LDM (2013) Cationic antimicrobial polymers and their assemblies. Int J Mol Sci 14(5):9906–9946
41. Kuroda K, Caputo GA, Degrado WF (2008) The role of hydrophobicity in the antimicrobial and hemolytic activities of polymethacrylate derivatives. Chem A Eur J 15(5):1123–1133
42. Langer R, Vacanti JP (1993) Tissue engineering. Science 260:920–926
43. Howard D, Buttery LD, Shakesheff KM, Roberts SJ (2008) Tissue engineering: strategies, stem cells and scaffolds. J Anat 213(1):66–72
44. Xue Y, Xiao H, Zhang Y (2015) Antimicrobial polymeric materials with quaternary ammonium and phosphonium salts. Int J Mol Sci 16(2):3626–3655
45. Martins AF, Facchi SP, Follmann HDM, Pereira AGB, Rubira AF, Muniz EC (2014) Antimicrobial activity of chitosan derivatives containing N-quaternized moieties in its backbone: a review. Int J Mol Sci 15(11):20800–20832
46. Strempel N, Strehmel J, Overhage J (2014) Potential application of antimicrobial peptides in the treatment of bacterial biofilm infections. Curr Pharm Des 21(1):67–84
47. Fuente-Nunez CDL, Korolik V, Bains M, Nguyen U, Breidenstein EBM, Horsman S, Lewenza S, Burrows L, Hancock REW (2012) Inhibition of bacterial biofilm formation and swarming motility by a small synthetic cationic peptide. Antimicrob Agents Chemother 56(5):2696–2704
48. Jenssen H, Hamill P, Hancock RE (2006) Peptide antimicrobial agents. Clin Microbiol Rev 19:491–511
49. Jacobsen AS, Jenssen H (2012) Human cathelicidin LL-37 prevents bacterial biofilm formation. Future Med Chem 4(12):1587–1599
50. Kargupta R, Bok S, Darr CM, Crist BD, Gangopadhyay K, Gangopadhyay S, Sengupta S (2014) Coatings and surface modifications imparting antimicrobial activity to orthopedic implants. Wiley Interdiscip Rev Nanomed Nanobiotechnol 6(5):475–495

Engineering Approaches to Create Antibacterial Surfaces on Biomedical Implants and Devices

Ruwen Tan, Jin Yoo, and Yeongseon Jang

Abstract Bacterial adhesion and biofilm formation on biomedical surfaces remain the annoying problems in global public health, causing severe infectious diseases and increasing health care costs. Moreover, the continued increase in the number of multidrug-resistant bacteria and their fast evolution induce a serious concern with the lack of development of new antimicrobials. These problems have initiated numerous research efforts to develop more effective antimicrobial surfaces through different engineering approaches to prohibit bacterial adhesion and subsequent biofilm formation. In this review, we summarize the engineering technologies for constructing antibacterial surfaces from the conventional to the cutting-edge strategies. Most of the traditional methods are based on the antifouling coatings and the release of toxic biocides from the chemically modified substrates. Antimicrobial nanoparticles can actively inhibit biofilm formation or other essential processes in the drug resistance mechanisms of bacteria. Thus, the combined use of bactericidal nanoparticles and antifouling polymers for functionalized organic–inorganic platforms has been investigated to enhance antibacterial performance. In recent years, unique surface topographies of antibacterial, natural surfaces have been discovered and studied with the increased understanding of the interaction between bacteria and substrates. We introduce various natural surfaces and artificial implantable biomaterials, which present the bactericidal surface topographies, along with their bactericidal mechanisms and efficiency. The use of biomimetic, nanotextured surfaces is a promising approach to overcome the current challenges for the treatment of multidrug-resistant bacteria.

Keywords Antimicrobial · Antifouling · Bactericidal · Implant · Coating · Surface engineering · Functional surface · Nanotechnology · Nanostructure

R. Tan · Y. Jang (✉)
Department of Chemical Engineering, University of Florida, Gainesville, FL, USA
e-mail: y.jang@ufl.edu

J. Yoo
School of Chemical and Biological Engineering, Seoul National University, Seoul, Republic of Korea

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_14

Introduction

Surgical procedures with implantation of biomedical devices have saved and improved the quality of life of numerous patients. The implantable medical devices are being used in many different parts of the body including orthopedic, cardiovascular, ophthalmic, or gastroenterological implants for various applications [1]. Given applications, diverse types of materials used for the medical implants and devices have been developed, ranging from pure metals, metal alloys, ceramics to polymers. Mechanical properties, corrosion resistance and biocompatibility of the materials, as well as fabrication methods and processing costs, are the key parameters to determine the success of the biomedical implants and devices, which the engineers must consider when designing advanced biomaterials.

Despite considerable efforts in developing implantable biomedical devices, the infectious problems persist accompanied by bacterial adhesion and growth on the surfaces. Bacterial infections are considered a challenge in the global health care units, which can lead to life-threatening problems or incurring substantial costs. Therefore, preventing bacterial adhesion and colonization of the surfaces of biomedical implants and devices is essential to mitigate pathogenic bacteria-associated infections. To create antibacterial properties of biomaterials, many researchers mainly focus on the development of surface features that are unfavorable for bacterial attachment and growth by engineering surface chemistry or physical textures. Herein, we review several engineering approaches to develop biomedical implants and devices for antibacterial performance by using diverse surface treatments with chemical or physical ways. The antibacterial surfaces can be achieved by three major categories: (1) sustained release of antibacterial agents, (2) repelling bacterial adhesion (antifouling), and (3) contact-killing.

First, antibacterial coatings with antibiotics, antimicrobial nanoparticles, or antifouling polymers have been widely used as one of the global strategies to inhibit bacterial infections by mitigating colonization [2]. We introduce several surface coatings using functionalized polymers (section “Surface Coatings Using Functionalized Polymers”), antimicrobial nanoparticles, and inorganic-organic hybrids (section “Surface Modification with Antimicrobial Nanoparticles and/or Inorganic–Organic Hybrids”), from traditional to recent approaches. The coating methods with functional molecules aim at preventing bacterial adhesion through antifouling surfaces or the controlled release of antibacterial agents from a chemical point of view. The chemical modification has advantages of the versatility to apply diverse materials, regardless of substrate macrostructure, and relatively easy and low-cost fabrication process. Nonetheless, coatings can have challenges of the possibility of drug resistance, delamination, and/or functionality loss due to thermal, hydrolytic, or solvent-induced degradation.

We can learn the lessons from Nature to design antibacterial surfaces without additional chemical treatments. Indeed, biomimetics has inspired many researchers in an interdisciplinary field of engineering, chemistry, and biology to develop new advanced materials that mimic outstanding biological, natural functions. In section “Biomimicry Toward Advanced Antimicrobial Surfaces,” we review several gripping

natural surfaces (e.g., insect wings, herb leaves, and animal skin), which display excellent antimicrobial properties, and summarize critical features that result in the reduced bacterial adhesion or the increased killing efficacy. We also discuss recent engineering techniques to fabricate artificial structures that mimic such natural surfaces with high antibacterial efficiency in section "Nature-Inspired, Nanostructured Surface Development for Antibacterial Properties." Several studies to understand the relationship between surface structure and bacterial adhesion are introduced along with the efforts to discover the most appropriate materials and methods for each practical use.

It would be feasible to apply the approaches discussed in this chapter for making a perfectly sterile environment in the surgical operating rooms by realizing desired surface properties of surgical tools and tables, monitoring equipment, injection tubes, and drapes. However, translational studies to utilize the surface engineering strategies for the clinical studies and the practical uses remain, which requires a collaborative effort from material researchers, engineers, medical doctors, and regulatory agencies. The multidisciplinary research and development will be the next step toward advanced antibacterial biomaterials design and application. Next-generation biomedical implants and devices should also exhibit multi-functionalities, long-term stability, and enhanced therapeutic properties, in addition to antibacterial properties. We review the current challenges in the latest generation of antibacterial surfaces and propose future respective in section "Prospective Approaches."

Engineering Strategies to Create Antibacterial Surfaces on Biomedical Implants and Devices

Advances in biomedical engineering have been driven by the development of new biomaterials including therapeutic agents, implants with desired mechanical properties and improved corrosion resistance, and de novo functional small molecules or polymers. Notably, the biological properties of materials, which include biocompatibility, biofouling effect, biodegradability, or cytotoxicity, are directly linked to the surface properties. In other words, the interaction of bacterial cells with biomaterials at surfaces dictates the cell fates, which controls adhesion, colonization, and the corresponding infections [3]. Given a systematic understanding of the surface–bacteria interactions, we can rationally design new classes of biomedical implants and devices and engineer their surface properties to inhibit bacterial cell adhesion and growth while promoting profitable cell growth.

This chapter reviews a variety of engineering strategies to create antibacterial surfaces on biomedical implants and devices in the two main categories: (1) chemical surface coatings and modifications and (2) physical surface texture developments. We summarize the surface fabrication methods from traditional approaches to recent events with a discussion of possible mechanisms of cell adhesion and growth on the engineered surfaces. Moreover, we highlight several examples of antibacterial surfaces in nature, which inspire to the next-generation engineers for advanced antifouling, bactericidal surface developments on artificial materials.

Surface Coatings Using Functionalized Polymers

Polymers have been actively explored as advanced coating materials for a myriad of biomedical applications, based on the molecular tunability (i.e., molecular weights, chemical properties of pendent groups, chain flexibility, and so on) and the corresponding diverse functionalities [4]. Several coating techniques of polymers on metals or metal alloys, for instance, layer-by-layer deposition [4], conventional organic coatings (e.g., dipping, spinning, spraying) [5], or brush formation [6], enable functionalization and protection of the surfaces from corrosive or other stimuli attacks.

Proteins are adsorbed on the implanted material surfaces by forming a thin layer in blood, leading to the promotion of bacterial cell attachment. Thus, for an effective antibacterial surface, it is essential to impart antifouling properties that repel proteins and bacteria at the surfaces. First, we highlight widely used polymers as coatings to prevent nonspecific protein adhesions on medical implants in section "Antifouling Polymer Coatings to Prevent Bacterial Adhesion" (i.e., passive approaches). Additionally, we can avoid bacterial infections by coating the surfaces with antibiotics or antimicrobial nanoparticles that can kill adhered, pathogenic microorganisms (i.e., active approaches). Functionalized polymer coatings that release such antibacterial agents can improve the bactericidal performance of the biomedical surfaces. In section "Bactericidal Activity of Functionalized Polymer Coatings," we introduce surface coating strategies based on functionalized polymers with such active antibacterial properties.

Antifouling Polymer Coatings to Prevent Bacterial Adhesion

To achieve an effective antifouling coating for the bacteria-repellent property, antifouling polymers such as zwitterionic [7], peptidomimetic polymers [8–9], or poly(ethylene glycol) (PEG) [10] have been widely employed, as shown in Fig. 1. The strategy to utilize such antifouling polymers for surface repellency against bacteria can be rationalized by the steric repulsion effect and the formation of the hydration layer [11]. Entropic instability induces the steric repulsion that prevents bacterial adhesion, while the hydrogen bonding interaction with PEG molecules or the electrostatic interaction with zwitterionic molecules forms a hydration layer to deter nonspecific interaction between cell and substrate. The attachment and binding of fouling agents to the surface are energetically unfavorable due to the energy barrier which must be overcome to disrupt the hydration layer [11].

PEG is a nontoxic, non-immunogenic, and uncharged polymer that is soluble in aqueous and many organic solvents, leading to broad utilization in a number of studies [12]. Since the PEG chains are hydrophilic, highly mobile and attain huge exclusion volumes, they prohibit adsorption of cell and protein at the surfaces [13]. Antifouling PEG polymers can be coated on surfaces *via* diverse techniques including self-assembled monolayer (SAM), physical adsorption, or chemical grafting.

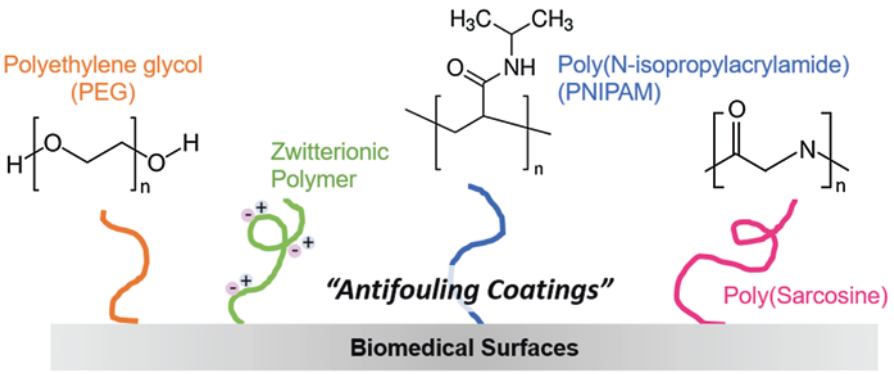


Fig. 1 Representative chemical structures of antifouling polymers commonly used for the surface modification of biomedical devices

Immobilizing PEG through SAM formation is one of the most commonly used approaches to impart passive antifouling activity to a surface. Prime et al. reported that SAMs made from PEG showed remarkable protein resistance [14]. Also, PEG molecules can be chemically grafted on surfaces using grafting-to or grafting-from methods. An important parameter in determining the antifouling performance of the PEG layer is the density of polymer molecules and the chain length. In general, the increase of chain length (i.e., polymer molecular weight) leads to a decreased number of adhering bacterial cells and higher grafting density results in more effective antifouling surfaces [15].

Apart from PEG, zwitterionic antifouling polymers have attracted significant attention due to their remarkable biofouling resistance, based on the high degrees of ionic hydration [7]. Zwitterionic polymers composed of both cationic and anionic groups with a unique molecular structure exhibit overall charge neutrality with high hydrophilicity. A broad spectrum of zwitterionic polymers can be synthesized with different chemical structures, whereas PEGs share the same repeating units. In this regard, the targeted library of zwitterionic brushes with varying densities of charge, hydration, chain lengths, and grafted chain densities has been quantitatively evaluated for their antifouling properties [16].

A few other hydrophilic polymer brushes have also been developed as antifouling coating materials. For example, poly N-isopropylacrylamide (PIPAAM), a thermo-responsive polymer with the lower critical solution temperature (LCST) behavior, which is grafted on Ti surfaces successfully induced significant detachment of bacteria upon rinsing at room temperature [17]. Polyacrylamide (PAAm) brushes coated on silicon rubber surfaces also showed effective resistance against attachment of proteins and bacteria. Furthermore, peptide-based coatings, such as self-assembled monolayers made from oligopeptide and serum albumin blocking layers, have been proposed for antifouling applications [18, 19].

Bactericidal Activity of Functionalized Polymer Coatings

Although the passive antifouling approaches have shown broad applicability, inherent limitations to these approaches remain when dealing with proliferative fouling due to their inability to suppress the colonization of bacteria. An alternative strategy is to construct a surface that can actively inhibit microbial colonization by killing bacteria. The active approach can be divided into the controlled release of antimicrobial agents and non-release-based antimicrobial systems.

Antibiotic- or antiseptic-releasing coatings are prepared either by soaking the carrier material coated with polymers in a solution containing antibiotics or by directly impregnating into the coating material. The release of the antibiotics can be controlled by manipulating the composition and concentration of the coating formulation. For example, Hammond and co-workers have used layer-by-layer (LbL) deposition technique to fabricate polyelectrolyte multilayer films containing an antibiotic agent, gentamicin. The LbL multilayered heterostructure was composed of hydrolytically degradable poly(ß-amino ester), biocompatible polyanionic hyaluronic acid, and gentamicin [20]. The gentamicin loading density, as well as its release rate, can be controlled by tuning of hydrophilicity and electrostatic interactions between the polymeric components in the films. In a similar approach, antimicrobial peptides were incorporated into a microgel by electrostatic interactions. Bactericidal efficiency of the peptide-loaded microgels was achieved *via* both direct contact-killing and release of incorporated peptides. The antimicrobial effects were governed by the release rate of the bactericidal peptides from the microgel, controlled by ionic strength in the solution that affects the electrostatic interactions of the chain scaffold components [21].

Despite many useful applications of the antibacterial systems that release antimicrobial agents, there remains limitation such as the difficulty of long-term use of bleaching agents, i.e., eventual depletion of agents. To circumvent the issues, surface-mounted antibacterial agents that kill bacteria by contact have served as a viable alternative. Cationic polymeric materials with cationic antimicrobial groups (e.g., quaternary ammonium (QA), phosphonium (QP) and guanidinium groups, etc.) have been designed and applied to fabricate surfaces with bacterial contact-killing features [22]. Besides, alkyl pyridinium was reported by Tiller et al. as an active antibacterial agent, resulting in effective contact-killing against bacteria [23]. Specifically, poly(vinyl-*N*-pyridinium bromide) covalently attached to various surfaces was reported to show 99% killing efficiency of both Gram-negative and Gram-positive bacteria [24].

Various bio-based polymers, such as chitosan and cellulose, are well known to have antimicrobial compounds of biological, chemical origins [25]. Coating with nanofibers of such biopolymers has been developed in this field to increase antimicrobial performance with high surface area. Recently, Correia et al. demonstrated that the chitosan nanofiber scaffolds grafted with antimicrobial oligomers induced efficient contact-killing of bacteria [26]. The electrostatic interaction between polycationic chitosan and the anionic exterior surface of microbes leads to the disruption

of the cell membrane and leakage of intracellular components. At the same time, DNA transcription and protein synthesis are interrupted by the penetration of chitosan into the cell membrane [27]. While chitosan shows effectively bactericidal efficacy against both Gram-negative and Gram-positive bacterial cell, chitosan is biocompatible to mammalian cells [25]. Chitosan-based nanofibers can be fabricated by electrospinning technique, which uses electric fields to make fibers in the order of hundreds of nanometers in diameter from charged polymer solutions or melts. Chitosan and its derivatives are successfully electrospun into antibacterial nanofibers as shown in several examples [28–30]. In addition, cellulose-based nanofibers formed *via* electrospinning or surface graft polymerization exhibited antimicrobial property with incorporation of antimicrobial agents or grafted functional groups [31–33]. Besides, diverse nanofibers made from different antimicrobial polymers have been created for bactericidal applications with high surface exposure area [34–36].

In addition to polymeric coatings, some nanomaterials, mainly inorganic nanoparticles including metals and metal oxides can also impart fouling resistance to surfaces. They have also attracted considerable attention because of their superior antibacterial activity with ameliorating the fouling resistance property. We will study the surface modification strategies to utilize antimicrobial inorganic nanoparticles in hybrid form in the next section.

Surface Modification with Antimicrobial Nanoparticles and/or Inorganic–Organic Hybrids

Bacterial cells primarily exist in robust communities by colonization on surfaces, known as biofilm, which is highly resistant to treatment with antibiotics. Biofilm formation of multidrug-resistant bacteria is a huge issue in a global health care system. Therefore, development of novel engineering approaches to actively prohibit the biofilm formation as well as the related infections is highly in demand rather than conventional antibiotic release from antifouling surfaces. This part focuses on the recent advances in antimicrobial nanoparticles (NPs) and inorganic–organic hybrid platforms, which may offer a promising solution for developing long-lasting antibacterial surfaces.

Antibacterial Nanoparticles

Antibacterial nanoparticles (NPs) are talent materials, as they can not only combat bacteria by themselves but also play a role as carriers for other biocidal agents. Two general advantages of antibacterial NPs are the distinctive antibacterial efficacy achieved by high surface to volume ratios due to the ultrasmall size and the potential to functionalization with different (bio)molecules [37].

The antimicrobial NPs exert bactericidal activity *via* different and extensive mechanisms. In other words, antibacterial NPs do not present the monotonous actions like standard antibiotics. They can directly interact with the cell wall of bacteria while preventing biofilm formation and triggering immune responses in the body. Ultimately, antibacterial NPs dispersed in a solution phase trigger reactive oxygen species (ROS) generation and intercellular damage of bacterial cells by interactions with nucleic acids and proteins [38].

Among different antibacterial NPs, silver NPs (AgNPs) are considered the most effective antibacterial agent [39]. Several mechanisms have been proposed to explain AgNPs antimicrobial activity [40, 41]. The adsorption of AgNPs leads to the depolarization of the bacterial cell wall, thereby inducing cell membrane disruption. Then, penetration of AgNPs produces ROS that inhibits ATP production and DNA replication. Furthermore, there is an evidence that an oxidative dissolution of AgNPs involves the release of Ag^+ species, known to exhibit antibacterial activity. Since the Ag^+ possesses a high affinity with amines, phosphates, and most thiols, it ultimately weakens protein functions that contain amine, phosphates, and thiols by the formation of a quasi-covalent bond. At the same time, AgNP exhibits bactericidal action itself by disrupting cell membranes, in parallel to Ag^+ release.

Other metallic NPs, such as ZnO, TiO_2, Au, CeO_2, CaO, and CuO, have also demonstrated bactericidal effects [42–44]. Although some controversy still exists, some studies have utilized AuNPs as antibacterial agents [45–46]. It has been also reported that AuNPs can be utilized as delivery vehicles of antibiotics attributing to nontoxicity for the human body and easy surface modification *via* diverse conjugation chemistry of AuNPs [47, 48]. Metal oxide NPs are also known to effectively inhibit the growth of a wide range of bacteria due to their intrinsic photocatalytic activity, generating ROS.

Inorganic–Organic Hybrids

In section "Antibacterial Nanoparticles," we highlighted several functional inorganic NPs that exhibit antibacterial properties. Surface modification of biomedical implants and devices with the antimicrobial NPs is useful and productive to prevent biofilm formation of bacteria, mainly presenting high antibiotic resistance. However, decoration of the medical device surfaces only with NPs will have challenges due to easy loss of NPs by adsorption to floating proteins, platelets, dead cells, and cell debris [49]. To resolve the problem, we can integrate the bactericidal inorganic NPs with antifouling organic materials into a single coating platform.

Surface coatings of antifouling polymers combined with antimicrobial NPs can be used for modification of medical implants [50]. Silver-based coatings hybridized with organic materials showed excellent bactericidal activity against both Gram-positive and Gram-negative bacteria. Indeed, many studies regarding organic–inorganic hybrid coatings containing AgNPs have been reported [6, 7, 51, 52].

The main advantage of using the inorganic–organic hybrid platform is in the versatility to create multi-functionalities of the material surfaces based on the beauty of chemistry. For instance, Yoo et al. have recently demonstrated that a combination of antifouling polymeric brushes and AgNPs on a Ti results in 100% bacterial killing

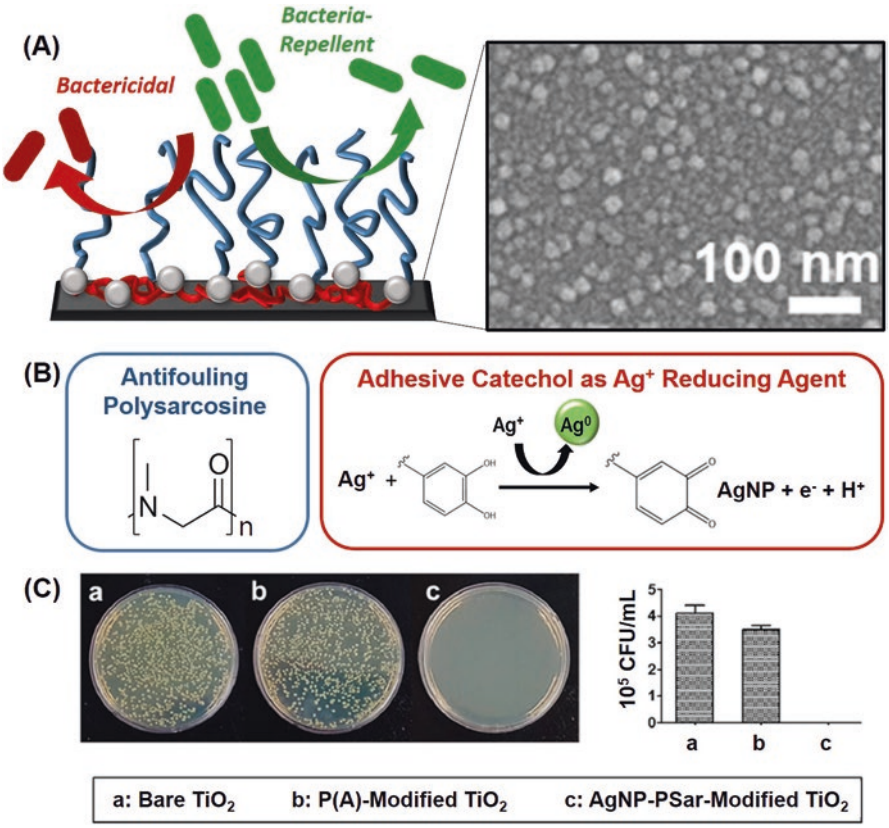


Fig. 2 (**A**) Schematic representation of surface modification with inorganic–organic hybridization on biomedical implants and devices. AgNPs are formed through reduction by catechol groups of polysarcosine (p(Sar)) brushes modified on a TiO_2 surface (inset: a scanning electron micrograph of the inorganic–organic hybrid surface) (**B**) Representative chemical structure of p(Sar) and catechol-mediated AgNP reduction process. (**C**) Photographs and the number of *E. coli* colonies with non-modified, p(Sar)-modified, and AgNPs-decorated p(Sar) TiO_2 surfaces. (Reprinted with permission from [6]. Copyright 2018 American Chemical Society)

efficiency. Protein-resistant polysarcosine brushes at the surface played a dual role in inhibiting adsorption of biofoulants and mediating formation of bactericidal AgNPs at the coated surfaces. This approach does not only yield antifouling properties but also introduce a pronounced bactericidal activity, thereby leading to far more improved antibacterial surfaces (Fig. 2) [6]. AgNPs can be conjugated with polymers *via* photoreduction or by using reducing agents. Recently, "green" synthesis of AgNPs using plant extracts as reducing agents has been spotlighted instead of using chemical agents [53, 54]. Similarly, other bacteria-repelling, antifouling polymers (e.g., zwitterionic polymer, per-fluoro polymer, and PEG) can also be demonstrated to improve the prevention efficacy of biofilm formation with the incorporation of AgNPs by the conjugation chemistry.

It would also be worthy to note that we can decorate the surfaces of antimicrobial NPs with organic materials to create a synergistic effect. Metal oxide photocata-

lysts, such as TiO_2, ZnO, CeO_2, and so on, generate ROS under light irradiation, thereby resulting in bactericidal effects. However, the surfaces of metal oxides are inherently hydrophilic, which is vulnerable to bacterial colonization. Surface modification of hydrophilic metal oxides with polydimethylsiloxane (PDMS) under UV light enables to create hydrophobic surfaces with retention of the natural photocatalytic activity [55].

Furthermore, biocompatible biopolymers to mammalian cells combined with antimicrobial inorganic NPs can improve the biocompatibility of implantable materials as well as facilitate the controlled release of biocidal agents. For example, the gelatin and ZnO NPs composite films have been applied for food preservation, showing excellent antibacterial activity against foodborne, pathogenic bacteria [56].

Biomimicry Toward Advanced Antimicrobial Surfaces

Living organisms in Nature have evolved their structure and functions over a geological period to survive extreme and various environmental conditions, which offer engineering solutions to overcome many challenges in new material development. For instance, the lotus effect, known as self-cleaning properties of surfaces to remove dirt particles with water droplets *via* superhydrophobicity based on hierarchical surface roughness, has inspired researchers in the fields of antifouling paints, clothes, anti-stiction coatings, and low friction surfaces [57].

Indeed, we can easily find functional, natural surfaces from plants, animals, and insects that display antifouling and/or bactericidal properties with the delicate nanostructure. Gecko skin [58], sharkskin [59], many insect wings [60, 61], and plant leaves [62] possess antibacterial properties with unique surface patterns. Also, we recently discovered nanoprotrusive natural surfaces, Such as gecko skin [63], cicada wing [64], and dragonfly wings [65], exhibit high bactericidal efficiency.

Motivated by such antifouling and bactericidal surfaces in nature, researchers have been actively trying to mimic and engineer the surface structure of human-made materials used in biomedical implants and devices [66]. In section "Biomimicry Toward Advanced Antimicrobial Surfaces," we introduce *natural antimicrobial surfaces* classified into two categories based on the resistance mechanism against bacterial cells: (1) reduction of cell attachment (antifouling surfaces), and (2) rupture of the attached cell membrane (bactericidal surfaces).

Natural, Antifouling Surfaces with Reduced Bacterial Adhesion

Preventing adhesion of contaminants on surfaces can be achieved *via* the creation of superhydrophobic surfaces with nano/micro hierarchical structures that mimic different natural, antifouling surfaces observed in various herbaceous plants [62], insect species [60–65], and animals [58, 59].

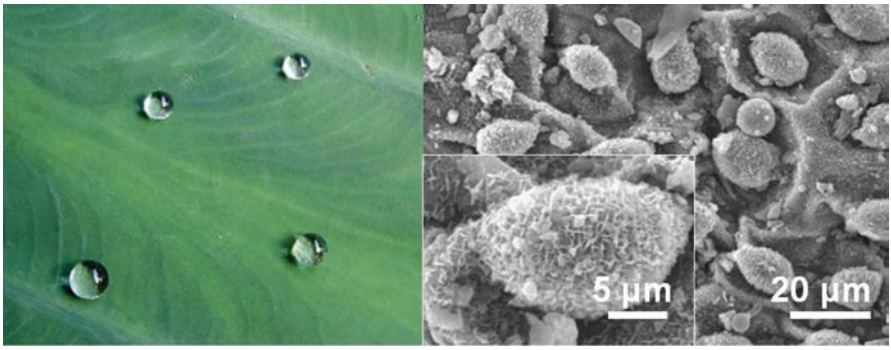


Fig. 3 Antifouling, superhydrophobic taro leaves. A photograph and scanning electron micrographs of taro leaves. (Reprinted from [68] with permission from Elsevier. Copyright 2007 Elsevier)

For examples, rice, lotus, and taro leaves display hierarchical surface layers with dense, nanometer-scale wax crystalloids on micrometer-sized, convex epidermal cells (Fig. 3) [68]. Such unique surface structural and chemical properties of leaves result in very high surface contact angles with low water sliding angles [69]. Hence, bacteria and any contaminant particles can be easily picked up by near water droplets and removed with the rolling-off-droplets on the natural superhydrophobic, antifouling surfaces.

The antifouling effect of superhydrophobic surfaces is based on air traps between the surface structure and bacterial cell membrane. The hierarchical nanofeatures on microstructures, which enable retaining the trapped air under water, have a critical role in fluid drag reduction and biofouling prevention [67]. Cassie–Baxter model suggests that the air layer captured under the droplet serves as another substrate in the system, leading to reduced surface tension of solid and vapor [70]. A heterogeneous surface composed of air and solid results in meager adhesive force between water and the solid surface, which induces "self-cleaning, antifouling effect."

In addition to the plenty of superhydrophobic plant leaves, animal skin also present great antifouling property. Sharkskin is one of the representative examples to demonstrate excellent antifouling efficacy against bacteria, algae, and barnacles. The superior antifouling performance of sharkskin originates from a combination of a unique surface structure composed of micrometer-scale riblets and dermal denticles. In conjunction with the unique surface patterns, superior mechanical flexibility and mucous surface layers of sharkskin significantly reduce friction drag and increase aerodynamics in water [59]. Many research efforts have been devoted to designing a new type of surface to attack bacteria with the sharkskin-mimicking, patterned diamond-like surface texture [71]. We will discuss the engineering approaches developed to mimic natural structure in the next section "Nature-Inspired, Nanostructured Surface Development for Antibacterial Properties" in more detail.

Other interesting natural surfaces that present antifouling effect can be found from a variety of insects living in an extreme environment like dusty soil and damp tropical jungle. Wings of butterflies, moths, alderflies, antlions, fishflies, dobsonflies, or snakeflies presented excellent antifouling properties, which were demonstrated by testing the efficiency to remove particles of different sizes with water droplets during fogging. It was reported that only less than 5% particles, initially applied to the surfaces, remain on the *Lepidoptera* and *Planipennia* wings [61]. Moreover, *Lepidoptera* wings have hierarchical microgrooves on an array of shingle-like structures, which is useful to create engineering surfaces by obtaining a replica of the surface for enhanced antifouling properties [72, 73]. Truly, insects have inspired many researchers to develop marvelous surface structure using advanced nanotechnology toward antifouling surfaces while overcoming the hostile environmental disadvantages.

Natural, Bactericidal Surfaces to Induce Membrane Rupture of Bacterial Cell

Another effective strategy to prevent biofilm formation is to kill and physically interfere interactions of attached microbes with surface protrusive topography. In this regard, cicada (e.g., *Psaltoda claripennis*) and dragonfly wings (e.g., *Diplacodes bipunctata*) have recently received an intense attention due to their antifouling as well as high bactericidal effectiveness. Herein, we summarize several reports of natural surfaces showing the excellent bactericidal performance.

Ivanova et al. reported the first example of natural cicada wings that kill Gram-negative pathogenic bacteria, *Pseudomonas aeruginosa* (*P. aeruginosa*), which causes severe infections at different sites within the body such as urinary and respiratory tracts or wounded skin [64]. The authors demonstrated that the nanopillars on the cicada wings could rupture and penetrate the bacterial membrane within approximately 3 min, resulting in high bactericidal efficiency. The physical and mechanical bactericidal ability was further demonstrated by altering the cicada surface into deposited gold to eliminate the chemical effect, which also confirmed that the nanopillar structure has a significant role to kill the adhered bacteria. A biophysical model to explain the interaction of bacterial cell and surface nanopillar structure supported that mechanical properties, especially rigidity of cells, are the important factors to determine bactericidal efficacy on the cicada wing [74]. These findings provide scientific insights into the bactericidal mechanism underlying natural antimicrobial materials. However, it is worth noting that the cicada wings are only lethal to Gram-negative bacteria but less effective to more rigid Gram-positive bacteria with a thicker layer of peptidoglycan. Also, the bactericidal efficacy on cicada wings varies from different cicada species [75, 76].

Dragonfly wings (*Diplacodes bipunctata*) are effective to kill both Gram-positive and Gram-negative bacterial cells, which originates from the high-aspect ratio nanoprotrusion on the surfaces [65]. The protrusive nanopillars or their clusters on dragonfly wings (e.g. *Hemianax papuensis, Sympetrum vulgatum*) are primarily

composed of aliphatic hydrocarbon and a fatty acid outer layer. The size of the nanoscale surface structures varies between 83 and 195 nm in diameter according to species [77, 78]. The bactericidal efficiency is dependent on the topography of the nanoprotrusion on their wings, which implies that different dragonfly species would show different bactericidal efficiency against pathogenic bacteria [79].

The sharp nanopillars on insect wings with high-aspect-ratio exhibit great bactericidal activity while bringing about by cell membrane deformation and lysis. Bandara et al. demonstrated that the cell membrane rupture is caused by a combination of the strong adhesion between nanopillars and extracellular polymeric substance (EPS) of attached bacteria as well as the shear force when immobilized bacterium tries to move on the nanotextured surface (Fig. 4) [80]. Most notably, the nanoprotrusive surface structure does play the main role in the bactericidal performance. A recent study confirmed that the black silicon surfaces which mimic dragonfly wings effectively killed both of Gram-negative and Gram-positive bacteria, despite low production of EPS [81].

For animal surfaces presenting the bactericidal effect, gecko skin (e.g., *L. steindachneri*) with sub-micro-spaced, hairy spinules on hexagonal-patterned dome-like structures demonstrated the self-cleaning property as well as bactericidal efficacy against both Gram-negative and Gram-positive bacteria [58, 63, 82]. In addition to terrestrial insect and animal surfaces, the cuticle of the aquatic larva of the drone fly (e.g., *Eristalis tenax*) is reported to have a potential bactericidal property [83]. The authors observed the spine-like nanopillars on the cuticle layers made it difficult for bacteria to attach and colonize the surfaces. Considered the larvae living in bacteria-, fungi- and algae-rich environments, mimicking an array of the nanopillars (typically <100 nm in diameter) would facilitate to enhance antibacterial efficiency of engineered surfaces in a harsh environment.

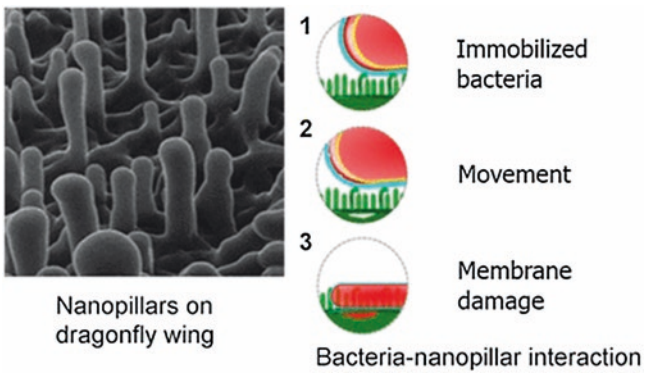


Fig. 4 SEM image and schematics to show the bactericidal effects of natural dragonfly wings with nano-topography. (Reprinted with permission from [80]. Copyright 2017 American Chemical Society)

Nature-Inspired, Nanostructured Surface Development for Antibacterial Properties

Advances in nanofabrication techniques facilitate the development of such functional, engineered surfaces on various materials. Recently, the creation of biomimetic, antibacterial surfaces using nanotechnology has gathered strong attention in surface sciences, material developments, and biomedical applications. In section "Nature-Inspired, Nanostructured Surface Development for Antibacterial Properties," we review current engineering approaches to create biomimicking, antibacterial nanostructures, classified into three categories based on the structural characteristics: (1) nano/microscale roughness, (2) nanoprotrusion, and (3) nanopores (Fig. 5).

Engineered Surfaces with Surface Roughness or Pattern at the Nano and Micro Scale

Inspired by the antimicrobial structure in the nature surfaces (e.g., plant leaves, insect wings, and animal skin), researchers have tried to fabricate analogous structures on the surfaces of biomedical implants or devices to prevent bacterial infections. Herein, we highlight widely used engineering techniques to create the nature-mimicking structures on surfaces of artificial materials, in particular, the fabrication methods to make nano/micro surface roughness, hierarchical structure, or regular patterns.

The lotus leaf-inspired hierarchical nanostructure has been created for wettability control on the diverse types of materials by using many advanced engineering techniques including self-assembly, laser processing, etching, and electrodeposition. The approaches to mimic superhydrophobic, antifouling lotus leaves can also be used for modification of biomedical surfaces [69]. Recent work reported that the lotus leaf-mimicking, fluorinated polypropylene surfaces significantly reduced *E. coli* adhesion, compared to the untreated, flat control surface, based on structural and chemical properties [84].

One of the easiest, feasible ways to mimic natural surfaces would be to obtain the direct replication of natural antibacterial surfaces by molding, embossing, and printing of polymers. The polymer replication techniques broadly require the following

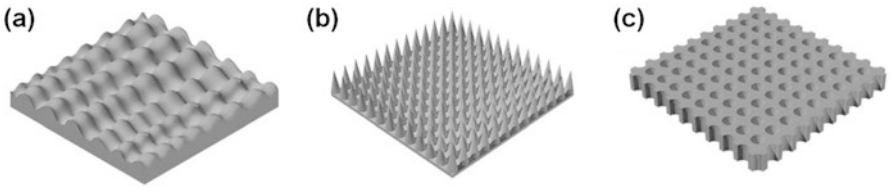


Fig. 5 Schematic representations of engineered surfaces with (**a**) nano-/microscale roughness, (**b**) protrusive structures with high-aspect-ratio nanopillars, and (**c**) pores at the nanometer scale

necessary steps: a master mold (e.g., a natural surface from which replicas are formed), replication of the master using moldable polymer, and transfer and registration of the replica to a functional material (e.g., implants or surgical tools) [85]. Using this technology, engineers have tried to make replicas of sharkskin, rice leaf, and butterfly wing by diverse types of polymeric substrates (e.g., polyurethane, polypropylene, and polydimethylsiloxane), which also demonstrated good antifouling effect against both Gram-positive and Gram-negative bacteria [73, 86]. Notably, some polymeric replicas of sharkskin exhibited a better self-cleaning and antifouling performance against microorganisms including zoospores and *E. coli* than the primary micropattern [87, 88]. The molding or casting methods are useful to fabricate the nature-mimicking surfaces in a high throughput and low cost with a good resolution at the microscale, but they can be limited to the higher resolution of the nanoscale range [66]. This challenge can be overcome with different engineering strategies. For example, polyethylene terephthalate was fabricated into nanocones or sharp nanopillars by colloidal lithography or inductively coupled plasma, which also mitigated bacterial adhesion and colonization activity [89, 90].

For the direct implementation of surface roughness on implantable metal or metal alloy materials, researchers have tried to apply sputtering or shot peening and tested the antimicrobial properties of the modified surfaces. For example, a titanium alloy (Ti_6Al_4V) has been coated with nanocolumnar structures by the glancing angle deposition using magnetron sputtering technique for lotus leaf effect [91]. The nano-roughened Ti alloy surfaces inhibited bacterial attachment and biofilm formation of *S. aureus* while showing good biocompatibility to osteoblasts [91]. The nano-roughened Ti surfaces with nanocolumns reduce the available area for bacterial cells to attach, leading to a limited number of anchoring points between the bacteria and nanorough surface. Nevertheless, nanoroughness on the Ti surface has less impact on osteoblasts due to a more deformable membrane and the large size of mammalian cells.

Such nano-roughened Ti surfaces can also be fabricated by physical vapor deposition. Jandt and others demonstrated the nanorough Ti surfaces reduced adhesion of *E. coli* and *S. aureus* with the increase of surface roughness, which originated from the decrease of adhesion points of the cells to the surfaces [92]. However, the degree of reduction of bacterial attachment on the nanorough surface may vary in an individual bacterial cell type for a given specific surface. The ability of bacterial attachment reduction could be affected by many factors, like shape or spatial distribution of surface features, surface chemistry, and bacterial type [93, 94]. To our best knowledge, up to date, there is no substrate possessing the universal ability to reduce the adhesion of all types of bacteria.

Biomimetic Surfaces with Nanoprotrusion

Since the first report of the antifouling and the bactericidal efficacy of the cicada wings with high-aspect-ratio nanoprotrusion [64], many efforts have been devoted to creating the bioinspired, bactericidal surfaces with nanoprotrusion on medical devices or implants.

Stainless steel 316L (SS316L) is one of the most extensively used metal alloys for food processing equipment and biomedical devices such as implants and surgical tools due to its biocompatibility, corrosion resistance, and mechanical strength [95]. Therefore, it is critical to developing a facile method to attain nano-topography on SS316L surfaces to inhibit bacterial adhesion. Surface-roughened SS316L can be created *via* severe shot peening [96], abrasive flow finishing [97], or electrochemical etching methods [98]. Jang and Choi et al. evaluated the antibacterial nature of nano-textured SS316L surfaces fabricated by electrochemical etching, which possess pronounced, nanoporous, and protrusive structures on the surface (Fig. 6) [98]. The SS316L surfaces with ~20 nm pores and protrusive nanospikes exhibited a significant reduction in surface adhesion of both of Gram-negative *E. coil* and Gram-negative *S. aureus*. Compared to other surface finishing techniques, the electrochemical etching process is affordable and scalable as well as has exquisite control of surface structures by electrochemical parameters such as potential and current density. Furthermore, the electrochemical surface modification produced a superior passive film with enrichment of Chromium (Cr) and Molybdenum (Mo) at the SS316L surface for corrosion resistance in physiological solution, which would be another advantage to use this method for biomedical applications.

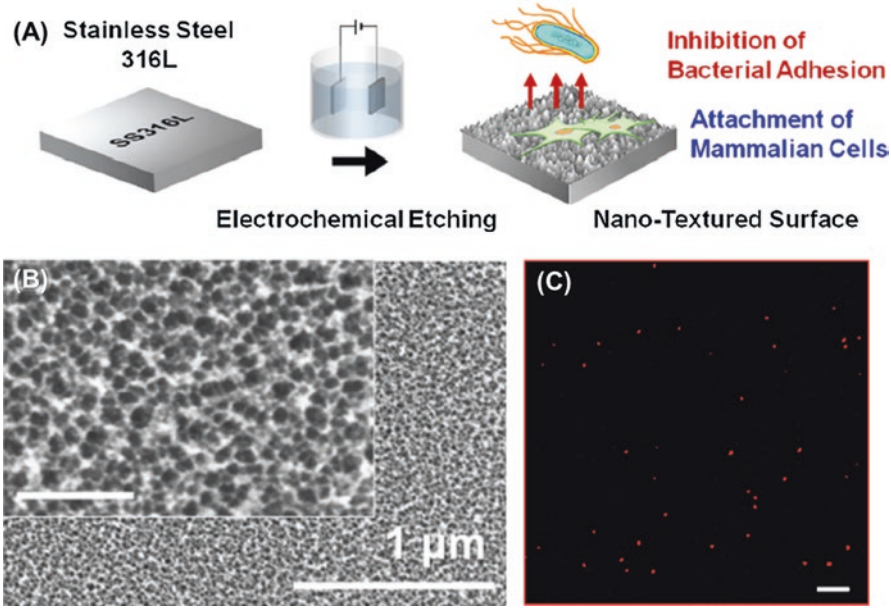


Fig. 6 (**A**) Schematic illustration of development of biocompatible, nanoporous, and protrusive stainless steel 316L (SS316L) surfaces by electrochemical etching to inhibit bacterial adhesion. (**B**) Scanning electron micrographs of nanotextured SS316L (NT-SS316L) surface. (**C**) A representative fluorescent micrograph of dead *E. coli* on NT-SS316L. (Reprinted with permission from [98]. Copyrights at American Chemical Society 2018)

Ti and its alloys are also one of the most popular choices of implant materials due to their excellent chemical and corrosion resistance, biocompatibility, and osseointegration [99–101]. Several different engineering approaches have been applied for the fabrication of protrusive structures on implantable Ti surfaces to prevent the microbial-induced infection problems. For instance, a cicada wing-mimicking nanocolumnar structure on Ti was fabricated by glancing angle sputter deposition, leading to selective bactericidal activity with 50% mortality of *E. coli* [102]. Using hydrothermal etching, Bhadra and coworkers created perpendicular-oriented nanowire-like structure on Ti surfaces. The engineered Ti surfaces with nanopatterned arrays, mimicking dragonfly wings, showed high efficiency in killing *P. aeruginosa* [103]. Another nanopillar structure, made from a chlorine-based reactive ion etching on Ti surface, also presented excellent bactericidal efficacy against Gram-negative bacterial cells [104]. In addition, all the aforementioned nanotextured surfaces possess good cytocompatibility allowing the proliferation and growth of mammalian cells. Some other works related to Ti and Ti alloys etched by the hydrothermal process [105], anodization [106], or thermal oxidation [107] also demonstrated specific bactericidal efficacy and good cytocompatibility as well.

Silicon (Si) is a good candidate to fabricate the nanoprotrusion, while enabling to control the aspect ratio systematically, with high throughput and low fabrication costs. The nanotextured silicon substrate created *via* reactive ion etching, called "black silicon," presents sharper, more discretely distributed, and lower clustered nanoprotrusive features [81, 108]. The black silicon resulted in higher efficiency to kill endospores, Gram-negative and Gram-positive bacteria than natural dragonfly wings [65]. Besides, in vivo implant study demonstrated that the biocompatibility of black silicon surfaces to eukaryotic cells without inflammatory response in ocular and general tissue environment of the host [109].

When it comes to the mechanism of bactericidal efficacy, the mechanical rupture of the cell membrane caused by the interaction between the cell membrane and surface topography is generally accepted as reported in several papers [74, 110–112]. The effect of topographic geometry on bactericidal efficiency has been systematically investigated by tuning the surface protrusion dimension (i.e., height, tip sharpness, and pillar diameter) and testing the various material surfaces, such as silicon, polymer, and metals [81, 105–114]. The nanoprotrusive surface topography and the bacterial motility are believed as the two paramount factors to result in the bactericidal efficacy [115, 116]. For the bactericidal effect underlying bacterial motility, several recent studies indicated that the strong focal adhesion of bacterial cells on the tips of nanopillars, enabled by the generation of extracellular polymeric substances (EPS), enhances the cell membrane tension during movement, thereby leading to the death of the bacterial cell [80, 114]. However, no universal mechanism regarding all the factors affecting the bactericidal properties of a nanotextured surface is reported so far. There are some controversial studies regarding the cell rupture mechanism emerged [80, 81, 115], which imply that we need more comprehensive studies to understand the universal mechanism behind bacteria adhesion on the engineered surfaces with nanoprotrusive structure.

Antifouling Nanoporous Surface Formation

An alternative strategy to enhance the antibacterial property is to lower the bacterial attachment *via* nanopore formation on the substrates. The nanoporous surface tends into more hydrophobic based on the Cassie–Baxter model, which leads to the reduction of attached bacterial cells with the increased wettability.

Anodized aluminum oxide with nanoscale pores in diameters of 15 and 20 nm displayed a significant reduction of bacterial attachment and biofilm formation of *E. coli* and *Listeria innocua*. The nanoporous surfaces inhibited flagella-dependent attachment of *E. coli* by suppressing expression of appendages. The antifouling effect of nanoporous surfaces can be explained by the increased net repulsion forces between bacterial cell and substrate, in combination with electrostatic repulsion and surface effective free energy [117]. The report about simple and robust anodizing method to develop nanoporous structure on alumina surfaces brought significant scientific benefits for understanding antibacterial mechanism of the surfaces with nanopores. However, porous anodic aluminum oxide is not applicable for biomedical implants or devices due to its toxicity, mechanical weakness, and low corrosion resistance as compared to other materials.

Implantable polymeric material, polymethyl methacrylate (PMMA), has been tested with the nanoporous surface structure fabricated by nanoimprint lithography for antibacterial properties. The nanoimprinted PMMA surfaces demonstrated the reduced attachment of bacteria as compared to the flat surfaces [118]. However, only a few works have been demonstrated in this area up to date. Some studies of the influence of pore size on the bacterial adhesion yielded conflicting results according to the types of materials and cells [90]. Therefore, development of potential fabrication methods to create nanopores on other biocompatible materials in tunable scale is worth to be investigated, along with a systematic study to understand the adhesion of different types of bacteria.

In section "Nature-Inspired, Nanostructured Surface Development for Antibacterial Properties," we highlighted several different engineering approaches to create nature-inspired, nanostructured surfaces for antibacterial activities, ranging from the nano- or microscale roughness/patterns, protrusion, and to pores. According to the material types of biomedical surfaces and target functionalities, we need to design the surface engineering methods rationally. Herein, we summarize the types of materials, fabrication methods, characteristic surface features, and corresponding antibacterial properties in Table 1.

Prospective Approaches

While many advances toward developing antibacterial surfaces have been achieved for several decades, the practical applications of the surfaces for in vivo implants or industrial fabrications are still in early stages. So far, material scientists have focused on the development of new surface properties of implantable biomaterials and dem-

Table 1 A summary of engineering approaches to create biomimetic antibacterial surfaces on diverse types of materials used for biomedical devices

	Nano/micro roughness	Nanoprotrusions	Nanopores
Materials	**Polymer:** Polypropylene [84, 86] Polyurethane [73] Polydimethylsiloxane (PDMS) [87, 88] Polyethylene terephthalate (PET) [90] **Metal:** Titanium [91, 92]	**Polymer:** Epoxy resin [63] Polymethyl methacrylate [113] Silicon [65, 81, 108, 109] **Metal:** Stainless steel [98] Titanium [102–107]	**Polymer:** Polymethyl methacrylate [118] **Metal:** Alumina [117]
Fabrication methods	Reactive ion etching (RIE) [84] Soft lithography [73, 86] Micro-molding [87, 88] Inductively coupled plasma etching [90] Glancing angle deposition [91] Physical vapor deposition [92]	Soft lithography [63] Nanoimprint lithography [113] RIE [65, 81, 104, 108, 109] Electrochemical etching [98] Glancing angle sputter deposition [102] Hydrothermal etching [103, 105] Anodization [106] Thermal oxidation [107]	Nanoimprint lithography [118] Anodization [117]
Surface features	Lotus, Rice leaf-mimicking hierarchical structure [73, 84, 86] Sharkskin riblet-like structure [73, 87, 88] Butterfly wing-mimicking shingle-like structure [73] Nanopillars with low aspect ratios [90, 92]	Gecko skin-mimicking hierarchical hairy structure [63] Insect wing-mimicking nanopillars with various dimensions (diameters: 20 nm–2 μm, Spacing gaps: 50 nm–1800 nm, Heights: 210 nm–2 μm) [65, 80, 98, 102, 104, 106, 108, 109, 113] Protrusive nanospikes [107]	Nanopores with diameters from tens to hundreds of nanometers [117, 118]
Antifouling/ bactericidal activities against	**Gram-positive bacteria:** *S. aureus* [86, 90–92] **Gram-negative bacteria:** *E. coli* [84, 86, 87, 90, 92] *H. pylori* [90] Others: Zoospore [88], Silicon carbide particles [73]	**Gram-positive bacteria:** *S. mutan* [63] *S. aureus* [65, 98, 103, 104, 106, 109] *B. subtilis* [65] *M. smegmatis* [104] **Gram-negative bacteria:** *P. gingivalis* [63] *E. coli* [81, 98, 102, 104, 107, 108, 113] *P. aeruginosa* [65, 81, 103–105, 108, 109]	**Gram-positive bacteria:** *L. innocua* [117] **Gram-negative bacteria:** *E. coli* [118] *P. aeruginosa* [118]
Summary	Nano-roughened or patterned surfaces can reduce the bacterial adhesion, but the efficiency varies from materials and dimension of surface structures	Nanoprotrusive surfaces can kill both Gram-negative and Gram-positive bacteria by increasing cell membrane stress. The bactericidal efficiency is dependent on the dimension and geometry of protrusive structures	Nanoporous surfaces can inhibit bacterial adhesion while showing excellent antifouling efficacy. More case studies are necessary to understand the relationship between porous dimension and bacterial adhesion with different cell types

onstration of antibacterial properties in lab scale. The biological, fundamental studies on the interactions between microorganisms and surfaces have been conducted separately. Meanwhile, medical doctors have faced challenges to find applicable medical devices that present advanced antibacterial properties without frequent replacement or additional antibiotic treatments. Moving forward, we believe that the multidisciplinary, cooperative efforts will be the primary focus of continued research. Engineers need to advance strategies to create functional surfaces with target antibacterial properties of biomedical devices in a practical scale by active discussions with surgeons and biomedical industry, as well as to improve understanding of the bacteria–surface interactions with collaboration with microbiologists.

The recent accomplishments in mimicking the excellent bactericidal, antifouling natural surfaces *via* different surface treatment techniques offer futuristic biomedical implants and devices. We have reviewed the diverse structural features of the natural surfaces displaying antibacterial activities (i.e., plant leaves, insect wings, and animal skins) on account of the relationship between the surface nanostructure and bactericidal mechanism. We realize that significant variations in the structural dimension and configuration of these natural surfaces, which implies that there is no universal surface structure to exhibit antimicrobial property against all types of bacteria. Current understanding on the pathogenesis of bacteria adhered on the nanotextured surfaces is still limited, and the biomimetic approaches have been recently suggested in this field. Since the future of biomimicking surfaces is promising, establishing the surface–bacteria correlation will be necessary while varying surface structure systematically and testing multiple microorganisms. Also, studies about the long-term effects of the surface textures on biomedical implants in the body are essential before the new methods can be used to inhibit bacterial adhesion.

Given the fast evolution of microorganisms with increasing antibiotic resistance, the development of functional surfaces inspired by nature is of great significance to inhibit pathogenic bacteria adhesion and growth without antibiotic or other chemical treatments. We will need a more comprehensive investigation to develop engineering methods to create the biomimetic structure in large-scale and rapid production for clinical demonstration. Ultimately, such cumulative attempts toward advanced antibacterial surfaces created *via* different engineering approaches will serve not only to enable the development of practical biomedical devices displaying excellent antimicrobial performance but also enhance our understanding of the complex bacteria–surface interactions.

Summary

We have discussed many facets of surface engineering approaches to create antibacterial properties for biomedical implants and devices, highlighting chemical and physical aspects to inhibit bacterial adhesion and growth, through this chapter.

Traditionally, surface coatings of antifouling polymers using PEG, PNIPAAM, or zwitterionic polymers have been widely employed to prevent protein adsorption ultimately leading to reduced bacterial attachment. However, these polymer coating

approaches can have inherent limitations due to the lack of long-term suppression ability against bacterial colonization. For contact-killing of adhered bacteria, researchers have also developed functionalized polymer coatings that release antibiotics or bleaching agents *via* several different surface modification techniques, which include layer-by-layer deposition, brush formation, and electrospinning. Recent advances in the synthesis of new types of functional polymers or peptides are promising to develop chemically active antimicrobial surfaces with enhanced performance. The fast evolution of bacteria that show high resistance to antibiotics is a growing problem. As another engineering approach to actively inhibit biofilm formation of multidrug-resistant bacteria, we have highlighted several antibacterial inorganic nanoparticles (e.g., Ag, ZnO, CuO, and TiO_2) and their bactericidal mechanisms. To carry the antimicrobial functions of nanoparticles at the surfaces of biomedical devices, engineers have developed several strategies for designing inorganic–organic hybrid platforms that can present dual activities of antifouling and contact-killing.

The challenges of chemical coatings of the delamination and functionality loss by degradation can be overcome through physical approaches to develop antibacterial physical structure at the surfaces. Inspired by living organisms in nature, which have evolved their structure to survive extreme conditions, we can establish new engineering strategies toward advanced antibacterial materials. Thus, we have reviewed various natural surfaces that present excellent antifouling and bactericidal properties, such as plant leaves, insect wings, and animal skins. Depending on the dimension and configuration of the natural surface structures, they presented bacterial-resistant mechanisms by inhibiting adhesion and rupturing the attached cell membranes. The biomimetic attempts and advances in nanofabrication techniques in recent years have recently accelerated the development of antibacterial surfaces on biomedical implants and devices while gathering intense attention in surface and materials science and engineering. We have reviewed cutting-edge reports in this field to create antifouling, bactericidal surfaces with biomimicking nanostructures (i.e., nano-/microscale roughness, hierarchical structure, protrusive nanopillars, and nanopores) *via* different types of surface treatment methods. Considerable efforts to advance diverse surface engineering approaches will facilitate the development of the next-generation implantable biomedical devices perfectly preventing bacterial infections.

Acknowledgments This work is partially supported by Dr. Jang's startup funds provided from Department of Chemical Engineering and Herbert Wertheim College of Engineering at the University of Florida. The authors also gratefully acknowledge the helpful comments and suggestions of the reviewers, which have improved the presentation.

References

1. Arsiwala A, Desai P, Patravale V (2014) Recent advances in micro/nanoscale biomedical implants. J Control Release 189:25–45. https://doi.org/10.1016/j.jconrel.2014.06.021
2. Cloutier M, Mantovani D, Rosei F (2015) Antibacterial coatings: challenges, perspectives, and opportunities. Trends Biotechnol 33:637–652. https://doi.org/10.1016/j.tibtech.2015.09.002

3. Tuson HH, Weibel DB (2013) Bacteria-surface interactions. Soft Matter 9:4368–4380. https://doi.org/10.1039/c3sm27705d
4. Jang Y, Park S, Char K (2011) Functionalization of polymer multilayer thin films for novel biomedical applications. Kor J Chem Eng 28:1149–1160. https://doi.org/10.1007/s11814-010-0434-x
5. Son H, Jang Y, Koo J, Lee JS, Theato P, Char K (2016) Penetration and exchange kinetics of primary alkyl amines applied to reactive poly(pentafluorophenyl acrylate) thin films. Polym J 48:487–495. https://doi.org/10.1038/pj.2016.6
6. Yoo J, Birke A, Kim J, Jang Y, Song SY, Ryu S, Kim BS, Kim BG, Barz M, Char K (2018) Cooperative catechol-functionalized polypept(o)ide brushes and Ag nanoparticles for combination of protein resistance and antimicrobial activity on metal oxide surfaces. Biomacromolecules 19:1602–1613. https://doi.org/10.1021/acs.biomac.8b00135
7. Xie Y, Tang C, Wang Z, Xu Y, Zhao W, Sun S, Zhao C (2017) Co-deposition towards mussel-inspired antifouling and antibacterial membranes by using zwitterionic polymers and silver nanoparticles. J Mater Chem B 5:7186–7193. https://doi.org/10.1039/c7tb01516j
8. Lau KHA, Ren C, Park SH, Szleifer I, Messersmith PB (2012) An experimental-theoretical analysis of protein adsorption on peptidomimetic polymer brushes. Langmuir 28:2288–2298. https://doi.org/10.1021/la203905g
9. Rosu C, Jang Y, Jiang L, Champion J (2018) Nature-Inspired and "Water-Skating" Paper and Polyester Substrates Enabled by the Molecular Structure of Poly(γ-stearyl-α, l-glutamate) Homopolypeptide. Biomacromolecules 19:4617–4628. https://doi.org/10.1021/acs.biomac.8b01312
10. Chen L, Zeng R, Xiang L, Luo Z, Wang Y (2012) Polydopamine-graft-PEG antifouling coating for quantitative analysis of food proteins by CE. Anal Methods 4:2852–2859. https://doi.org/10.1039/c2ay25129a
11. Zhang RN, Liu YN, He MR, Su YL, Zhao XT, Elimelech M, Jiang ZY (2016) Antifouling membranes for sustainable water purification: strategies and mechanisms. Chem Soc Rev 45:5888–5924. https://doi.org/10.1039/c5cs00579e
12. Banerjee I, Pangule RC, Kane RS (2011) Antifouling coatings: recent developments in the design of surfaces that prevent fouling by proteins, bacteria, and marine organisms. Adv Mater 23:690–718. https://doi.org/10.1002/adma.201001215
13. Kingshott P, Thissen H, Griesser HJ (2002) Effects of cloud-point grafting, chain length, and density of PEG layers on competitive adsorption of ocular proteins. Biomaterials 23:2043–2056. https://doi.org/10.1016/S0142-9612(01)00334-9
14. Prime KL, Whitesides GM (1993) Adsorption of proteins onto surfaces containing end-attached oligo(ethylene oxide)—a model system using self-assembled monolayers. J Am Chem Soc 115:10714–10721. https://doi.org/10.1021/ja00076a032
15. Roosjen A, van der Mei HC, Busscher HJ, Norde W (2004) Microbial adhesion to poly(ethylene oxide) brushes: influence of polymer chain length and temperature. Langmuir 20:10949–10955. https://doi.org/10.1021/la0484691
16. Lau KHA, Sileika TS, Park SH, Sousa AML, Burch P, Szleifer I, Messersmith PB (2015) Molecular design of antifouling polymer brushes using sequence-specific peptoids. Adv Mater Interfaces 2:1400225. https://doi.org/10.1002/Admi.201400225
17. Lee SJ, Heo DN, Lee HR, Lee D, Yu SJ, Park SA, Ko WK, Park SW, Im SG, Moon JH, Kwon IK (2015) Biofunctionalized titanium with anti-fouling resistance by grafting thermo-responsive polymer brushes for the prevention of peri-implantitis. J Mater Chem B 3:5161–5165. https://doi.org/10.1039/c5tb00611b
18. Chelmowski R, Koster SD, Kerstan A, Prekelt A, Grunwald C, Winkler T, Metzler-Nolte N, Terfort A, Woll C (2008) Peptide-based SAMs that resist the adsorption of proteins. J Am Chem Soc 130:14952–14953. https://doi.org/10.1021/ja8065754
19. Le NCH, Gubala V, Gandhiraman RP, Daniels S, Williams DE (2011) Evaluation of different nonspecific binding blocking agents deposited inside poly(methyl methacrylate) microfluidic flow-cells. Langmuir 27:9043–9051. https://doi.org/10.1021/la2011502

20. Chuang HF, Smith RC, Hammond PT (2008) Polyelectrolyte multilayers for tunable release of antibiotics. Biomacromolecules 9:1660–1668. https://doi.org/10.1021/bm800185h
21. Nystrom L, Stromstedt AA, Schmidtchen A, Malmsten M (2018) Peptide-loaded microgels as antimicrobial and anti-inflammatory surface coatings. Biomacromolecules 19:3456–3466. https://doi.org/10.1021/acs.biomac.8b00776
22. Kaur R, Liu S (2016) Antibacterial surface design—contact kill. Prog Surf Sci 91:136–153. https://doi.org/10.1016/j.progsurf.2016.09.001
23. Tiller JC, Liao C-J, Lewis K, Klibanov AM (2001) Designing surfaces that kill bacteria on contact. Proc Natl Acad Sci 98:5981–5985. https://doi.org/10.1073/pnas.111143098
24. Tiller JC, Lee SB, Lewis K, Klibanov AM (2002) Polymer surfaces derivatized with poly(vinyl-N-hexylpyridinium) kill airborne and waterborne bacteria. Biotechnol Bioeng 79:465–471. https://doi.org/10.1002/bit.10299
25. Wang J, Vermerris W (2016) Antimicrobial nanomaterials derived from natural products—a review. Materials (Basel) 9:255. https://doi.org/10.3390/ma9040255
26. Correia VG, Ferraria AM, Pinho MG, Aguiar-Ricardo A (2015) Antimicrobial contact-active oligo(2-oxazoline)s-grafted surfaces for fast water disinfection at the point-of-use. Biomacromolecules 16:3904–3915. https://doi.org/10.1021/acs.biomac.5b01243
27. Rabea EI, Badawy MET, Stevens CV, Smagghe G, Steurbaut W (2003) Chitosan as antimicrobial agent: applications and mode of action. Biomacromolecules 4:1457–1465. https://doi.org/10.1021/bm034130m
28. Ohkawa K, Minato KI, Kumagai G, Hayashi S, Yamamoto H (2006) Chitosan nanofiber. Biomacromolecules 7:3291–3294. https://doi.org/10.1021/bm0604395
29. Elsabee MZ, Naguib HF, Morsi RE (2012) Chitosan based nanofibers, review. Mater Sci Eng C 32:1711–1726. https://doi.org/10.1016/j.msec.2012.05.009
30. Torres-Giner S, Ocio MJ, Lagaron JM (2008) Development of active antimicrobial fiber based chitosan polysaccharide nanostructures using electrospinning. Eng Life Sci 8:303–314. https://doi.org/10.1002/elsc.200700066
31. Fernandes SCM, Sadocco P, Alonso-Varona A, Palomares T, Eceiza A, Silvestre AJD, Aki Mondragon I, Freire CSR (2013) Bioinspired antimicrobial and biocompatible bacterial cellulose membranes obtained by surface functionalization with aminoalkyl groups. ACS Appl Mater Interfaces 5:3290–3297. https://doi.org/10.1021/am400338n
32. Roemhild K, Wiegand C, Hipler U, Heinze T (2013) Novel bioactive amino-functionalized cellulose nanofibers. Macromol Rapid Commun 34:1767–1771. https://doi.org/10.1002/marc.201300588
33. Roy D, Knapp JS, Guthrie JT, Perrier S (2008) Antibacterial cellulose fiber via RAFT surface graft polymerization. Biomacromolecules 9:91–99. https://doi.org/10.1021/bm700849j
34. Heunis T, Bshena O, Klumperman B, Dicks L (2011) Release of bacteriocins from nanofibers prepared with combinations of poly(D,L-lactide) (PDLLA) and poly(ethylene oxide) (PEO). Int J Mol Sci 12:2158–2173. https://doi.org/10.3390/ijms12042158
35. Viana JFC, Carrijo J, Freitas CG, Paul A, Alcaraz J, Lacorte CC, Migliolo L, Andrade CA, Falcão R, Santos NC, Gonçalves S, Otero-González AJ, Khademhosseini A, Dias SC, Franco OL (2015) Antifungal nanofibers made by controlled release of sea animal derived peptide. Nanoscale 7:6238–6246. https://doi.org/10.1039/c5nr00767d
36. Gatti JW, Smithgall MC, Paranjape SM, Rolfes RJ, Paranjape M (2013) Using electrospun poly(ethylene-oxide) nanofibers for improved retention and efficacy of bacteriolytic antibiotics. Biomed Microdevices 15:887–893. https://doi.org/10.1007/s10544-013-9777-5
37. Baptista PV, McCusker MP, Carvalho A, Ferreira DA, Mohan NM, Martins M, Fernandes AR (2018) Nano-strategies to fight multidrug resistant bacteria—"A Battle of the Titans". Front Microbiol 9:1441. https://doi.org/10.3389/fmicb.2018.01441
38. Wang L, Hu C, Shoa L (2017) The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int J Nanomed 12:1227–1249. https://doi.org/10.2147/IJN.S121956
39. Le Ouay B, Stellacci F (2015) Antibacterial activity of silver nanoparticles: a surface science insight. Nano Today 10:339–354. https://doi.org/10.1016/j.nantod.2015.04.002
40. Ramalingam B, Parandhaman T, Das SK (2016) Antibacterial effects of biosynthesized silver nanoparticles on surface ultrastructure and nanomechanical properties of gram-negative bac-

teria viz Escherichia coli and Pseudomonas aeruginosa. ACS Appl Mater Interfaces 8:4963–4976. https://doi.org/10.1021/acsami.6b00161
41. Duran N, Marcato PD, De Conti R, Alves OL, Costa FTM, Brocchi M (2010) Potential use of silver nanoparticles on pathogenic bacteria, their toxicity and possible mechanisms of action. J Braz Chem Soc 21:949–959. https://doi.org/10.1590/S0103-50532010000600002
42. Salem W, Leitner DR, Zingl FG, Schratter G, Prassl R, Goessler W, Reidl J, Schild S (2015) Antibacterial activity of silver and zinc nanoparticles against Vibrio cholerae and enterotoxic Escherichia coli. Int J Med Microbiol 305:85–95. https://doi.org/10.1016/j.ijmm.2014.11.005
43. Hemeg HA (2017) Nanomaterials for alternative antibacterial therapy. Int J Nanomedicine 12:8211–8225. https://doi.org/10.2147/Ijn.S132163
44. Slavin YN, Asnis J, Hafeli UO, Bach H (2017) Metal nanoparticles: understanding the mechanisms behind antibacterial activity. J Nanobiotechnol 15:56. https://doi.org/10.1186/s12951-017-0308-z
45. Cui Y, Zhao YY, Tian Y, Zhang W, Lu XY, Jiang XY (2012) The molecular mechanism of action of bactericidal gold nanoparticles on Escherichia coli. Biomaterials 33:2327–2333. https://doi.org/10.1016/j.biomaterials.2011.11.057
46. Shamaila S, Zafar N, Riaz S, Sharif R, Nazir J, Naseem S (2016) Gold nanoparticles: an efficient antimicrobial agent against enteric bacterial human pathogen. Nanomaterials 6:71. https://doi.org/10.3390/nano6040071
47. Rastogi L, Kora AJ, Arunachalam J (2012) Highly stable, protein capped gold nanoparticles as effective drug delivery vehicles for amino-glycosidic antibiotics. Materials Science and Engineering: C 32(6):1571–1577. https://doi.org/10.1016/J.MSEC.2012.04.044
48. Roshmi T, Soumya KR, Jyothis M, Radhakrishnan EK (2015) Effect of biofabricated gold nanoparticle-based antibiotic conjugates on minimum inhibitory concentration of bacterial isolates of clinical origin. Gold Bulletin 48(1–2):63–71. https://doi.org/10.1007/s13404-015-0162-4
49. Zeng Q, Zhu Y, Yu B, Sun Y, Ding X, Xu C, Wu YW, Tang Z, Xu FJ (2018) Antimicrobial and antifouling polymeric agents for surface functionalization of medical implants. Biomacromolecules 9:2805–2811. https://doi.org/10.1021/acs.biomac.8b00399
50. Qayyum S, Oves M, Khan AU (2017) Obliteration of bacterial growth and biofilm through ROS generation by facilely synthesized green silver nanoparticles. PLoS One 12:e0181363. https://doi.org/10.1371/journal.pone.0181363
51. Jankauskaite V, Lazauskas A, Griskonis E, Lisauskaite A, Zukiene K (2017) UV-curable aliphatic silicone acrylate organic-inorganic hybrid coatings with antibacterial activity. Molecules 22:964. https://doi.org/10.3390/molecules22060964
52. Xu DQ, Su YL, Zhao LL, Meng FC, Liu C, Guan YY, Zhang JY, Luo JB (2017) Antibacterial and antifouling properties of a polyurethane surface modified with perfluoroalkyl and silver nanoparticles. J Biomed Mater Res Pt A 105:531–538. https://doi.org/10.1002/jbm.a.35929
53. Prasannaraj G, Venkatachalam P (2017) Enhanced antibacterial, anti-biofilm and antioxidant (ROS) activities of biomolecules engineered silver nanoparticles against clinically isolated gram positive and gram negative microbial pathogens. J Clust Sci 28:645–664. https://doi.org/10.1007/s10876-017-1160-x
54. Gupta K, Barua S, Hazarika SN, Manhar AK, Nath D, Karak N, Namsa ND, Mukhopadhyay R, Kalia VC, Mandal M (2014) Green silver nanoparticles: enhanced antimicrobial and anti-biofilm activity with effects on DNA replication and cell cytotoxicity. RSC Adv 4:52845–52855. https://doi.org/10.1039/c4ra08791g
55. Wooh S, Butt HJ (2017) A photocatalytically active lubricant-impregnated surface. Angew Chem Int Ed 56:4965–4969. https://doi.org/10.1002/anie.201611277
56. Shankar S, Teng XN, Li GB, Rhim JW (2015) Preparation, characterization, and antimicrobial activity of gelatin/ZnO nanocomposite films. Food Hydrocoll 45:264–271. https://doi.org/10.1016/j.foodhyd.2014.12.001
57. Solga A, Cerman Z, Striffler BF, Spaeth M, Barthlott W (2007) The dream of staying clean: lotus and biomimetic surfaces. Bioinspir Biomim 2:S126–S134. https://doi.org/10.1088/1748-3182/2/4/S02

58. Watson GS, Green DW, Schwarzkopf L, Li X, Cribb BW, Myhra S, Watson JA (2015) A gecko skin micro/nano structure—a low adhesion, superhydrophobic, anti-wetting, self-cleaning, biocompatible, antibacterial surface. Acta Biomater 21:109–122. https://doi.org/10.1016/j.actbio.2015.03.007
59. Bixler GD, Bhushan B (2013) Fluid drag reduction with shark-skin riblet inspired microstructured surfaces. Adv Funct Mater 23:4507–4528. https://doi.org/10.1002/adfm.201203683
60. Watson GS, Cribb BW, Watson JA (2010) How micro/nanoarchitecture facilitates anti-wetting: an elegant hierarchical design on the termite wing. ACS Nano 4:129–136. https://doi.org/10.1021/nn900869b
61. Wagner T, Neinhuis C, Barthlott W (1996) Wettability and contaminability of insect wings as a function of their surface sculptures. Acta Zool 77:213–225. https://doi.org/10.1111/j.1463-6395.1996.tb01265.x
62. Neinhuis C, Barthlott W (1997) Characterization and distribution of water-repellent, self-cleaning plant surfaces. Ann Bot 79:667–677. https://doi.org/10.1006/ANBO.1997.0400
63. Li X, Cheung GS, Watson GS, Watson JA, Lin S, Schwarzkopf L, Green DW (2016) The nanotipped hairs of gecko skin and biotemplated replicas impair and/or kill pathogenic bacteria with high efficiency. Nanoscale 8:18860–18869. https://doi.org/10.1039/c6nr05046h
64. Ivanova EP, Hasan J, Webb HK, Truong VK, Watson GS, Watson JA, Baulin VA, Pogodin S, Wang JY, Tobin MJ, Löbbe C, Crawford RJ (2012) Natural bactericidal surfaces: mechanical rupture of pseudomonas aeruginosa cells by cicada wings. Small 8:2489–2494. https://doi.org/10.1002/smll.201200528
65. Ivanova EP, Hasan J, Webb HK, Gervinskas G, Juodkazis S, Truong VK, Wu AHF, Lamb RN, Baulin VA, Watson GS, Watson JA, Mainwaring DE, Crawford RJ (2013) Bactericidal activity of black silicon. Nat Commun 4:2838. https://doi.org/10.1038/ncomms3838
66. Jaggessar A, Shahali H, Mathew A, Yarlagadda PKDV (2017) Bio-mimicking nano and micro-structured surface fabrication for antibacterial properties in medical implants. J Nanobiotechnol 15:64. https://doi.org/10.1186/s12951-017-0306-1
67. Ma J, Sun Y, Gleichauf K, Lou J, Li Q (2011) Nanostructure on Taro Leaves Resists Fouling by Colloids and Bacteria under Submerged Conditions. Langmuir 27 (16):10035–10040
68. Guo Z, Liu W (2007) Biomimic from the superhydrophobic plant leaves in nature: binary structure and unitary structure. Plant Sci 172:1103–1112. https://doi.org/10.1016/j.plantsci.2007.03.005
69. Feng L, Li S, Li Y, Li H, Zhang L, Zhai J, Song Y, Liu B, Jiang L, Zhu D (2002) Superhydrophobic surfaces: from natural to artificial. Adv Mater 14:1857–1860. https://doi.org/10.1002/adma.200290020
70. Cassie ABD, Baxter S (1944) Wettability of porous surfaces. Trans Faraday Soc 40:546–551. https://doi.org/10.1039/tf9444000546
71. Dundar Arisoy F, Kolewe KW, Homyak B, Kurtz IS, Schiffman JD, Watkins JJ (2018) Bioinspired photocatalytic shark-skin surfaces with antibacterial and antifouling activity via nanoimprint lithography. ACS Appl Mater Interfaces 10:20055–20063. https://doi.org/10.1021/acsami.8b05066
72. Bixler GD, Bhushan B (2015) Rice and butterfly wing effect inspired low drag and antifouling surfaces: a review. Crit Rev Solid State Mater Sci 40:1–37. https://doi.org/10.1080/10408436.2014.917368
73. Bixler GD, Bhushan B (2012) Bioinspired rice leaf and butterfly wing surface structures combining shark skin and lotus effects. Soft Matter 8:11271–11284. https://doi.org/10.1039/c2sm26655e
74. Pogodin S, Hasan J, Baulin VA, Webb HK, Truong VK, Phong Nguyen TH, Boshkovikj V, Fluke CJ, Watson GS, Watson JA, Crawford RJ, Ivanova EP (2013) Biophysical model of bacterial cell interactions with nanopatterned cicada wing surfaces. Biophys J 104:835–840. https://doi.org/10.1016/j.bpj.2012.12.046
75. Hasan J, Webb HK, Truong VK, Pogodin S, Baulin VA, Watson GS, Watson JA, Crawford RJ, Ivanova EP (2013) Selective bactericidal activity of nanopatterned superhydrophobic cicada Psaltoda claripennis wing surfaces. Appl Microbiol Biotechnol 97:9257–9262. https://doi.org/10.1007/s00253-012-4628-5

76. Kelleher SM, Habimana O, Lawler J, O'reilly B, Daniels S, Casey E, Cowley A (2016) Cicada wing surface topography: an investigation into the bactericidal properties of nanostructural features. ACS Appl Mater Interfaces 8:14966–14974. https://doi.org/10.1021/acsami.5b08309
77. Ivanova EP, Nguyen SH, Webb HK, Hasan J, Truong VK, Lamb RN, Duan X, Tobin MJ, Mahon PJ, Crawford RJ (2013) Molecular organization of the nanoscale surface structures of the dragonfly Hemianax papuensis wing epicuticle. PLoS One 8:e67893. https://doi.org/10.1371/journal.pone.0067893
78. Selvakumar R, Karuppanan KK, Pezhinkattil R (2012) Analysis on surface nanostructures present in hindwing of dragon fly (Sympetrum vulgatum) using atomic force microscopy. Micron 43:1299–1303. https://doi.org/10.1016/J.MICRON.2011.10.017
79. Mainwaring DE, Nguyen SH, Webb H, Jakubov T, Tobin M, Lamb RN, Wu AHF, Marchant R, Crawford RJ, Ivanova EP (2016) The nature of inherent bactericidal activity: insights from the nanotopology of three species of dragonfly. Nanoscale 8:6527–6534. https://doi.org/10.1039/c5nr08542j
80. Bandara CD, Singh S, Afara IO, Wolff A, Tesfamichael T, Ostrikov K, Oloyede A (2017) Bactericidal effects of natural nanotopography of dragonfly wing on Escherichia coli. ACS Appl Mater Interfaces 9:6746–6760. https://doi.org/10.1021/acsami.6b13666
81. Linklater DP, Juodkazis S, Rubanov S, Ivanova EP (2017) Comment on "Bactericidal effects of natural nanotopography of dragonfly wing on Escherichia coli". ACS Appl Mater Interfaces 9:29387–29393. https://doi.org/10.1021/acsami.7b05707
82. Watson GS, Cribb BW, Schwarzkopf L, Watson JA (2015) Contaminant adhesion (aerial/ground biofouling) on the skin of a gecko. J R Soc Interface 12:20150318. https://doi.org/10.1098/rsif.2015.0318
83. Hayes MJ, Levine TP, Wilson RH (2016) Identification of nanopillars on the cuticle of the aquatic larvae of the drone fly (diptera: Syrphidae). J Insect Sci 16:1–7. https://doi.org/10.1093/jisesa/iew019
84. Kayes MI, Galante AJ, Stella NA, Haghanifar S, Shanks RMQ, Leu PW (2018) Stable lotus leaf-inspired hierarchical, fluorinated polypropylene surfaces for reduced bacterial adhesion. React Funct Polym 128:40–46. https://doi.org/10.1016/J.REACTFUNCTPOLYM.2018.04.013
85. Wolfe DB, Love JC, Whitesides GM (2004) Nanostructures replicated by polymer molding. Marcel Dekker, New York
86. Jafari Nodoushan E, Ebrahimi NG, Ayazi M (2017) An anti-bacterial approach to nanoscale roughening of biomimetic rice-like pattern PP by thermal annealing. Appl Surf Sci 423:1054–1061. https://doi.org/10.1016/J.APSUSC.2017.06.193
87. Munther M, Palma T, Angeron IA, Salari S, Ghassemi H, Vasefi M, Beheshti A, Davami K (2018) Microfabricated biomimetic placoid scale-inspired surfaces for antifouling applications. Appl Surf Sci 453:166–172. https://doi.org/10.1016/j.apsusc.2018.05.030
88. Carman ML, Estes TG, Feinberg AW, Schumacher JF, Wilkerson W, Wilson LH, Callow ME, Callow JA, Brennan AB (2006) Engineered antifouling microtopographies—correlating wettability with cell attachment. Biofouling 22:11–21. https://doi.org/10.1080/08927010500484854
89. Liu W, Liu X, Fangteng J, Wang S, Fang L, Shen H, Xiang S, Sun H, Yang B (2014) Bioinspired polyethylene terephthalate nanocone arrays with underwater superoleophobicity and anti-bioadhesion properties. Nanoscale 6:13845–13853. https://doi.org/10.1039/c4nr04471a
90. Jin L, Guo W, Xue P, Gao H, Zhao M, Zheng C, Zhang Y, Han D (2015) Quantitative assay for the colonization ability of heterogeneous bacteria on controlled nanopillar structures. Nanotechnology 26:055702. https://doi.org/10.1088/0957-4484/26/5/055702
91. Izquierdo-Barba I, García-Martín JM, Álvarez R, Palmero A, Esteban J, Pérez-Jorge C, Arcos D, Vallet-Regí M (2015) Nanocolumnar coatings with selective behavior towards osteoblast and Staphylococcus aureus proliferation. Acta Biomater 15:20–28. https://doi.org/10.1016/J.ACTBIO.2014.12.023

92. Lüdecke C, Roth M, Yu W, Horn U, Bossert J, Jandt KD (2016) Nanorough titanium surfaces reduce adhesion of Escherichia coli and Staphylococcus aureus via nano adhesion points. Colloids Surf B Biointerfaces 145:617–625. https://doi.org/10.1016/J.COLSURFB.2016.05.049
93. Wu S, Zhang B, Liu Y, Suo X, Li H (2018) Influence of surface topography on bacterial adhesion: a review (review). Biointerphases 13:060801. https://doi.org/10.1116/1.5054057
94. Desrousseaux C, Sautou V, Descamps S, Traoré O (2013) Modification of the surfaces of medical devices to prevent microbial adhesion and biofilm formation. J Hosp Infect 85:87–93. https://doi.org/10.1016/J.JHIN.2013.06.015
95. DEWIDAR MM, KHALIL KA, LIM JK (2007) Processing and mechanical properties of porous 316L stainless steel for biomedical applications. Trans Nonferrous Met Soc Chin 17:468–473. https://doi.org/10.1016/S1003-6326(07)60117-4
96. Bagherifard S, Hickey DJ, de Luca AC, Malheiro VN, Markaki AE, Guagliano M, Webster TJ (2015) The influence of nanostructured features on bacterial adhesion and bone cell functions on severely shot peened 316L stainless steel. Biomaterials 73:185–197. https://doi.org/10.1016/j.biomaterials.2015.09.019
97. Sundararaj K, Bangaru M, Mohan B (2017) In vitro biocompatibility study on stainless steel 316L after nano finishing. ASME Paper No. IMECE2017-72606: V003T04A064. https://doi.org/10.1115/IMECE2017-72606
98. Jang Y, Choi WT, Johnson CT, García AJ, Singh PM, Breedveld V, Hess DW, Champion JA (2018) Inhibition of bacterial adhesion on nanotextured stainless steel 316L by electrochemical etching. ACS Biomater Sci Eng 4:90–97. https://doi.org/10.1021/acsbiomaterials.7b00544
99. Lin Y, Gallucci GO, Buser D, Bosshardt D, Belser UC, Yelick PC (2011) Bioengineered periodontal tissue formed on titanium dental implants. J Dent Res 90:251–256. https://doi.org/10.1177/0022034510384872
100. Michelle Grandin H, Berner S, Dard M (2012) A review of titanium zirconium (TiZr) alloys for use in endosseous dental implants. Materials (Basel) 5:1348–1360. https://doi.org/10.3390/ma5081348
101. Neoh KG, Hu X, Zheng D, Kang ET (2012) Balancing osteoblast functions and bacterial adhesion on functionalized titanium surfaces. Biomaterials 33:2813–2822. https://doi.org/10.1016/j.biomaterials.2012.01.018
102. Sengstock C, Lopian M, Motemani Y, Borgmann A, Khare C, Buenconsejo PJS, Schildhauer TA, Ludwig A, Köller M (2014) Structure-related antibacterial activity of a titanium nanostructured surface fabricated by glancing angle sputter deposition. Nanotechnology 25:195101. https://doi.org/10.1088/0957-4484/25/19/195101
103. Bhadra CM, Khanh Truong V, Pham VTH, Al Kobaisi M, Seniutinas G, Wang JY, Juodkazis S, Crawford RJ, Ivanova EP (2015) Antibacterial titanium nano-patterned arrays inspired by dragonfly wings. Sci Rep 5:16817. https://doi.org/10.1038/srep16817
104. Hasan J, Jain S, Chatterjee K (2017) Nanoscale topography on black titanium imparts multi-biofunctional properties for orthopedic applications. Sci Rep 7:41118. https://doi.org/10.1038/srep41118
105. Tsimbouri PM, Fisher L, Holloway N, Sjostrom T, Nobbs AH, Meek RMD, Su B, Dalby MJ (2016) Osteogenic and bactericidal surfaces from hydrothermal titania nanowires on titanium substrates. Sci Rep 6:36875. https://doi.org/10.1038/srep36857
106. Hizal F, Zhuk I, Sukhishvili S, Busscher HJ, Van Der Mei HC, Choi CH (2015) Impact of 3D hierarchical nanostructures on the antibacterial efficacy of a bacteria-triggered self-defensive antibiotic coating. ACS Appl Mater Interfaces 7:20304–20313. https://doi.org/10.1021/acsami.5b05947
107. Sjöström T, Nobbs AH, Su B (2016) Bactericidal nanospike surfaces via thermal oxidation of Ti alloy substrates. Mater Lett 167:22–26. https://doi.org/10.1016/j.matlet.2015.12.140
108. Wang X, Bhadra CM, Yen Dang TH, Buividas R, Wang J, Crawford RJ, Ivanova EP, Juodkazis S (2016) A bactericidal microfluidic device constructed using nano-textured black silicon. RSC Adv 6:26300–26306. https://doi.org/10.1039/c6ra03864f

109. Pham VTH, Truong VK, Orlowska A, Ghanaati S, Barbeck M, Booms P, Fulcher AJ, Bhadra CM, Buividas R, Baulin V, James Kirkpatrick C, Doran P, Mainwaring DE, Juodkazis S, Crawford RJ, Ivanova EP (2016) Race for the surface: eukaryotic cells can win. ACS Appl Mater Interfaces 8:22025–22031. https://doi.org/10.1021/acsami.6b06415
110. Li X (2015) Bactericidal mechanism of nanopatterned surfaces. Phys Chem Chem Phys 18:1311–1316. https://doi.org/10.1039/c5cp05646b
111. Xue F, Liu J, Guo L, Zhang L, Li Q (2015) Theoretical study on the bactericidal nature of nanopatterned surfaces. J Theor Biol 385:1–7. https://doi.org/10.1016/j.jtbi.2015.08.011
112. Nowlin K, Boseman A, Covell A, LaJeunesse D (2014) Adhesion-dependent rupturing of Saccharomyces cerevisiae on biological antimicrobial nanostructured surfaces. J R Soc Interface 12:20140999. https://doi.org/10.1098/rsif.2014.0999
113. Dickson MN, Liang EI, Rodriguez LA, Vollereaux N, Yee AF (2015) Nanopatterned polymer surfaces with bactericidal properties. Biointerphases 10:021010. https://doi.org/10.1116/1.4922157
114. Michalska M, Gambacorta F, Divan R, Aranson IS, Sokolov A, Noirot P, Laible PD (2018) Tuning antimicrobial properties of biomimetic nanopatterned surfaces. Nanoscale 10:6639–6650. https://doi.org/10.1039/c8nr00439k
115. Lin N, Berton P, Moraes C, Rogers RD, Tufenkji N (2018) Nanodarts, nanoblades, and nanospikes: mechano-bactericidal nanostructures and where to find them. Adv Colloid Interface Sci 252:55–68. https://doi.org/10.1016/j.cis.2017.12.007
116. Elbourne A, Crawford RJ, Ivanova EP (2017) Nano-structured antimicrobial surfaces: from nature to synthetic analogues. J Colloid Interface Sci 508:603–616. https://doi.org/10.1016/j.jcis.2017.07.021
117. Feng G, Cheng Y, Wang SY, Hsu LC, Feliz Y, Borca-Tasciuc DA, Worobo RW, Moraru CI (2014) Alumina surfaces with nanoscale topography reduce attachment and biofilm formation by Escherichia coli and Listeria spp. Biofouling 30:1253–1268. https://doi.org/10.1080/08927014.2014.976561
118. Kim S, Jung UT, Kim SK, Lee JH, Choi HS, Kim CS, Jeong MY (2015) Nanostructured multifunctional surface with antireflective and antimicrobial characteristics. ACS Appl Mater Interfaces 7:326–331. https://doi.org/10.1021/am506254r

Antibacterial Coatings on Medical Implants

Sheetal Khatri, Yingchao Su, and Donghui Zhu

Abstract Bacterial contamination has been a serious problem in every field from space missions to medicine and implants. Implant surfaces have been a hazardous site for bacterial adhesion and microbial contamination. This contamination leads to prosthetic infection which results in the necessity of continued antibiotic therapy, eventually leading to removal of the device which comes with long hospitalization time, costs, stress, and pain. Antibacterial coatings have been used as a solution to bacterial contamination. Biofilm formation, antibacterial mechanisms, and types of coating methods on medical implants are briefly described in the present review. Then, the typical antibacterial coatings, including metallic nano-coatings, ceramic coatings, and polymeric coatings, are described.

Keywords Biofilm · Antibacterial coating · Hydroxyapatite · Nanoparticle · Biodegradable coating

Introduction

Medical implants are the core of today's health care system. Implants for different parts of body are constantly in use. Dental implants, orthopedic implants, and vascular implants like catheters, stents, vascular grafts, and bone screws are not only used to save lives or treat disorders but are also used to reinstate a quality of life. Regardless of the wide utility of medical implants, infection is still an obstacle [1–3].

Bacterial contamination is threatening medical implants as it can create an infection in a patient who may already have a poor immune system. About two million occurrences of health-related infections have been recorded each year in the USA,

S. Khatri
Department of Biomedical Engineering, University of North Texas, Denton, TX, USA

Y. Su (✉) · D. Zhu (✉)
Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY, USA
e-mail: yingchao.su@stonybrook.edu; donghui.zhu@stonybrook.edu

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_15

and approximately 100,000 of them lead to mortality [4, 5]. When bacteria adhere to an implant surface, biofilm formation takes place. Gram-positive and Gram-negative bacteria are a source of device-related infections [6]. Although only 1% of total prosthetics involve device-related infections, in case of revision surgery, it rises intensely [7–9]. This results in a requirement for antibiotic therapy for a long time and ultimately the removal of an implant. A growth in antibiotic-resistant strains of bacteria has been observed in patients because of antibiotic therapy. This also includes postoperative complications, costs, time, and pain [10].

A brief description of biofilm formation and the antibacterial effect is described in the first section of this chapter. Antibacterial coatings have a crucial role for preventing biofilm formation; however, how it is coated is also vital. The next part focuses on various methods used to coat different antibacterial agents. Then, different coatings in medical implants are summarized, including hydroxyapatite coatings, polymer coatings, nanoparticle coatings, and biodegradable coatings on different substrates of medical implants [11, 12].

Biofilm Formation on Implants

Cells play various roles as they start to differentiate where they increase adaptation, help intercellular communication, and divide tasks (which results in a multicellular body insuring better efficiency and effective usage of scarce resources) [13]. However, the proliferation of cells is initiated from a single cell in biofilm, but they are not replicas of the original cell. They show different phenotypical features relying on different genetic materials which depend on the environment of cell division, i.e., it may depend on nutrients and oxygen that are accessible to a specific cell in a biofilm gradient. Hence, there is eminent heterogeneity in extracellular material (ECM) formation of a biofilm, yet all bacteria are proficient in biofilm formation [14, 15]. The heterogenous distribution of a biofilm is related to its foundation where a biofilm is formed from various spatially divided microcolonies as various bacterial species can be developed by its basic unit. With the growing colonies, a biofilm spreads taking different bacterial species to which eventually fungi could also join. Hence, we can find heterogenous gene expression within and across different species. After cells get bounded by a biofilm, it starts preserving itself; a defense system starts secreting a slimy ECM which consists of insoluble polysaccharides and proteins, lipids, pili, nucleic acid, and flagella [16].

The first process for biofilm formation is attachment of bacteria to a substrate. This process is very vital in biofilm formation. Planktonic bacteria residing in a liquid are a source of the first colonizers. Bacteria being adhered to the surface is not a full phenomenon for the first stage of biofilm formation. Bacteria can always detach from the surface as several factors influence adhesion of the colonizers. Adsorption is weak and does not remain for long in the first stage; in the case of an unfavorable condition, this step turns out to be reversible adsorption too. Contrasting to the first stage, the second stage is nonreversible where bacteria secrete an extracellular polymeric substrate (EPS) which leads to the formation of microcolonies

[17–19]. Gradually, microcolonies grow and form multilayered shaped clusters in further stages. Independently aroused numerous microcolonies go through the process of maturation which builds a three-dimensional structure, not only for the bacteria in a biofilm but also others from the environment join the growing biofilm. Eventually, with biofilm maturation, the first colonizer is released in the last stage, allowing the cycle to endure [20, 21]. The whole phase of biofilm formation is shown in Fig. 1 [20].

Antibacterial Mechanism

Biomedical implants are critical for health and life and it is very important that bacteria should not get to them. Bacteria can easily colonize these surfaces and protect themselves with a biofilm [16, 22].

Different factors may influence bacterial adhesion like wettability, which is clearly seen in Fig. 2 [23]. It is very crucial to prevent micro colonization and biofilm formation on the surface of implantable devices. Surface chemical modification or by adjusting topography can be used to prevent colonization.

Antifouling is one of the approaches to prevent attachment of bacteria to a surfaces which is also known as a fouling-resistant approach. Basically, it is an anti-adhesive principle which deals with the reduction of bacteria adhesion on the surface

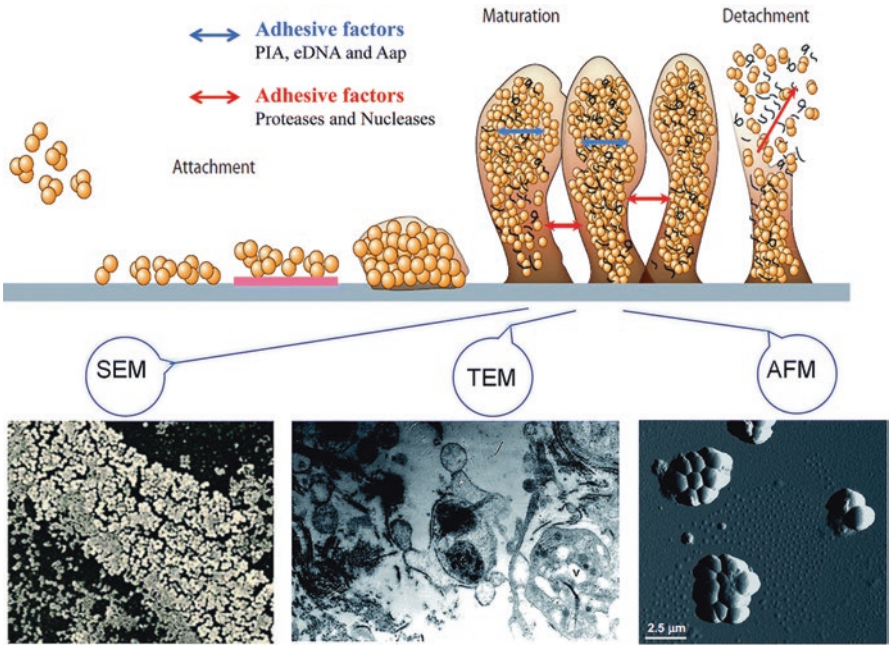


Fig. 1 Several stages for the biofilm formation and the biofilm characterization on the implant surface [20]. (Published by The Royal Society of Chemistry)

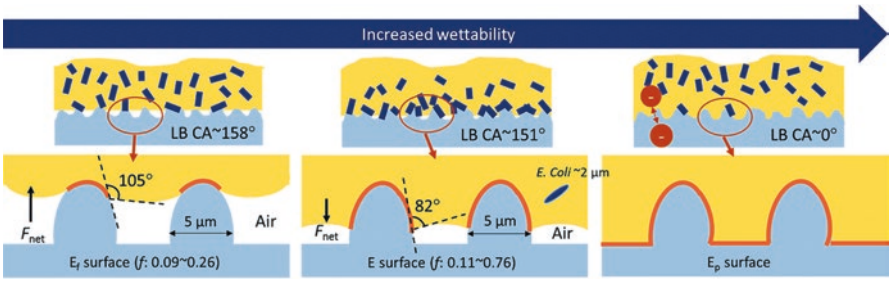


Fig. 2 Different interactions between the bacteria *Escherichia coli* and the rough surfaces with different wettability [23]. (Published by The Royal Society of Chemistry)

of implantable devices [23, 24]. There are fouling-resistant and fouling-release coatings. Reversible changes in surface properties like topography, wettability, and charge are due to fouling-releasing polymer coatings. Moreover, hydrogels, superhydrophobic surfaces, and passive polymers are fouling-resistant [25, 26].

The bactericidal effect is different from the fouling effect. The fouling effect prevents the adhesion of bacteria on the surface whereas the bactericidal effect is killing of bacteria. This strategy involves the use of antibiotics and germicides [27]. Besides them, peptides, quaternary ammonium compounds, active cationic polymer chains are used in this effect. Some coatings contain both bacteria-repellent and bactericidal effect which is more fruitful [28].

Coating Methods

Spray Coating

Spray coating is such a technology which can also be used in drug release and osseointegration coating. Drugs release can be controlled by different parameters of this technology. Spray coating is done by the deposition of different active agents like drugs, herbs, and polymers on biomedical devices. Several types of implants like catheters, pacemakers, heart valves, drug-eluting stents, orthopedic surgical implants, balloons, and sensors have gone through spray coating for antibacterial properties or drug release.

Ultrasonic Spray Nozzle

Among numerous technologies considered as spray coating, ultrasonic spray nozzle is the most widespread. A fine mist spray is prepared using ultra sound (i.e., high-frequency) sound vibrations which constitute an atomized coating solution. Electrical input is converted into high-frequency sound vibration (i.e., mechanical energy)

through piezoelectric transducers. When introduced into the nozzle, ultrasound creates capillary waves in the solution. An amplitude of vibration of an atomized surface should be controlled meticulously for the liquid to atomize. Production of an atomized drop depends on amplitude, as the energy produced below a critical amplitude will not be able to produce atomized drops of coatings, whereas, extremely high amplitude results in cavitation. Cavitation is the condition where the solution is ripped apart resulting in the ejection of large chunks of fluid instead of atomized drops [29]. Hence, the production of low-velocity mist with a fine nozzle and ideal amplitude is possible only within a narrow range of input. In this procedure, flow rates are on the range of 20–100 mL/min and tiny median drop diameters and spray diameters on the order of 0.5–2 mm result [30].

Stents are highly coated with an ultrasonic spray nozzle. A stent kept on a mandrel is attached to a rotating shaft in this procedure. A solution to be sprayed constitutes the drug or polymer which is dissolved in an appropriate organic solvent. A stent is placed below where the nozzle is mounted. For rapid drying, a high vapor pressure organic solvent is used. To produce a good quality coating and supreme material transfer efficiency, traverses should be repeated coupled with low flow rates.

Aerosol

Aerosol is also used as a spray coating technique. In a chamber which is filled with loose powder, gas is passed which results in production of a fluidized bed. This is the process for aerosol generation. These particles are then carried from aerosol chamber to deposition chamber. This process is driven by pressure difference. At high speeds, substrates collide with particles via a focused jet. The aerosol was deposited by spraying with a vacuum on AZ31 Mg alloy samples with hydroxyapatite (HA)—chitosan powder at room temperature by Hahn et al. A nozzle was rotated in the x- and y-direction of the sample which was kept opposite to the nozzle. The nozzle was not rotated in the z-direction. In this way, antiseptic HA and 4-hexylresorcinol were effectively coated onto a titanium surface [27, 30].

Thermal Spray

The thermal spray coating technique is another spray coating procedure that is excessively applicable to coat orthopedic implants with HA. The coating material is heated and melted to spray on the surface of implants. Using plasma, arc (electrical means) or combustion (chemical means), a coating material is heated [31].

High-Velocity Oxygen Fuel Coatings

In a combustion chamber, fuel and oxygen are compressed in a continuous flow to produce a high-speed jet of combustion. High-speed jet production particles are inserted into a gas stream and augmented to high velocity. It is high velocity which

matters instead of high temperature for fusion. The high-velocity oxygen fuel (HVOF) procedure is completed in an ambient environment [32]. For coating of implants with HA, HVOF is used. Implants coated with HA have improved shear strength, Young's modulus, and fracture toughness after being coated by the HVOF method. However, a minor decrease in Young's modulus was observed when titanium content was increased from 10 to 20% volume. Furthermore, a linear correlation was observed between phase composition, residual stress, and microhardness from the impact of velocity and particle temperature [33].

Pulsed Laser Deposition

In pulsed laser deposition (PLD), molecular beam epitaxy and sputter deposition share process characteristics. This procedure is carried out in a vacuum system. A pulsed laser is used for deposition which is focused on the target material to be piled. Sufficient high laser energy density is ensured as vaporization of each laser pulse produces a plasma plume. In a highly forward directed plume, ablated material ejection is through the target. This offers material flux which enhances film growth. Different parameters of thermodynamics of the substrate and condensing plasma fluxes may affect film growth. Particle energy, density, and ionization degree in the case of condensing plasma fluxes and activation energy of surface deposition, temperature, diffusion, and density of absorption sites for thermodynamic may effect film growth [34].

PLD is a technique having potential for HA coatings with great quality and high-performance coatings. PLD has been used to coat titanium substrates with a thickness ranging from 0.5 to 5 mm. PLD-HA coating properties are depended on deposition parameters. HA smooth and uniform films were obtained by the PLD method on titanium substrates by Torrisi and Setola in 1993 at 400 °C. However, the same procedure at room temperature may result in mixed morphologies, i.e., granular and uniform. Similarly, in 1995 Jelinek et al. got a uniform PLD-HA film in the same substrate but at different temperatures which ranged from 200 to 400 °C. PLD-HA coated samples were made at a higher temperature, i.e., 600–700 °C, in a substrate which resulted in increased roughness, heterogeneity, and buckling [34, 35].

Chemical Vapor Deposition

Any universal equipment has not been reported till now for chemical vapor deposition (CVD), but the equipment is usually custom-made for the particular deposition of materials, implant surface structure, etc. Three main components are used for

CVD equipment. The chemical vapor predecessor supply structure, reactor, and effluent gas supervision arrangement. The chemical vapor predecessor supply structure produces vapor precursors and carries it to the reactors. There is a reaction chamber in the reactor with a load-lock for carriage. It also consists of a place for the implant in the chamber, holder, and temperature control system. A CVD reactor may be either a hot or cold wall reactor which basically heats the substrates to the deposition temperature. Indirect heating is carried out in a hot-wall reactor where a heated furnace is used; however, the cold-wall reactor's wall remains cold where only the substrate is heated. An effluent gas handling system comprises a neutralizing portion for a vacuum system or exhaust gas to deliver a necessary reduced pressure which acts at a high vacuum or low pressure for CVD processing. The primary purpose of effluent gas supervision arrangement is elimination of dangerous by-products and poisonous unreacted precursors carefully. For implants, CVD is more appropriate for aluminum oxide, titanium nitride, and titanium carbonitride. However, several materials can be deposited through CVD [36].

Sputter Coating

A gaseous plasma is produced aiming an ion bombardment to a target (source material). The target is eroded by incoming ions through the transfer of energy which is evicted as neutral particles as individual atoms, molecules, or a cluster of atoms [37]. These neutral particles travel in straight lines until any particles or surface are encountered. So, by placing a substrate on this path of ejected particles, a film of source material is coated on it. This coating is widely used for HA and CaP coating [37–39]. Generally, sputtering practice, uniform and dense coatings are observed. Depending on deposition time, the thickness of the coating could be lower than 10 nm. Moreover, a CaP coating can expedite osseointegration even in osteoporotic conditions. This technique has also been used for zinc oxide deposition to regulate and avoid the dispersion and perseverance of bacterial infections [40, 41].

Inkjet Printing

A set of material deposition strategies are comprised of inkjet printing. A nozzle is used to spread a system of solvents which is evicted onto a surface. Being an entire automated process, the pros of this method in comparison to traditional coating practices is that there is more accuracy and precision to regulate droplet size which in turn reduces material losses, permits design of several deposition patterns, and decreases contamination of the coating [42, 43].

Layer-by-Layer Coating

A layer-by-layer (LBL) coating includes the dipping of a substrate repeatedly in polyelectrolyte solutions having opposite charges. This method has numerous applications in the biomaterial arena as it allows for the regulation of the nanometer level composed of thin films and production of highly advanced, custom-made coating compositions. For the deposition of growth factors, LBL has been used on different implant substrates [44, 45].

Metallic Nano-Coatings

Nanoparticle coatings are growing as antibacterial agents in the research field. Silver (Ag) is gaining much attention nowadays. Silver nanoparticles (AgNPs) are kept in contact with cell walls through which the localized release of Ag^+ ions by dissolution can occur [46]. Silver nanoparticles are clusters of silver atoms. Silver is considered to have eminent antibacterial property. Silver has the most suitable combination of properties for medical devices [46, 47]. The efficiency and properties of nanoparticles usually depend on their size and shape. If certain nanoparticles do not have expected properties using nanotechnology, one may control material parameters. Silver ions have shown strong antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* at concentrations of one-tenth of a ppm. For 2 h, tens of ppm silver ion solutions were applied to *S. aureus*. This caused cell lysis confirming the bactericidal property of silver ions. Morphology of *E. coli* also changed when treated with silver ions [46, 48].

Coatings play a vital role on medical implant as only implant substrate is not enough for resisting bacteria. AgNPs are identified to have antibacterial properties. One research study inspected the insertion of AgNPs on titanium dioxide nanotubes (TiO_2 NTs) on Ti-6Al-4V discs. The TiO_2 NTs were developed on a Ti alloy via an electrochemical method, which was adorned with AgNPs. AgNPs were produced using δ-gluconolactone by chemical reduction. The Ag-TiO_2 composites were examined by scanning electron microscopy (SEM); meanwhile, elements were evaluated by energy dispersive X-ray spectroscopy (EDS). SEM images of AgNPs forming micro-clusters on TiO_2 nanotubes grown on medical grade titanium alloy, along with the images of the composite coating, are shown in Fig. 3 [46]. The prepared coating was incubated in simulated body fluid to provide a released amount of Ag in 24 h. Release of Ag from micron-sized bunches was better than nanosized bunches while 0.015 M of silver ammonia was used. The antibacterial activity was tested against *S. aureus* and both the micron- and nano-sized bunches of the AgNPs showed antibacterial properties using the Live/Dead assay [46, 48].

Similar research was done where a catheter was coated with AgNPs and tested for antimicrobial properties. *S. aureus*, *E. coli*, *P. aeruginosa*, and coagulase-negative staphylococci were used as pathogens that are prevalent microorganisms in catheter-related infections. The AgNPs-coated catheter had significant antibacterial properties, and also prevented biofilm formation [49].

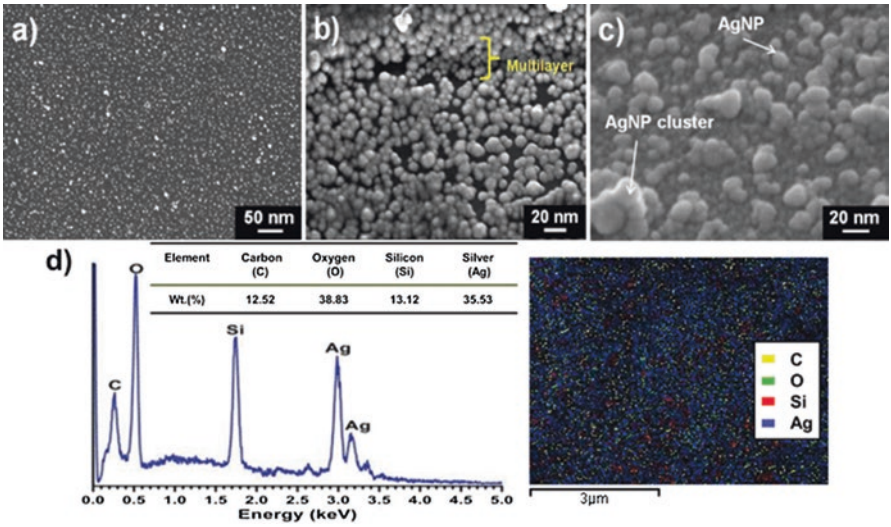


Fig. 3 SEM images and the corresponding EDS results of silver nanoparticles forming micro-clusters on the amine-modified glass surfaces [46]. (Published by The Royal Society of Chemistry)

Ceramic Coatings

Hydroxyapatite Coatings

As a well-known calcium phosphate ceramic, HA has been extensively studied as a biomaterial with high biocompatibility. It has osteogenic properties which could connect or have strong bonds with bone tissues. Sharing similitudes to bone and mineral of teeth, HA coatings has been broadly applied in orthopedic implants. The structure of HA provides a scope to incorporate with foreign ions which boost properties of HA and relevancy to orthopedic and dental implant application. It has been found that cerium (III)-HA shows antibacterial properties like silver. One of the captivating topics in biomedical science is the exchange of Ca ions in the HA lattice with cerium ions [50].

Zinc Oxide Coating

Zinc oxide (ZnO) is one of the well-known ceramic antibacterial materials and has been utilized as one component in biocompatible composite materials. Using a chemical solution deposition method trailed by heating at 650 °C, HA with a ZnO precipitate layer in the top surface coating was formed on a titanium substrate. Altering ZnO concentration, ZnO precipitates can be controlled in the deposition solution. Moreover, the release rate of Zn from the surface can be steered changing

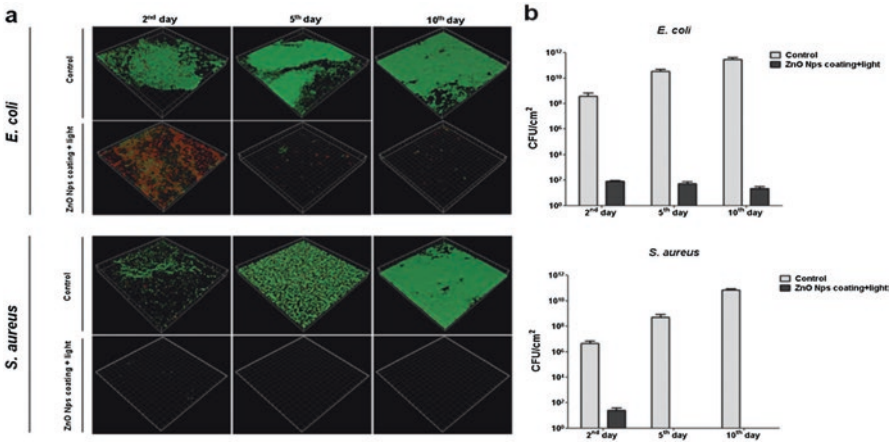


Fig. 4 Antibiofilm formation property of ZnO nanoparticle coating on glass surfaces: (**a**) the biofilm images of *E. coli* and *S. aureus*, (**b**) bacterial colony forming units [51]. (Published by The Royal Society of Chemistry)

ZnO amount. This coating showed outstanding antibacterial properties against *E. coli* and *Staphylococcus epidermidis* strains, as shown in Fig. 4 [51]. No connection was observed between the degree of efficacy and Zn release rate [52].

Other research was done to scrutinize the antibacterial properties of ZnO nanoparticles and how they react against the pathogen *E. coli* O157:H7 [53]. Antibacterial tests were conducted against different concentrations and sizes of nanoparticles of zinc oxide. Results showed an increase in inhibition on the growth of *E. coli* O157:H7 with the increase in concentration of ZnO nanoparticles. At the concentration of 12 mmol, a complete inhibition of microbial growth was attained. Significant antimicrobial properties were observed against *E. coli* O157:H7. Antibacterial properties increased with an increase in concentration of zinc oxide nanoparticles. Moreover, ZnO nanoparticles damage the bacterial cell membrane leading to cell death. This result recommends ZnO nanoparticles as a promising agent to be used for antibacterial activity [52, 53].

Polymer Coatings

Polymers are not only used for coatings but also are carriers for antibiotics which show their dual property as a coating on implants. In 2006, different polymers were used by Harris et al to explore cytocompatibility for human telomerase reverse transcriptase fibroblasts (hTERT), *S. aureus*, and *S. epidermidis*. Poly(D, L-lactide) (PDLLA), polyurethane (PU), polyterefate(PTF), polyvinylpyrollidone (PVP) were applied on titanium alloys. The study found that the release kinetics changed from

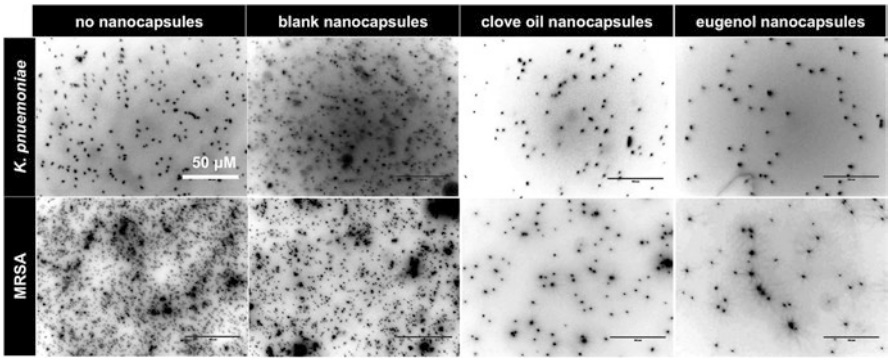


Fig. 5 Improved antibacterial performance of the PA13-coated coverslips loaded with two different nano-capsules (no cross-linker) when compared with control groups [55]. (Published by The Royal Society of Chemistry)

slow to excessive release [10, 54]. PDLLA and PTF were promising coatings for drug delivery passing mechanical tests and being more cytocompatible to hTERT fibroblasts [10, 54]. Antimicrobial polymers could inhibit the growth of bacterial, fungi, or protozoa. Venkateswaran et al. prepared poly(lauryl acrylate) nano-capsules containing eugenol (4-allyl-2-methoxyphenol), loading in the polymer coating, to achieve a slow release of eugenol and a long-term bacterial performance, as shown in Fig. 5 [55]. In addition, polymers are made to mimic antibacterial peptides which are used by the immune system of living beings for killing bacteria [56].

There are implants in the biomedical market which are biofilm resistant, nontoxic, and biocompatible. Hydrophilic polymer chains which are covalently grafted relate to a series of antimicrobial peptides (AMPs). An exceptional wide spectrum antibacterial activity was observed in vitro and in vivo because of tethered AMPs. This specifically designed coating was tremendously effective in counterattacking biofilm formation. The point to be focused on is biofilm resistance, which is dependent on the design and properties of the connecting peptides. No toxicity was observed to bone cells (like osteoblasts) which helped in bone regeneration [57, 58].

Polymer-coated devices are extensively used by interventional physicians and vascular surgeons to treat clinical problems. Even though polymer coating shows potential for infection resistance, polymer wear and its embolism have been a problem in different clinical presentations. Polymer coating integrity is dependent on coating material, substrate, coating application procedure, and thickness [58]. Hydrophilic polymer gel compounds are slippery because of high water absorption. One of the cons of hydrophilic polymer is erosion of the polymer coating during interventional processes. Polymer embolism is seen in the vascular path and through the bloodstream to nearby organs. Proper coating application, device, and characterization is further needed in this arena of polymer embolism [59].

Biodegradable polymers are used in various sectors today including implants, food packaging, and drug carriers. Blood compatible biodegradation implants are

now used in wound dressings, tissue engineering scaffolds, and bone cement. Polylactic acid (PLA) and polyglycolic acid (PGA) have currently fascinated the biomaterial market not only because they have the capacity to degrade totally but also due to their material properties. They have widely taken the place of research for surgical products and implants. They have the potential to be developed as orthopedic implants as they may solve the problem of re-surgery [59].

Collagen Coatings

Coatings have been completed on biomaterials for different purposes. Among all, collagen specifically has been deposited on implants for its antibacterial properties. Cerium-doped collagen was deposited on titanium substrates using a biomimetic method. A supersaturated calcification solution which incorporated a cerium source and collagen was prepared as a coating. At first, surface modification was done. An alkali by thermal oxidation pretreatment was applied to guarantee that the bioactivity increased on the substrate. Examination of the coatings was done by Fourier transformed infrared spectroscopy (FTIR), X-ray diffraction (XRD), SEM, and X-ray spectroscopy (EDX). Cerium combination with HA lattice was observed from EDX and XRD. Moreover, Fourier-transform infrared spectroscopy (FTIR) analysis proved the presence of collagen. The coating was tested against *E. coli* and *S. aureus* bacteria for its antibacterial properties. The cerium-doped HA/collagen coatings were more bacteriostatic meanwhile, they showed a better result against *E. coli* than *S. aureus*. These coatings have the potential to be used on orthopedic and dental implants [50].

Another study used HA/collagen to test bone formation and rapid osteointegration. Titanium implants were coated by HA/collagen and HA/collagen nanocomposites. HA/collagen was coated by dipping a titanium rod a couple of times. Further, specimens were sputter coated with gold and the prepared implants were observed through SEM images. Coated implants were placed under the periosteum of rat calvaria. New bone formation was observed 4 weeks after surgery in contact to the HA/collagen coated implant. The HA/collagen coating reabsorbed at the implant's surface where bone formation occurred. No serious infection was observed among rats and all of them survived the research period [60].

A similar experiment was carried out which used osteoprogenitor cells with biomaterials for bone grafting. A collagen–HA scaffold was made which degrades more easily than a ceramic scaffold. Material properties were characterized, and cell attachment and cell viability were completed. New bone formation was observed within 3 weeks in vivo. No serious infection was observed. Collagen–HA has been promising in the field of bone formation, osteointegration, and bone grafting. It has shown potential in orthopedics either used as a scaffold or as a coating for orthopedic implants [61].

PLA-Based Coatings

PLA is a biodegradable aliphatic polyester which is expensive, stable, and harder than other polymers. It is thermoplastic derived from natural organic acid. As mentioned earlier, PLA is biodegradable which degrades in physiological conditions. PLA and its copolymers (being aliphatic polyesters) are more biocompatible. It degrades by enzyme and hydrolysis under physiological conditions. PLA-based biodegradable nanocomposite coatings were prepared to check against antibacterial properties through an active gas phase which is generated by low energy electron beam dispersion of powdered norfloxacin and silver nitrate in a vacuum where silver nitrate is used for its antibacterial properties. Different parameters like optical properties, chemical states, morphology, and molecular structure were examined by transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS), ultraviolet visible spectroscopy (Uv-Vis), and attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR). PLA-based coatings on various surfaces were verified against *E. coli* and *S. aureus* by means of an agar diffusion method. ATR-FTR spectrum analysis was verified by polymer formation, doped antibacterial components and showed communication between them [53, 59].

Heparin Coatings

A new approach to use heparin as a nanoparticle was developed. In studies, a novel strategy was advanced to make pH-responsive films which were implanted with polymeric micelles as nano-vehicles loaded with weakly charged antibiotic drugs. Negatively charged tobramycin (Tob)-embedded heparin micelles (HET) and positively charged chitosan (CHT) were used as a LBL multilayer building block correspondingly. The characterization of the morphologies, chemical compositions, and hydrophilicity of the altered surface assured the effective deposition of the Tob-loaded CHT/HET coatings on the polydopamine altered substrate. Antibacterial tests showed that the Tob deposited CHT/HET nanostructured multilayers strongly repressed initial bacterial adhesion and distort biofilm formation [62]. Heparin nanoparticles are also used to treat a peptic ulcer. Helicobacter pylori bacterium was discovered from peptic ulcer patients. The bactericidal effect shown by heparin nanoparticles is promising to heal ulcers [62, 63].

Conclusion and Perspectives

To prevent the high infection risk of biomedical implants, surface coatings are some of the effective solutions to be applied on the implant materials. Different materials have been used as biomaterial coatings like metallic nano-coatings, ceramic coatings, and polymeric coatings. The coating materials are significant for antibacterial property, e.g., incorporation of AgNPs in the coatings resulted in a high antibacterial per-

formance. Different coating methods and the applied parameters, as well as the coating morphology also matter during the antibacterial process. Although many previous studies have been performed on the antibacterial mechanism of different antibacterial materials, a further detailed mechanism is also necessary to understand the interaction between materials and bacterial cells. In addition, balanced cytocompatibility and antibacterial properties, especially the in vivo dynamic performances, are still highly required during the different stages of implantation in the clinical practice.

References

1. Eggimann P, Sax H, Pittet D (2004) Catheter-related infections. Microbes Infect 6(11):1033–1042
2. Engelsman AF, van der Mei HC, Ploeg RJ, Busscher HJ (2007) The phenomenon of infection with abdominal wall reconstruction. Biomaterials 28(14):2314–2327
3. Van Houdt R, Mijnendonckx K, Leys N (2012) Microbial contamination monitoring and control during human space missions. Planet Space Sci 60(1):115–120
4. Kanematsu H, Barry DM (2015) Biofilm and materials science. Springer, Berlin
5. Nelson GN, Davis DE, Namdari S (2016) Outcomes in the treatment of periprosthetic joint infection after shoulder arthroplasty: a systematic review. J Shoulder Elbow Surg 25(8):1337–1345
6. Bandyk DF, Novotney ML, Back MR, Johnson BL, Schmacht DC (2001) Expanded application of in situ replacement for prosthetic graft infection. J Vasc Surg 34(3):411–420
7. Moran E, Byren I, Atkins B (2010) The diagnosis and management of prosthetic joint infections. J Antimicrob Chemother 65(suppl_3):iii45–iii54
8. Papagelopoulos PJ, Partsinevelos AA, Themistocleous GS, Mavrogenis AF, Korres DS, Soucacos PN (2006) Complications after tibia plateau fracture surgery. Injury 37(6):475–484
9. Campoccia D, Montanaro L, Arciola CR (2006) The significance of infection related to orthopedic devices and issues of antibiotic resistance. Biomaterials 27(11):2331–2339
10. Zhao L, Chu PK, Zhang Y, Wu Z (2009) Antibacterial coatings on titanium implants. J Biomed Mater Res B Appl Biomater 91(1):470–480
11. Simchi A, Tamjid E, Pishbin F, Boccaccini A (2011) Recent progress in inorganic and composite coatings with bactericidal capability for orthopaedic applications. Nanomed Nanotechnol Biol Med 7(1):22–39
12. Cheang P, Khor K (1996) Addressing processing problems associated with plasma spraying of hydroxyapatite coatings. Biomaterials 17(5):537–544
13. Hori K, Matsumoto S (2010) Bacterial adhesion: from mechanism to control. Biochem Eng J 48(3):424–434
14. Lindsay D, Von Holy A (2006) Bacterial biofilms within the clinical setting: what healthcare professionals should know. J Hosp Infect 64(4):313–325
15. Arciola CR, Campoccia D, Speziale P, Montanaro L, Costerton JW (2012) Biofilm formation in Staphylococcus implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials. Biomaterials 33(26):5967–5982
16. Hsu LC, Fang J, Borca-Tasciuc DA, Worobo RW, Moraru CI (2013) Effect of micro-and nanoscale topography on the adhesion of bacterial cells to solid surfaces. Appl Environ Microbiol 79(8):2703–2712
17. Chehroudi B, Brunette DM (2002) Subcutaneous microfabricated surfaces inhibit epithelial recession and promote long-term survival of percutaneous implants. Biomaterials 23(1):229–237
18. Gristina AG (1987) Biomaterial-centered infection: microbial adhesion versus tissue integration. Science 237(4822):1588–1595

19. Walkowiak-Przybyło M, Klimek L, Okrój W, Jakubowski W, Chwiłka M, Czajka A, Walkowiak B (2012) Adhesion, activation, and aggregation of blood platelets and biofilm formation on the surfaces of titanium alloys Ti6Al4V and Ti6Al7Nb. J Biomed Mater Res A 100(3):768–775
20. Zhao X, Zhao F, Wang J, Zhong N (2017) Biofilm formation and control strategies of foodborne pathogens: food safety perspectives. RSC Adv 7(58):36670–36683
21. Hou S, Gu H, Smith C, Ren D (2011) Microtopographic patterns affect Escherichia coli biofilm formation on poly (dimethylsiloxane) surfaces. Langmuir 27(6):2686–2691
22. Dhir S (2013) Biofilm and dental implant: the microbial link. J Indian Soc Periodontol 17(1):5
23. Yuan Y, Hays MP, Hardwidge PR, Kim J (2017) Surface characteristics influencing bacterial adhesion to polymeric substrates. RSC Adv 7(23):14254–14261
24. Lemire JA, Harrison JJ, Turner RJ (2013) Antimicrobial activity of metals: mechanisms, molecular targets and applications. Nat Rev Microbiol 11(6):371
25. Hassan MS, Amna T, Kim HY, Khil M-S (2013) Enhanced bactericidal effect of novel CuO/TiO2 composite nanorods and a mechanism thereof. Compos Part B Eng 45(1):904–910
26. Li K, Xie Y, Huang L, Ji H, Zheng X (2013) Antibacterial mechanism of plasma sprayed Ca 2 ZnSi 2 O 7 coating against Escherichia coli. J Mater Sci Mater Med 24(1):171–178
27. Choi J, Jang BN, Park BJ, Joung YK, Han DK (2014) Effect of solvent on drug release and a spray-coated matrix of a sirolimus-eluting stent coated with poly (lactic-co-glycolic acid). Langmuir 30(33):10098–10106
28. Thomas JG, Nakaishi LA (2006) Managing the complexity of a dynamic biofilm. J Am Dent Assoc 137:S10–S15
29. Gadow R, Killinger A, Stiegler N (2010) Hydroxyapatite coatings for biomedical applications deposited by different thermal spray techniques. Surf Coat Technol 205(4):1157–1164
30. Hahn B-D, Park D-S, Choi J-J, Ryu J, Yoon W-H, Choi J-H, Kim H-E, Kim S-G (2011) Aerosol deposition of hydroxyapatite–chitosan composite coatings on biodegradable magnesium alloy. Surf Coat Technol 205(8-9):3112–3118
31. Lima RS, Marple BR (2007) Thermal spray coatings engineered from nanostructured ceramic agglomerated powders for structural, thermal barrier and biomedical applications: a review. J Therm Spray Technol 16(1):40–63
32. Li H, Khor K, Cheang P (2002) Titanium dioxide reinforced hydroxyapatite coatings deposited by high velocity oxy-fuel (HVOF) spray. Biomaterials 23(1):85–91
33. Hearley J, Little J, Sturgeon A (2000) The effect of spray parameters on the properties of high velocity oxy-fuel NiAl intermetallic coatings. Surf Coat Technol 123(2-3):210–218
34. Zeng H, Lacefield WR (2000) XPS, EDX and FTIR analysis of pulsed laser deposited calcium phosphate bioceramic coatings: the effects of various process parameters. Biomaterials 21(1):23–30
35. Torrisi L, Setola R (1993) Thermally assisted hydroxyapatite obtained by pulsed-laser deposition on titanium substrates. Thin Solid Films 227(1):32–36
36. Mungkalasiri J, Bedel L, Emieux F, Doré J, Renaud FN, Maury F (2009) DLI-CVD of TiO2–Cu antibacterial thin films: growth and characterization. Surf Coat Technol 204(6-7):887–892
37. Wolke J, Van Dijk K, Schaeken H, De Groot K, Jansen J (1994) Study of the surface characteristics of magnetron-sputter calcium phosphate coatings. J Biomed Mater Res 28(12):1477–1484
38. Yang Y, Kim K-H, Ong JL (2005) A review on calcium phosphate coatings produced using a sputtering process—an alternative to plasma spraying. Biomaterials 26(3):327–337
39. Thian E, Huang J, Best S, Barber Z, Bonfield W (2005) Magnetron co-sputtered silicon-containing hydroxyapatite thin films—an in vitro study. Biomaterials 26(16):2947–2956
40. Ando E, Miyazaki M (2008) Durability of doped zinc oxide/silver/doped zinc oxide low emissivity coatings in humid environment. Thin Solid Films 516(14):4574–4577
41. Zhou Y, Kelly P, Postill A, Abu-Zeid O, Alnajjar A (2004) The characteristics of aluminium-doped zinc oxide films prepared by pulsed magnetron sputtering from powder targets. Thin Solid Films 447:33–39
42. Le HP (1998) Progress and trends in ink-jet printing technology. J Imaging Sci Technol 42(1):49–62
43. Jang D, Kim D, Moon J (2009) Influence of fluid physical properties on ink-jet printability. Langmuir 25(5):2629–2635

44. Shah NJ, Macdonald ML, Beben YM, Padera RF, Samuel RE, Hammond PT (2011) Tunable dual growth factor delivery from polyelectrolyte multilayer films. Biomaterials 32(26):6183–6193
45. Goodman SB, Yao Z, Keeney M, Yang F (2013) The future of biologic coatings for orthopaedic implants. Biomaterials 34(13):3174–3183
46. Agnihotri S, Mukherji S, Mukherji S (2013) Immobilized silver nanoparticles enhance contact killing and show highest efficacy: elucidation of the mechanism of bactericidal action of silver. Nanoscale 5(16):7328–7340
47. Rai M, Yadav A, Gade A (2009) Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv 27(1):76–83
48. Polívková M, Hubáček T, Staszek M, Švorčík V, Siegel J (2017) Antimicrobial treatment of polymeric medical devices by silver nanomaterials and related technology. Int J Mol Sci 18(2):419
49. Marassi V, Di Cristo L, Smith SG, Ortelli S, Blosi M, Costa AL, Reschiglian P, Volkov Y, Prina-Mello A (2018) Silver nanoparticles as a medical device in healthcare settings: a five-step approach for candidate screening of coating agents. R Soc Open Sci 5(1):171113
50. Ciobanu G, Harja M (2019) Cerium-doped hydroxyapatite/collagen coatings on titanium for bone implants. Ceram Int 45(2):2852–2857
51. Applerot G, Lellouche J, Perkas N, Nitzan Y, Gedanken A, Banin E (2012) ZnO nanoparticle-coated surfaces inhibit bacterial biofilm formation and increase antibiotic susceptibility. RSC Adv 2(6):2314–2321
52. Ohtsu N, Kakuchi Y, Ohtsuki T (2018) Antibacterial effect of zinc oxide/hydroxyapatite coatings prepared by chemical solution deposition. Appl Surf Sci 445:596–600
53. Liu Y, He L, Mustapha A, Li H, Hu Z, Lin M (2009) Antibacterial activities of zinc oxide nanoparticles against Escherichia coli O157: H7. J Appl Microbiol 107(4):1193–1201
54. Harris L, Mead L, Müller-Oberländer E, Richards R (2006) Bacteria and cell cytocompatibility studies on coated medical grade titanium surfaces. J Biomed Mater Res A 78(1):50–58
55. Venkateswaran S, Dos Santos ODH, Scholefield E, Lilienkampf A, Gwynne PJ, Swann DG, Dhaliwal K, Gallagher MP, Bradley M (2016) Fortified interpenetrating polymers–bacteria resistant coatings for medical devices. J Mater Chem B 4(32):5405–5411
56. Mowery BP, Lee SE, Kissounko DA, Epand RF, Epand RM, Weisblum B, Stahl SS, Gellman SH (2007) Mimicry of antimicrobial host-defense peptides by random copolymers. J Am Chem Soc 129(50):15474–15476
57. Gao G, Lange D, Hilpert K, Kindrachuk J, Zou Y, Cheng JT, Kazemzadeh-Narbat M, Yu K, Wang R, Straus SK (2011) The biocompatibility and biofilm resistance of implant coatings based on hydrophilic polymer brushes conjugated with antimicrobial peptides. Biomaterials 32(16):3899–3909
58. Skovdal SM, Jørgensen NP, Petersen E, Jensen-Fangel S, Ogaki R, Zeng G, Johansen MI, Wang M, Rohde H, Meyer RL (2018) Ultra-dense polymer brush coating reduces Staphylococcus epidermidis biofilms on medical implants and improves antibiotic treatment outcome. Acta Biomater 76:46–55
59. He C, Chen Q, Yarmolenko M, Rogachev A, Piliptsou D, Jiang X, Rogachev A (2018) Structure and antibacterial activity of PLA-based biodegradable nanocomposite coatings by electron beam deposition from active gas phase. Prog Org Coat 123:282–291
60. Villa MM, Wang L, Huang J, Rowe DW, Wei M (2015) Bone tissue engineering with a collagen–hydroxyapatite scaffold and culture expanded bone marrow stromal cells. J Biomed Mater Res B Appl Biomater 103(2):243–253
61. Uezono M, Takakuda K, Kikuchi M, Suzuki S, Moriyama K (2013) Hydroxyapatite/collagen nanocomposite-coated titanium rod for achieving rapid osseointegration onto bone surface. J Biomed Mater Res B Appl Biomater 101(6):1031–1038
62. Zhou W, Jia Z, Xiong P, Yan J, Li M, Cheng Y, Zheng Y (2018) Novel pH-responsive tobramycin-embedded micelles in nanostructured multilayer-coatings of chitosan/heparin with efficient and sustained antibacterial properties. Mater Sci Eng C 90:693–705
63. Rodriguez-Torres MdP, Acosta-Torres LS, Diaz-Torres LA (2018) Heparin-based nanoparticles: an overview of their applications. J Nanomater 2018:1–8

Metal- and Polymer-Based Nanoparticles for Advanced Therapeutic and Diagnostic System Applications

Nicole J. Bassous and Thomas J. Webster

Abstract Solutions for distinct clinical conditions that arise due to the application of nanotechnology, pertaining to refined diagnostics and therapeutics, are steadily revolutionizing the medical field. Presently, distinct modalities have emerged which advocate the manipulation of nanomaterials to produce medical devices. While several of these constructs are actively being used in the clinic, a greater number are being audited for clinical safety and efficacy, and many more are under various stages of development. Nanomaterials that are frequently investigated and that have been approved for clinical use include capsules, dendrimers, polymeric nanoparticles, nanocages, nanoshells, biopolymer nanocarriers, fullerenes, carbon nanotubes, and various inorganic materials. Due to the vibrancy of the nanomedical field, novel solutions are continuously being developed and adapted to meet standard patient needs and to exceed the capabilities of antiquated hospital diagnostic and treatment systems. In this review, the integration of biomaterials and nanotechnology, to yield nanomaterial building blocks, is investigated, especially with pertinence to the fabrication of contemporary medical devices that can be used to treat or diagnose a broad range of bacterial infections. Although nanotechnology has been credited with advancing numerous clinical breakthroughs, substantial efforts must be directed toward extensive cytotoxicity, biodegradation, administration, distribution, and metabolic analyses, among other performance identifiers, prior to the adoption of nanoparticles and/or nanomaterials as dependable drug substitutes, carriers, implants, or sensor elements.

Keywords Nanotechnology · Biomaterials · Metal nanoparticles · Mechanisms of action · Antimicrobial · Drug delivery vehicles · Polymers · Advanced imaging systems · Surface-enhanced Raman spectroscopy (SERS)

N. J. Bassous · T. J. Webster (✉)
Department of Chemical Engineering, Northeastern University, Boston, MA, USA
e-mail: th.webster@neu.edu

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_16

Introduction to Nanotechnology

Types of Nanomaterials

Presently, discoveries in biomaterials are enriched by breakthroughs in nanotechnology, and the medical research and clinical scene have been propelled substantially forward through the foundation of technologies based on nanomaterials [1]. Since the inception of the US National Nanotechnology Initiative in 2000, the adoption of nanotechnologies for the enhancement of consumer or manufacturing products has been thoroughly well received on the world stage. Significantly, within the past two decades, various principles of nanotechnology have permeated into diverse sectors integral to societal advancement, including computing, manufacturing, energy, electronic devices, and, pertinent to the current review, health [2]. Such solutions are dependent on the manipulation of elementary constituents on the nanoscale, where the *nano-* prefix is dimensionally indicative of 10^{-9} units of measure.

In particular, as the result of a ubiquitous understanding that optimal quantum effects and enhanced surface area-to-volume ratios are associated with dimensions on the order of 100 nm or less, the nanoscale designation in scientific literature is generally assigned to constituents that meet this standard in size, from extremity to extremity [3, 4]. A variety of shapes, sizes, chemical compositions, and surface functionalities are, nevertheless, attributable to nanomaterials [5]. Commonly researched contemporary nanomaterials are classified according to structural properties as zero dimensional (0D), one dimensional (1D), or two dimensional (2D). 0D, 1D, and 2D nanomaterials include nanoparticles; nanorods, nanotubes, and nanowires; and nanocoatings, nanofilms, and nanolayers, respectively [6]. Different nanomaterial configurations are presented in Fig. 1. Materials that possess any of

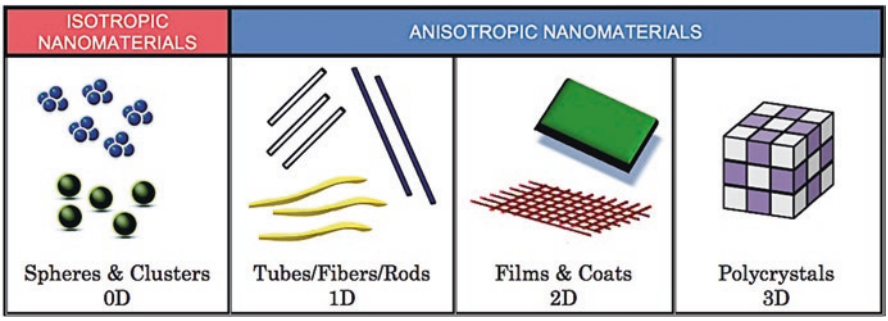


Fig. 1 0D, 1D, and 2D nanomaterials are naturally or synthetically derived [7, 8]. Common types of 0D, 1D, 2D, and 3D materials include organic nanoparticles like lipid micelles; inorganic nanorods; polymeric nanocoatings; and the carbon allotrope for diamond, respectively [9]

these size distributions generally display excellent tunability with regard to their anticipated function, whether biological, electrical, optical, thermal, or magnetic in nature [5]. Innovative strategies, such as self-assembly or electrospinning, are continuously being adapted by scientists to generate complex higher-order architectures from basic nanostructural units [6].

Applying Nanotechnology to Resist Infectious Diseases

The application of nanotechnology has become prevalent among researchers as a unique strategy for counteracting antibiotic resistance in bacteria. Prions, parasites, fungi, bacteria, and viruses are the most widely recognized classes of virulent pathogens that contribute to transmissible infectious diseases worldwide, and over 1400 microorganisms have been known to prompt disease in humans [10, 11]. Due to the prevalence of pathogenic illness among a global population, and as the result of the significant mortality rates attributable to these organismal types, infectious diseases are classified as a major health and well-being risk to humans. Actions are perpetually underway to counteract the prevalence, sustainability, and communicableness of many major pathogens. Advanced pasteurization approaches, novel vaccines, and antibacterial agents are continuously studied and generated [2]. Despite these efforts, microorganisms are steadily evolving to overcome risks to their own security, and unique solutions are being sought. In recent years, the rapid maturation of antibiotic-resistant bacteria, set on by genetic mutations and propagated by horizontal gene transfer, has weakened the efficacy of antibiotic therapies that have in the past demonstrated clinical feasibility [12]. As this evolutionary element continues to confer robustness to bacterial strains, new clinical practices that supplement or supplant antibiotics are needed to counter probable societal ill effects.

The scientific literature has been saturated with numerous studies investigating a diversity of approaches for utilizing nanomaterials to diagnose and treat infections, especially those caused by antibiotic-resistant strains of bacteria. Indeed, noble metal nanoparticles, metal oxide nanoparticles, carbon-based nanomaterials, bionanomaterials, and polymeric nanomaterials have all been demonstrated to aid in the detection or treatment of illnesses across a broad spectrum of disease states [13]. Acute or chronic bacterial infections are among the human afflictions that have been shown to be responsive to these nanomedicine therapies. This is an existential result of the ancillary properties conferred to nanoparticles and materials due to their engineered shape and size characteristics. Modifications in the synthesis parameters or conditions of nanomaterials frequently result in unique architectures that combat disease through elaborate and sometimes uncommon mechanisms. In the following sections, a variety of nanomaterials with the capacity to confer medically applied devices with capabilities ranging from sensing to diagnostics, therapeutics, and targeted therapy will be examined.

Noble Metal and Metal Oxide Nanoparticles as Antibacterial Agents

Metal Nanoparticle-Induced Pathogenic Toxicity: Mechanisms and Actions

Among the classes of noble metal and metal oxide nanoparticles, gold (Au), silver (Ag), platinum (Pt), palladium (Pd), zinc oxide (ZnO), cerium oxide (CeO_2), iron oxide (Fe_2O_3), and copper oxide (CuO) are distinguished due to the antibacterial effects either directly or indirectly associated with their application within in vitro and in vivo environments [13]. Common metal-based nanoparticle types are tabulated in Table 1, and a few of their unique applications, besides antimicrobial efficacy, are summarized [14–21]. Strategies for preparing nanoparticles include traditional hydrothermal and solvothermal syntheses, thermal decomposition, spray pyrolysis, ball milling, and chemical precipitation [14]. Emerging green methods are nutrient, plant, fungus, or polymer mediated [14].

Table 1 Common metal nanoparticle identities and their physical properties are tabulated

Nanoparticle identity	Group	Classification	Crystal structure	Select biomedical applications	References
Cerium oxide (CeO_2)	Ce 3	Lanthanoid	HCP	Alteration of mitochondrial metabolism Reshaping immune microenvironment Reduction in tumor growth	[14]
Copper oxide (CuO)	Cu 11	Transition metal	FCC	Targeted cancer therapy Wound healing	[15]
Gold (Au)	Au 11	Transition metal	FCC	Antifungal and anticancer Excellent catalytic activity Plasmonic properties	[16]
Iron oxide (Fe_3O_4, Fe_2O_3)	Fe 8	Transition metal	BCC	MRI contrast agent Magnetic hyperthermia for cancer treatment Tissue repair	[17]
Palladium (Pd)	Pd 10	Transition metal	FCC	Anticancer and anti-tumor Electrocatalytic uses	[18]
Platinum (Pt)	Pt 10	Transition metal	FCC	Catalytic activity Anti-inflammatory	[19]
Silver (Ag)	Ag 11	Transition metal	FCC	Catalytic redox properties Surface-enhanced Raman scattering Calorimetric Sensing	[20]
Zinc oxide (ZnO)	Zn 12	Transition metal	*HCP*	Use in positron emission tomography Gene delivery	[21]

Select applications are additionally outlined [14–21]
Abbreviations: *FCC* face centered cubic, *HCP* hexagonal close packed, *BCC* body centered cubic

Researchers have described extensively within the literature many strategies for experimentally inhibiting bacterial proliferation using elementally pure or hybridized metallic nanoparticles, and modifications have been investigated which facilitate these inhibition effects. The presentation of bacteriostatic and bactericidal properties by noble metal and metal oxide nanoparticles is attributable to their unique physiochemical properties, pertaining to size, morphology, and electronic responsiveness [22]. Often times, nanoparticle interactions with bacterial membranes prompt, or invoke, critical processes that significantly deplete cells of their energy sources or disrupt internal mechanistic channels. Biomolecular impairment and ATP depletion are common responses that suppress bacterial activity [23].

Membrane damage and depolarization are significant stimulants of microbial cell degradation, and these are often brought on by antagonistic interactions with metallic nanoparticles. Frequently, the cationic nature of a number of metallic nanoparticle types, or of their constituents, promotes selective electrostatic interactions with anionic bacterial membranes. Positively charged nanoparticles have demonstrated the capability to invoke physical damage to the bacterial wall and to eventually exhaust the electron transport chain [2]. In the case of magnesium oxide (MgO) and magnesium hydroxide ($Mg(OH)_2$) nanoparticles, for example, the main modes of action are adsorption onto and destruction of the cell wall [24, 25]. Here, preeminently in the case of $Mg(OH)_2$, it is believed that extracellular nanoparticle aggregation damages the bacterial cell envelope and alters the normal texture or thickness of the cell. However, aggregation is not therapeutically admissible, due to the potential adverse impact on healthy human cells, blockage of normal blood flow, and the possible reduction in bacterial cell–particle interactions [26]. Penetration or endocytosis of adsorbed nanoparticles across the bacterial wall is common among alternative particles types, leading to selective intracellular damage and cytotoxicity effects [2].

Cellular damage caused by nanoparticles is characteristically propagated by a variety of processes, especially those involving the release of metal ions and oxidative or non-oxidative stresses [22]. Metallic nanoparticles that are suspended in an aqueous environment can release charged ions as the result of a naturally imposed electrochemical potential. It is regularly observed among researchers that a greater degree of nanoparticle dissolution yields a higher concentration of ions, which is directly correlated with mammalian cell toxicity [27–30]. Enhanced dissolution rates, and hence toxicity effects, are defined among smaller and rougher nanoparticles [29]. Ions are soluble and therefore suited for uniformly confining bacterial cells [22]. The localization potential of intact, electrostatically interactive nanoparticles about a membrane domain promotes the regional generation of ions. High, localized ion concentrations facilitate cell membrane disengagement. For instance, in the case of Ag nanoparticles, the uptake of silver particles or ions by the cell can be observed by the presence of irregular pits on the bacterial surface [31]. Alternative hypotheses, again with reference to silver, predict that Ag^+ ions transcend the cellular wall through cation-selective pathways [32]. The association of Ag nanoparticles with bacterial walls has demonstrated an interplay in which the bacterial wall deteriorates or is transcended, to enable the influx of cytotoxic Ag^+ ions into the cellular cytosol [33, 34].

Evidence supporting the claim that metal ions incontrovertibly impose toxicity on bacterial cells is profound in the literature. For instance, exposing *Escherichia coli* (*E. coli*) cells to titanium dioxide (TiO_2) or aluminum oxide (Al_2O_3) nanoparticles, under uniform conditions, contributes to the body of evidence that substantiates that Al_2O_3 is a more potent membrane disruptor than TiO_2 [26]. This correlates with the assertion that ions contribute to bacterial death, in that Al_2O_3 releases Al^{3+} ions in solution, whereas Ti^{4+} ions are not detected in the case of the TiO_2 formulation [26]. To corroborate this theory, and to eliminate the possibility of alternative contributing factors, comparisons can be drawn between the effect of nanoparticle control treatments on bacterial cells and the effect of nanoparticle suspensions that are leached of their ions. Research has shown that, upon the removal of impurities from nanoparticle systems, especially of metal ions, nanoparticle toxicity is significantly reduced [35].

Nevertheless, other factors come into play when evaluating the agents responsible for nanoparticle efficacy. For example, although CuO nanoparticles yield a higher ion count than Ag nanoparticles in solution, Ag nanoparticles are more bactericidal. In this specific case, the cause is attributed to the understanding that Cu is an essential element and, as such, can be eliminated from the intracellular environment by pathways maintained due to natural homeostasis [36, 37]. In contrast, Ag is nonessential to cellular stability and can bind irreversibly to cysteine molecules [22]. This cysteine binding can impair intracellular enzymatic mechanisms, leading to possible perturbation of the electron transport chain or energy production.

Cellular leakage is a common disruption process that is compelled by metal nanoparticles and their ions. When examined under an electron microscope, bacteria lysed by nanoparticle–cell interactions may exhibit partial or complete disengagement of the intracellular environment from the cell wall, depending on the nature of the applied nanoparticle treatment [38]. Electron-dense inessential matter or granules, presumably generated by the interactions of anionic compounds found inside the bacterial cell wall with cationic species, are often detected in regions surrounding the lysed cells [38, 39]. It is predicted that the release of ions destabilizes the bacterial wall and compels membrane dislocation. These processes are emulative of the phenomena involved in plasmolysis, in which cells are depleted of their water sources [40]. Bacterial membrane impairment, resulting in leakage, has been observed from exposing gram-positive and gram-negative bacterial cells primarily to Ag, ZnO, MgO, and TiO_2 nanoparticles [41–43]. By performing temporal growth and electron imaging experiments, researchers have concluded that, generally, nanoparticles such as Ag are more effective at cleaving the bacterial walls of gram-negative bacteria than of gram-positive bacteria. This is attributed to the thick peptidoglycan layer that protects the bacterial cytoplasmic membrane in gram-positive organisms [29, 35, 38].

Membrane damage and cellular leakage due to nanoparticle agglomeration, adsorption, and ion production are not the only mechanisms responsible for bacterial cell death, and nanoparticles are very multifaceted in their efficacy routes. Commonly, chemically responsive reactive oxygen species (ROS) are generated upon the interaction of metal nanoparticles and cell walls [41]. ROS, including

superoxides, peroxides, and free radicals, are produced naturally during basic metabolic processes in which oxygen is passed through one or several states of reduction, and have critical roles in maintaining homeostasis and cell signaling pathways. However, the generation of ROS in excess, due to external or environmental stresses, could invoke cellular and biomolecular damage [44]. In fact, this effect is pronounced with the adoption of nanoparticle regimens, since a variety of metal nanoparticle types have been proven experimentally to stimulate the production of high levels of ROS, especially free radicals. This outcome, of elevated ROS generation, is observed even among *Cupriavidus metallidurans* (*C. metallidurans*) strains upon exposure to nanoparticles, despite their known survivability in a heavy metal stress environment [26]. Moreover, nanoparticles can be subjected to auxiliary stresses to activate the production of more ROS. For example, light- and UV-activated ZnO nanoparticles that are introduced into a water-rich environment split the water molecules to produce H^+ and OH^-, which react to form H_2O_2 [45]. Similar phenomena have been widely observed with Ag nanoparticles. The chemical processes involved in ROS production, and the activation of Ag nanoparticles to impose antibacterial toxicity, are outlined in Fig. 2.

ROS that are present in excess within the vicinity of a cell, either intra- or extracellularly, will alter the cell membrane by a number of mechanisms. Typically, the peroxidation of membrane lipids inhibits bacterial growth [46]. Moreover, DNA replication and ATP generation are impeded in the presence of ROS [47]. Nevertheless, the mechanisms involved in ROS toxicity are complex, and insufficient evidence is available to distinguish the primary mode of killing. Indicative of this complexity is the research observation that although DNA damage is imposed in the presence of ROS, it is not uncommon to distinguish intact bacterial membranes among cells that are thus affected [48]. Moreover, research evidence indecisively marks the dominant antimicrobial mechanism as the cause of oxidative stresses or thiol-containing protein inactivation. In support of the oxidative or catalytic stress

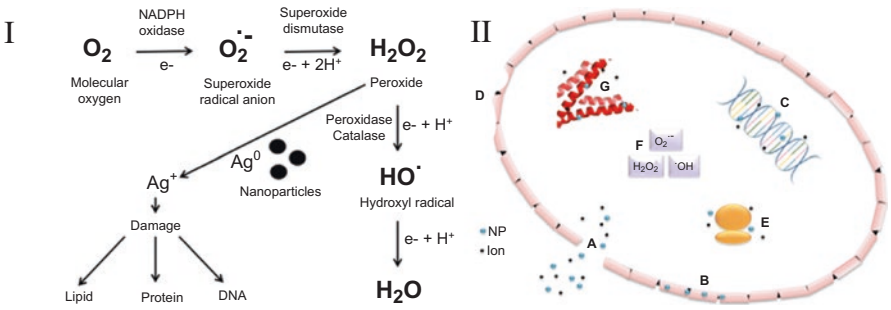


Fig. 2 In Part **I**, the reaction mechanisms involved in ROS production and Ag nanoparticles toxicity are represented. Part **II** outlines the impact of Ag nanoparticles on bacterial cells, including (A) cell wall disintegration, (B) periplasmic space separation, (C) DNA damage, (D) cell pit formation, (E) ribosomal inhibition, (F) ROS production, and (G) protein interactions [22]. Permissions for figure reuse were assumed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/)

theory, a study was performed by Wang et al. that magnified the role of H_2O_2 in debilitating *E. coli* cells [49]. Here, it was also demonstrated that Ag^+ ions generated by Ag nanoparticles aid in intracellular ROS production, although not to the extent in which extracellular ROS stimulate the production of intracellular ROS. The protein inactivation proposition was derived earlier in the research of Xiu et al., who observed that Ag^+ ions demonstrated no significant differences in toxicity under aerobic versus anaerobic conditions [50]. From these contradictory hypotheses, it is practicable to conclude that the circumstances involved in metal nanoparticle–bacterial interactions are so complex that a diversity of interrelated events contributes to cellular toxicity. It is difficult to identify a single species, activity, or mechanism that predominantly compels bacterial cell death.

Strategies for Modifying and Encapsulating Nanoparticles for Disease Applications

A variety of strategies have been devised to augment the properties, functions, and delivery of metallic nanoparticles. These include structural and surface modifications, such as doping, capping, and halogen treating; or encapsulation routines, in which one or more nanospecies are incorporated into polymer- or biomolecule-based nano delivery vehicles. Several cases examining the optimization of metal nanoparticles for utilization in the clinic are highlighted here.

Doping, Capping, and Halogenating

Physical and chemical fabrication methods for the preparation of doped metallic nanoparticles are described extensively within the literature. Physical techniques for the conversion of metal-organic precursors are classified according to the primary process utilized in nanoparticle preparation, including spray pyrolysis [51], thermochemical/flame decomposition [52, 53], and vapor condensation [54]. Likewise, chemical routes entail the application of at least one characteristic technique such as sol–gel transition [55], thermal hydrolysis [56], or hydrothermal processing [57]. Due to the well-defined nature of conventional doping strategies, the process of incorporating different species into a crystal structure is quite feasible, despite the complications involved in synthesis. The inclusion of supplements that can complement the activity of, or positively alter, pure substances has been scrutinized extensively in nanomedicine.

Recently, Azam et al. demonstrated that the doping of ZnO nanoparticles, using cobalt (Co) and/or magnesium (Mg), yielded enhanced antibacterial efficacy, in comparison to non-doped ZnO nanoparticles [2]. Moreover, the antibacterial properties of cobalt, for instance as a stable metal coordination complex [4], and magnesium, even in its pure elemental form, have been demonstrated in the literature [58].

Quantitative and qualitative analyses were designed by Azam et al. to assess the auxiliary and synergistic properties afforded to ZnO species upon complexation [2]. Empirical trends indicated that the antibacterial characteristics of nanocrystalline ZnO were significantly enhanced by the addition of Co or Mg to the crystal lattice. This effect was slightly more pronounced in the circumstance of Co doping versus Mg doping. In vitro experiments were designed based on standard temporal growth curve and zone of inhibition procedures. The growth behaviors of four types of bacteria, including gram-negative *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*), and gram-positive *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*), in the presence of the different nanoparticle types were analyzed [2]. The data indicated that all bacterial types underwent a perceptible and significant reduction in cell viability when treated with ZnO or doped ZnO nanoparticles. In particular, a positive trend was observed, in which a higher Co or Mg concentration within the primary aggregate ZnO nanostructure produced an increase in the antimicrobial activity. The minimum inhibitory concentration (MIC) of the doped ZnO particles was lower than the MIC of the non-doped particles, and the zone of inhibition increased with nanoparticle doping. These observations align well with the characterization measurements, which indicate that an increase in the nanoparticle Co or Mg concentration corresponds with a decrease in particle size. The higher surface area-to-volume ratio attributed to smaller particle sizes promotes interactions between the nanoparticles and bacterial cells, which improves nanoparticle efficacy. Refer to Fig. 3 for relevant data representations.

It is postulated that several factors are at play in conferring bacteriostatic function to ZnO nanoparticles. Electrostatic interactions between the ZnO particles and the bacterial wall compel a complex series of processes that invoke cellular dysfunction. Zn^{+} ions invoke bacterial cell wall or membrane damage, which is aggravated by an influx of nanoparticles and ions into the cell body. This influx stimulates cellular swelling and consequential bursting. Concomitantly, ZnO nanoparticles produce high yields of ROS, especially hydroxyl radicals and peroxides, which contribute to cell damage and death [59–62]. Further, modifications to the ZnO crystal lattice, that is, doping, can be applied to improve in the antimicrobial efficacy of the composite nanospecies. Due to this wide range of action associated with pure ZnO nanoparticles, and due to their potential for optimization through structural modification, ZnO nanoparticles hold great promise for the clinical obstruction of infections. Moreover, doping has emerged as a practicle means for enhancing the properties of metal-based nanoparticles, although additional data on the in vivo interactions of such species must be obtained prior to their being applied as medical devices.

Alternative approaches to modifying the precise configurations of metallic nanoparticles involve the incorporation of capping agents or halogens into the composite nanostructure. Nanoparticle fabrication strategies, implicating the use of a wide selection of capping agents are abundant. Generally, capping agents are associated with the enhanced stability and good dispersion of nanoparticles in suspension [22]. Due to the tendency of capped nanoparticles to remain as separate entities for extended time periods, with minimal agglomeration, nanoparticle tox-

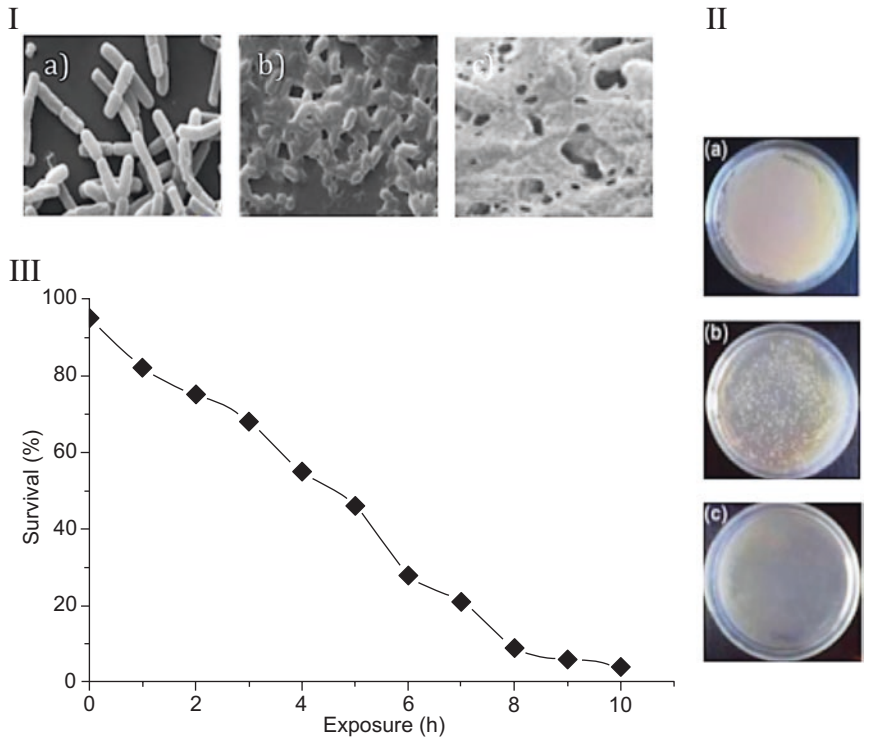


Fig. 3 A survival assay of *Bacillus subtilis* bacteria in the presence of ZnO nanoparticles was performed [2]. Parts **I** and **II** show SEM micrographs and nutrient agar plates, respectively. Different time points were tested, in which (*a*) corresponds to 2 h, (*b*) 6 h, and (*c*) 10 h incubation of bacteria and ZnO nanoparticles. A cell survival curve is represented in Part **III**

icity in comparison to the standard, uncapped form is altered. Specifically, metal nanoparticles such as Ag significantly inhibit bacterial growth when applied in combination with common capping agents like chitosan, citrate, and polyvinyl acetate. In the case of Ag nanoparticle capping, the antimicrobial activity is significantly enhanced through the incorporation of chitosan and citrate, potentially due to the increase in the generation of Ag^{+} ions that is propelled by the inclusion of a capping material [63]. 11-mercaptoundecanoic acid demonstrates improved virulence in comparison to other types of organic layers such as citrate [64]. Generally, this effect may be attributed to the poor stability of 11-mercaptoundecanoic acid in solution, which promotes the release of destructive metal ions from the core nanoparticle body; the occurrence of Cd^{2+} ions in solution indicate the general instability of 11-mercaptoundecanoic acid. Moreover, in the case of Ag nanoparticles capped by 11-mercaptoundecanoic acid, for example, adherence to the hydrophilic cell wall of *P. aeruginosa* indicates that specific interactions or affinities

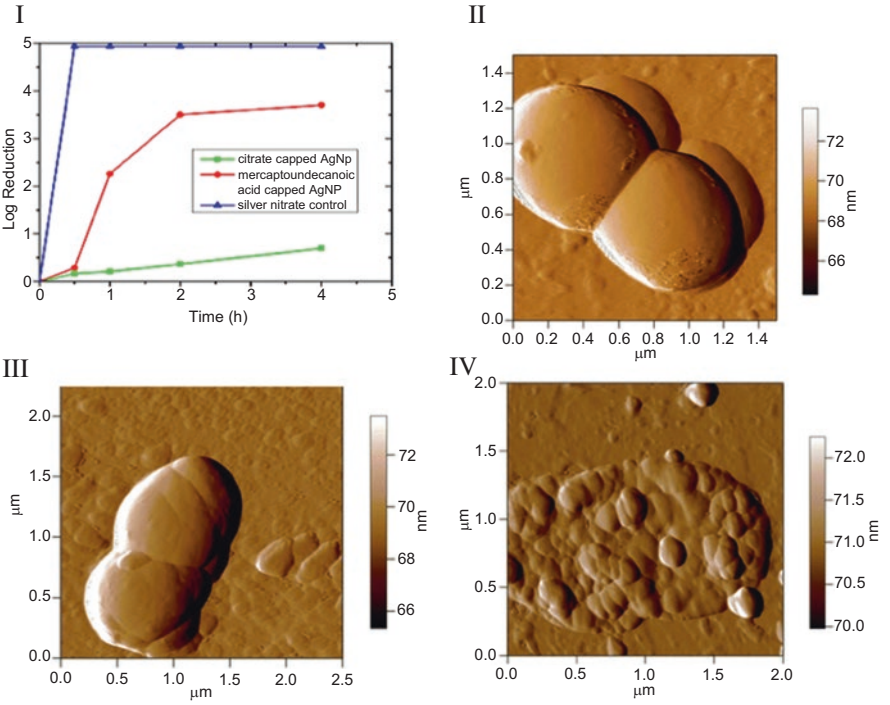


Fig. 4 A log reduction plot was produced examining the effect of citrate capped and 11-mercaptoundecanoic acid-capped Ag nanoparticles on *P. aeruginosa*, in comparison to a silver nitrate control (**I**). AFM representations of *P. aeruginosa* are shown in (**II**) before treatment, (**III**) after citrate-capped Ag treatment, and (**IV**) after 11-mercaptoundecanoic acid-capped Ag treatment [64]

may be involved in the induction of 11-mercaptoundecanoic acid toxicity [64]. The microbicidal activities of citrate- and 11-mercaptoundenanoic acid-capped Ag nanoparticles, in comparison to a silver nitrate control, are outlined in Fig. 4.

Other types of stabilizers utilize the principles of green technology. In most cases, biogenic silver has been produced through routes involving the application of natural plant materials composed of -OH, -SH, $-NH_2$, and -COOH functional groups. For instance, the *Ocimum sanctum* (Tulsi) aqueous extract and gum arabic have been applied as capping agents, and their reception has been favorable, especially due to their overall efficacy and omission of hazardous organic solvents during synthesis [65]. Surfactants such as cetyltrimethylammonium bromide (CTAB) are additionally utilized during such syntheses in order to direct morphological arrangements during particle formation [65]. Besides chemical- and green-based capping agents, halogens have been investigated as feasible functional groups for improving the antimicrobial behaviors of metal nanoparticles. The ability of halogens to confer potency to nanospecies is attributed principally to their oxidizing potential [42].

Polymeric Nanomaterials and their Usefulness as Drug or Particle Carriers

In addition to surface modifying metal nanoparticles in order to achieve enhanced efficacy or stability, nanoparticle encapsulation has been widely investigated as a means for providing adequate delivery or promoting therapeutic synergism. Variable particle types can be tuned to accommodate distinct functionalities and to address issues of biodegradability and biostability as they pertain to a particular end operation. In particular, carrier micro- and nanoparticles preferentially adopt dendrimer, micelle, liposome, polymersome, or other capsule-based morphologies. These particles typify possible cell body architectures. Particle selection is dependent on the intended final use or distribution route. Regularly, biomaterials that are facilely expelled from the body after performing their function are desired in particle assembly, and biodegradable substances are favored in the fabrication of such nanomaterials.

For example, poly(lactic-*co*-glycolic acid) (PLGA), polylactide (PLA)-based enantiomers, and poly(*ε*-caprolactone) (PCL) are biodegradable and suitable for use in solid drug delivery vehicles, polymersomes, micelles, or other particles that assemble using synthetic polymers as their main body [66]. Nanoparticles assembled using these or any of an array of biocompatible polymers commonly unload their therapeutic contents through cross-membrane diffusion, controlled polymer degradation (i.e., temperature, pH, or electrical sensitivity or stimulation), or vesicular dissociation. Localized accumulation of these particles at the diseased site(s) may involve extended exposure of affected tissue to the therapeutic load. Particles can further be designed to accommodate a diverse group of therapeutic molecules, depending on their membrane properties.

It is possible to incorporate hydrophilic and/or hydrophobic species into several synthetic cell body types. One conceivable advantage of particle configurations, that is, capsules, retaining a hydrophobic core is the potential for solubilizing hydrophobic drugs within the core and, as a result, increasing the concentration and enhancing the effectiveness of this hydrophobic drug in an aqueous environment. Generally, excipients like Kolliphor EL, which cause autoimmunity or hypersensitivity, have been used to deliver poorly water-soluble drugs [67–71]. The solubilization and delivery of hydrophobic drugs utilizing a hydrophobic particle core would help circumvent comparable undesirable reactions or side effects.

In the fabrication of nano drug carriers, terminal particle size distributions must be optimized to satisfy the physical restrictions imposed by administration and discharge routes. Cell bodies injected into or otherwise taken up by the vasculature should be designed to cap at 1–2 μm in their outer diameter in order to bypass possible agglomeration in small blood vessels and capillaries [72]. The in vivo fate of these particles is further subjected to dimensionality constraints. Literature sources suggest that particles having a diameter of 200 nm or less experience an extended stay in circulation as compared to larger particles, due to a reduced rate of clearance from the body [72]. Extended circulation times may be attributed to a

decline in the efficiency of opsonin binding. The high radius of curvature characteristic of smaller particles has been directly correlated with this phenomenon.

The intrinsic dimensionality of moderately sized nanoparticles brings about diminished renal clearance [73]. As a reference, a 10 nm effective pore size cutoff and a 30–50 kDa molecular weight cutoff are typically associated with glomerular filtration [74]. Therefore, prior to biodegradation, particles <200 nm in the outer diameter will avoid filtration by the kidneys. This general coupling, of a sufficiently small particle diameter and extended circulation times, has been demonstrated to enhance therapeutic reservoir discharge within tissue that is presumptively diseased. Within this context, presumptively diseased tissue constitutes regions of abnormal lymphatic drainage, as in tumor, inflammation, or infection sites, or otherwise excessively permeable vascular structures. This drug targeting strategy passively assists in the identification of disease and the concurrent evasion of healthy tissue, and is conventionally termed the enhanced permeability and retention (EPR) effect [75, 76]. As a result of this discussion, the majority of artificial cells considered as part of the current research will be nanoparticles, with diameters in the 1–1000 nm range, and preferentially in the 50–500 nm range. Dimensions will be defined, or the *micro-* prefix attached, for vesicles having spherical or longitudinal diameters exceeding 1000 nm.

From the literature, it is apparent that several materials can successfully self-assemble, and form the fundamental framework for guided biological, chemical, or sensory delivery [66, 77–81]. Proteins including albumin, collagen, and gelatin, and polysaccharides including alginate, starch, and dextran have all been utilized as solid particle drug carriers. Alternatively, phospholipids like phosphatidylcholine have been used to form lipid bilayer-containing vesicles called liposomes. Contemporary research predispositions favor the use of synthetic polymers instead of naturally extracted polymers or materials. This is due in part to documented or anticipated improved particle stability, tunability, and chemical versatility when select synthetic polymers are employed in place of other organic or inorganic materials. These synthetic polymers include the previously cited PLGA, PLA, and PCL, in addition to poly(vinyl alcohol) (PVA), poly(vinyl pyrrolidone) (PVP), poly(hydroxyethyl methacrylate) (pHEMA), poly(butadiene) (PBD), polyphosphazene, silicones, and polyanhydrides [66, 77–81]. Common polymers used to fabricate drug delivery vehicles are outlined in Table 2.

Amphiphilic di- or tri-block copolymers are commonly used to synthesize a wide array of multifaceted nanoparticles. Block copolymers are derived via polymerization of multiple monomers within the same system. Preferentially, this results in a polymer with interconnected chains that have a local aversion or attraction toward aqueous mixtures or solutions. This dual hydrophobic and hydrophilic character enables subsequent facile production of functionalizable nanoparticles. Typically, when intended for use in clinical applications, these copolymers are constructed using biocompatible and biodegradable hydrophobic blocks made of poly(amino acids) or polyesters, for example. Ideally, the selected hydrophobic polymer block is covalently linked to a hydrophilic block that is similarly biocompatible. Although polyethylene glycol (PEG), or polyethylene oxide (PEO), is fre-

Table 2 The chemical structures, names, and abbreviations of select polymers that are used to fabricate organic nanoparticles

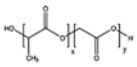
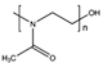
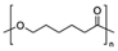
Hydrophilic Polymer Blocks			Hydrophobic Polymer Blocks		
	poly(vinyl pyrrolidone)	**PVP**		poly(hydroxyethyl methacrylate)	**pHEMA**
	polyethylene glycol/ polyethylene oxide	**PEG/PEO**		poly(lactic-co-glycolic acid)	**PLGA**
	poly(acrylic acid)	**PAA**		poly(D,L-lactide)	**PDLLA**
	poly(2-ethyl-2-oxazoline)	**PEtOx**		poly(dimethylsiloxane)	**PDMS**
	poly(2-methyl-2-oxazoline)	**PMOXA**		polycaprolactone	**PCL**

Table 3 Synthetic methods for producing polymeric vesicles

Method of preparation	Type	Size	Additives	Polydispersity
Film rehydration	Solvent free	SUV, MLV	–	High
Solid rehydration	Solvent free	SUV, MLV	–	High
Electroformation	Solvent free	GUV	–	Low
Gel-assisted hydration	Solvent free	GUV	Agarose or PVA	Low
Solvent injection	Solvent displacement	SUV	Solvent	High
Emulsion phase transfer	Solvent displacement	GUV	Solvent, surfactant	Low
Microfluidics	Solvent displacement	SUV or GUV	Solvent, surfactant, polymer, and so on	Low

Abbreviations: *SUV* small unilamellar vesicles, *MLV* multilamellar vesicles, *GUV* giant unilamellar vesicles. This table is adapted from the work of Rideau et al. [85]

quently adopted as the hydrophilic block of choice, suitable alternatives include PVP, poly(acrylic acid) (PAA), and poly(2-ethyl-2-oxazoline) (PEtOx) [82]. It is generally observed that particle circulation times are augmented by the incorporation of PEG, which has been until recently described as a "stealth" molecule—or an immune system evader [83], into nano drug delivery devices. Scientific evidence does demonstrate that water in the body forms a dense barrier around PEGylated surfaces that impedes opsonin adhesion [83]. However, recent literature by Abu Lila et al. suggests that anti-PEG antibodies are evolving in synchrony with the heightened utility of PEG in medical or other products. This anomaly is associated with the resulting so-called accelerated blood clearance (ABC) phenomenon [84], and alternatives to PEG are frequently investigated in the development of nanomaterials that will be applied or administered internally.

Several block copolymers have been studied that integrate an assortment of hydrophobic and hydrophilic blocks of variable block lengths and molecular weights. Often, amphiphilic block composition and synthesis routine influence the terminal structure of a polymeric nanoparticle. Refer to Table 3 for a summary of common methods of fabrication.

Hydrophobic block identity further governs the release of therapeutic loads. For example, poly(D,L-lactide) (PDLLA) degrades at an accelerated rate at physiological temperatures of 37 °C [86]. Similarly, poly(diethylaminoethylmethacrylate) (PDEAEM)-based assemblies will release their cargo as a result of abrupt changes in pH that are common, for example, at tumor sites [87]. A few amphiphilic block copolymers, especially mPEG-b-PCL, have the capacity to modulate species efflux across membranes that have P-glycoprotein expression [88]. This has significant implications for the transfer of therapeutic agents, for instance, across the blood–brain barrier, drug-resistant tumors, or intestinal epithelia.

Researchers have described extensively within the literature many instances of successfully encapsulating polymer-based nanoparticles for various disease or imaging applications. Synthetic polymeric bodies are rendered biofunctional through the integration of drugs, metal nanoparticles, proteins (including antibodies, peptides, and enzymes), DNA, fluorescent molecules, or other species that demonstrate the capacity to interact with local physiological events or conditions [89–93].

Spulber et al. generated ceria nanoparticle-loaded nanoreactors made from poly(*N*-vinylpyrrolidone)-block-poly(dimethylsiloxane)-block-poly(*N*-vinylpyrrolidone) (PDMS-PNVP) that possess negligible cytotoxicity, in comparison to free ceria nanoparticles, toward HeLa cells [94]. The ceria-loaded vesicles were highly stable and possessed exceptional superantioxidant activity. In a similarly translatable study, Geilich et al. developed dual Ag nanoparticle- and ampicillin-loaded mPEG-b-PDLLA polymersome vesicles, by a phase inversion strategy, that synergistically inhibited the proliferation of a multiple-drug resistant *Escherichia coli* (*E. coli*) strain that was mutated to express the *bla* gene for ampicillin resistance [95]. Here, in vitro bacterial proliferation assays confirmed that the dual delivery of metal nanoparticles and antibiotics inside a robust delivery vehicle effectively hindered bacterial proliferation. There are a few mechanistic validations for this outcome [95]. First, the bilayer membrane protected the antibiotics from hydrolysis by β-lactamase enzymes released by bacteria and promoted the sustained contact between the antibiotic and the bacterial membrane. Second, the Ag nanoparticles disrupted the bacterial cell membranes and weakened lipopolysaccharide permeability barriers. This morphological debilitation was aggravated by the reactive oxygen species (ROS) of Ag ions released from the Ag nanoparticles.

A similar study was performed in which the mPEG-b-PDLLA polymersomes were encapsulated by superparamagnetic iron oxide nanoparticles (SPIONs) and methicillin [96]. This dual therapy was targeted toward *Staphylococcus epidermidis* (*S. epidermidis*) biofilms that often occur along the surfaces of medical devices post-implantation. Following the application of an external magnetic field to an in vitro system containing *S. epidermidis* and co-functionalized polymersomes, bacterial biofilm permeation associated with cellular killing was significant, with 20 μm of penetration depth ascribed to the interaction. Figure 5 illustrates the advantages of using dual-functionalized SPION–antibiotic polymersomes that are directed by a magnetic field.

Thus, fabricated nano drug carriers may be bound to medical devices including orthopedic prosthetics to counteract the likelihood of rejection due to autoimmunity or bacterial seeding onto the implant. The principle of self-defending surfaces by

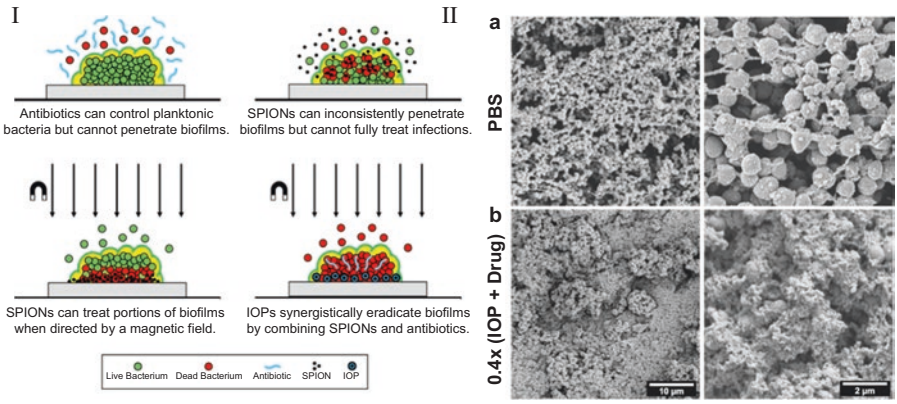


Fig. 5 Combination strategies for inhibiting bacterial biofilms are shown in Part **I** [96]. Summarily, SPIONs and antibiotics, delivered concurrently within IOPs, can penetrate and eradicate bacterial biofilms when directed by a magnetic field. Abbreviations: *IOP* iron oxide-encapsulating polymersome, *SPION* superparamagnetic iron oxide nanoparticle. TEM images in Part **II** illustrate the morphology of *S. epidermidis* biofilms and the surrounding polymer matrix (**a**) before and (**b**) after IOP treatment

the immobilization of PMOXA-b-PDMS-PMOXA polymersomes was explored by Langowska et al. [97]. Specifically, vesicles loaded with the biocatalyst penicillin acylase were anchored onto solid support surfaces by Schiff base formation and reductive amination [97]. The resulting surfaces were bioactive, stable, and antibacterial, enzymatically releasing regulated levels of antibiotics that effectively restricted *E. coli* cell growth [97]. Such models can be feasibly adapted to satisfy various clinical objectives related to implant antifouling or biosensing.

Disease Detection Through the Application of Imaging Methods and Nanoparticles

Nanoparticles for treating bacterial infections and for delivering antimicrobial agents have been reviewed. A novel possibility that has been explored in the scientific literature is the application of these nanoparticles as integral components of combination therapies, and in conjunction with microscopy and imaging methods, to diagnose or sense infections before they intractably progress. Augmented disease diagnostics is crucial for the management of infectious agents, as the early detection of pathogens could aid hospital workers to subdue interperson transmission and to more effectively treat affected individuals. Conventional diagnostic approaches can be time-consuming, inefficient, and inaccurate; and they typically involve some form of microscopy, cell culture, enzyme-linked immunoassay (ELISA), lateral flow immunoassay (LFA), or polymerase chain reaction (PCR), applied either independently or in combination. Novel approaches facilitate the use of nanomaterials,

such as quantum dots, gold nanoparticles, and magnetic nanoparticles for diagnostic purposes. Moreover, various detection modalities have emerged recently, involving for example fluorescence-, electrochemical-, or thermometry-based biosensing techniques in combination with various nanoparticle formulations. A few of the nanoparticle types reported in this chapter will be reintroduced as potential diagnostic aids, and detection modes will be briefly examined.

Nanoparticles with unique physiochemical properties, including magnetic iron (III) oxide (Fe_3O_4) and gold (Au), serve as good contrast agents for imaging applications [98, 99]. Nanoparticles decorated with ligands enable the enhanced, non-invasive imaging of diseased regions and provide a platform for the targeted delivery of large therapeutic and diagnostic loads. These nanocarriers effectively function as molecular imaging probes, maintaining some capacity for quantifying the pervasiveness of an infection and the efficacy of targeted treatments. Ideally, the operability of discrete imaging modalities is reinforced by the incorporation of compatible probes.

Ultrafine Fe_3O_4 nanoparticles, otherwise classified as SPIONs, have been shown to enhance the resolution of images obtained using magnetic resonance imaging (MRI) and to aid in adaptability studies of targeted cells or molecules [98, 99]. SPIONs can be further modified to track tissue abnormalities and to detect the onset of inflammation or disease, to serve as gene therapy or magnetic hyperthermia devices for the treatment of medical conditions, and to aid with the sequencing of biomolecules that require magnetic separation. In fact, with their high magnetization potential and unique properties, SPIONs greatly enhance the resolution of MR images without the emergence of acute side effects in vivo. In relation to infection monitoring, Lefevre et al. demonstrated the usefulness of ultrasmall SPIONs in the MRI monitoring of macrophage levels in vivo [100, 101]. Septic arthritis was induced by the inoculation of methicillin-susceptible *S. aureus* into the joints of adult female New Zealand White rabbits. It was determined noninvasively that, in comparison to control organisms that underwent antibiotic treatment, macrophage infiltration was more highly detectable within the synovium of rabbits that were not administered an antibiotic [100]. Indeed, SPION formulations (e.g., Feridex I.V. and Combidex) have been previously approved by the Food and Drug Administration (FDA) for applications in MRI contrast imaging, although currently many of these formulations have been discontinued clinically until further validations of their safe administration [102]. Alternatively, empirical evidence corroborates the usefulness of colloidal Au nanoparticles (AuNPs) in optical imaging, surface-enhanced Raman imaging (SERS), and in dark-field imaging. Qian et al. developed PEGylated Au nanoparticles modified by Raman reporter molecules and conjugated with anti-epidermal growth factor receptor (EGFR) antibodies [103]. These tumor-targeting probes applied under SERS are capable of identifying human cancer cells with high specificity and, in animal models, of localizing along tumor xenografts containing EGFRs. Au nanoparticles coated with Raman reporter molecules have been utilized in the detection of Rift Valley and West Nile fever viruses, by the specific identification of capsid and surface envelope antigens. Numerous research studies further validate the application of SERS-based methods for the detection of bacterial biomarkers,

such as those indicating pathogenic assault in common urinary tract or periprosthetic joint infections, for example [104, 105].

These principles can be extended to the detection of bacterial infections. Stimuli-responsive micelles, made out of polypyrrole, have been self-assembled and coated onto the surfaces of implantable devices that are designed to sense the proliferation of bacteria [106]. Antibacterial or anticancer drugs and nanoparticles incorporated into the cores of such micelles may be exploited as in vivo imaging probes. For better diffusion and transport properties, the polypyrrole micelles have been embedded into temperature-sensitive hydrogels. The properties described here are applicable to other drug delivery systems.

Recall that Ag nanoparticles indent bacterial membranes and facilitate the controlled influx of antibiotics into bacterial cells when used as part of Ag–antibiotic combination therapies. Similarly, formulated polymersomes can be utilized dually in disease treatment and prognosis. Ag nanoparticles are viable agents for molecular labeling using SERS [98]. This is a direct result of the optical properties, including surface plasmon resonance (SPR) and extensive light scattering effects, associated with Ag nanoparticles. Alternate imaging modalities that are enhanced by the incorporation of metallic nanoparticles include computed tomography (CT), X-ray, ultrasound (US), and positron emission tomography (PET).

Due to the versatility and potential for novel applicability in the clinic attributed to Raman spectroscopy, this technique, in addition to inherent shortcomings and enrichment strategies, will be discussed in greater detail. Raman spectroscopy is an optical and analytical tool for evaluating the chemical constitution of the cellular matrices, and fluids that occupy biological moieties [107, 108]. In particular, a spectrum is generated when incident light strikes vibrating molecules in the region of interest (i.e., the sample), which prompts inelastic light scattering. The user maintains the ability to retrieve diverse spectra obtained from the analysis of similar samples and to develop a cumulative multivariate model that supports precise diagnostics of independent specimens. An important feature of Raman spectroscopy is its capacity to generate precise molecular data with minimal sample preparation or perturbation (i.e., staining, labeling, etc.). Due to characteristic light backscattering, light transmission through a specimen is not required, and Raman spectroscopy can be applied effectively for direct in vivo imaging or in the analysis of dense or otherwise bulky tissue samples.

The applicability of Raman spectroscopy as an imaging modality is both qualitatively and lucratively feasible, and it is anticipated that Raman spectroscopy will achieve autonomy as a diagnostic tool when critical deficiencies have been redressed. Classically, Raman spectroscopy is associated with poor signals and protracted acquisition periods, which could be circumvented by spatial under-sampling and poor signal-to-noise fractions [107, 108]. Strategies have been devised to counteract these base infrastructural defects and to enable a clinically pertinent imaging technology. For instance, Raman signals have been augmented using nonlinear optics and metallic nanoparticles. Further, integration of photonic devices including miniature lasers and optical fibers improve intrinsic performance and acquisition times. SERS generally refers to the use of metallic nanoparticles for improving poor

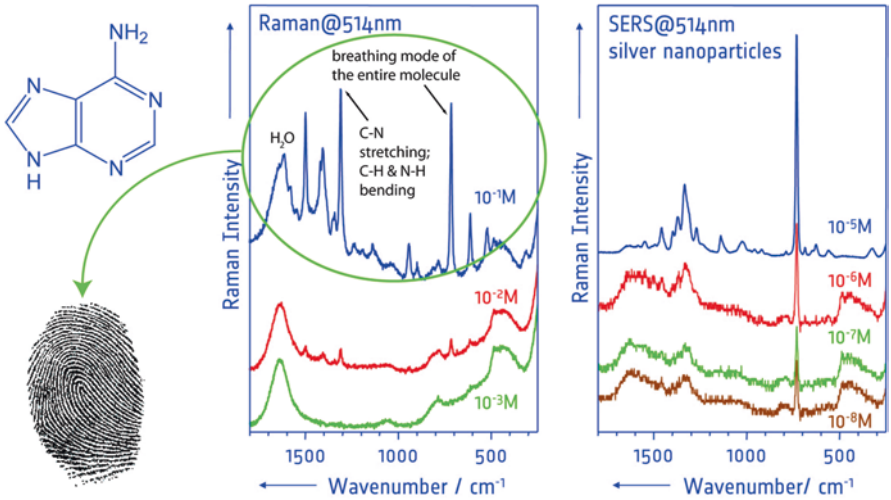


Fig. 6 From the Raman bands for adenine under non-SERS and SERS conditions, the detection limit is improved by the application of SERS and Ag nanoparticles [109]

Raman signals. Metallic nanoparticles that reside along the sample region excite surface plasmons that interact resonantly with incident light and generate an enhanced electric field in the area being investigated. Raman bands for the molecules under inspection are effectually amplified about 10^5–10^6 fold. Figure 6 shows the Raman bands of the biomolecule adenine, where an improvement in the Raman spectroscopy detection limit is observed upon the addition of Ag nanoparticles. Chemisorption of sample molecules and metallic nanoparticles complements this effect. Chemisorption may additionally be useful in the attachment of Raman reporter molecules to metallic nanoparticles prior to imaging, which propagates extrinsic SERS processing. Thus, decorated nanoparticles may further be PEGylated and appended by homing devices.

Raman microscopy has been implemented in vivo and in vitro by researchers investigating the molecular anomalies associated with brain, breast, lung, skin, esophageal, prostate, and colorectal cancers; subtle protein chemistry changes caused by bone diseases like osteoarthritis; blood serum or biofluid constitutions that aid in the diagnosis of asthma or other diseases, including malaria, and enable the quantification of disease severity; blood glucose changes in diabetics; fibrinogen and heparin levels in blood samples obtained from patients undergoing surgery or medical procedures that require enhanced or reduced blood coagulation; and concentration fluctuations of C-reactive protein (CRP) in the blood plasma that are indicative of a developing or subsiding inflammatory response [107, 108]. This final consideration, regarding blood plasma CRP levels, can be synchronized with research that investigates the efficacy of antimicrobial polymersomes or other drug delivery vehicles, for example. CRP concentration in the blood plasma is generally correlated with the intensity of a bacterial infection, and a strong intensity grade

obtained by Raman spectroscopy suggests the presence of an infection. In addition, precise biomarkers have been identified using Raman spectroscopy that discriminate, with an 80% success rate, between physiological sepsis and systemic inflammatory response syndrome (SIRS) [107, 108]. In the orthopedics research scene specifically, SERS has been adapted for the rapid and precise diagnosis of prosthetic joint infections and osteoarthritis [110, 111]. Fargašová et al. demonstrated the efficacy of this detection route in their SERS-based analysis of knee joint fluid infected by *Staphylococcus aureus* and *Streptococcus pyogenes* [110].

Future Perspectives in Nanoparticle Research

The biomedical applications of nanoparticles have spanned the delivery of therapeutics, genes, and contrast agents for the treatment of various disease conditions, and imaging and tissue engineering practices have been enhanced through the incorporation of nanostructured materials [112–115]. Moreover, characteristics have been defined which correlate the efficacy of varying nanospecies with their morphologies [116, 117]. Reported in the literature are several in vitro and animal studies recommending the use of nanoparticles in the clinic to treat pathogenic infections and various cancers, among other conditions, and nanoparticle treatments are already being actively administered to patients [118, 119]. For instance, albumin- and liposomal carriers loaded with anticancer drugs have been introduced to the market since 1995, starting with Doxil®, and are available for intravenous or intramuscular applications [120].

However, a noticeable margin of difference exists between the extent of nanoparticle research performed, and the volume of clinically authorized nanoproducts that are approved for human use. This could be attributed to several factors, including the nature of the mechanisms involved in nanoparticles syntheses, the considerable toxicity effects that nanoparticles have been determined to impose on healthy mammalian cells in vitro or in vivo, and the uncertainty with respect to the long-term consequences of nanoparticle treatments [121]. Conventionally, for example, strategies involved in the production of nanoparticles regularly incorporate the use of harsh chemicals, and the energy expenditure associated with synthesis is often quite high. Interestingly, green synthesis is currently being adapted to aid in overcoming these issues [122]. Biogenic methods that utilize bacteria, plant extracts, fungi, or yeasts are prominent in the nanoparticle fabrication scene. Further research is additionally anticipated to better define the morphological elements of varying nanoparticle types in guiding cellular toxicity. This latter aim is of particular interest since nanoparticles tend to impose cytotoxicity to healthy mammalian cells, in addition to disease-causing target cells. Ideally, an optimal nanoparticle architecture could be engineered that would diminish healthy cell toxicity while promoting the perturbation and destruction of disease-producing cells. Indeed, an ingrained challenge must be overcome in the early stages of nanoparticle research before nanotechnology can evolve into a common applicatory mode for disease diagnostics and treatment.

Guidelines are available which prescribe the efficacy and safety standards that therapeutic or diagnostic agents must comply with in order to obtain approval for clinical use [123]. General and directed toxicity studies are often time-intensive, involving variable dose injections over 28 days in at least two different large animal species. Histological and other evaluations must be conducted to assess the general effect of the agents on mammalian tissues and to define the concentration limits that produce nephrotoxicity, neurotoxicity, reprotoxicity, genotoxicity, immunotoxicity, and carcinogenicity in an organism. For nanoparticles that are too large for clearance by the excretory system, biodegradation studies must be performed to define the temporal behaviors of non-endogenous particles or particle fragments and to identify their potential side effects prior to discharge. Contingent on the particle excretion or degradation rate, these studies could be short term or long term, and in the latter case, developmental study costs could rise sharply. Administration, distribution, and metabolic analyses must be performed to supplement the excretion data. These validations would all supersede comprehensive physiochemical characterizations that would ensure the uniformity and standard marketability of nanoparticle products.

The relative newness of the nanotechnology field and the ongoing drive toward nanoparticle modifications that would eliminate their toxicity effects on healthy mammalian cells, combined with the need for immediate procedural and time-demanding evaluations, directly implies that nanoproducts will one day play a significant role in medicine, although time and testing are vital before major changes are introduced to the clinic. This is especially true when considering the current context in which diagnostics and treatments are administered. Since a multitude of medicinal agents are readily available to patients, novel solutions must be proven to match or supersede current therapeutics in terms of efficacy or safety, or a combination thereof. Risk–benefit analyses must be performed extensively for any new medical product that would potentially enter the market, and intellectual property rights must be resolved, although the complexity of this often accrues with the complexity of the product. From an economic perspective, nanotechnology in medicine would be recommended upon the development of cost-effective and efficient processes that enable the production of nanodevices in high yields. Regarding these issues and established practices, nanotechnology is currently within a developmental stage, and continuing research is anticipated to prompt the widespread adoption of nanomedicine. Within the next few years, drug delivery or enhancement tools, diagnostic systems, sensor technologies, and self-healing materials, among other aspects of medical pertinence, may benefit, in terms of efficacy, safety, and reduced clinical expenditure, by the adaptation of nanotechnology to existing therapeutics.

Conclusions

Advances in nanotechnology will inevitably yield mature devices that greatly outperform primitive treatment and diagnostic tools currently utilized in the clinic. Specifically, natural and synthetic materials, modified through variable nanoassembly

approaches, are anticipated to supplant current medical devices or medications used the in the treatment of pathogenic infections, especially those related to bacteria. In this review, noble metal and metal oxide nanoparticle types are examined, and their mechanisms of action in disrupting the colonization of in vitro or in vivo systems by bacteria are elaborated. Strategies that address the improvement of metal nanoparticle functional and structural features, especially by the consolidation of distinct materials by doping, capping, or functionalization techniques, are outlined. Nanoparticle encapsulation within nanocarriers, such as nanospheres, nanocapsules, and micelles, was explored as a viable method for the targeted delivery of therapeutic loads. This is especially relevant for the elimination of antibiotic-resistant bacteria and bacterial biofilms, which require innovative and potent treatment strategies that are reciprocally non-cytotoxic toward healthy human cells. The desired end result is an index of safe and versatile nanoparticle treatments that functionally exceed current regimens or that warrant clinical solutions to otherwise untreatable complications or conditions acquired through exposure to bacteria and other pathogens.

References

1. Sethuraman S, Krishnan UM, Subramanian A (2016) Biomaterials and nanotechnology for tissue engineering. CRC Press, Boca Raton. ISBN: 9781498743747
2. Azam A, Arshad M, Dwivedi S, Ashraf MT (2017) Antibacterial applications of nanomaterials. Adv Struct Mater 83:143–158
3. Bardosova M, Wagner T (2015) Nanomaterials and nanoarchitectures: a complex review of current hot topics and their applications. In: Nanomaterials and Nanoarchitectures: a complex review of current hot topics and their applications. Springer, Dordrecht, Netherlands, pp 1–343. ISBN: 9789401799218
4. Williams D (2008) The relationship between biomaterials and nanotechnology. Biomaterials 29:1737–1738. https://doi.org/10.1016/j.biomaterials.2008.01.003
5. Kim J, Mohamed MAA, Zagorovsky K, Chan WCW (2017) State of diagnosing infectious pathogens using colloidal nanomaterials. Biomaterials 146:97–114
6. Caldorera-Moore M, Peppas NA (2009) Micro- and nanotechnologies for intelligent and responsive biomaterial-based medical systems. Adv Drug Deliv Rev 61:1391–1401
7. Saito Y, Luo X, Zhao C, Pan W, Chen C, Gong J, Matsumoto H, Yao J, Wu H (2015) Filling the gaps between graphene oxide: a general strategy toward nanolayered oxides. Adv Funct Mater 25:5683–5690. https://doi.org/10.1002/adfm.201501358
8. Richards DA, Maruani A, Chudasama V (2017) Antibody fragments as nanoparticle targeting ligands: a step in the right direction. Chem Sci 8:63–77. https://doi.org/10.1039/C6SC02403C
9. Sajanlal PR, Sreeprasad TS, Samal AK, Pradeep T (2011) Anisotropic nanomaterials: structure, growth, assembly, and functions. Nano Rev 2:5883. https://doi.org/10.3402/nano.v2i0.5883
10. Barreto ML, Teixeira MG, Carmo EH (2006) Infectious diseases epidemiology. J Epidemiol Community Health 60:192–195
11. Woolhouse MEJ, Gowtage-Sequeria S (2005) Host range and emerging and reemerging pathogens. Emerg Infect Dis 11:1842–1847. https://doi.org/10.3201/eid1112.050997
12. Andersson DI (2003) Persistence of antibiotic resistant bacteria. Curr Opin Microbiol 6:452–456

13. Maduraiveeran G, Sasidharan M, Ganesan V (2018) Electrochemical sensor and biosensor platforms based on advanced nanomaterials for biological and biomedical applications. Biosens Bioelectron 103:113–129
14. Dhall A, Self W (2018) Cerium oxide nanoparticles: a brief review of their synthesis methods and biomedical applications. Antioxidants 7:97. https://doi.org/10.3390/antiox7080097
15. Grigore ME, Biscu ER, Holban AM, Gestal MC, Grumezescu AM (2016) Methods of synthesis, properties and biomedical applications of CuO nanoparticles. Pharmaceuticals 9(4):75. https://doi.org/10.3390/ph9040075
16. Elahi N, Kamali M, Baghersad MH (2018) Recent biomedical applications of gold nanoparticles: a review. Talanta 184:537–556
17. Ali A, Zafar H, Zia M, ul Haq I, Phull AR, Ali JS, Hussain A (2016) Synthesis, characterization, applications, and challenges of iron oxide nanoparticles. Nanotechnol Sci Appl 9:49–67
18. Leso V, Iavicoli I (2018) Palladium nanoparticles: toxicological effects and potential implications for occupational risk assessment. Int J Mol Sci 19(2)
19. Pedone D, Moglianetti M, De Luca E, Bardi G, Pompa PP (2017) Platinum nanoparticles in nanobiomedicine. Chem Soc Rev 46:4951–4975
20. Caro C, Castillo P, Klippstein R, Pozo D, Zaderenko AP (2010) Silver nanoparticles: sensing and imaging applications. Silver Nanoparticles, pp 201–225. ISBN: 978-953-307-028-5
21. Zhang Y, Nayak T, Hong H, Cai W (2013) Biomedical applications of zinc oxide nanomaterials. Curr Mol Med 13:1633–1645. https://doi.org/10.2174/15665240136661311111130058
22. Slavin YN, Asnis J, Häfeli UO, Bach H (2017) Metal nanoparticles: understanding the mechanisms behind antibacterial activity. J Nanobiotechnol 15:65
23. Karakoti AS, Hench LL, Seal S (2006) The potential toxicity of nanomaterials—the role of surfaces. JOM 58:77–82
24. Leung YH, Ng AMC, Xu X, Shen Z, Gethings LA, Wong MT, Chan CMN, Guo MY, Ng YH, Djurišič AB, Lee PKH, Chan WK, Yu LH, Phillips DL, Ma APY, Leung FCC (2014) Mechanisms of antibacterial activity of MgO: non-ROS mediated toxicity of MgO nanoparticles towards Escherichia coli. Small 10:1171–1183. https://doi.org/10.1002/smll.201302434
25. Pan X, Wang Y, Chen Z, Pan D, Cheng Y, Liu Z, Lin Z, Guan X (2013) Investigation of antibacterial activity and related mechanism of a series of nano-Mg(OH)2. ACS Appl Mater Interfaces 5:1137–1142. https://doi.org/10.1021/am302910q
26. Simon-Deckers A, Loo S, Mayne-L'Hermite M, Herlin-Boime N, Menguy N, Reynaud C, Gouget B, Carriere M (2009) Size-, composition- and shape-dependent toxicological impact of metal oxide nanoparticles and carbon nanotubes toward bacteria. Environ Sci Technol 43:8423–8429. https://doi.org/10.1021/es9016975
27. Ivask A, Elbadawy A, Kaweeteerawat C, Boren D, Fischer H, Ji Z, Chang CH, Liu R, Tolaymat T, Telesca D, Zink JI, Cohen Y, Holden PA, Godwin HA (2014) Toxicity mechanisms in Escherichia coli vary for silver nanoparticles and differ from ionic silver. ACS Nano 8:374–386. https://doi.org/10.1021/nn4044047
28. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT, Yacaman MJ (2005) The bactericidal effect of silver nanoparticles. Nanotechnology 16:2346–2353. https://doi.org/10.1088/0957-4484/16/10/059
29. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, Cho MH (2007) Antimicrobial effects of silver nanoparticles. Nanomed Nanotechnol Biol Med 3:95–101. https://doi.org/10.1016/j.nano.2006.12.001
30. Soltani Nezhad S, Rabbani Khorasgani M, Emtiazi G, Yaghoobi MM, Shakeri S (2014) Isolation of copper oxide (CuO) nanoparticles resistant Pseudomonas strains from soil and investigation on possible mechanism for resistance. World J Microbiol Biotechnol 30:809–817. https://doi.org/10.1007/s11274-013-1481-3
31. Pal S, Tak YK, Song JM (2015) Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium Escherichia coli. J Biol Chem 290:1712–1720. https://doi.org/10.1128/AEM.02218-06.
32. McQuillan JS, Shaw AM (2014) Differential gene regulation in the Ag nanoparticle and Ag(+)-induced silver stress response in Escherichia coli: a full transcriptomic profile. Nanotoxicology 5390:1–8. https://doi.org/10.3109/17435390.2013.870243.

33. McQuillan JS, Infante HG, Stokes E, Shaw AM (2012) Silver nanoparticle enhanced silver ion stress response in Escherichia coli K12. Nanotoxicology 6:857–866
34. Lok C-N, Ho C-M, Chen R, He Q-Y, Yu W-Y, Sun H, Tam PK-H, Chiu J-F, Che C-M (2007) Silver nanoparticles: partial oxidation and antibacterial activities. J Biol Inorg Chem 12:527–534. https://doi.org/10.1007/s00775-007-0208-z
35. El Badawy AM, Silva RG, Morris B, Scheckel KG, Suidan MT, Tolaymat TM (2011) Surface charge-dependent toxicity of silver nanoparticles. Environ Sci Technol 45:283–287. https://doi.org/10.1021/es1034188
36. Linder MC, Hazegh-Azam M (1996) Copper biochemistry and molecular biology. Am J Clin Nutr 63:797S–811S
37. Berthon G (2009) Critical evaluation of the stability constants of metal complexes of amino acids with polar side chains. Pure Appl Chem 67:1117–1240
38. Jung WK, Koo HC, Kim KW, Shin S, Kim SH, Park YH (2008) Antibacterial activity and mechanism of action of the silver ion in Staphylococcus aureus and Escherichia coli. Appl Environ Microbiol 74:2171–2178. https://doi.org/10.1128/AEM.02001-07
39. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO (2000) A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. J Biomed Mater Res 52:662–668. https://doi.org/10.1002/1097-4636(20001215)52:4<662::AID-JBM10>3.0.CO;2-3
40. Tamayo LA, Zapata PA, Vejar ND, Azócar MI, Gulppi MA, Zhou X, Thompson GE, Rabagliati FM, Páez MA (2014) Release of silver and copper nanoparticles from polyethylene nanocomposites and their penetration into Listeria monocytogenes. Mater Sci Eng C 40:24–31. https://doi.org/10.1016/j.msec.2014.03.037
41. Ramalingam B, Parandhaman T, Das SK (2016) Antibacterial effects of biosynthesized silver nanoparticles on surface ultrastructure and nanomechanical properties of Gram-negative Bacteria viz. Escherichia coli and Pseudomonas aeruginosa. ACS Appl Mater Interfaces 8:4963–4976. https://doi.org/10.1021/acsami.6b00161
42. Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ (2002) Metal oxide nanoparticles as bactericidal agents. Langmuir 18:6679–6686. https://doi.org/10.1021/la0202374
43. Sohm B, Immel F, Bauda P, Pagnout C (2015) Insight into the primary mode of action of TiO2 nanoparticles on Escherichia coli in the dark. Proteomics 15:98–113. https://doi.org/10.1002/pmic.201400101
44. Manke A, Wang L, Rojanasakul Y (2013) Mechanisms of nanoparticle-induced oxidative stress and toxicity. Biomed Res Int 2013(1):942916
45. Padmavathy N, Vijayaraghavan R (2008) Enhanced bioactivity of ZnO nanoparticles—an antimicrobial study. Sci Technol Adv Mater 9:035004. https://doi.org/10.1088/1468-6996/9/3/035004
46. Madl AK, Plummer LE, Carosino C, Pinkerton KE (2014) Nanoparticles, lung injury, and the role of oxidant stress. Annu Rev Physiol 76:447–465. https://doi.org/10.1146/annurev-physiol-030212-183735
47. Mukha IP, Eremenko AM, Smirnova NP, Mikhienkova AI, Korchak GI, Gorchev VF, Chunikhin AY (2013) Antimicrobial activity of stable silver nanoparticles of a certain size. Appl Biochem Microbiol 49:199–206. https://doi.org/10.1134/S0003683813020117
48. Choi O, Hu Z (2008) Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. Environ Sci Technol 42:4583–4588. https://doi.org/10.1021/es703238h
49. Wang L, He H, Yu Y, Sun L, Liu S, Zhang C, He L (2014) Morphology-dependent bactericidal activities of Ag/CeO2 catalysts against Escherichia coli. J Inorg Biochem 135:45–53. https://doi.org/10.1016/j.jinorgbio.2014.02.016
50. Xiu Z-M, Ma J, Alvarez PJJ (2011) Differential effect of common ligands and molecular oxygen on antimicrobial activity of silver nanoparticles versus silver ions. Environ Sci Technol 45:9003–9008. https://doi.org/10.1021/es201918f
51. Kodas TT (1989) Generation of complex metal oxides by aerosol processes: superconducting ceramic particles and films. Adv Mater 1:180–192
52. Ulrich GD, Rieh JW (1982) Aggregation and growth of submicron oxide particles in flames. J Colloid Interface Sci 87:257–265. https://doi.org/10.1016/0021-9797(82)90387-3

53. Skandan G, Chen YJ, Glumac N, Kear BH (1999) Synthesis of oxide nanoparticles in low pressure flames. Nanostruct Mater 11:149–158. https://doi.org/10.1016/S0965-9773(99)00028-8
54. Granqvist CG, Buhrman RA (1976) Log-normal size distributions of ultrafine metal particles. Solid State Commun 18:123–126. https://doi.org/10.1016/0038-1098(76)91415-0
55. Vorkapic D, Matsoukas T (1998) Effect of temperature and alcohols in the preparation of titania nanoparticles from alkoxides. J Am Ceram Soc 81:2815–2820. https://doi.org/10.1111/j.1151-2916.1998.tb02701.x.
56. Park HK, Kim DK, Kim CH (1997) Effect of solvent on titania particle formation and morphology in thermal hydrolysis of TiCl4. J Am Ceram Soc 80:743–749. https://doi.org/10.1111/j.1151-2916.1997.tb02891.x
57. Smiya S (2003) Hydrothermal processing in ceramics. In: Handbook of advanced ceramics: materials, applications, processing and properties, vol 1–2, pp 471–513. ISBN: 9780080532943
58. Chang EL, Simmers C, Knight DA (2010) Cobalt complexes as antiviral and antibacterial agents. Pharmaceuticals 3:1711–1728
59. Sawai J, Kawada E, Kanou F, Igarashi H, Hashimoto A, Kokugan T, Shimizu M (1996) Detection of active oxygen generated from ceramic powders having antibacterial activity. J Chem Eng Jpn 29:627–633. https://doi.org/10.1252/jcej.29.627
60. Sawai J, Kojima H, Igarashi H, Hashimoto A, Shoji S, Takehara A, Sawaki T, Kokugan T, Shimizu M (1997) Escherichia coli damage by ceramic powder slurries. J Chem Eng Jpn 30:1034–1039. https://doi.org/10.1252/jcej.30.1034
61. Sawai J, Shoji S, Igarashi H, Hashimoto A, Kokugan T, Shimizu M, Kojima H (1998) Hydrogen peroxide as an antibacterial factor in zinc oxide powder slurry. J Ferment Bioeng 86:521–522. https://doi.org/10.1016/S0922-338X(98)80165-7
62. Applerot G, Lipovsky A, Dror R, Perkas N, Nitzan Y, Lubart R, Gedanken A (2009) Enhanced antibacterial activity of nanocrystalline ZnO due to increased ROS-mediated cell injury. Adv Funct Mater 19:842–852. https://doi.org/10.1002/adfm.200801081
63. Cavassin ED, de Figueiredo LFP, Otoch JP, Seckler MM, de Oliveira RA, Franco FF, Marangoni VS, Zucolotto V, Levin ASS, Costa SF (2015) Comparison of methods to detect the in vitro activity of silver nanoparticles (AgNP) against multidrug resistant bacteria. J Nanobiotechnol 13:64. https://doi.org/10.1186/s12951-015-0120-6
64. Dorobantu LS, Fallone C, Noble AJ, Veinot J, Ma G, Goss GG, Burrell RE (2015) Toxicity of silver nanoparticles against bacteria, yeast, and algae. J Nanopart Res 17:172. https://doi.org/10.1007/s11051-015-2984-7
65. Aazam ES, Zaheer Z (2016) Growth of Ag-nanoparticles in an aqueous solution and their antimicrobial activities against Gram positive, Gram negative bacterial strains and Candida fungus. Bioprocess Biosyst Eng 39:575–584. https://doi.org/10.1007/s00449-016-1539-3
66. Quintanar-Guerrero D, Allémann E, Fessi H, Doelker E (1998) Preparation techniques and mechanisms of formation of biodegradable nanoparticles from preformed polymers. Drug Dev Ind Pharm 24:1113–1128. https://doi.org/10.3109/03639049809108571
67. Simon-Gracia L, Hunt H, Scodeller PD, Gaitzsch J, Braun GB, Willmore a-M a, Ruoslahti E, Battaglia G, Teesalu T (2016) Paclitaxel-loaded polymersomes for enhanced intraperitoneal chemotherapy. Mol Cancer Ther 15:670–680. https://doi.org/10.1158/1535-7163.MCT-15-0713-T
68. Shuai X, Merdan T, Schaper AK, Xi F, Kissel T (2004) Core-cross-linked polymeric micelles as paclitaxel carriers. Bioconjug Chem 15:441–448. https://doi.org/10.1021/bc034113u
69. Xie J, Wang CH (2005) Self-assembled biodegradable nanoparticles developed by direct dialysis for the delivery of paclitaxel. Pharm Res 22:2079–2090. https://doi.org/10.1007/s11095-005-7782-y
70. Kim SY, Lee YM (2001) Taxol-loaded block copolymer nanospheres composed of methoxy poly(ethylene glycol) and poly(ε-caprolactone) as novel anticancer drug carriers. Biomaterials 22:1697–1704. https://doi.org/10.1016/S0142-9612(00)00292-1
71. Zhang X, Jackson JK, Burt HM (1996) Development of amphiphilic diblock copolymers as micellar carriers of taxol. Int J Pharm 132:195–206. https://doi.org/10.1016/0378-5173(95)04386-1

72. Ishida O, Maruyama K, Sasaki K, Iwatsuru M (1999) Size-dependent extravasation and interstitial localization of polyethyleneglycol liposomes in solid tumor-bearing mice. Int J Pharm 190:49–56. https://doi.org/10.1016/S0378-5173(99)00256-2
73. Jones MC, Leroux JC (1999) Polymeric micelles—a new generation of colloidal drug carriers. Eur J Pharm Biopharm 48:101–111
74. Gagliardini E, Conti S, Benigni A, Remuzzi G, Remuzzi A (2010) Imaging of the porous ultrastructure of the glomerular epithelial filtration slit. J Am Soc Nephrol 21:2081–2089. https://doi.org/10.1681/ASN.2010020199
75. Yokoyama M, Kwon GS, Okano T, Sakurai Y, Kataoka K (1994) Development of micelle-forming polymeric drug with superior anticancer activity. In: Polymeric drugs and drug administration, vol 545, pp 126–134. ISBN: 0097-6156r0-8412-2744-6
76. Hao Y-L, Deng Y-J, Chen Y, Wang K-Z, Hao A-J, Zhang Y (2005) In-vitro cytotoxicity, in-vivo biodistribution and anti-tumour effect of PEGylated liposomal topotecan. J Pharm Pharmacol 57:1279–1287. https://doi.org/10.1211/jpp.57.10.0006
77. Fujioka K (1998) Protein release from collagen matrices. Adv Drug Deliv Rev 31:247–266. https://doi.org/10.1016/S0169-409X(97)00119-1
78. Tabata Y, Ikada Y (1998) Protein release from gelatin matrices. Adv Drug Deliv Rev 31:287–301. https://doi.org/10.1016/S0169-409X(97)00125-7.
79. Dumitriu S, Chornet E (1998) Inclusion and release of proteins from polysaccharide-based polyion complexes. Adv Drug Deliv Rev 31:223–246. https://doi.org/10.1016/S0169-409X(97)00120-8.
80. Lambert G, Fattal E, Couvreur P (2001) Nanoparticulate systems for the delivery of antisense oligonucleotides. Adv Drug Deliv Rev 47:99–112
81. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE (2001) Biodegradable polymeric nanoparticles as drug delivery devices. J Control Release 70:1–20
82. Brinkhuis RPRP, Rutjes FPJT, van Hest JCM (2011) Polymeric vesicles in biomedical applications. Polym Chem 2:1449. https://doi.org/10.1039/c1py00061f
83. Gref R, Minamitake Y, Peracchia MT, Trubetskoy V, Torchilin V, Langer R (1994) Biodegradable long-circulating polymeric nanospheres. Science 263:1600–1603. https://doi.org/10.1126/science.8128245
84. Ishida T, Kiwada H (2008) Accelerated blood clearance (ABC) phenomenon upon repeated injection of PEGylated liposomes. Int J Pharm 354:56–62
85. Rideau E, Dimova R, Schwille P, Wurm FR, Landfester K (2018) Liposomes and polymersomes: a comparative review towards cell mimicking. Chem Soc Rev 47:8572–8610. https://doi.org/10.1039/c8cs00162f
86. Jia L, Zheng JJ, Jiang SM, Huang KH (2010) Preparation, physicochemical characterization and cytotoxicity in vitro of gemcitabine-loaded PEG-PDLLA nanovesicles. World J Gastroenterol 16:1008–1013. https://doi.org/10.3748/wjg.v16.i8.1008
87. Liu J, Huang Y, Kumar A, Tan A, Jin S, Mozhi A, Liang XJ (2014) PH-sensitive nano-systems for drug delivery in cancer therapy. Biotechnol Adv 32:693–710
88. Zastre JA, Jackson JK, Wong W, Burt HM (2008) P-glycoprotein efflux inhibition by amphiphilic diblock copolymers: relationship between copolymer concentration and substrate hydrophobicity. Mol Pharm 5:643–653
89. Bruni S, Chang TM (1989) Hepatocytes immobilised by microencapsulation in artificial cells: effects on hyperbilirubinemia in Gunn rats. Biomater Artif Cells Artif Organs 17:403–411. https://doi.org/10.3109/10731198909118855
90. Chang TMS (1972) Artificial cells. Charles C. Thomas, Springfield
91. Lopes de Menezes DE, Pilarski LM, Allen TM (1998) In vitro and in vivo targeting of immunoliposomal doxorubicin to human B-cell lymphoma. Cancer Res 58:3320–3330
92. Trubetskoy VS (1999) Polymeric micelles as carriers of diagnostic agents. Adv Drug Deliv Rev 37:81–88
93. Delgado A, Soriano I, Sánchez E, Oliva M, Évora C (2000) Radiolabelled biodegradable microspheres for lung imaging. Eur J Pharm Biopharm 50:227–236. https://doi.org/10.1016/S0939-6411(00)00109-0.

94. Spulber M, Baumann P, Liu J, Palivan CG (2015) Ceria loaded nanoreactors: a nontoxic superantioxidant system with high stability and efficacy. Nanoscale 7:1411–1423. https://doi.org/10.1039/C4NR02748E
95. Geilich BM, Singleton GL, Van De Ven AL, Sridhar S, Webster TJ (2014) Silver nanoparticle-embedded polymersome nanocarriers for the treatment of antibiotic-resistant infections. In: Proceedings of the IEEE annual northeast bioengineering conference, NEBEC, vol 2014-Decem
96. Geilich BM, Gelfat I, Sridhar S, van de Ven AL, Webster TJ (2017) Superparamagnetic iron oxide-encapsulating polymersome nanocarriers for biofilm eradication. Biomaterials 119:78–85. https://doi.org/10.1016/j.biomaterials.2016.12.011
97. Langowska K, Palivan CG, Meier W (2013) Polymer nanoreactors shown to produce and release antibiotics locally. Chem Commun 49:128–130. https://doi.org/10.1039/c2cc36345c
98. Mody VV, Siwale R, Singh A, Mody HR (2010) Introduction to metallic nanoparticles. J Pharm Bioallied Sci 2:282–289. https://doi.org/10.4103/0975-7406.72127
99. Blasiak B, Van Veggel FCJM, Tomanek B (2013) Applications of nanoparticles for MRI cancer diagnosis and therapy. J Nanomater 2013:148578
100. Lefevre S, Ruimy D, Jehl F, Neuville A, Robert P, Sordet C, Ehlinger M, Dietemann J-L, Bierry G (2011) Septic arthritis: monitoring with USPIO-enhanced macrophage MR imaging. Radiology 258:722–728. https://doi.org/10.1148/radiol.10101272
101. Neuwelt A, Sidhu N, Hu CAA, Mlady G, Eberhardt SC, Sillerud LO (2015) Iron-based superparamagnetic nanoparticle contrast agents for MRI of infection and inflammation. Am J Roentgenol 204:W302–W313. https://doi.org/10.2214/AJR.14.12733
102. Wang YXJ (2015) Current status of superparamagnetic iron oxide contrast agents for liver magnetic resonance imaging. World J Gastroenterol 21:13400–13402. https://doi.org/10.3748/wjg.v21.i47.13400
103. Qian W, Murakami M, Ichikawa Y, Che Y (2011) Highly efficient and controllable PEGylation of gold nanoparticles prepared by femtosecond laser ablation in water. J Phys Chem C 115:23293–23298. https://doi.org/10.1021/jp2079567
104. Hamm L, Gee A, Indrasekara ASDS (2019) Recent advancement in the surface-enhanced Raman spectroscopy-based biosensors for infectious disease diagnosis. Appl Sci 9:1448. https://doi.org/10.3390/app9071448
105. Alvand A, Rezapoor M, Parvizi J (2017) The role of biomarkers for the diagnosis of implant-related infections in orthopaedics and trauma. Adv Exp Med Biol 971:69–79
106. Ge J, Neofytou E, Cahill TJ, Beygui RE, Zare RN (2012) Drug release from electric-field-responsive nanoparticles. ACS Nano 6:227–233. https://doi.org/10.1021/nn203430m
107. Kong K, Kendall C, Stone N, Notingher I (2015) Raman spectroscopy for medical diagnostics—from in-vitro biofluid assays to in-vivo cancer detection. Adv Drug Deliv Rev 89:121–134
108. Stewart S, Priore RJ, Nelson MP, Treado PJ (2012) Raman Imaging. Annu Rev Anal Chem 5:337–360. https://doi.org/10.1146/annurev-anchem-062011-143152
109. Ackermann K, Bohme R, Cialla D, Dorfer T, Marz A, Moller R, Popp J, Strelau K. Surface enhanced Raman spectroscopy. http://www.photonics4life.eu/index.php/layout/set/print/Consortium/P4L-DB/All-items/Surface-enhanced-Raman-Spectroscopy
110. Fargašová A, Balzerová A, Prucek R, Sedláková MH, Bogdanová K, Gallo J, Kolář M, Ranc V, Zbořil R (2017) Detection of prosthetic joint infection based on magnetically assisted surface enhanced Raman spectroscopy. Anal Chem 89:6598–6607. https://doi.org/10.1021/acs.analchem.7b00759
111. Pavlou E, Zhang X, Wang J, Kourkoumelis N (2018) Raman spectroscopy for the assessment of osteoarthritis. Ann Joint 3:83–83. https://doi.org/10.21037/aoj.2018.09.10
112. Parveen S, Misra R, Sahoo SK (2012) Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. Nanomedicine 8(2):147–166
113. Jin S, Leach JC, Ye K (2009) Nanoparticle-mediated gene delivery. Methods Mol Biol. https://doi.org/10.1007/978-1-59745-483-4_34

114. Cormode DP, Naha PC, Fayad ZA (2014) Nanoparticle contrast agents for computed tomography: a focus on micelles. Contrast Media Mol Imaging 9(1):37–52. https://doi.org/10.1002/cmmi.1551
115. Goldberg M, Langer R, Jia X (2007) Nanostructured materials for applications in drug delivery and tissue engineering. J Biomater Sci Polym Ed 18(3):241–268
116. Elkady MF, Shokry Hassan H, Hafez EE, Fouad A (2015) Construction of zinc oxide into different morphological structures to be utilized as antimicrobial agent against multidrug resistant bacteria. Bioinorg Chem Appl 2015:536854. https://doi.org/10.1155/2015/536854
117. Khodashenas B, Ghorbani HR (2015) Synthesis of silver nanoparticles with different shapes. Arab J Chem. https://doi.org/10.1016/j.arabjc.2014.12.014
118. Shaalan MI, El-Mahdy MM, Theiner S, El-Matbouli M, Saleh M (2017) In vitro assessment of the antimicrobial activity of silver and zinc oxide nanoparticles against fish pathogens. Acta Vet Scand 59:49. https://doi.org/10.1186/s13028-017-0317-9
119. Goldman E, Zinger A, Da Silva D, Yaari Z, Kajal A, Vardi-Oknin D, Goldfeder M, Schroeder JE, Shainsky-Roitman J, Hershkovitz D, Schroeder A (2017) Nanoparticles target early-stage breast cancer metastasis in vivo. Nanotechnology 28(43):43LT01. https://doi.org/10.1088/1361-6528/aa8a3d
120. Barenholz Y (2012) Doxil®—the first FDA-approved nano-drug: lessons learned. J Control Release 160(2):117–134
121. Ventola CL (2012) The nanomedicine revolution. P T 37(9):512–517, 525
122. Parveen K, Banse V, Ledwani L (2016) Green synthesis of nanoparticles: their advantages and disadvantages. AIP Conf Proc 1724:020048
123. Skotland T, Iversen T, Sandvig K (2014) Development of nanoparticles for clinical use. Nanomedicine (Lond) 9:1295–1299

Battling Bacteria with Free and Surface-Immobilized Polymeric Nanostructures

Serena Rigo, Myrto Kyropoulou, Cora-Ann Schoenenberger, and Cornelia G. Palivan

Abstract With the discovery of antibiotics, bacterial infections and previously fatal diseases suddenly became curable. During the golden era of antibiotics, new classes of antibiotics were discovered. However, antibiotic-resistant bacteria rapidly evolved while fewer new antimicrobial drugs were discovered and marketed. Today, a growing number of infections are becoming harder to treat as the bacterial resistance is spreading and antibiotics become less effective. Evidently, there is an urgent demand for new strategies that efficiently battle pathogenic bacteria. Among emerging technologies, those involving polymeric nanostructures, especially polymersomes, offer many features that make them attractive candidates for battling infections. Polymersomes can be designed to be biocompatible and respond to various environmental signals. They are more robust than liposomes and can host hydrophobic and hydrophilic antimicrobial compounds, which can be released and act locally. Last but not least, they are biodegradable. Moreover, platforms comprising polymeric nanostructures can be designed as sensors for diagnosing infections. Many of these approaches require the immobilization of the antimicrobial nanostructures on a surface whereby the activity is localized to a specific region. Several recent examples of polymeric nanostructures with antimicrobial activity, both free in solution or immobilized on surfaces, are highlighted and discussed in this chapter.

Keywords Polymers · Self-assembly · Antimicrobial nanocompartments · Catalytic nanocompartments · Antimicrobial surfaces · Immobilization · Active surfaces

S. Rigo · M. Kyropoulou · C.-A. Schoenenberger (✉) · C. G. Palivan (✉)
Department of Chemistry, University of Basel, Basel, Switzerland
e-mail: cora-ann.schoenenberger@unibas.ch; cornelia.palivan@unibas.ch

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_17

Introduction

Bacteria can be both beneficial or harmful to our health. A well-known example are the 500–1000 unique bacterial strains in the human colon. This personal community of bacterial strains, referred to as the gut microbiota, is vital for many aspects of health, including physiology, resistance to disease and digestion, among others. In addition, there is a tight link of the human gut microbiota with the host central nervous system [1]. The microbiome or genetic content of gut bacteria changes in individuals with disease, such as inflammatory bowel disease, obesity, or diabetes compared to unaffected individuals. Advances in sequencing technology enabled the exploration of the role of the gut microbiota in a broad range of neurological and psychiatric disorders and diseases including Alzheimer's disease [2] and depression [3]. In addition, evidence to a causal role for the microbiota in disease acquisition is increasing. Similarly, the microbes of the skin are fundamental to skin physiology and prevent pathogens from entering the body.

Besides probiotics, there are also numerous pathogenic bacteria which, when they proliferate uncontrolled in or on our body, cause a lot of distress, harm, and in the worst case, kill the patient. With the discovery of the first antibiotics, hitherto deadly infections could finally be treated and were no longer life-threatening [4]. In the following years, different classes of antibiotics were developed with different mechanisms of action. However, the antibiotic era was soon marred by the emergence of antibiotic resistance which in the meantime has turned into a serious global problem. Besides improving the discovery models for new antimicrobial agents that are successful in the combat of antibiotic resistance, the need for developing new strategies such as the design of more effective preventive measures is urgent.

In today's life, medical devices play an important role in extending life and/or improving its quality. Bone fractures can be repaired using metal screws, pins, and plates; hip and knee joints can be replaced; pacemakers control the heartbeat; implants help reconstructing breasts after mastectomy; and different catheters are used in diagnosis and treatment. Despite all medical progress, there is always the risk that infections occur following surgery. Pathogenic bacteria may spread within the body and proliferate on the implanted device. Of particular concern is the threat of bacterial biofilm development, since biofilm-mediated infections are difficult to diagnose and effective treatments are lacking. Moreover, biofilm formation not only occurs on the implant, but also affects adjacent soft tissues and bone. Preventive antibacterial treatment is essential to reduce possible bacterial contamination in the wound. With the dramatic increase in the emergence of antibiotic-resistant bacterial strains, there is an urgent need for more efficient antibiotic treatments. Another important research field involves antimicrobial surfaces which actively or passively prevent bacterial colonialization by reducing the bacteria's ability to adhere and grow into a biofilm on implants [5].

New strategies for the delivery and release of antibiotics as well as the ability to sense pathogenic bacteria are becoming tangible by nanoscience approaches. Polymer-based nanocompartments, in particular polymeric vesicles known as

polymersomes, micelles, and nanoparticles, offer the advantage of highly diverse materials whose physical and chemical properties can be tuned toward specific applications. Depending on the target function, e.g., antimicrobial drug production and delivery or sensing, self-assembled polymeric nanocompartments can be designed to be biocompatible, stable, responsive to different triggers (i.e., pH, light, and redox potential), and biodegradable. In addition, they can be equipped with specific functional groups that enable the coupling of ligands such as targeting moieties or mediate their immobilization on specific surfaces. Nanocompartments resulting from the self-assembly of amphiphilic block copolymers are particularly suited for antimicrobial drug delivery systems. Among the advantages of polymeric nanocompartments is that they shield antibiotics or other drugs and are able to ferry them to distinct locations in the body [6]. In this case, the overall drug dosage can be reduced thereby decreasing unwanted side effects such as unbalancing the intestinal flora. Polymer-based micelles and nanoparticles (NP) as antimicrobial agents or delivery systems have been extensively reviewed elsewhere [7, 8]. Antimicrobial surfaces can be designed via various nanoscience-based approaches including the immobilization of antimicrobial polymeric nanocompartments [5]. Surfaces patterned with a distinct micro- or nanostructure that reduces bacterial attachment and surface coatings that actively kill bacteria by means of their intrinsic properties or agent release are reviewed elsewhere [9–12].

In this chapter, we focus on current aspects of free and surface-immobilized polymeric self-assembled nanocompartments as key players in delivery systems and in designing surfaces with antimicrobial activity. In the first part, we present how polymersomes can produce, carry, and release antimicrobial drugs and/or antimicrobial agents such as inorganic NPs, and expand on why polymersomes offer suitable and efficient alternatives to conventional antibiotic therapy. We then introduce some exciting examples where polymersomes are used for treating specific bacterial infections and describe how they can be used as carriers, as catalytic nanocompartments producing antibiotics, and as sensors for the detection of pathogenic bacteria. In the second part, we discuss different covalent and non-covalent immobilization techniques as well as lithography methods for immobilizing polymeric nanocompartments in spatially defined patterns. We present selected examples of active surfaces with a particular emphasis on surfaces where immobilized nanocompartments fight bacteria and reduce biofilms.

Amphiphilic Block Copolymers: The Essential Building Blocks

Amphiphilic molecules are composed of both hydrophilic and hydrophobic parts. A prominent example in nature are lipids with a hydrophilic head group and a hydrophobic tail. Together with membrane proteins, lipids make up the membrane boundary of natural vesicles including extracellular vesicles [13, 14], endosomes [15], and lysosomes [16], which store, transport, produce, or protect molecules such as enzymes by generating compartmentalized reaction spaces.

Natural or synthetic lipids are used to design simple systems that mimic biological membranes [17]. Due to their amphiphilic character, lipids are able to self-assemble into various structures, such as micelles or vesicles (liposomes) that are often applied as delivery systems in the cosmetic and pharmaceutical industries [18, 19]. However, one major drawback of liposomes and other lipid-based structures is their instability in the body. Functionalizing lipids with polymers, for example by PEGylation [20], was found to improve liposome stability and robustness. This prompted the development of membrane mimics composed purely of amphiphilic polymers [21, 22] that contain at least one hydrophilic and one hydrophobic block. Depending on how many blocks are used, these amphiphilic polymers are referred to as di- or triblock copolymers, AB or ABA, respectively, with A being the hydrophilic and B the hydrophobic block [23, 24]. Triblock copolymers with different hydrophilic blocks A and C on either side of the hydrophobic block B are designed to create asymmetric membranes [25–27]. Like lipids, amphiphilic polymers can self-assemble into structures that allow mimicking biological compartmentalization strategies [28]. However, block copolymers are much more versatile than lipids because they can be composed of a wide range of building blocks synthesized from chemically distinct monomers, and lend themselves to chemical modifications. In addition to the possibility of functionalization, which allows for tuning the surface properties, polymer-based nanocompartments have thicker membranes and are therefore more stable than lipid-based systems [24, 29–32]. These features are particularly important for in vivo applications because they help to prolong circulation time in the body [33]. The robustness and mechanical stability of the polymer membrane also protects the cargo against adverse effects from the environment. For example, an otherwise poorly soluble and unstable antimalarial compound showed increased solubility and retained activity when provided as a polymer formulation [6].

Amphiphilic block copolymers with specific molecular weight and dispersity ($Đ$) can be synthesized by different approaches such as (1) reversible addition–fragmentation chain transfer (RAFT) polymerization, (2) atom transfer radical polymerization (ATRP), (3) anionic living polymerization, or (4) ring-opening polymerizations (ROPs), each one having its own advantages and disadvantages. In living polymerization techniques, the reaction stops once the monomer in solution has been consumed and restarts upon the addition of fresh monomer. Living polymerizations are often used for synthesizing distinct block copolymers (ABA, ABC, ABCA, etc.) because they offer precision and control over molar mass and end-groups [34].

1. RAFT polymerization is a versatile polymerization technique where many different vinyl monomers and different solvents including water can be used and reaction conditions are moderate. For example, thermoresponsive poly[(glycerol monomethacrylate-*stat*-glycidyl methacrylate)]-*block*-poly(2-hydroxypropyl methacrylate) (P(GMA-*stat*-GlyMA)-PHPMA) block copolymers were synthesized via RAFT [35]. Although RAFT is a controlled polymerization, reinitiation is required once monomers are completely consumed. One major advantage

of RAFT polymerization is its compatibility with various functional groups [36, 37]. RAFT agents typically consist of a thiocarbonylthio group with two substituents, denoted R and Z. The Z group determines the stability of the intermediate radical adduct while the R group primarily affects the rate at which the RAFT agent is consumed. A wide range of groups can be introduced as substituents on "R" or "Z" groups including functionalities that can be used in "click" reactions.

2. ATRP is another method that can be applied to synthesize polymers from a wide selection of vinyl monomers. For example, poly(2-dimethylaminoethyl methacrylate) (PDMAEMA) was synthesized by ATPR [38]. This positively charged polymer is photosensitive. Under UV irradiation, PDMAEMA changes to its neutral form, which as a constituent of a self-assembled nanocompartment enables the slow release of a compound from the compartment. However, ATRP typically requires a transition metal catalyst, albeit metal-free catalysts are emerging [39, 40]. The toxicity that is frequently associated with the metal catalyst and its ligands requires careful purification of the polymer and thus, limits the potential of polymers synthesized via ATRP in biomedical applications [37].
3. In anionic living polymerization, the variety of possible vinyl monomers is reduced as the negative charge needs to be stabilized and delocalized with the help of stabilizing substituents.
4. ROPs are other living polymerization techniques [34] with which biodegradable polymers can be synthesized [37]. As the name ROP suggests, cyclic monomers are used and react with the reactive polymer end by opening its ring system and turning itself into the reactive end. There exist radical, cationic, or anionic ROP techniques depending on the nature of the propagation center. ROPs are sensitive to impurities including water and oxygen. For example, radical ROP was used to synthesize dimethylated poly(carbolactone) (PdmCL), which is biodegradable in the presence of esterase [41]. Anionic ROP can be used for the synthesis of polyesters, polyamides like nylon 6, polycarbonates, polyurethanes, and polyphosphates in a controlled fashion, while cationic ROP is one of the techniques used for the synthesis of polyoxazolines like poly(2-methyl-2-oxazolin (PMOXA) [23, 42]. PMOXA is a biocompatible and bioinert polymer, which decreases blood clotting, protein adsorption, and bacterial colonization [43, 44]. Furthermore, PMOXA is a peptidomimetic but is known to be more stable toward degradation [43, 45].

The polymerization techniques mentioned above result in a statistical distribution of different chain lengths. This variation is described by the dispersity $Đ$ (Eq. 1), defined by the ratio between the weight average molecular weight (M_w) (the molecular mass of each polymer chain is assessed by its mass fraction of the whole mass of the sample) and the number average molecular weight (M_n) (the arithmetic mean value) for the polymer [46]. A narrow mass distribution of the polymer gives a low $Đ$, which is always greater than one because M_w is always bigger than M_n, as M_n is more sensitive to lower mass molecules. Thus, if all polymers have the same mass, $Đ$ would equal 1 [39, 47].

$$Đ = \frac{M_w}{M_n} \quad (1)$$

Self-Assembly of Polymeric Nano-Architectures

Amphiphilic block copolymers can self-assemble in aqueous solution into various nano- or micrometer sized structures such as spherical micelles, cylindrical micelles, tubes, lamellar structures, or vesicles (polymersomes) (Fig. 1) [24]. There are different procedures to obtain self-assembled structures. In the cosolvent [27], solvent switch [48], and the water addition/solvent evaporation [27] procedure, the amphiphilic block copolymer is dissolved in an organic solvent. The ultimately desired solvent system in which only the hydrophilic block is soluble, i.e., an aqueous buffer, is added to the polymer solution (solvent switch and water addition/solvent evaporation). Alternatively, the polymer solution is added dropwise to the aqueous solvent (cosolvent). One disadvantage of these methods is that traces of organic

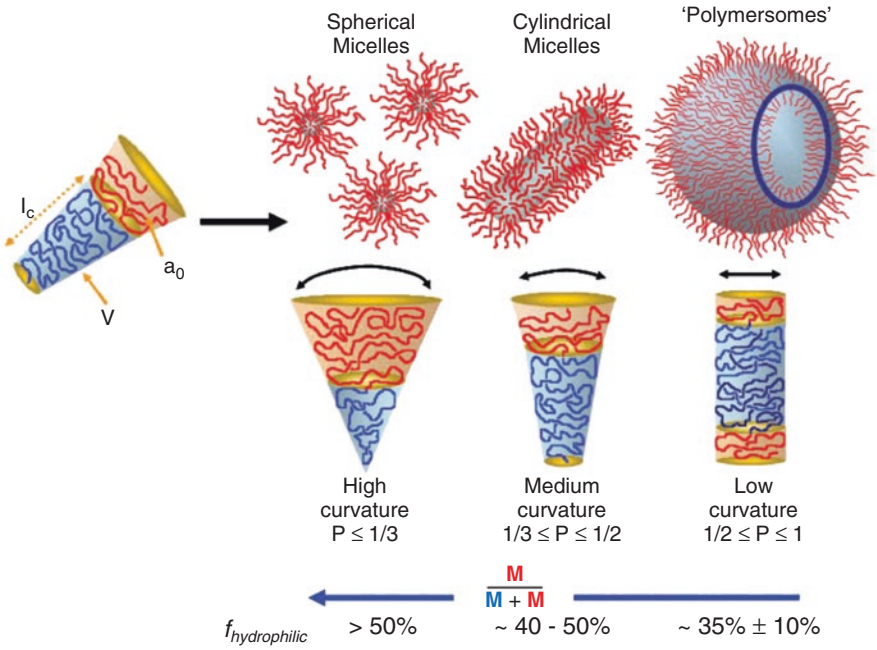


Fig. 1 Different structures self-assemble from amphiphilic block copolymers. The hydrophilic (soluble) fraction of amphiphilic block copolymers (bottom panel) and their packing parameter (top panel) influence the outcome of the self-assembly. v = volume of the hydrophobic (insoluble) fraction, l_c = length of the hydrophobic (insoluble) volume, a_0 = area between the hydrophobic (insoluble) volume and the hydrophilic (soluble) volume. (Top panel from Blanazs A, Armes SP, Ryan AJ (2009) Macromol Rapid Commun 30: 267–277 [22] with permission from WILEY)

solvent might remain in the system which might be toxic for downstream applications. This is not the case in the film rehydration method [27, 49], where the polymer dissolved in the organic solvent is first completely dried to a thin film, whose subsequent rehydration with aqueous buffer results in self-assembly. The type of polymer, the solvent(s), and the method applied for self-assembly affect what structure will be obtained. The nature of the polymer, its molecular weight (M_w), its hydrophilic fraction ($f_{hydrophilic}$) (Eq. 2; Fig. 1, bottom), and the packing parameter (P) (Eq. 3; Fig. 1, top [22]), are crucial parameters that determine the structures resulting from self-assembly.

$$f_{\text{hydrophilic}}\left[\%\right] = \frac{M_{\text{w hydrophilic}}}{M_{\text{w hydrophilic}} + M_{\text{w hydrophobic}}} \tag{2}$$

$$P = \frac{v}{a_0 l_c} \tag{3}$$

v describes the volume occupied by the hydrophobic, water-insoluble block, and l_c is its length. a_0 is the optimum area between the volumes occupied by the soluble and insoluble blocks. All these parameters (v, l_c, and a_0) are defined in an equilibrated state where the interactions between the two blocks are balanced. The curvature which arises due to the relative size ratio of the two blocks is reflected in P, and is an important determinant to estimate the resulting structure.

Self-assembly is based on intramolecular and intermolecular interactions, mostly driven by non-covalent hydrophobic interactions. In order to initiate self-assembly, the polymer concentration in solution needs to exceed the critical micelle concentration (CMC). In aqueous media, the hydrophobic blocks tend to align with each other and are shielded from the water by interacting hydrophilic blocks which happily dissolve in the aqueous media. As a result of this self-assembly, the total free energy will be minimized. To obtain polymersomes, the hydrophilic, water-soluble fraction of the block copolymer should be around 35% and $^1/_2 \leq P \leq 1$. Polymers with a hydrophilic fraction of 40–50% and $^1/_3 \leq P \leq {}^1/_2$ tend to self-assemble into rod-like aggregates. Theoretically, the most stable condition for self-assembled structures would be infinitely long cylinders and infinitely large membranes. However, in order to avoid contact between the insoluble fraction and the solvent, the cylinders bend and form cylindrical micelles while the membranes close to polymersomes [22]. Block copolymers with a hydrophilic fraction larger than 50% and $P \leq {}^1/_3$ tend to form spherical micelles. Along with the polymer nature, the concentration and solvent properties (including pH, polarity, viscosity, osmolarity, and temperature) also have an impact on which structure is preferentially formed.

An important parameter to characterize the self-assembled structures is the shape factor (ρ), which is the ratio of the radius of gyration (R_g) and the hydrodynamic radius (R_h) (Eq. 4) of the nanocompartment. R_g and R_h are obtained by static light scattering (SLS) and dynamic light scattering (DLS) measurements, respectively. For homogeneously spherical and dense structures, like micelles or nanoparticles, R_g is smaller than R_h and therefore, $\rho < 1$. For a hollow sphere, ρ equals 1, since

there is no mass in the cavity. For extended structures such as worms, $\rho > 1$. The combination of SLS/DLS is very useful, because it gives precise information about the size and architecture of the formed structures using a noninvasive method.

$$\rho = \frac{R_g}{R_h} \tag{4}$$

Micrographs obtained by transmission electron microscopy (TEM) also provide information on the morphology of self-assembled structures. However, the harsh conditions of sample preparation might interfere with structure preservation. In particular, depending on the stiffness of their membrane, some polymersomes collapse and will appear as deflated balls in negatively stained TEM images.

Polymersomes as Nanocarriers for Antimicrobial Applications

The main advantages of polymersomes as nanocarriers with antimicrobial applications are: (1) they can host a wide range of hydrophilic molecules including hydrophilic antibiotics in their cavity [50] and hydrophobic compounds in their membrane; (2) the diversity of building blocks allows them to be chemically tuned to respond to various stimuli, either internal (i.e., pH, enzymes, oxidative stress, etc.) or external (i.e., temperature, magnetic field, light, ultrasound) [51]; (3) they are usually biocompatible and have low toxicity [52, 53]; and (4) they are very robust and thus, protect the integrity and activity of the encapsulated compounds [31]. In the following sections, we will focus on emerging strategies which involve polymersomes as model systems in the battle against harmful bacteria.

Polymersomes with Intrinsic Antimicrobial Features

Some polymersomes intrinsically exhibit antibacterial activity based on the chemical nature of their constituents. For example, the antimicrobial activity of polymersomes based on the thermoresponsive block copolymer Poly[2-(2-methoxyethoxy) ethyl methacrylate]-Poly[2-(tert-butylaminoethyl) methacrylate] ($PMeO_2MA$-*b*-PTA) is related to the amino groups in the PTA block [54]. These amino groups bear a positive charge and thus can interact with Ca^{2+} and/or Mg^{2+} ions of the bacterial membrane and consequently damage it. These polymersomes were tested on both Gram-negative *Escherichia coli* (*E. coli*) and Gram-positive *Staphylococcus aureus* (*S. aureus*) at neutral pH 7.4. When solutions of polymersomes were added to bacterial cultures at concentrations of 0.05, 0.1, and 0.25 mg/mL, the counting of bacteria colonies after a 48 h incubation at 37 °C revealed that bacterial growth was prevented from 91.8% to 99.9% by the presence of $PMeO_2MA$-*b*-PTA polymersomes.

In a different approach, the conjugation of a synthetic, biodegradable block copolymer poly(ε-caprolactone) (PCL) with the antibacterial block poly[phenylalanine-*stat*-lysine-*stat*-(lysine-folic acid)] (Phe_{12}-*stat*-Lys_9-*stat*-$(Lys\text{-}FA)_6$) resulted in a polymer able to form antibacterial polymersomes in aqueous solution [55]. Similar to what was reported for the PTA amino groups, lysine residues that become positively charged in water mediate attachment to the bacterial membrane, which in turn enables membrane penetration by the phenylalanine residues and the subsequent death of *E. coli* and *S. aureus*.

Polymersomes as Nanocompartments for Antimicrobial Drugs and Their Production

Polymersomes can serve as compartments or carriers for antimicrobial agents. They are considered catalytic nanocompartments when reactions take place inside and educts and products are exchanged across the polymer membranes [56]. For example, polymersomes assembled from the amphiphilic block copolymer poly(2-methyloxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-methyloxazoline) (PMOXA-*b*-PDMS-*b*-PMOXA) loaded with the enzyme penicillin acylase (PA) were able to locally produce antibiotics [57]. To obtain these vesicular catalytic nanocompartments, the polymer was dried together with the bacterial porin outer membrane protein F (OmpF) to a thin film which was then rehydrated in a buffer containing PA. This way, OmpF was inserted in the membrane of the resulting polymersomes, forming a protein gateway that allows the diffusion of molecules up to 600 Da. In parallel, PA was encapsulated in the hydrophilic cavity of the polymersomes. When the externally added substrates 7-aminodesacetoxycephalosporanic acid (7-ADCA) and phenylglycine methyl ester (PGME) diffused into the polymersomes through OmpF, PA catalyzed the production of cephalexin inside the cavity which was then released into the environment through OmpF. Cephalexin is a well-known antibiotic disrupting the growth of the bacterial cell wall of *S. aureus*, *S. epidermidis*, *E. coli*, and *Proteus mirabilis* [58, 59]. To confirm the antibiotic activity of the produced cephalexin, the effects of PA-loaded polymersomes on the growth of *E. coli* were monitored in the presence and absence of substrate. Notably, the growth of *E. coli* was inhibited by the presence of the antibiotic producing polymersomes which remained active (i.e., they kept producing cephalexin) for 7 days under physiological conditions.

Similarly, a light-sensitive, water-soluble tetra-alkylpyridinium porphyrin (TpyCP) was encapsulated into PMOXA-*b*-PDMS-*b*-PMOXA polymersomes and evaluated for its antimicrobial activity against *E. coli* [49]. TpyCP is a photosensitizer, that upon irradiation (e.g., LED light of 660 nm wavelength) is able to induce reactive oxygen species (ROS) production and cause oxidative stress in target cells in vitro. The particular advantage of this system is that only the ROS produced in the aqueous cavity of the polymersomes diffuse across the polymer membrane and

reach the bacteria of interest, whereas TpyCP remains encapsulated. This compartmentalization allows for a selective and controlled process of ROS release on demand. The antimicrobial activity of light-induced ROS was demonstrated by irradiating *E. coli* cultures that were incubated with porphyrin containing polymersomes. Counting colony forming units (CFU) and imaging of corresponding *E. coli* cultures by confocal laser scanning microscopy (CLSM) strongly indicated that TpyCP-polymersomes caused a significant decrease of the *E. coli* population only when irradiated (Fig. 2).

Another example of applying polymersomes to battle bacteria is the efficient treatment of *Porphyromonas gingivalis* (*P. gingivalis*) infected oral keratinocytes with drug-loaded polymersomes [60]. Metronidazole and doxycycline antibiotics were encapsulated in poly[2-(methacryloyloxy)ethyl phosphorylcholine] (PMPC)-poly[2-(diisopropylamino)ethyl methacrylate] (PDPA) polymersomes. The PMPC block interacts with specific plasma membrane receptors that promotes endocytosis while the PDPA block is pH-responsive and serves as a trigger for antibiotic release. Accordingly, polymersomes disintegrate at the acidic pH of the endosomal–lysosomal compartment (pH 6.5–4.5) and their cargo is released. The effect of antibiotic-loaded PMPC-PDPA polymersomes was tested in vitro. H357 and TR146 human oral squamous cell carcinoma cells, and NOK cells (immortalized oral keratinocytes) were infected with *P. gingivalis* and then incubated with antibiotic-loaded polymersomes. Apart from these three cell lines, a tissue-engineered oral mucosa that more closely represents the physiological conditions of a living organism was used as a test model. Metronidazole-loaded polymersomes reduced the bacterial

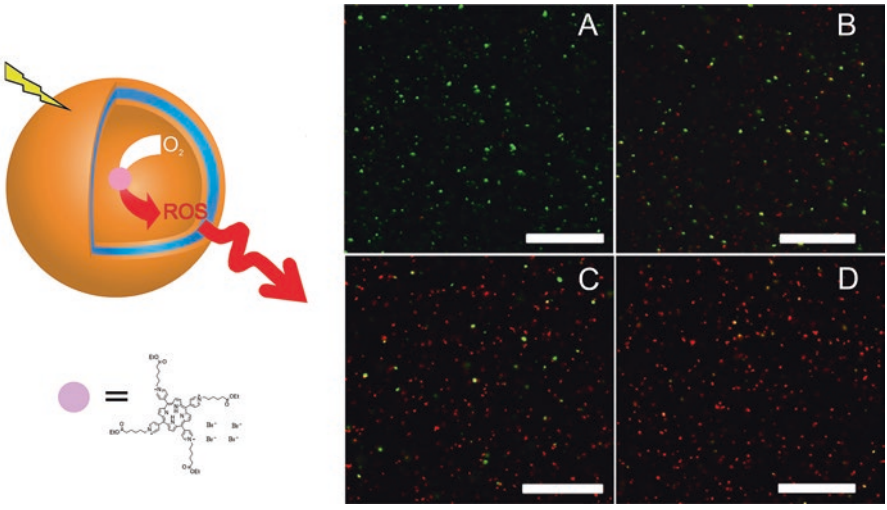


Fig. 2 Left panel, schematic of porphyrin containing polymersomes. Right panel, *E. coli* bacteria incubated in presence of 200 μM TpyCP-loaded polymersomes were stained with SYTO 9 (considered alive, green) and propidium iodide (considered dead, red) after (**a**) 0 min, (**b**) 30 min, (**c**) 120 min, and (**d**) 360 min of illumination with red LED light (λ_{max} = 660 nm). Scale bars: 10 μm. (Reprinted from the original [49] according to https://creativecommons.org/licenses/by/4.0/)

infection up to 80%. Even though *P. gingivalis* were not completely eradicated from either of the test systems, the results strongly indicated that metronidazole- and doxycycline-loaded polymersomes were taken up by the cells, where they disintegrated in the endosomal compartment and developed antibacterial activity.

Subsequently, pH responsive PMPC-PDPA polymersomes were loaded with a number of antimicrobial drugs including gentamicin, lysostaphin, vancomycin, rifampicin, and isoniazid [61]. Their potential to reduce intracellular pathogens was tested both in vitro and in vivo, in monocyte-derived macrophages (THP-1 cells) and embryos of zebra fish (*Danio rerio*), respectively. Both THP-1 cells and the zebra fish embryos were infected with either *S. aureus*, *M. bovis*-attenuated Bacillus Calmette–Guérin (BCG), *M. tuberculosis*, or *M. marinum* bacteria. After screening all possible combinations of cargoes and infected model systems, antimicrobial-loaded polymersomes were found to inhibit the bacterial growth both in vitro and in vivo in all the cases. Notably, antimicrobial-loaded polymersomes were more efficient than when the corresponding drug was added in solution. In some cases, they were even able to completely eradicate the intracellular microorganisms.

Moreover, copolymerization of 2-hydroxyethyl methacrylate (HEMA) and poly(ethylene glycol) methyl ether methacrylate (O950) yielded a library of block copolymers. Selected copolymers were pH responsive and spontaneously formed polymersomes [62]. These pH responsive polymersomes were loaded with the hydrophilic antibiotic drug ceftazidime and incubated with RAW 264.7 murine macrophages previously infected with *B. thailandensis* (AH183) containing the pMLS7-eGFP plasmid encoding genes for both green fluorescence protein and trimethoprim resistance. In order to evaluate the impact of the ceftazidime containing polymersomes on bacterial growth, infected RAW 264.7 cells were treated with free and encapsulated ceftazidime and examined after 6 and 24 h by fluorescence microscopy. Compared to untreated control cells, infected RAW 264.7 treated with free ceftazidime and loaded polymersomes showed low levels of fluorescent bacteria, both intra- and extracellular even after 24 h. Microscopy findings indicated that ceftazidime was released when the pH-responsive polymersomes had reached the endosomal compartment and inhibited bacterial growth to a noticeable extent. A coculture bacterial challenge assay with *B. thailandensis* and RAW 264.7 cells showed that colony-forming units per well (CFU/Well) were similarly reduced from 1.3×10^7 to around 35,000 CFU/Well by free ceftazidime and lysates from ceftazidime-polymersome-treated cells, however, at much lower ceftazidime concentrations (0.05 and 0.1 mg/mL) for polymersome-treated cells compared to free ceftazidime (0.2 mg/mL). At a dose of 0.2 mg/mL, the intracellular bacteria load was reduced to undetectable levels in lysates from ceftazidime-polymersome treated cells.

An alternative approach is based on the co-assembly of polymersomes derived from poly(ε-caprolactone)-*block*-poly(lysine-*stat*-phenylalanine) [PCL-*b*-P(Lys-*stat*-Phe)] copolymers and hydrogels made from dibenzaldehyde-functionalized PEG (DF-PEG) block copolymers that had "quick" as well as "long-term" antibacterial function (Fig. 3) [63]. Here, both the polymersomes and the hydrogels contain the antibiotic penicillin G from *Penicillium chrysogenum*. The porous structure of

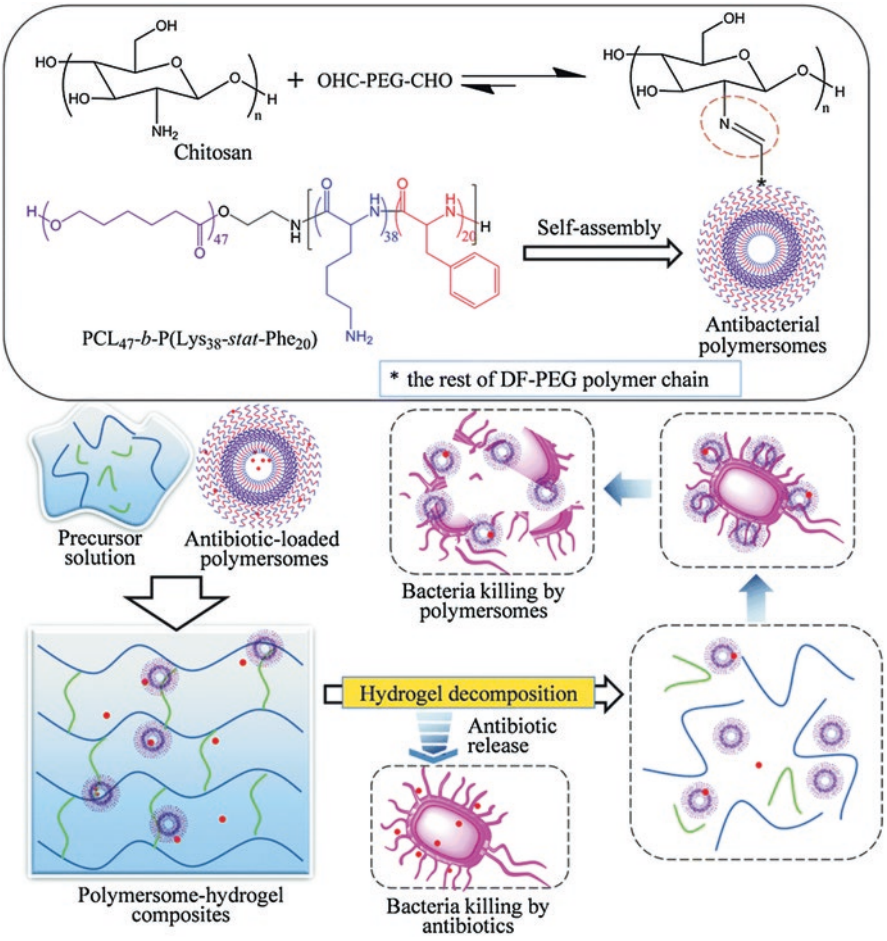


Fig. 3 Schematic representation of an antibiotic containing polymersome—hydrogel system which is capable of "quick" and "long-term" antibiotic release. (Reproduced from [63] with permission from The Royal Society of Chemistry)

the hydrogels allows for the release of penicillin G as well as the release of antibiotic containing polymersomes. The presence of penicillin G within the hydrogels allows for fast antibacterial action ("quick" release). However, the half time of penicillin G is rather short, up to 30 min. By incorporation into polymersomes, penicillin G is protected and its activity is extended through long-term release. At the same time, these specific polymersomes exhibit intrinsic antimicrobial activity: the positively charged lysine residues of the surface-exposed P(Lys-*stat*-Phe) corona can bind to the negatively charged bacterial membranes and disrupt them. In addition, the interaction of the polymer membrane with the bacteria facilitates the release of the antibiotic cargo. The hydrogel–polymersome system was tested on *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) bacteria cultures and was shown to

effectively reduce their viability. Hardly any bacterial colonies managed to grow in the presence of polymersomes at the concentration of 250 mg/mL. More specifically, 99.7% of *S. aureus* and 99.6% of *E. coli* were killed. Furthermore, these studies revealed that the coexistence of hydrogels and polymersomes was critical to significantly extend the duration of penicillin G release.

Polymersomes Loaded with NPs

Inorganic NPs were introduced as promising antibacterial agents but they faced the limitation of low specificity and high toxicity in eukaryotic cells [64]. Conceivably, these limitations can be overcome by encapsulating NPs in polymer-based assemblies such as polymersomes or micelles. Hence, the antibacterial activity of inorganic NPs is combined with the enhanced stability and biocompatibility of polymer nanostructures.

Silver nanoparticles (AgNPs) were incorporated into the membrane of polymersomes and were tested for antimicrobial activity in vitro [65]. Specifically, a PEO-*b*-P(DMA-*stat*-tBA) block copolymer was synthesized and self-assembled into polymersomes in aqueous solution. The polymersome solution was then mixed with an $AgNO_3$ solution and solid $NaBH_4$. As a result, AgNPs formed within the polymersome membrane. The Ag-decorated polymersomes were evaluated for their antibacterial efficacy in vitro. When Gram-negative *E. coli* were exposed to the Ag-decorated polymersomes, the Minimum Inhibitory Concentration (MIC), which is the lowest concentration of an antibacterial agent required for the visible inhibition of growth, and the Minimum Bactericidal Concentration (MBC), defined as the minimum concentration of an antibacterial agent that results in bacterial death, were low. The inhibition of bacterial growth was rather high. In a next step, methoxypoly(ethelyne glycol)-poly(D)-(L)-lactic acid (mPEG-PDLLA), a biodegradable block copolymer, was used to produce polymersomes with AgNPs incorporated into the hydrophobic part of the membrane. Additionally, the hydrophilic cavities of the mPEG-PDLLA polymersomes were loaded with ampicillin.

The potential antimicrobial activity of the AgNPs and antibiotic containing mPEG-PDLLA polymersomes was tested by monitoring the proliferation (CFU/mL) of a suspension of ampicillin-resistant *E. coli* [66]. The bacterial growth was monitored for 48 h after treatment with free ampicillin, AgNPs-containing polymersomes without ampicillin, and ampicillin-containing polymersomes without AgNPs. From this study, it resulted that only the combination of Ag and ampicillin in polymersomes was able to significantly inhibit bacterial growth, whereas with the other treatments, the bacteria were able to proliferate. In the presence of AgNPs-containing polymersomes containing 55 μg/mL ampicillin there was no bacterial proliferation.

Other examples of inorganic NPs in the membrane of polymersomes and antibiotics in the aqueous cavity include methoxy poly(ethylene glycol)-*b*-poly(D,L-lactic acid) based polymersomes hosting the antibiotic methicillin and hydrophobic

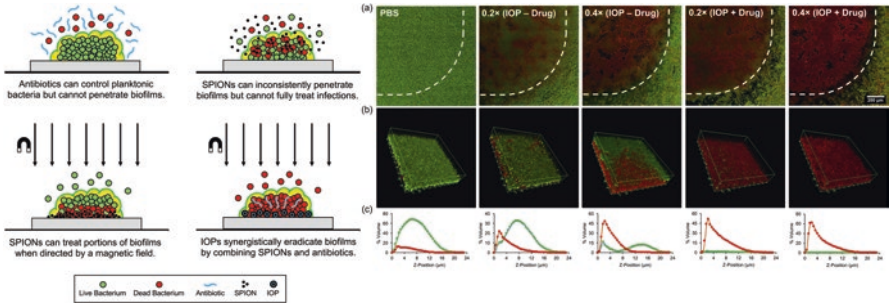


Fig. 4 Left: Current strategies for biofilm treatment using SPIONs and/or antimicrobials. *IOP* iron oxide-encapsulating polymersome, *SPION* superparamagnetic iron oxide nanoparticle. Right: Iron oxide-containing polymersomes preventing biofilm formation. CLSM micrographs of LIVE/DEAD staining of biofilms treated with different dilutions of IOPs (1 × stock = 100 μg/mL SPION; 50 μg/mL methicillin) for 24 h. Bacteria stained green are considered alive and red are considered dead. (**a**) Tile scans collected halfway through the biofilm show a dependency between the concentration of the IOP-Drug and the bacteria death (**b**) 3D reconstructions of z-stacks collected across the biofilm thickness inside the magnetic field. (**c**) The percentage of biofilm volume occupied by live (green) and dead (red) bacteria as a function of biofilm depth (0 μm = bottom) as quantified from image slices. (Reprinted from Superparamagnetic iron oxide-encapsulating polymersome nanocarriers for biofilm eradication[119], Geilich BM, Gelfat I, Sridhar S, van de Ven AL, Webster TJ (2017):78–85. Copyright (2017), with permission from Elsevier)

superparamagnetic iron oxide nanoparticles (SPIONs) in the membrane (Fig. 4) [67]. These assemblies are called iron oxide-encapsulating polymersomes (IOPs). Biofilms formed by *S. epidermidis* RP62a grown on glass cover slips were incubated with IOPs. Taking advantage of the magnetic properties of the SPIONs, a magnet placed underneath the coverslip was used to attract the IOPs and make them penetrate the biofilm. All IOP treatments resulted in a decrease of biofilm volume. Neither SPIONs by themselves nor drug-free IOPs were able to reduce the live bacteria by more than 60%. For drug containing IOPs, the extent of biofilm inhibition correlated with the concentration of polymersomes. Complete eradication of methicillin-resistant *S. epidermidis* biofilms was observed with IOPs containing 40 mg/mL SPIONs and 20 mg/mL of methicillin. Thus, the unique combination of SPIONs and antibiotic within polymersomes that are able to uniformly penetrate a biofilm upon exposure to a magnet represents a weapon of great potential against drug-resistant bacteria.

Polymersomes for Sensing Pathogenic Bacteria

Polymersomes lend themselves to the development of platforms for sensing pathogenic bacteria. More specifically, poly(ethylene glycol)-*block*-poly(lactic acid) (PEG-*b*-PLA) polymersomes equipped with poly-saccharide hyaluronic acid (HYA) which is selectively degraded by the enzyme hyaluronidase, can serve as biosensors

for the detection of the pathogenic *S. aureus* [68]. Like many pathogenic bacteria able to establish infections at the mucosal or skin surface, *S. aureus* secrete hyaluronidase that is likely to degrade nearby polymersomes with surface-exposed HYA. In order to assess the degradation, a wide range of reporter dye molecules (e.g., carboxyfluorescein, CF) as well as antimicrobials (e.g., gentamicin) were encapsulated into the polymersomes during the self-assembly process. The enzyme-triggered degradation of HYA-polymersomes resulted in the release of the respective dye or an increase of cargo-dependent activity. Since the majority of *S. aureus* strains secrete hyaluronidase, HYA-*b*-PCL block copolymer systems offer a promising indicator system for the detection of these bacteria.

The interest in polymer-based nanostructures has been boosted by the fact that they can be designed to release the active agent on demand as exemplified above. If such nanostructures are immobilized on the surface of a medical device, not only would the active agent be released at the site of interest, but the release could be controlled over longer periods of time. These benefits have made immobilized polymeric nanoparticles a hot topic in nanomedicine.

Immobilized Nanocompartments

Immobilization Techniques

Various methods exist to immobilize nanostructures, including covalent and non-covalent strategies. In most cases, the nanostructures are equipped with surface modifications complementary to those of the surface onto which they are immobilized. Non-covalent interactions include the receptor ligand pair biotin–streptavidin, where different immobilization strategies are employed. For example, streptavidin is added to a biotinylated surface and biotinylated polymersomes are then immobilized on the surface by binding to streptavidin [69–71]. Using a plasma-polymerized acrylic acid surface that exposes streptavidin to interact with biotinylated polymersomes also leads to successful immobilization [72]. Another non-covalent immobilization method is based on the adamantane–β-cyclodextrin host–guest complexation. Here the adamantane moiety on polymersomes fits tightly into the cavity of β-cyclodextrin which is accessible on the substrate surface [73]. Electrostatic interactions enable the reversible immobilization of polymersomes via Mg^{2+} bridges [74] or the immobilization of negatively charged (COO^-) polymersomes on positively charged (NH_3^+) surfaces [75].

A prominent example of covalent immobilization procedures is the Schiff base formation between aldehyde functionalized surfaces and amine functionalized polymersomes [76] or vice versa [77] with possible further reductive amination [78]. In the thiol-ene click reaction, the double bond of methacrylate exposed on polymersomes covalently binds to thiol functionalized surfaces [79]. With the Copper (I) catalyzed alkyne azide cycloadditon (CuAAC), azide functionalized polymersomes can covalently bind to alkyne functionalized surfaces, forming a stable triazole linker [80].

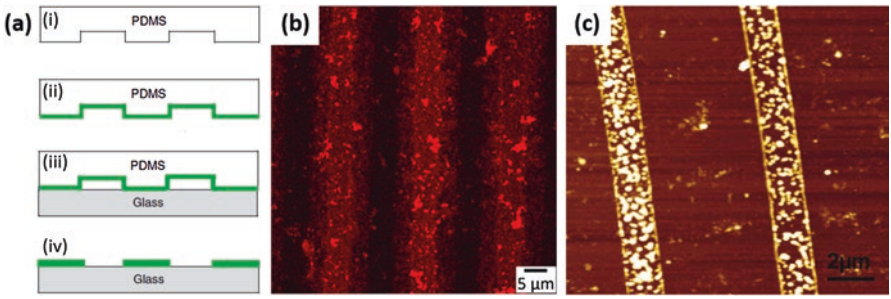


Fig. 5 (**a**) Schematic representation of how to perform μCP: (i) the PDMS micro-stamp is (ii) inked with the material of interest and then (iii) brought in contact with the surface. (iv) The pattern is transferred after removing the micro-stamp. μCP of (**b**) azide exposing polymersome covalently bound onto DBCO functionalized surfaces [83], and (**c**) positively charged liposomes onto negatively charged glass substrates [84]. (Reprinted with permission from [83] Copyright (2018) and [84] Copyright (2005) American Chemical Society)

However, the cytotoxicity of Cu(I) represents a significant limitation of this well-established reaction in biological applications. With the strain promoted azide alkyne click (SPAAC) reaction, a catalyst free reaction pathway was developed that overcomes this limitation. Due to the high ring strain (18 kcal/mol), the alkynes in an 8-membered ring are highly reactive which allows the reaction to proceed without any catalyst [81, 82]. Azide exposing polymersomes were covalently immobilized on dibenzocycloocyne (DBCO) functionalized surfaces [83].

Soft lithography techniques can be used to immobilize nanostructures in a spatially defined pattern. Micro-molding in capillaries (MIMIC) takes advantage of the capillary forces induced by channel-like spaces between the surface and the stamp which are formed when a micro-stamp is pressed on the surface. Accordingly, immobilization of lipid vesicles was achieved by adding a solution of vesicles in front of the channel opening. The vesicle solution then moved into the defined space between the stamp and the surface by capillary action [75, 84]. Classical micro-contact printing (μCP) is a different soft lithography technique where poly(dimethyl-siloxane) (PDMS) micro-stamps are used. In μCP, connected patterns on the stamp are not required to create channels. Instead, the substrate is directly inked on the patterned side of the stamp and then brought in contact with the surface, thereby transferring the pattern onto the surface [85]. By using corresponding solutions of nanostructures as ink on the PDMS stamp, polymersomes [83], liposomes [84], or inorganic nanoparticles [86] were immobilized on a surface of interest in a defined pattern (Fig. 5).

Active Surfaces

The immobilization procedures can be applied to the catalytic nanocompartments or drug-loaded nanocompartments that have been described free in solution in the previous section. This leads to the design of “active surfaces” where the activity of

the nanostructures becomes locally concentrated. For polymersomes to be functional on a surface, i.e., create an active surface, it is crucial that they remain responsive to a redox state, light, pH, etc. despite being immobilized. To show that immobilized vesicles with a disulfide cross-linked polymer shell maintain their redox responsive release properties, polymersomes with encapsulated carboxyfluorescein were exposed to the reducing agent (tris(2-carboxyethyl)phosphine) TCEP [71]. The resulting change in redox potential triggered the release of carboxyfluorescein as visualized by a 10 h increase of fluorescence. To evaluate pH responsiveness, polymersomes were loaded with pyranine, a pH sensitive dye, and immobilized on a glass substrate [87]. Depending on the pH of the surrounding buffer, the fluorescence was either increased (pH 8) or quenched (pH 6). Compared to free polymersomes, the immobilized ones retained their responsiveness although their physical behavior may change. If polymersomes are free in solution, their swelling, which is induced by a change of pH, occurs equally in all directions. Interestingly, immobilization causes them to swell more in the z direction than in the lateral direction [73]. This indicates that immobilization of nanostructures on a surface has an influence on the physical properties.

By going a step further to immobilized catalytic nanocompartments the possible design of "active surfaces" gets more versatile. Surfaces coated with catalytic nanocompartments can be used as detecting platforms. For example, ribitol, a model sugar alcohol was detected by means of surface immobilized polymersomes loaded with the enzyme ribitol dehydrogenase (RDH) [88]. To allow selective diffusion of sugar alcohols across the membrane into the cavity where RDH was encapsulated, the *E. coli* glycerol facilitator (GlpF) had been incorporated into the membrane of these polymersomes. Immobilization in a distinct pattern as introduced in the previous section can also be applied to catalytic nanocompartments. For example, a patterning of immobilized nanostructures was achieved for acid phosphatase encapsulated in polymersomes [69]. Here, the surface was first micro-contact printed with biotin and then streptavidin was added to immobilize the biotinylated catalytic nanocompartment. These patterned catalytic nanocompartments successfully dephosphorylated the fluorogenic substrate ELF 97.

Above, we introduced AgNPs and their great potential as antimicrobial agents. Intriguingly, it has been shown that immobilized cationic nanoparticles are more effective in killing bacteria than free nanoparticles in solution [89]. This could be due to the lack of movement of the nanoparticles during the interaction with the bacteria. Poly(L-lactic acid) (PLLA) nanoparticles containing AgNPs and the detergent polyvinyl alcohol (PVA) as a stabilizer were immobilized through electrostatic interactions between negatively charged COO^- groups on the PLLA nanoparticles and positively charged NH_3^+ groups on the substrate [90]. This surface was able to reduce biofilm formation up to 98% compared to immobilized PLLA nanoparticles which are lacking the AgNP. PLLA itself does not have any influence on biofilm growth. However, it might facilitate silver ion availability as lactic acid, a degradation product of PLLA, enhances the permeabilization through the bacterial cell wall. Additionally, the decrease in local pH due to lactic acid might enhances the oxidation and dissolution of the AgNP.

The previously discussed catalytic nanocompartments that are able to produce the antibiotic cephalexin [57] have been immobilized through Schiff base formation with further reductive amination on silica surfaces (Fig. 6a) [78]. After immobilization, the catalytic nanocompartments remained active and produced and released the antibiotic up to 7 days. This is a strong indication for a prolonged activity of immobilized nanocompartments. Bacterial attachment and proliferation were reduced by around 75% on surfaces with immobilized catalytic nanocompartments producing cephalexin compared to immobilized non-active nanocompartments.

Antimicrobial peptides (AMP), which are often natural peptides, as alternatives to conventional antibiotics have obtained a lot of attention in recent years [91–93]. On the one hand they are less likely to evoke bacterial resistance [94], and on the other hand, they are able to selectively destroy bacterial membranes [92]. The AMP clavanin A (clavA) was attached on iron oxide (Fe_3O_4) nanoparticles functionalized with dimercaptosuccinic acid (DMSA) [95]. These Fe_3O_4-DMSA-clavA nanoparticles were then immobilized on the inner wall of a central venous catheter (CVC) which was previously functionalized by aminopropyl trimethoxysilane (APTS) to create an amino surface (Fig. 6b). The clavA on the immobilized nanoparticles was able to disrupt bacterial membranes. The CVC modified with the Fe_3O_4-DMSA-clavA nanoparticles reduced Gram-negative bacteria attachment by nearly 90% compared to non-modified CVC. Furthermore, laser irradiation of iron oxide nanoparticles causes local hyperthermia and thermoablation of already formed biofilms.

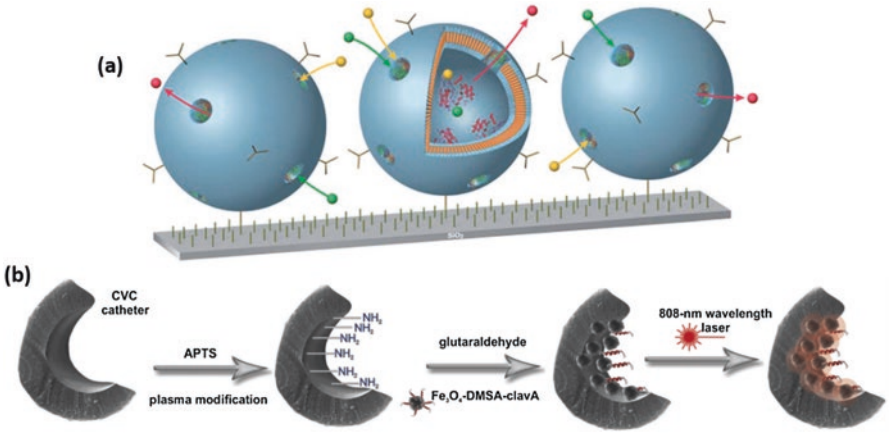


Fig. 6 Schematic representation of two strategies to design antimicrobial surfaces by immobilization of different active nanostructures. (**a**) Catalytic nanocompartments encapsulating the enzyme PA are immobilized on silica wafers. OmpF is used to create pores in the polymersome through which the educts 7-ADCA and PGME (yellow and green dots) as well as the product cephalexin (red dots) can diffuse [78]. (**b**) CVC are modified with APTS to obtain amino-functionalized surfaces on which iron oxide nanoparticles containing the AMP clavA are immobilized. An 808 nm wavelength laser triggers local hyperthermia and ablation [95]. (Reproduced with permission from [78] Copyright (2014) The Royal Society of Chemistry and Reprinted from Clavanin A-bioconjugated Fe_3O_4/Silane core-shell nanoparticles for thermal ablation of bacterial biofilms[169], Ribeiro KL, Frias IAM, Franco OL, Dias SC, Sousa-Junior AA, Silva ON, Bakuzis AF, Oliveira MDL, Andrade CAS (2018): 72–81. Copyright (2018), with permission from Elsevier)

Conclusion and Perspectives

In this chapter, we provide an insight into the importance and potential of polymeric nanocompartments, both as 3D structures in solution and immobilized on surfaces, for active and passive antimicrobial applications. Polymers offer many advantages including robustness, biocompatibility, and a broad spectrum of possible functional moieties, which enable them to act in a spatial and temporal controlled manner. The versatile properties of polymers that self-assemble into polymersomes open different avenues for battling bacteria. Polymersomes can be intrinsically antimicrobial, form catalytic nanocompartments that produce active agents in situ or create ROS which indirectly kill bacteria through toxic stress. Alternatively, polymersomes can be designed as drug containers which are able to battle intracellular infections by releasing cargoes in response to pH changes inside the cell. Moreover, we discussed how to increase the antimicrobial potential of polymersomes by exploiting the hydrophobic membrane and the aqueous cavity. Finally, we showed that polymersomes, owing to their surface properties, can also function as sensors of bacterial infections.

We presented different approaches for the immobilization of polymersomes which is key to the design of a new generation of smart coatings. By directly immobilizing nanostructures on the surface of medical devices, a concentration of the antimicrobial activity at the most vulnerable site, the place of the implant, could be achieved. Moreover, immobilization should prevent rapid clearance of the catalytic nanocompartments and thereby promote their sustained activity. Not only is a local drug release and/or antimicrobial activity more efficient but distant parts of the body are less affected.

Despite the obvious advantages of polymeric nanocompartments as drug carriers or as antimicrobial drug containers on surfaces, many challenges need to be resolved before they can be fully exploited for applications in vivo. Each system needs to be optimized for maximum loading of nanocompartments with antimicrobial agents, desired release kinetics of the active agents while preventing non-desired leakage, and the concentration of immobilized nanocompartments in case of antimicrobial surfaces. Major efforts are devoted to the scale-up of nanocontainer production and to the ability to sterilize the surfaces without destroying them. Besides sterility and biocompatibility, the shelf life of the formulations and durability in vivo are key concerns in developing polymersome-based surfaces for medical devices.

With the present chapter, we hope that we have provided an overview of emerging antibacterial strategies that involve polymersomes. Although many challenges remain, the advances made with model systems in the battle against bacteria raise our hopes for producing polymersome-based surfaces with ideal antimicrobial properties in the future. What requirements should such a surface fulfill? The obvious ones include biocompatibility and a stable immobilization of different kinds and arrangements of polymersomes at high density. The polymersomes should be loaded with potent cargoes that are specifically and rapidly released for as long as their activity is required but with as few side effects as possible. The possibility to simultaneously encapsulate hydrophilic and hydrophobic drugs and exploit their synergistic effects is another advantage offered by polymersomes. From the production point of view,

surface fabrication should be easy, reproducible, and inexpensive. Provided these specifications are met, one can picture to further increase efficiency by combining sensing properties with antimicrobial activity. The dream surface prevents biofilm formation by selectively blocking bacteria attachment and actively battling local infections.

Acknowledgments The authors gratefully acknowledge the generous financial support from the SNSF, NCCR-MSE, and the University of Basel.

References

1. Valles-Colomer M, Falony G, Darzi Y, Tigchelaar EF, Wang J, Tito RY, Schiweck C, Kurilshikov A, Joossens M, Wijmenga C, Claes S, Van Oudenhove L, Zhernakova A, Vieira-Silva S, Raes J (2019) The neuroactive potential of the human gut microbiota in quality of life and depression. Nat Microbiol 4(4):623–632
2. Hill JM, Clement C, Pogue AI, Bhattacharjee S, Zhao YH, Lukiw WJ (2014) Pathogenic microbes, the microbiome, and Alzheimer's disease (AD). Front Aging Neurosci 6:127
3. Zheng P, Zeng B, Zhou C, Liu M, Fang Z, Xu X, Zeng L, Chen J, Fan S, Du X, Zhang X, Yang D, Yang Y, Meng H, Li W, Melgiri ND, Licinio J, Wei H, Xie P (2016) Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. Mol Psychiatry 21(6):786–796
4. Aminov RI (2010) A brief history of the antibiotic era: lessons learned and challenges for the future. Front Microbiol 1:134
5. Rigo S, Cai C, Gunkel-Grabole G, Maurizi L, Zhang X, Xu J, Palivan CG (2018) Nanoscience-based strategies to engineer antimicrobial surfaces. Adv Sci:1700892
6. Najer A, Wu DL, Nussbaumer MG, Schwertz G, Schwab A, Witschel MC, Schafer A, Diederich F, Rottmann M, Palivan CG, Beck HP, Meier W (2016) An amphiphilic graft copolymer-based nanoparticle platform for reduction-responsive anticancer and antimalarial drug delivery. Nanoscale 8(31):14858–14869
7. Abed N, Couvreur P (2014) Nanocarriers for antibiotics: a promising solution to treat intracellular bacterial infections. Int J Antimicrob Agents 43(6):485–496
8. Lam SJ, Wong EHH, Boyer C, Qiao GG (2018) Antimicrobial polymeric nanoparticles. Prog Polym Sci 76:40–64
9. Rodríguez-Hernández J (2017) Nano/microstructured antibacterial surfaces. In: Polymers against microorganisms: on the race to efficient antimicrobial materials. Springer International, Cham, pp 125–154
10. Meng JX, Zhang PC, Wang ST (2014) Recent progress in biointerfaces with controlled bacterial adhesion by using chemical and physical methods. Chem Asian J 9(8):2004–2016
11. Krishnamoorthy M, Hakobyan S, Ramstedt M, Gautrot JE (2014) Surface-initiated polymer brushes in the biomedical field: applications in membrane science, biosensing, cell culture, regenerative medicine and antibacterial coatings. Chem Rev 114(21):10976–11026
12. Hadjesfandiari N, Yu K, Mei Y, Kizhakkedathu JN (2014) Polymer brush-based approaches for the development of infection-resistant surfaces. J Mater Chem B 2(31):4968–4978
13. Raposo G, Stoorvogel W (2013) Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol 200(4):373–383
14. Hessvik NP, Llorente A (2018) Current knowledge on exosome biogenesis and release. Cell Mol Life Sci 75(2):193–208
15. Cullen PJ, Steinberg F (2018) To degrade or not to degrade: mechanisms and significance of endocytic recycling. Nat Rev Mol Cell Biol 19(11):679–696

16. Cabukusta B, Neefjes J (2018) Mechanisms of lysosomal positioning and movement. Traffic 19(10):761–769
17. Zhang XY, Tanner P, Graff A, Palivan CG, Meier W (2012) Mimicking the cell membrane with block copolymer membranes. J Polym Sci Pt A Polym Chem 50(12):2293–2318
18. Traversier M, Gaslondes T, Milesi S, Michel S, Delannay E (2018) Polar lipids in cosmetics: recent trends in extraction, separation, analysis and main applications. Phytochem Rev 17(5):1179–1210
19. Tran S, DeGiovanni PJ, Piel B, Rai P (2017) Cancer nanomedicine: a review of recent success in drug delivery. Clin Transl Med 6
20. Abu Lila AS, Ishida T (2017) Liposomal delivery systems: design optimization and current applications. Biol Pharm Bull 40(1):1–10
21. Gunkel-Grabole G, Sigg S, Lomora M, Lorcher S, Palivan CG, Meier WP (2015) Polymeric 3D nano-architectures for transport and delivery of therapeutically relevant biomacromolecules. Biomater Sci 3(1):25–40
22. Blanazs A, Armes SP, Ryan AJ (2009) Self-assembled block copolymer aggregates: from micelles to vesicles and their biological applications. Macromol Rapid Commun 30(4-5):267–277
23. Lorcher S, Meier W (2017) Cosolvent fractionation of PMOXA-b-PDMS-b-PMOXA: bulk separation of triblocks from multiblocks. Eur Polym J 88:575–585
24. Mai YY, Eisenberg A (2012) Self-assembly of block copolymers. Chem Soc Rev 41(18):5969–5985
25. Konishcheva EV, Daubian D, Rigo S, Meier WP (2019) Probing membrane asymmetry of ABC polymersomes. Chem Commun 55(8):1148–1151
26. Konishcheva EV, Zhumaev UE, Meier WP (2017) PEO-b-PCL-b-PMOXA triblock copolymers: from synthesis to microscale polymersomes with asymmetric membrane. Macromolecules 50(4):1512–1520
27. Konishcheva EV, Zhumaev UE, Kratt M, Oehri V, Meier W (2017) Complex self-assembly behavior of bis-hydrophilic PEO-b-PCL-b-PMOXA triblock copolymers in aqueous solution. Macromolecules 50(18):7155–7168
28. Belluati A, Craciun I, Meyer CE, Rigo S, Palivan CG (2019) Enzymatic reactions in polymeric compartments: nanotechnology meets nature. Curr Opin Biotechnol 60:53–62
29. Itel F, Chami M, Najer A, Lorcher S, Wu DL, Dinu IA, Meier W (2014) Molecular organization and dynamics in polymersome membranes: a lateral diffusion study. Macromolecules 47(21):7588–7596
30. Itel F, Najer A, Palivan CG, Meier W (2015) Dynamics of membrane proteins within synthetic polymer membranes with large hydrophobic mismatch. Nano Lett 15(6):3871–3878
31. Discher BM, Won YY, Ege DS, Lee JCM, Bates FS, Discher DE, Hammer DA (1999) Polymersomes: tough vesicles made from diblock copolymers. Science 284(5417):1143–1146
32. Malinova V, Nallani M, Meier WP, Sinner EK (2012) Synthetic biology, inspired by synthetic chemistry. FEBS Lett 586(15):2146–2156
33. Discher DE, Eisenberg A (2002) Polymer vesicles. Science 297(5583):967–973
34. Penczek S, Pretula J, Lewinski P (2017) Dormant polymers and their role in living and controlled polymerizations; influence on polymer chemistry, particularly on the ring opening polymerization. Polymers (Basel) 9(12):–E646
35. Ratcliffe LPD, Bentley KJ, Wehr R, Warren NJ, Saunders BR, Armes SP (2017) Cationic disulfide-functionalized worm gels. Polym Chem 8(38):5962–5971
36. Moad G (2017) RAFT polymerization to form stimuli-responsive polymers. Polym Chem 8(1):177–219
37. Palmiero UC, Sponchioni M, Manfredini N, Maraldi M, Moscatelli D (2018) Strategies to combine ROP with ATRP or RAFT polymerization for the synthesis of biodegradable polymeric nanoparticles for biomedical applications. Polym Chem 9(30):4084–4099
38. Dinu IA, Duskey JT, Car A, Palivan CG, Meier W (2016) Engineered non-toxic cationic nanocarriers with photo-triggered slow-release properties. Polym Chem 7(20):3451–3464

39. Matyjaszewski K, Xia JH (2001) Atom transfer radical polymerization. Chem Rev 101(9):2921–2990
40. Fantin M, Lorandi F, Gennaro A, Isse AA, Matyjaszewski K (2017) Electron transfer reactions in atom transfer radical polymerization. Synthesis 49(15):3311–3322
41. Gaitzsch J, Welsch PC, Folini J, Schoenenberger CA, Anderson JC, Meier WP (2018) Revisiting monomer synthesis and radical ring opening polymerization of dimethylated MDO towards biodegradable nanoparticles for enzymes. Eur Polym J 101:113–119
42. Konishcheva E, Daubian D, Gaitzsch J, Meier W (2018) Synthesis of linear ABC triblock copolymers and their self-assembly in solution. Helv Chim Acta 101(2):e1700287
43. Bai LC, Tan L, Chen LJ, Liu ST, Wang YM (2014) Preparation and characterizations of poly(2-methyl-2-oxazoline) based antifouling coating by thermally induced immobilization. J Mater Chem B 2(44):7785–7794
44. Zhang XY, Zhang PY (2017) Polymersomes in nanomedicine—a review. Curr Nanosci 13(2):124–129
45. Konradi R, Pidhatika B, Muhlebach A, Textor M (2008) Poly-2-methyl-2-oxazoline: a peptide-like polymer for protein-repellent surfaces. Langmuir 24(3):613–616
46. Atkins PW, de Paula J (2007) Physikalische Chemie. Oxford University Press, Oxford
47. Aoshima S, Kanaoka SA (2009) Renaissance in living cationic polymerization. Chem Rev 109(11):5245–5287
48. Najer A, Wu DL, Vasquez D, Palivan CG, Meier W (2013) Polymer nanocompartments in broad-spectrum medical applications. Nanomedicine 8(3):425–447
49. Lanzilotto A, Kyropoulou M, Constable EC, Housecroft CE, Meier WP, Palivan CG (2018) Porphyrin-polymer nanocompartments: singlet oxygen generation and antimicrobial activity. J Biol Inorg Chem 23(1):109–122
50. Palivan CG, Goers R, Najer A, Zhang XY, Car A, Meier W (2016) Bioinspired polymer vesicles and membranes for biological and medical applications. Chem Soc Rev 45(2):377–411
51. Onaca O, Enea R, Hughes DW, Meier W (2009) Stimuli-responsive polymersomes as nanocarriers for drug and gene delivery. Macromol Biosci 9(2):129–139
52. Nardin C, Widmer J, Winterhalter M, Meier W (2001) Amphiphilic block copolymer nanocontainers as bioreactors. Eur Phys J E 4(4):403–410
53. Broz P, Benito SM, Saw C, Burger P, Heider H, Pfisterer M, Marsch S, Meier W, Hunziker P (2005) Cell targeting by a generic receptor-targeted polymer nanocontainer platform. J Control Release 102(2):475–488
54. Zhang C, Zhu YQ, Zhou CC, Yuan WZ, Du JZ (2013) Antibacterial vesicles by direct dissolution of a block copolymer in water. Polym Chem 4(2):255–259
55. Wang MZ, Zhou CC, Chen J, Xiao YF, Du JZ (2015) Multifunctional biocompatible and biodegradable folic acid conjugated poly(epsilon-caprolactone)-polypeptide copolymer vesicles with excellent antibacterial activities. Bioconjug Chem 26(4):725–734
56. Belluati A, Craciun I, Liu J, Palivan CG (2018) Nanoscale enzymatic compartments in tandem support cascade reactions in vitro. Biomacromolecules 19(10):4023–4033
57. Langowska K, Palivan CG, Meier W (2013) Polymer nanoreactors shown to produce and release antibiotics locally. Chem Commun 49(2):128–130
58. Wick WE (1967) Cephalexin a new orally absorbed cephalosporin antibiotic. Appl Microbiol 15(4):765–769
59. Spratt BG (1975) Distinct penicillin binding-proteins involved in division, elongation, and shape of Escherichia coli-K12. Proc Natl Acad Sci U S A 72(8):2999–3003
60. Wayakanon K, Thornhill MH, Douglas CWI, Lewis AL, Warren NJ, Pinnock A, Armes SP, Battaglia G, Murdoch C (2013) Polymersome-mediated intracellular delivery of antibiotics to treat Porphyromonas gingivalis-infected oral epithelial cells. FASEB J 27(11):4455–4465
61. Rizzello L, Robertson JD, Elks PM, Poma A, Daneshpour N, Prajsnar TK, Evangelopoulos D, Ortiz Canseco J, Yona S, Marriott HM, Dockrell DH, Foster S, De Geest B, De Koker S, McHugh T, Renshaw SA, Battaglia G (2017) Targeting mononuclear phagocytes for eradicating intracellular parasites. bioRxiv. p 119297

62. Lane DD, Su FY, Chiu DY, Srinivasan S, Wilson JT, Ratner DM, Stayton PS, Convertine AJ (2015) Dynamic intracellular delivery of antibiotics via pH-responsive polymersomes. Polym Chem 6(8):1255–1266
63. Hong YX, Xi YJ, Zhang JX, Wang DD, Zhang HL, Yan N, He SS, Du JZ (2018) Polymersome-hydrogel composites with combined quick and long-term antibacterial activities. J Mater Chem B 6(39):6311–6321
64. Kurtjak M, Aničić N, Vukomanovicć M (2017) Inorganic nanoparticles: innovative tools for antimicrobial agents in antibacterial agents. IntechOpen, Rijeka, pp 39–60
65. Lu H, Fan L, Liu QM, Wei JR, Ren TB, Du JZ (2012) Preparation of water-dispersible silver-decorated polymer vesicles and micelles with excellent antibacterial efficacy. Polym Chem 3(8):2217–2227
66. Geilich BM, van de Ven AL, Singleton GL, Sepulveda LJ, Sridhar S, Webster TJ (2015) Silver nanoparticle-embedded polymersome nanocarriers for the treatment of antibiotic-resistant infections. Nanoscale 7(8):3511–3519
67. Geilich BM, Gelfat I, Sridhar S, van de Ven AL, Webster TJ (2017) Superparamagnetic iron oxide-encapsulating polymersome nanocarriers for biofilm eradication. Biomaterials 119:78–85
68. Haas S, Hain N, Raoufi M, Handschuh-Wang S, Wang T, Jiang X, Schonherr H (2015) Enzyme degradable polymersomes from hyaluronic acid-block-poly(epsilon-caprolactone) copolymers for the detection of enzymes of pathogenic bacteria. Biomacromolecules 16(3):832–841
69. Grzelakowski M, Onaca O, Rigler P, Kumar M, Meier W (2009) Immobilized protein-polymer nanoreactors. Small 5(22):2545–2548
70. Berthaud A, Quemeneur F, Deforet M, Bassereau P, Brochard-Wyart F, Mangenot S (2016) Spreading of porous vesicles subjected to osmotic shocks: the role of aquaporins. Soft Matter 12(5):1601–1609
71. De Vries WC, Tesch M, Studer A, Ravoo BJ (2017) Molecular recognition and immobilization of ligand-conjugated redox-responsive polymer nanocontainers. ACS Appl Mater Interfaces 9(48):41760–41766
72. Battaglia G, LoPresti C, Massignani M, Warren NJ, Madsen J, Forster S, Vasilev C, Hobbs JK, Armes SP, Chirasatitsin S, Engler AJ (2011) Wet nanoscale imaging and testing of polymersomes. Small 7(14):2010–2015
73. Iyisan B, Janke A, Reichenbach P, Eng LM, Appelhans D, Voit B (2016) Immobilized multifunctional polymersomes on solid surfaces: infrared light-induced selective photochemical reactions, PH responsive behavior, and probing mechanical properties under liquid phase. ACS Appl Mater Interface 8(24):15788–15801
74. Li F, Ketelaar T, Stuart MAC, Sudholter EJR, Leermakers FAM, Marcelis ATM (2008) Gentle immobilization of nonionic polymersomes on solid substrates. Langmuir 24(1):76–82
75. Chen Q, de Groot GW, Schonherr H, Vancso GJ (2011) Patterns of surface immobilized block copolymer vesicle nanoreactors. Eur Polym J 47(2):130–138
76. Choi JM, Yoon B, Choi K, Seol ML, Kim JM, Choi YK (2012) Micropatterning polydiacetylene supramolecular vesicles on glass substrates using a pre-patterned hydrophobic thin film. Macromol Chem Phys 213(6):610–616
77. Domes S, Filiz V, Nitsche J, Fromsdorf A, Forster S (2010) Covalent attachment of polymersomes to surfaces. Langmuir 26(10):6927–6931
78. Langowska K, Kowal J, Palivan CG, Meier W (2014) A general strategy for creating self-defending surfaces for controlled drug production for long periods of time. J Mater Chem B 2(29):4684–4693
79. Gunkel-Grabole G, Palivan C, Meier W (2017) Nanostructured surfaces through immobilization of self-assembled polymer architectures using thiol-ene chemistry. Macromol Mater Eng 302(4)
80. Rein C, Nissen S, Grzelakowski M, Meldal M (2016) Click-chemistry of polymersomes on nanoporous polymeric surfaces. J Polym Sci Pt A Polym Chem 54(13):2032–2039
81. Lutz JF (2008) Copper-free azide-alkyne cycloadditions: new insights and perspectives. Angew Chem Int Ed 47(12):2182–2184

82. Agard NJ, Prescher JA, Bertozzi CR (2005) A strain-promoted [3+2] azide-alkyne cycloaddition for covalent modification of biomolecules in living systems (vol 126, pg 15046, 2004). J Am Chem Soc 127(31):11196–11196
83. Rigo S, Gunkel-Grabole G, Meier W, Palivan CG (2019) Surfaces with dual functionality through specific coimmobilization of self-assembled polymeric nanostructures. Langmuir 35(13):4557–4565
84. Mahajan N, Lu RB, Wu ST, Fang JY (2005) Patterning polymerized lipid vesicles with soft lithography. Langmuir 21(7):3132–3135
85. Qin D, Xia YN, Whitesides GM (2010) Soft lithography for micro- and nanoscale patterning. Nat Protoc 5(3):491–502
86. Xin ZQ, Liu Y, Li X, Liu SL, Fang Y, Deng YQ, Bao C, Li LH (2017) Conductive grid patterns prepared by microcontact printing silver nanoparticles ink. Mater Res Express 4(1)
87. Craciun I, Denes AS, Gunkel-Grabole G, Belluati A, Palivan CG (2018) Surfaces decorated with polymeric nanocompartments for pH reporting. Helv Chim Acta 101(3)
88. Zhang XY, Lomora M, Einfalt T, Meier W, Klein N, Schneider D, Palivan CG (2016) Active surfaces engineered by immobilizing protein-polymer nanoreactors for selectively detecting sugar alcohols. Biomaterials 89:79–88
89. Fang B, Jiang Y, Nusslein K, Rotello VM, Santore MM (2015) Antimicrobial surfaces containing cationic nanoparticles: how immobilized, clustered, and protruding cationic charge presentation affects killing activity and kinetics. Colloids Surf B Biointerfaces 125:255–263
90. Taheri S, Baier G, Majewski P, Barton M, Forch R, Landfester K, Vasilev K (2014) Synthesis and surface immobilization of antibacterial hybrid silver-poly(l-lactide) nanoparticles. Nanotechnology 25(30)
91. Ali SM, Siddiqui R, Khan NA (2018) Antimicrobial discovery from natural and unusual sources. J Pharm Pharmacol 70(10):1287–1300
92. Fry DE (2018) Antimicrobial peptides. Surg Infect (Larchmt) 19(8):804–811
93. Riool M, de Breij A, Drijfhout JW, Nibbering PH, Zaat SAJ (2017) Antimicrobial peptides in biomedical device manufacturing. Front Chem 5:63
94. Riool M, de Breij A, de Boer L, Kwakman PHS, Cordfunke RA, Cohen O, Malanovic N, Emanuel N, Lohner K, Drijfhout JW, Nibbering PH, Zaat SAJ (2017) Controlled release of LL-37-derived synthetic antimicrobial and anti-biofilm peptides SAAP-145 and SAAP-276 prevents experimental biomaterial-associated Staphylococcus aureus infection. Adv Funct Mater 27(20):1606623
95. Ribeiro KL, Frias IAM, Franco OL, Dias SC, Sousa-Junior AA, Silva ON, Bakuzis AF, Oliveira MDL, Andrade CAS (2018) Clavanin A-bioconjugated Fe3O4/Silane core-shell nanoparticles for thermal ablation of bacterial biofilms. Colloids Surf B Biointerfaces 169:72–81

Polymeric Nanoparticulate Delivery Vehicles of Antimicrobials for Biofilm Eradication

Yuezhou Zhang, Luofeng Yu, Jianhong Zhang, and Peng Li

Abstract Given the challenge that the number of existing antibiotic resistant strains and species is increasing at an alarming rate, in particular these biofilm-associated microorganisms are often thought to be hard to eradicate by free antibiotics since they are embedded in a condensed polymeric matrix therefore hamper the effective killing. The develoment of nanotechnology enable the deeper penetration and targeting of antibiotics to noramlly unreached biofilm. Combined with the diversity of polymers, antibiotics has been formulated into different version of delivery vehicles with antimicrobials included to deplete biofilm. In addition, polymeric nanocomposite can also be assembled by incorporating other type of toxic nanoparticles to achieve synergetic biofilm eradication outcomes.

Keywords Biofilm · Eradication · Polymeric nanoparticles · Delivery vehicles · Antimicrobials · Tolerance

Introduction

Intracellular pathogenic bacteria often cause severe disease burden. They can invade, survive, and propagate in the host cells and either cause acute morbidity and mortality or hibernate over long period of time until the outbreak of life-threatening disease [1]. Bacteria may often inhabit locations where the free antimicrobials are poorly able to reach. Furthermore, the abuse of antimicrobials increases the development of resistance. To tackle the abovementioned challenges, an efficient

Y. Zhang · J. Zhang · P. Li (✉)
Xi'an Institute of Flexible Electronics & Xi'an Institute of Biomedical Materials and Engineering, Northwestern Polytechnical University (NPU), Xi'an, China
e-mail: iampli@nwpu.edu.cn

L. Yu
Key Laboratory of Flexible Electronics (KLOFE) and Institute of Advanced Materials (IAM), Jiangsu National Synergetic Innovation Center for Advanced Materials (SICAM), Nanjing Tech University (NanjingTech), Nanjing, China

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_18

antimicrobial delivery system is much needed. Compared to free drug administration, the delivery systems can improve antimicrobial usage in multiple aspects: (1) targeting the infection point [2]; (2) protect from inactivation; (3) efficient drug encapsulation; and (4) enable drugs to bypass cellular barriers. Nowadays, novel drug delivery strategies for efficient delivery of antibiotics have been extensively explored; one such strategy is represented by nanoparticulate delivery systems.

Nanoparticles (NPs), sized from 1 to 100 nm at least in one dimension, are of great scientific interest [3] as they promise significant potential applications within a variety of fields, particularly within the biomedical landscape. The combination of medicine and nanotechnology has boosted a new cross-disciplinary nanomedicine [4, 5]. Nanomedicine nowadays also helps to establish various nanoparticulate systems to favor the antimicrobial delivery to the infection sites [6–9]. The delivery of antimicrobials to biofilm and intracellular bacteria has been particularly in focus. The intracellular bacteria may escape the cell and mature into biofilms [10] and it is believed biofilm occurrence is in coexistence with antimicrobial tolerance and chronic bacterial infections. These biofilm-associated microorganisms are often thought to be hard to eradicate by free antibiotics since they are embedded in a condensed polymeric matrix with increased tolerance to antibiotics due to a range of mechanisms, when compared to their planktonic counterparts [11].

Bacteria are a universal component supporting life in many environments and the most natural mode of growth in many environments is as biofilms. The biofilms are a community of surface-attached microbial cells which are encircled by extracellular matrix made of polymeric substances (EPS) [12], such as polysaccharide, glycoprotein, and protein. Bacterial biofilms may coat on many medical devices, and therefore have a huge impact on healthcare since the biofilms are more recalcitrant to conventional antimicrobials and the human immune system. Short of effective antibiotics and antibiofilm pharmaceuticals delivery system have encouraged to develop novel strategies to overcome biofilm infections. Against this background, the development of nanomedicine and its application on targeting bacterial and antibiofilm has become more prominent.

Several aspects which underline the interactions between bacterial biofilm and NPs in the aquatic environment including NP–biofilm interactions, impact of NP characteristics and biofilm matrix components on NP–biofilm interactions, fate of NPs within biofilms have been reviewed [13] and could be summarized as follows: (1) The interactions between NPs and biofilm generally represent transportation of NPs to the biofilm edge, adhere to the biofilm surface, and move within the biofilm; (2) The shape, size, and charge of NPs influence their interactions with biofilm at all abovementioned steps; (3) The density, 3D network, highly hydrated feature, and composition heterogeneity of biofilm play important roles in the starting deposition and followed aggregation of NPs; (4) It is difficult to elucidate the exact fate of the NPs within biofilms; (5) Once NPs are introduced into the bacterial biofilms, they tend to accumulate into micro-sized agglomerates while the EPS may change in response to the appearance of NPs.

Due to the opportunity for increased antibacterial activity offered by NPs, the use of nanotechnology to delivery therapeutics deserve in-depth exploration, particularly

considering the global concern of developing multidrug resistant (MDR) cases and the unmet demand of biofilm eradication. The broader spectrum about the requirements and characteristics of nanotechnology-based antimicrobial delivery systems to control biofilm infection has been reviewed by Liu et al. [14] and others [15] recently. In this chapter, we narrow down our discussion to the application of polymer-oriented nanotechnology for biofilm destruction and annihilation since the polymeric NPs for drug delivery to bacterial biofilms have only been superficially discussed [16]. Their use as drug delivery vehicles is highly appealing because of their structural uniformity and stability, simple of production and functionalization, and are capable of controlled cargo release. The following text is organized according to the means by which the polymer and therapeutics are combined/incorporated.

Polymer-Based Antimicrobial Delivery for Biofilm Elimination

Due to the emergence of MDR pathogens, classical antimicrobials are gradually losing their efficacy. This trend is even compounded by the infecting bacteria which prefers to live in biofilms and hinders antimicrobial diffusion in biofilms. The NPs made of antimicrobial polymer have recently been discussed as new antibiotics [17].

NPs Consist of Polymers Exhibiting Antimicrobial Activities

Antimicrobial polymers by definition show antimicrobial activity, or impede the proliferation of microorganisms including bacteria and related biofilm [18]. Amphiphilic block copolymers represent one type of important antimicrobials which often typically form core–shell NPs through self-assembly. Nguyen et al. reported the antimicrobial amphiphilic ternary random copolymers (Fig. 1a), which formed single-chain polymeric NPs (SCPNs) (Fig. 1b) and showed tunable cell membrane wall disruption by changing hydrophobicity. The particles inhibited a variety of Gram-negative bacteria, for instance *Pseudomonas aeruginosa* at 1.4 μM concentrations and remarkably killed ≥99.99% bacteria in biofilm within 1 h (Fig. 1c). Besides, it also showed that incorporation of poly(ethylene glycol) (PEG) into antimicrobial polymers was able to prevent complex formation with proteins in vivo [19]. The evolution of formidable MDR and biofilms matrix has made the bacterial infection treatment worse, eradication of drug-resistant bacterial biofilms is difficult, but some advances have been achieved. Li et al. synthesized a block copolymer DA95B5 (Fig. 1d) which self-assembled into a nonfouling dextran shell and a cationic core NPs. The particles effectively removed preformed biofilms of multiple drug-resistant Gram-positive bacteria. These NPs penetrated into biofilms and adhered to bacteria but did not directly kill them; instead they dispersed the biofilm bacteria because the dextran shell of the NP enhanced the solubility of the bacteria–NP complex. The incorporation of an antimicrobial hydrogel could remove

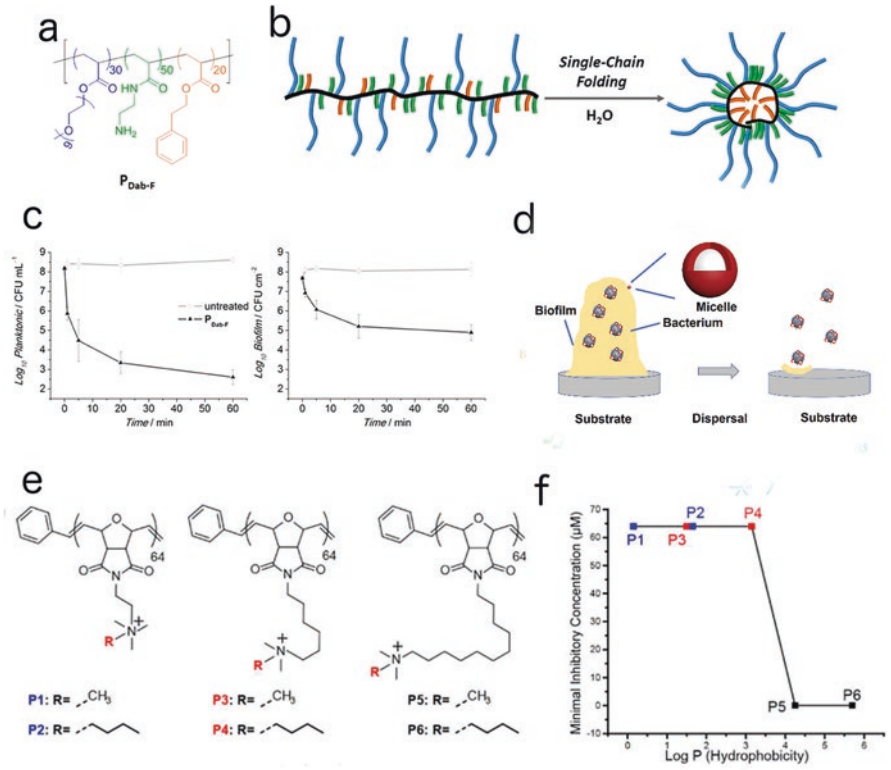


Fig. 1 Nanoparticles consisting of polymer themselves exhibit antimicrobial activity. (**a**) The compositional structures of the SCPNs including oligoethylene glycol side chains plus amino and hydrophobic groups. (**b**) Single-chain folding of amphiphilic random copolymers to form antimicrobial SCPNs in water. (**c**) Bactericidal activity of SCPNs **PDab-F** and **PDab-EH** (at 4× MIC in M9 medium) on planktonic cells and biofilm was assessed by colony-forming unit (CFU) analysis. Reproduced from [18]. Copyright American Chemical Society, 2017. (**d**) Mechanism of preformed biofilm removal by DA95B5 NPs (green, dextran, and light blue, poly(AMPTMA-*co*-BMA)) and the structure of DA95B5. Reproduced from [19]. Copyright American Chemical Society, 2018. (**e**) Molecular structures of oxanorbornene polymer derivatives. (**f**) MIC values of polymer derivatives with different hydrophobic chain lengths. Log P represents the calculated hydrophobic values of each monomer. (Reproduced from [21]. Copyright American Chemical Society, 2018)

methicillin-resistant *S. aureus* biofilm by 3.6 log reduction, while the 1.7 log reduction was observed using vancomycin [20]. The antibacterial behavior of polymeric NPs on biofilm formed is observable through field emission scanning electron microscopy and the bacteria viability treated with poly(lactic-co-glycolic acid) (PLGA) NPs was quantified with LIVE/DEAD BacLight kit [21], demonstrating the technique feasibility for antimicrobials development.

The superbug with treatment disappointment is further aggravated by chronic infections caused by antibiotic-resistant biofilms. It was believed that efficacious polymeric antimicrobial agents should include rational design of hydrophobic and

cationic domains. Therefore, Gupta et al. synthesized quaternary ammonium poly(oxanorborneneimides) (Fig. 1e) and found that the longer hydrophobic alkyl linker connecting the cationic headgroup and polymer backbone, the higher toxicity of polymer against planktonic bacteria, reflected by minimal inhibitory concentrations (MICs) (Fig. 1f). At the same time, these polymers had high therapeutic indices (TI) against red blood cells. More importantly, no resistance occurred against polymeric NPs for tested bacteria after 20 serial passages. Conventional antibiotics, by contrast, experienced significant resistance after only a few passages [22].

Nanosized Polymeric Carriers for Antimicrobial Delivery

Bacteria enmeshed in biofilms show increased antibiotic tolerance. Compounded by the rapid occurrence of MDR species, the existing antibiotics are failing to addressing the current problems. Without launching new drug discovery project, the wise alternative might be to develop novel drug delivery strategies to formulate commercialized medications for on-demand release. Not only polymeric NPs themselves retain antimicrobial profiles, but can also serve as vehicles to deliver antimicrobials, in particular when core–shell structured. The integration of antimicrobials with polymers is of great interests, largely due to the flexibility of polymeric materials. Improved antimicrobial performance was often observed with antimicrobials being encapsulated into polymeric vehicles.

Nanoencapsulation can improve the apparent solubility and sustained release characteristics of some drugs, exemplified by dihydromyricetin (DMY) which presents potent antimicrobial activity against a variety of bacteria, but low aqueous solubility and bioavailability limits clinical uses. Using polyacrylic acid, Dalcin et al. reported nanoencapsulated DMY formulations resulted in 8.1% higher biofilm eradication than free DMY within 72 h (Fig. 2a). The inhibition of biofilm by DMY nanocapsules was 26% more effective (Fig. 2b) compared to free DMY in 96 h [23], implying that sustained DMY release was achieved from nanoencapsulation (Fig. 2c). PLGA is widely used as an NP matrix for delivery of pharmaceutically relevant payloads. Emulsion evaporation process led to negatively charged nano-PLGA/clove-oil (CO) NPs. The NPs had sustained CO release inside dentinal tubules for more than 15 days (Fig. 2d, e) in vitro and in vivo. In addition, the formulations of nano-PLGA/CO effectively reduced oral biofilm formation after 24 h [24]. Cationic polymers, such as aminocellulose, are membrane active biocidal agents due to their membrane infusion with bacterial phospholipids biolayer may result in bacterial membrane disorder. Using layer-by-layer (LbL) technique, (Fig. 2f) Ivanova et al. coated multilayers of antimicrobial aminocellulose and antifouling polysaccharide hyaluronic acid on biocompatible polymer NPs. These biopolymer decorated NPs inhibited biofilm formation of *S. aureus* and *E. coli* by 94% and 40%, respectively [25].

In addition to the nanoencapsulation of single antibiotics into polymer, the combination of multiple agents, in particular macromolecules such as enzymes,

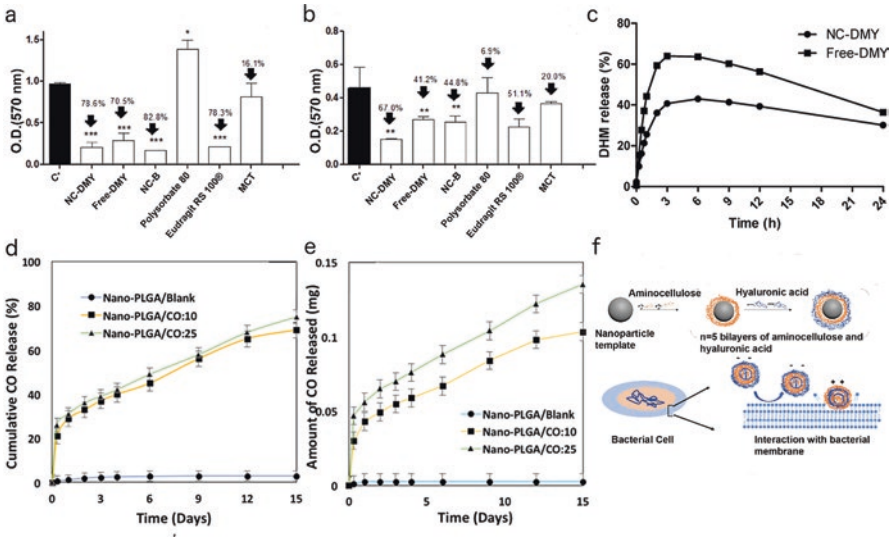


Fig. 2 Effect of the formulations evaluated on the *P. aeruginosa* biofilm formation (**a**) after 72 h of treatment and (**b**) after 96 h of treatment. Statistically significant comparing positive control with $***$ to $p < 0.001$ and $**$ to $p < 0.01$. (**c**) In vitro DMY release profiles from DMY-loaded nanocapsules and free DMY. (Reproduced from [22]. Copyright Elsevier, 2017.) (**d**) Mean ± standard deviation of % in vitro cumulative CO-release from nano-CO:10 and nano-CO:25 for 15 days. (**e**) Ex vivo CO cumulative release from nano-PLGA/CO:10 and nano-PLGA/CO:25 delivered to demineralized dentin substrates after 15 days. (Reproduced from [23]. Copyright IET, 2018.) (**f**) Schematic illustration of the polymeric NP template decoration in an LbL fashion. (Reproduced from [25]. Copyright American Chemical Society, 2018)

into nanosized polymeric particles represents a novel therapeutic strategy since current antibiotic delivery approaches often fail to eliminate biofilm-protected bacteria. Extracellular DNA (eDNA), one of the major components of biofilms, is vital to form biofilm, stabilize structure, and circulate pathogenicity. It is also a biofilm matrix crosslinker and chelator of cationic antimicrobials. Degradation of eDNA can change the behavior of biofilm to antimicrobials; therefore, it is an ideal target for biofilm control [26]. In this regard, deoxyribonuclease I (DNase I) functionalized ciprofloxacin (CIP)-loaded PLGA NPs (Fig. 3a) released the antimicrobial payload in a controlled fashion, but also enabled targeting and disassembled *P. aeruginosa* biofilms. Moreover, continuous administration for 3 days of DNase I-coated NPs encapsulating CIP eradicated more than 99.8% (Fig. 3b) of preformed biofilm, surpassed poly(lysine)-coated CIP NPs formulations and the free-soluble drug [27]. Oxacillin (Oxa) and DNase I were also co-loaded into positively charged chitosan NPs (CSNP-DNase-Oxa) (Fig. 3c) which displayed improved antibiofilm profile than Oxa-loaded NPs without DNase I and free Oxa at each experimental concentration, effectively inhibited biofilm formation; 2 days of treatment (Fig. 3d) resulted in 98.4% biofilm eradiation [28]. To achieve synergistic or improved antibiofilm treatment, it is necessary to combine physicochemically different agents into one

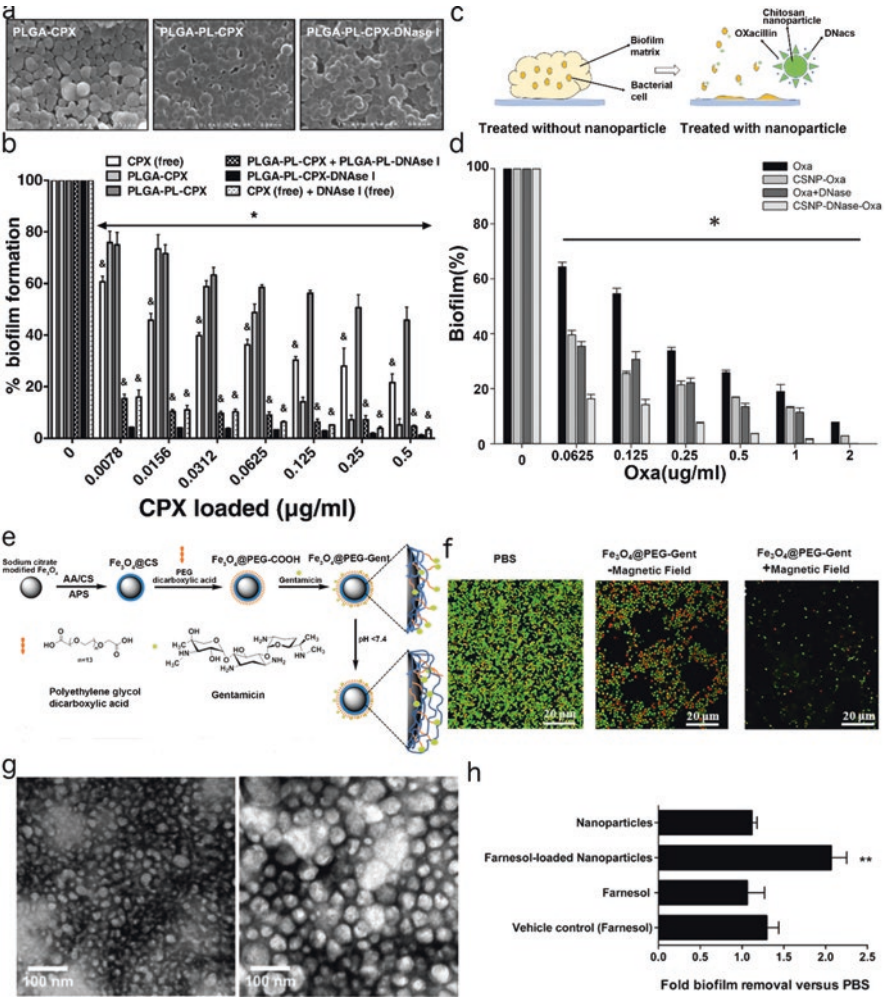


Fig. 3 (**a**) SEM micrographs of PLGA–CPX (left), PLGA–PL–CPX (center) and PLGA–PL–CPX–DNase I (right) NPs. (**b**) Disassembling the existing *P. aeruginosa* biofilms by CPX and PLGA NPs. (**c**) Schematics of CSNP-DNase-Oxa internalizing to bacterial biofilm. (**d**) Mature biofilm responses to two consecutive NPs treatments. (Reproduced from [28]. Copyright Elsevier, 2018.) (**e**) Schematic illustration of the formation of Fe_3O_4@PEG-Gent nanocomposites. (**f**) CSLM images of LIVE/DEAD staining of maturely formed *S. aureus* biofilms after the treatment with PBS, Fe_3O_4@PEG-Gent nanocomposites without and with an external magnetic field, respectively. (Reproduced from [29]. Copyright Springer, 2018.) (**g**) Transmission electron microscopy (TEM) images that demonstrate an increase in nanoparticle size upon loading; control (unloaded (left) and loaded with farnesol at 21 wt% (right)). (**h**) Antibiofilm effects of farnesol delivery via nanoparticles. (Reproduced from [30]. Copyright American Chemical Society, 2015)

unit which is often concomitantly a blend of different polymeric materials. For instance, the introduction of PEG to chitosan (CS)-coated Fe_3O_4 NPs improved the dispersity of NPs and facilitate the loading of free gentamicin (Gent) onto Fe_3O_4 NPs via electrostatic interaction (Fig. 3e). When magnetic Fe_3O_4 NPs were introduced into CS/PEG/Gent nanocarriers and applied to an external magnetic field, this nanocomposites achieved deeper penetration into preformed biofilms of *S. aureus*, allowing for effective Gent delivery (Fig. 3f) [29], demonstrating the advantage of combined strategies for antibiofilm application.

It is challenging to develop effective therapies for oral biofilm control since the therapeutics must prevent their quick clearance from tooth biofilm interfaces while being targeted to the biofilms. The problem is compounded by exopolysaccharides and acidification of the biofilm microenvironments. Benjamin et al. synthesized diblock copolymers which self-assembled into cationic NPs of ~21 nm in size (Fig. 3g). The size of the NPs increased upon drug loading; for instance, when loaded with 22 wt% of farnesol, the NPs enlarged from 21 nm before loading of farnesol to 60 nm. Attributed to ionic interactions between monomer tertiary amines, the obtained NPs showed excellent adsorption affinities (~244 L $mmol^{-1}$) to negatively charged EPS surfaces. Up to ~22 wt% of farnesol was loaded into the NPs and the drug was released in a pH-dependent manner and the NPs experienced core/shell destabilization at acidic pH. Importantly, the treatment of *Streptococcus mutans* with farnesol-loaded NPs was fourfold more effective than free parent drug (Fig. 3h) [30]. The SspB Adherence Region peptide encapsulated methoxy-polyethylene glycol-PLGA NPs hindered biofilm formation (IC_{50} = 0.7 μM) and interrupted established biofilms (IC_{50} = 1.3 μM) of *Porphyromonas gingivalis* in a dose-dependent profile [31].

Polymer–Lipid Hybrid Micellar Nanocarriers for Antibiotic Delivery to Bacterial Biofilms

Polymeric NPs have been intensively investigated for antimicrobial applications, and many undergo preclinical and clinical trials at different stages. Although some promising progresses have been described, various degrees of limitations such as low drug loading efficiency, low stability, and costly production often compromise these individual formulations. Yet, the combination of methods may offset some disadvantageous properties of one formulation while inherit desired profiles of the other and therefore obtain improved antimicrobial loading/delivery behavior. Baek et al. fabricated hybrid NPs (LPNs) with a solid polymer core and a cationic lipid shell hybrid with particle size of 100–130 nm, positively charged zeta potential of 15.2 ± 3.6 mV (Fig. 4a), and up to 95% antibiotics encapsulation. The obtained LPNs settled to the surfaces of a variety of bacterial biofilms and carried out localized and sustained drug release (Fig. 4b), reduced more than 95% of biofilm activity at concentrations of 8 to 32-time lower than antibiotics alone [32]. More importantly,

this PLGA/DOTAP NP formulation approach could be applied to formulate antibiotics with different solubility, from highly hydrophobic to amphiphilic to highly hydrophilic.

Micellar nanocarriers with PEG shell may fully penetrate bacterial biofilms, but lack pH-responsive feature. Co-formulation of PEG with poly(β-amino esters) (PAE) may offer micelles not only penetration ability, but also positively charged property in the acidic biofilm environment, therefore enabling their accumulation in negatively charged cell surfaces in biofilms. Liu et al. loaded protoporphyrin IX into such mixed-shell micelle nanocarriers (Fig. 4c) and applied light thereafter; it turned out PEG-PAE composite nanocarriers were superior than single-shell ones in terms of killing multidrug-resistant *staphylococcal* biofilms and the biofilms (Fig. 4d) were eradicated by daily injection of such photoactivatable micelle nanocarriers after about 5 days of treatment [33].

PEG-PAE made of mixed-shell-polymeric-micelles (MSPMs) were positively charged at pH 5.0, while being negatively charged at physiological pH (Fig. 4e) [34]; this means that MSPMs could be specifically attracted by negative charged bacterial surface in biofilms while be repelled in normal tissues, driven by electrostatic interaction (Fig. 4f). To avoid unwanted antimicrobial release, Liu et al. conjugated Triclosan into PEG-PAE micelles that could specifically enter biofilms, navigate themselves to bacterial cell surfaces, and release conjugated Triclosan through degradation by bacterial enzyme, and the concentration of PEG-PAE-Triclosan needed for oral biofilm clearance was 30–40 fold lower than Triclosan itself in solution (Fig. 4g) [35].

Comparing to synthetic polymeric material-based antibiotic transporter, protein-oriented nonvehicles are considered as more reliable delivery carriers owing to their superior permeation and retention property as well as biological friendliness. Antimicrobial peptides (AMPs) and their derivatives play a critical role in treating antibiotic-resistant infectious diseases. Due to the intermolecular electrostatic repulsion between the protonated amino acid residues, β-sheet folding peptide amphiphiles adopted a coil micellar structure with ≈198 nm in aqueous media. Acute in vivo toxicity testing of this peptides demonstrated not only efficient bacterial biofilm inhibition and biomass dispersion, but also higher intravenous LD_{50} values as compared to the commercialized drugs [36]. Driven by host–guest interaction between α-cyclodextrin and PEG, photosensitizer Chlorin e6 (Ce6) and AMP Magainin I were assembled into supramolecular micelles (Fig. 5a) with an average size of 55 nm in PBS and Zeta potential of -17.1 ± 2.2 mV. The obtained micelles exhibited not only excellent bacterial targeting effects, but enhanced biofilm killing ability against bacterial biofilms (Fig. 5b) [37]. Proteins are generally bigger polymeric macromolecules than peptides in size. Besides, proteins tend to be structurally organized, well-known as secondary, tertiary, and quaternary structures and have stable three-dimensional structures. They have also been considered as antimicrobial carriers. In particular, serum albumin is suitable alternative nanocarriers due to its immunogenic-free, biocompatible, nontoxic profile, and efficient encapsulation [38]. After confirmation of antibiofilm property of dipyridinium-based synthetic amphiphile alone and combination with other antibiotics, Goswami et al.

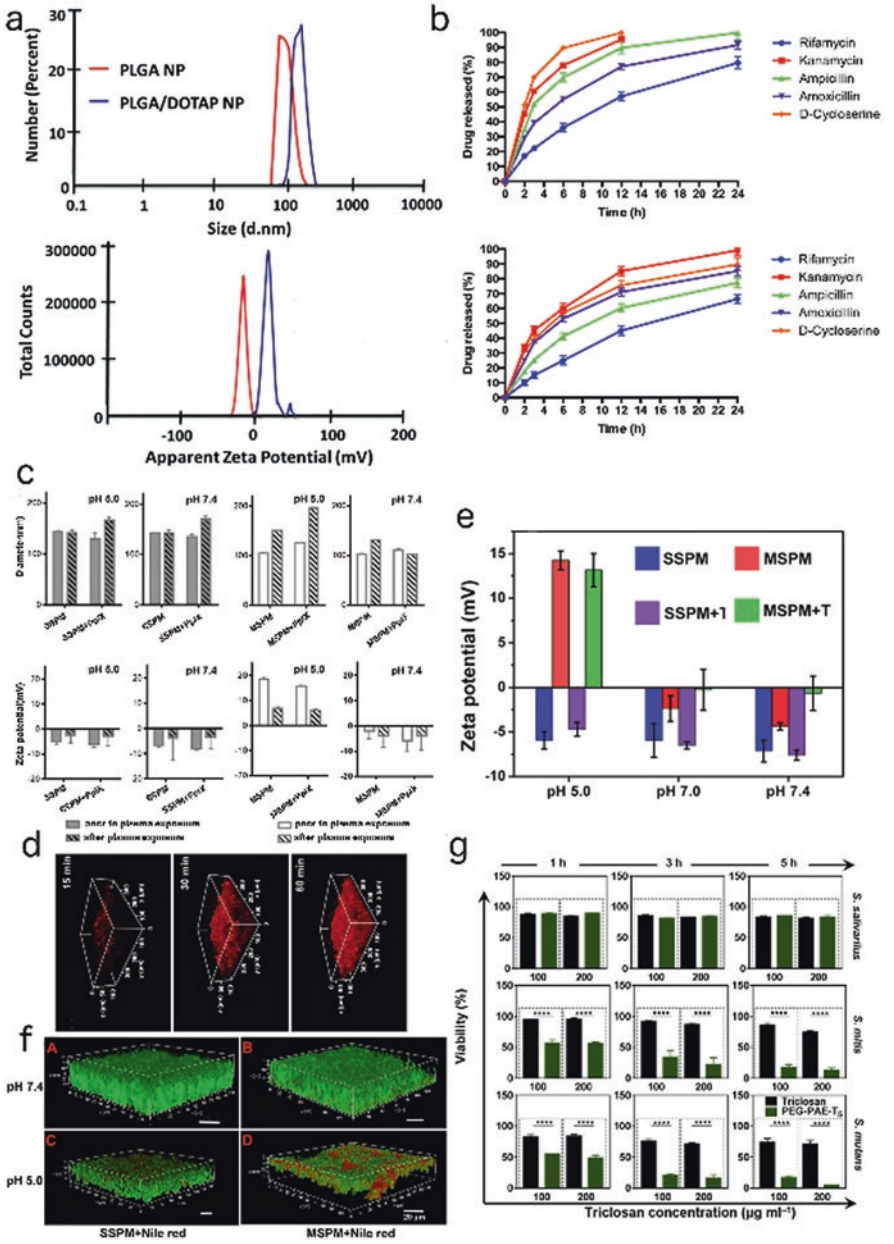


Fig. 4 (**a**) Dynamic laser scattering (top) and Zeta potential (bottom) of PLGA nanoparticles and PLGA/DOTAP nanoparticles. (**b**) The release profiles of antibiotics from PLGA nanoparticles and PLGA/DOTAP nanoparticles. In vitro release profiles of different antibiotics from (**a**) PLGA nanoparticles and (**b**) PLGA/DOTAP nanoparticles in PBS (pH 7.4) for 24 h at 37 °C. (Reproduced

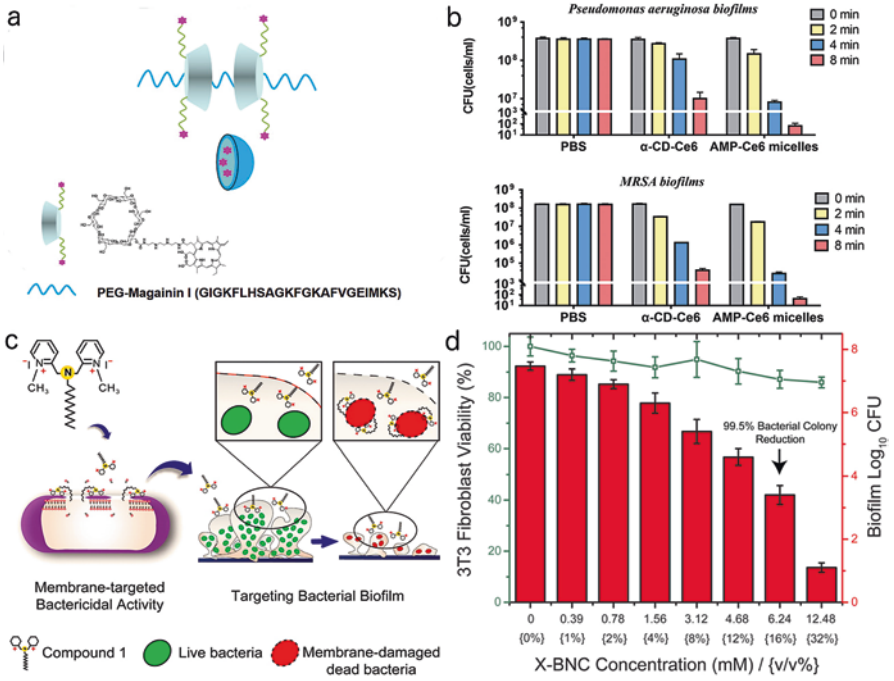


Fig. 5 (**a**) Schematic illustration of α-CD-Ce6/PEG-AMP supramolecular micelles. (**b**) Biofilm killing results of Gram(−) *Pseudomonas aeruginosa* and Gram(+) MRSA. Gray column: without irradiation. (Three parallel samples were tested per group.) Yellow column: 2 min irradiation. Blue column: 4 min irradiation. Red column: 8 min irradiation. (**c**) Cartoon illustrating the potential antibiofilm activity of the membrane-acting compound 1. (Reproduced from [39]. Copyright American Chemical Society, 2014.) (**d**) Viability of 3T3 fibroblast cells and *P. aeruginosa* biofilms in the coculture model after treating X-BNCs at different emulsion concentrations for 3 h. (Reproduced from [42]. Copyright American Chemical Society, 2018)

Fig. 4 (continued) from [32]. Copyright the Royal Society of Chemistry, 2018.) (**c**) Micelle characterization, Micelle diameters of SSPMs and MSPMs with or without PpIX loading measured using dynamic light scattering (up) and zeta potentials (down). (**d**) Examples of 3D confocal laser scanning microscopy micrographs of *S. aureus* WHGFP biofilms after penetration and accumulation for different time intervals of Nile red-loaded MSPMs. (Reproduced from [33]. Copyright Wiley-VCH Verlag GmbH & Co. KGaA, 2017.) (**e**) Zeta potentials in 10 mM phosphate buffer of MSPMs and SSPMs at different pH values with and without Triclosan loading. (**f**) Penetration and pH-dependent bacterial targeting of Nile red loaded micelles in a staphylococcal biofilm. (Reproduced from [34]. Copyright American Chemical Society, 2016.) (**g**) Percentage bacterial viability in *S. salivarius* HB, *S. mitis* ATCC9811, and *S. mutans* ATCC700610 biofilms after 1, 3, and 5 h of retention following 2 min exposure to Triclosan at different Triclosan-equivalent concentrations. (Reproduced from [35]. Copyright Elsevier, 2018)

developed a human serum albumin (HSA)-based nanocarrier loaded with this dipyridinium-containing compound, which displayed potent antibacterial properties (Fig. 5c) and could reduce preformed *S. aureus* biofilm on the surface of urinary catheter [39].

Plant-derived phytochemicals are a promising alternative to traditional antibiotics for MDR bacteria elimination [40]. However, the poor solubility of these compounds in aqueous media confine their clinical applications. Surfactants and polymer supplements can assist phytochemical delivery by forming oil-in-water droplets [41]. Landis et al. developed poly(oxanorborneneimide)-stabilized oil-in-water "nanosponges" (X-BNCs) supposed to be degradable in the presence of glutathione and esterase enzymes. Not only could X-BNCs easily penetrate and deliver carvacrol efficiently within biofilms, but also effectively eliminated multiple pathogenic bacterial biofilms (Fig. 5d) within 3 h [42].

Polymeric Nanogel/Hydrogel for Antimicrobial Delivery to Biofilms

Hydrogels with antibacterial properties are a main focus in biomedical research due to their excellent properties, including high aqueous swellability, smaller gas molecule permeability, enhanced biocompatibility, diversified structure, and convenient drug incorporation [43]. The loading of metal NPs, such as silver NPs (AgNPs) in polymeric hydrogels represents a novel strategy for biofilm elimination, since AgNPs functioned as an important antimicrobial/antibiofilm agent [44, 45]. Thermoplastic hydrogel nanofibrous webs consisting of multiblock hybrid polyurethanes have no antibacterial property in the absence of $AgNO_3$ while featured outstanding biofilm killing for more than 2 weeks (Fig. 6a) when $AgNO_3$ was contained [46].

Not only cationic silver but AgNPs themselves can be entrapped by hydrogels. In situ synthesis of AgNPs in a biocompatible nanogel (NG) comprising dextran and lysozyme. (Fig. 6b). By grafting target ligands on the surface NGs, they can be used to prevent biofilm formation and control bacterial infection [47]. ZnO NGs were also synthesized and studied for treatment of skin microbial infections affected by bacterial strains which may attach to the surface of the skin and form biofilms [48].

To anchor drug delivery vehicles on the infection site under high shear stress condition, Zhang et al. established a bioadhesive NP–hydrogel hybrid in which CIP was loaded into polymeric NPs and then implanted into a 3D hydrogel network (Fig. 6c) that offered superior stickiness and drug retention when high shear force was applied on bacterial film (Fig. 6d) [49]. AMP represents a key substitute to widely used antimicrobial agents, but the lack of pinpoint delivery to the target and the risk of unwanted toxicity compromise its clinical applications. Formulation of biopolymer NGs incorporating AMPs and lysine-based α-peptide/β-peptoid showed potent activity against *P. aeruginosa* grown within biofilms [50].

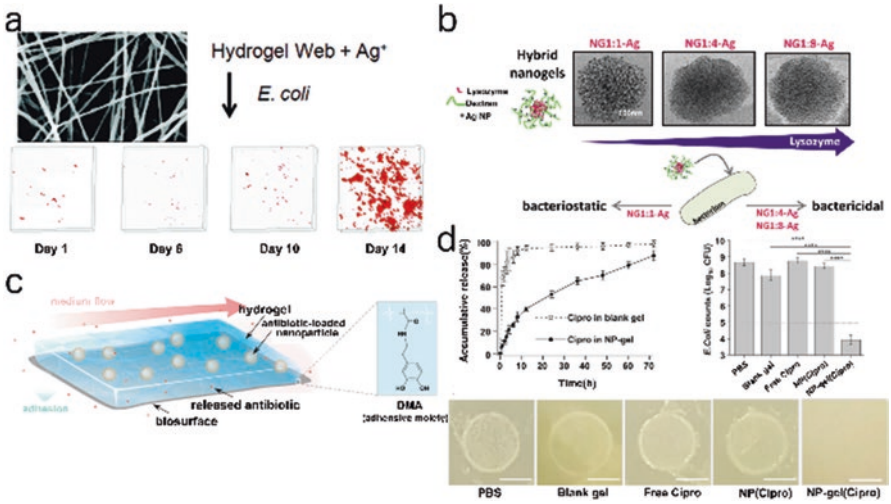


Fig. 6 (**a**) SEM micrographs of fiber diameter of electrospun fibrous scaffolds of TPUs made from 20 wt% DMF with 1 wt% $AgNO_3$ and representative 3D microscope images of *E. coli* biofilms formed. (Reproduced from [46]. Copyright American Chemical Society, 2009.) (**b**) cryo-TEM micrographs of the hybrid NG and schematic of NG inhibition toward bacterium. (**c**) Schematic illustration of such adhesive NP–gel system for localized antibiotic release to inhibit bacterial growth under flow conditions. In the formulation, dopamine methacrylamide (DMA) containing catechol functional group was used as an adhesive moiety. (**d**) Photographs of *E. coli* biofilm formation with the treatment of PBS, blank gel (without nanoparticles or Cipro), free Cipro, Cipro-loaded nanoparticles (NP(Cipro), without hydrogel), and Cipro-loaded NP–gel (NP–gel(Cipro)). (Reproduced with permission from [49]. , 2016)

Polymersome/Liposome Nanocarriers for Antimicrobial Delivery to Biofilm

Polymersomes are developed primarily as drug delivery vehicles and can be tailored for both hydrophilic and hydrophobic drug transportation [51] since they are made of amphiphilic block copolymers [52].

Benjamin et al. synthesized polymersomes in which AgNPs were in the hydrophobic part while ampicillin in the hydrophilic part (Fig. 7a); these polymersomes inhibited the growth of biofilm-forming ampicillin tolerance *E. coli* in a dose-dependent manner (Fig. 7b) while neither free ampicillin, AgNPs, nor AgNPs free ampicillin polymersomes had any effect on bacterial growth, implying the synergism between the AgNPs and ampicillin. In addition, silver-to-ampicillin ratio of 1:0.64 enable complete growth inhibition of the *E. coli* [53]. Although superparamagnetic iron oxide NPs (SPIONs) were antimicrobial agents, their individual utility is highly limited since the antibacterial action of SPIONs is inversely proportional to the particle size, while their magnetic positionability is proportional to particle

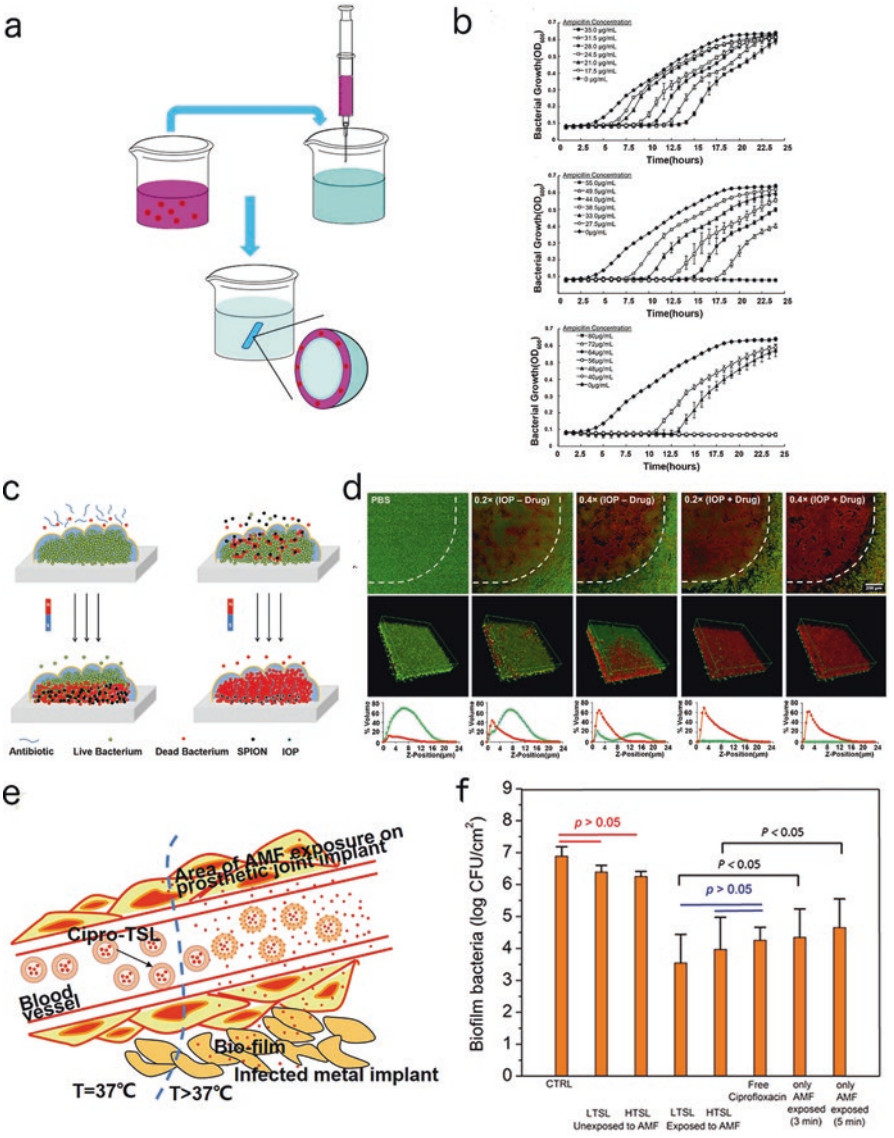


Fig. 7 (**a**) Polymersome nanocarriers synthesis. (**b**) Bacterial growth inhibition. The proliferation of a 10^6 CFU mL^{-1} suspension of antibiotic-resistant *E. coli* was measured over 24 h in the presence of different concentrations of AgNPs loaded with (top) 1 Ag:0.28 AMP, (middle) 1 Ag:0.44 AMP, and (bottom) 1 Ag:0.64 AMP. (Reproduced from [53]. Copyright Royal Society of Chemistry 2015.) (**c**) The strategies for biofilm treatment using SPIONs and/or antimicrobials. (**d**) Antibiofilm activity of iron oxide-encapsulating polymersomes: Tile scans collected halfway through the biofilm show concentration-dependent bacterial death within the boundary of the external applied magnetic field (dashed line) (up row), 3D reconstructions of z-stacks collected across the biofilm thickness inside the magnetic field (middle row), the percentage of biofilm volume occupied by live (green) and dead (red) bacteria as a function of biofilm depth (0 μm = bottom) as quantified from image slices (down row). (Reproduced from [54]. Copyright Elservier 2017.) (**e**) Schematic of ciprofloxacin release from TSL in the vicinity of an infected metal implant heated by exposure to alternating magnetic field (AMF). (**f**) The bactericidal effect of AMF exposures on biofilm-associated bacteria associated with a metal washer for cipro-TSL

size. In the clinic, a physician would prefer targeting ability as well. In addition, clinically used SPION is often decorated with hydrophobic oleic acid to fully utilize its superior structural and magnetic properties. Nevertheless, the monodispersity of these particles in aqueous biological fluids is often compromised; therefore, SPIONs are generally ineffective as a single antibacterial therapy. To enhance the internalization of SPION-based particles into biofilms, Benjamin et al. developed a polymersome nanocarrier containing multiple SPIONs and antibiotics methicillin to treat medical device-associated infections (Fig. 7c). The obtained vehicles penetrated 20 μm thick *Staphylococcus epidermidis* biofilms with high efficiency under the external magnetic field. The optimized formulation containing 40 μg/mL SPION and 20 μg/mL of methicillin thoroughly eliminated bacterial biofilm thickness (Fig. 7d) [54].

Liposomes have a similar structure as polymersomes, in which their hydrophilic core can carry hydrophilic antimicrobials and are exemplified by the first FDA approved liposomal formulation Doxil® [55]. Liposomes made of phospholipids may enable them readily to be integrated with bacterial phospholipid membrane to release loaded antimicrobials directly into a bacterium compartment. An overview of a variety of liposome-based drug delivery platform and their use for prevention and/or elimination of bacterial biofilms have been discussed few years ago [56]; therefore, only latest progresses will be discussed in this section.

Alternating magnetic fields (AMF) was applied to temperature-sensitive liposomal (TSL) to control antibiotic release (Fig. 7e), a 3 log reduction of bacteria quantity in biofilms (Fig. 7f) was observed [57], suggesting that the release of antibiotic from temperature-sensitive liposomes could be triggered by exposure of metal implants to AMF for potent antibiofilm effect.

Phytochemical terpinen-4-ol exhibits antimicrobial activity. However, its high volatilization and hydrophobicity are problematic for its application. Compring with other particulate carriers, lipid NPs are more tolerable against external impacts and biodegradable for drug loading. Terpinen-4-ol was fixed in lipid NPs through film sonication technology, and by modifying the lipid NPs with PEG, the lipid NPs obtained presented sustained release of terpinen-4-ol, and remarkably killed *C. albicans* ATCC 11231 in both suspension and biofilm [58]. It was also showed that the smaller unilamellar phospholipid liposomes penetrated deeper than larger multilamellars in bacteria biofilm, the cationic liposomes adhered better than their anionic ones [59].

Electrospun Nanofiber to Deliver Antimicrobials

Nanofiber, with diameters less than 100 nm, were first produced via electrospinning more than 400 years ago [60]. The combination of electrospun technology and biocompatible polymer has led to the polymeric fiber been of interest for antibacterial applications. For example, zwitterionic polymers like polysulfobetaine and polysulfabetaines (PSBs) were electrospun to form environmentally friendly antifouling

surfaces [61]. Thanks to the amenability of polymer and the flexibility of electrospun technology, it is possible to incorporate other components into the nanofiber system to achieve advanced functions. For instance, copper particles were loaded into electrospinning poly-D,L-lactide (PDLLA) and poly(ethylene oxide) (PEO) nanofibers (Fig. 8a). After 2 days of coculture, copper particle nanofibers reduced biofilm for-

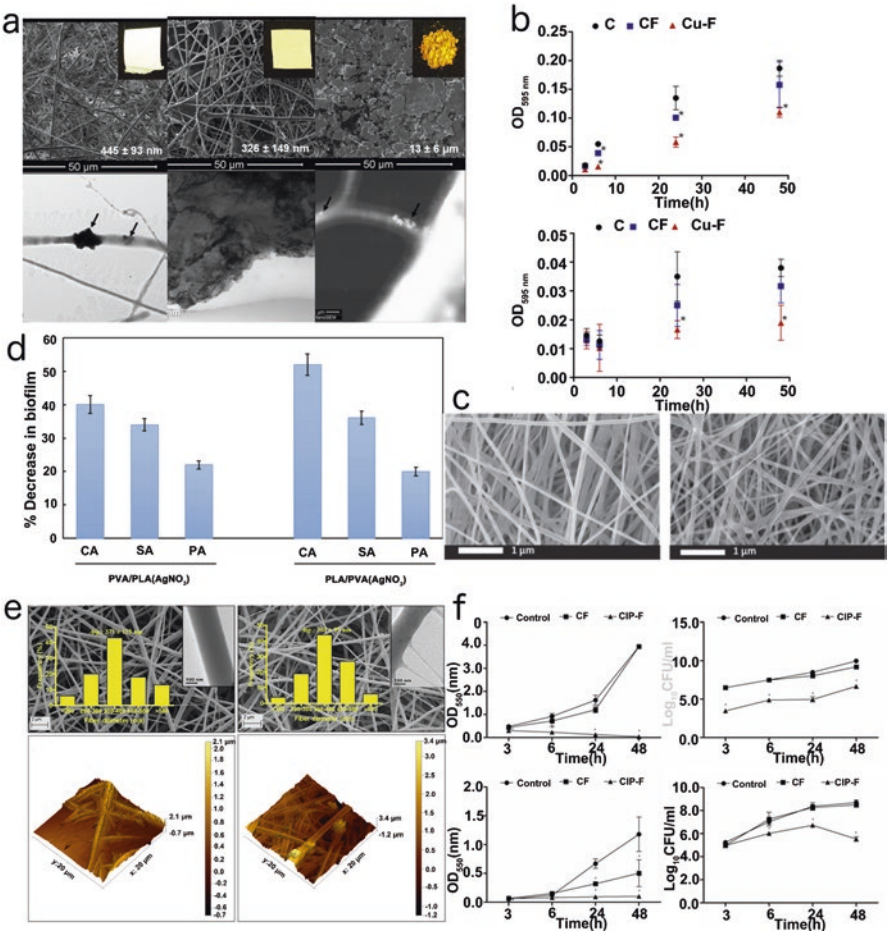


Fig. 8 (**a**) The SEM (up) and the TEM (down) images of control nanofibers, copper-containing nanofibers (Cu-F) and copper particles before electrospinning. (**b**) Total biofilm formation by cells of *P. aeruginosa* PA01 (up) and *S. aureus* Xen 30 (down). (**c**) SEM image of PLA/PVA($AgNO_3$) (left) and PVA/PLA($AgNO_3$) (right). (**d**) The biofilm formation on the core–shell nanofiber membranes synthesized with AgNPs compared to those without AgNPs against *Candida albicans* (CA), *Staphylococcus aureus* (SA), and *Pseudomonas aeruginosa* (PA). (Reproduced from [64]. Copyright IOP Publishing 2018.) (**e**) SEM (up) and Atomic force microscopy (AFM) (down) images of nanofibers without ciprofloxacin, CIP, and CIP-F. (**f**) Biofilm formation recorded for *P. aeruginosa* PA01 (left column) and *S. aureus* Xen 30 (right column), respectively. (Reproduced with permission from [65]. Copyright American Chemical Society 2016)

mation by *Pseudomonas aeruginosa* PA01 and *Staphylococcus aureus* (strain Xen 30) by 41% and 50% (Fig. 8b), respectively [62].

After improvement of the mechanical and cytocompatible properties of PLA and poly(vinyl alcohol) [63], (PVA)-made core-shell nanofibers, (Fig. 8c) inhibited the biofilm formation of bacteria as high as 52% (Fig. 8d) compare to bare polymeric nanofibers, with PLA/PVA($AgNO_3$) core-shell composites achieved 12% antifilm activity higher than PVA/PLA($AgNO_3$) did [64]. Nanofibers loaded with AgNPs, prepared in situ using Ag^+ ions in tetrahydrofuran solution by just adding poly(ε-caprolactone) (PCL) in the electrospinning solution, also showed good and specific antibacterial effect [65].

Besides electrospinning NPs into nanofibers, smaller organic ligands can also be incorporated. CIP is rarely effective to kill bacteria in biofilms mainly due to its slow diffusion into the biofilm cores. CIP was suspended into poly(D,L-lactide) (PDLLA) and poly(ethylene oxide) (PEO), and electrospun into nanofibers (CIP-F) (Fig. 8e) which inhibited 99% of *P. aeruginosa* PA01 and 91% of *S. aureus* Xen 30 biofilm formation (Fig. 8f). In addition, CIP levels maintained above MIC for 5 days [66]. Nanofibers can also be combined with polymeric films for delivery of multiple antibiotics. For instance, PLGA and PCL were electrospun into nanofibers to locally codeliver antibiotics of vancomycin and rifampin on the implant surfaces; the release of each antibiotic was tunable by loading individual drug into different polymers or by adjusting polymer ratio [67].

Conclusions and Future Perspective

Microbial biofilms, being complicated and extremely recalcitrant microbial amasses formed on surfaces of many objectives, are accounted for two-thirds of persistent infections and remain a serious risk to human well-being. Individuals who experienced implants, organ transplants, and those with uncured injuries or burns are particularly fragile to get infected. Antibiotics, the central player against bacterial infections, have often failed to fight with microbes when bacteria are embedded in biofilms. Elimination of biofilms needs researchers to think beyond the "antibiotics" box and it may be desirable to find alternative ways to pinpoint biofilms [68].

Given the observation that infectious resistance of infection to the therapy was mainly due to bacteria in biofilms, and compare this with the inefficient delivery of antibiotics into deeper biofilm plus the excellent penetration property of nanosized vehicles, the selection of NPs as antimicrobials has offered unique advantages over conventional administration and delivery vehicles. In particular, polymeric NPs are of great interest since they are structurally homogeneous, easy to prepare and functionalize, and may be released in a controlled pattern. Moreover, polymer-based nanocarriers can be readily combined with diverse agents to achieve a synergistic outcome. In general, the polymeric particles described in literature are typically formed by PLGA, chitosan, or a mixture of PLGA and lipids.

Although the great progresses in treating intracellular infections using nanoparticulate carriers has been made, some challenges still remain: (1) for specific infections induced by bacterial biofilms, the therapeutic efficiency of these NPs is usually compromised as they fail to penetrate the deep level of the biofilms and reach high enough therapeutic concentrations, and thus may lead to more persistent antimicrobial resistance; (2) poorly realized pinpointing drug delivery to bacteria/biofilm subcompartments and delivering synergistic drug combinations; (3) underexploited drug release in a controlled manner, in particular biofilm microenvironment-specific stimuli responsive ones are really desirable; (4) the selective toxicity toward drug-resistant biofilm cells but not mammalian cells should not be overlooked; (5) the current literature presenting data bias in which the in vitro antibiofilm activity of nanoformulations outnumbered in vivo clues.

Despite the aforementioned concerns and far beyond perfectness of nanoparticulate antimicrobial delivery vehicles for biofilm eradication, we and the others [69] are confident that continuous research input in this field will lead to fruitful achievements and will enrich the physicians' arsenal against biofilms.

Acknowledgments This research was supported by the Fundamental Research Funds for the Central Universities of China, grant number 31020190QD030, the National Key R&D Program of China (2018YFC1105402 and 2017YFA0207202).

References

1. Ladavière C, Gref R (2015) Toward an optimized treatment of intracellular bacterial infections: input of nanoparticulate drug delivery systems. Nanomedicine 10(19):3033–3055
2. Lu HD, Yang SS, Wilson BK, McManus SA, Chen CV-H, Prud'homme RK (2017) Nanoparticle targeting of Gram-positive and Gram-negative bacteria for magnetic-based separations of bacterial pathogens. Appl Nanosci 7(3–4):83–93
3. Ma X, Zhang Y, Weisensee K (2019) Conducting polymeric nanocomposites with a three-dimensional co-flow microfluidics platform. Micromachines 10(6):383
4. Khan H, Sakharkar M, Nayak A, Kishore U, Khan A (2018) Nanoparticles for biomedical applications: an overview. In: Nanobiomaterials. Elsevier, pp 357–384
5. Zhang Y, Tu J, Wang D, Zhu H, Maity SK, Qu X, Bogaert B, Pei H, Zhang H (2018) Programmable and multifunctional DNA-based materials for biomedical applications. Adv Mater 30(2):e1703658
6. Patel S, Singh D, Srivastava S, Singh M, Shah K, Chauahn DN, Chauhan NS (2017) Nanoparticles as a platform for antimicrobial drug delivery. Adv Pharmacol Pharm 5(3):31–43
7. Baptista PV, McCusker MP, Carvalho A, Ferreira DA, Mohan NM, Martins M, Fernandes AR (2018) Nano-strategies to fight multidrug resistant Bacteria—"a battle of the titans". Front Microbiol 9:1441
8. Biswaro LS, Sousa MGdC, Rezende TMB, Dias SC, Franco OL (2018) Antimicrobial peptides and nanotechnology, recent advances and challenges. Front Microbiol 9:855
9. Gao W, Thamphiwatana S, Angsantikul P, Zhang L (2014) Nanoparticle approaches against bacterial infections. Wiley Interdiscip Rev Nanomed Nanobiotechnol 6(6):532–547
10. Anderson GG, Palermo JJ, Schilling JD, Roth R, Heuser J, Hultgren SJ (2003) Intracellular bacterial biofilm-like pods in urinary tract infections. Science 301(5629):105–107
11. Niemira BA, Solomon EB (2005) Sensitivity of planktonic and biofilm-associated Salmonella spp. to ionizing radiation. Appl Environ Microbiol 71(5):2732–2736

12. Theocharis AD, Skandalis SS, Gialeli C, Karamanos NK (2016) Extracellular matrix structure. Adv Drug Deliv Rev 97:4–27
13. Ikuma K, Decho AW, Lau BL (2015) When nanoparticles meet biofilms—interactions guiding the environmental fate and accumulation of nanoparticles. Front Microbiol 6:591
14. Liu Y, Shi L, Su L, van der Mei HC, Jutte PC, Ren Y, Busscher HJ (2019) Nanotechnology-based antimicrobials and delivery systems for biofilm-infection control. Chem Soc Rev 48:428–446
15. Ramos MADS, Silva PBD, Spósito L, Toledo LGD, Bonifácio BV, Rodero CF, Santos KCD, Chorilli M, Bauab TM (2018) Nanotechnology-based drug delivery systems for control of microbial biofilms: a review. Int J Nanomedicine 13:1179–1213
16. Forier K, Raemdonck K, Smedt SCD, Demeester J, Coenye T, Braeckmans KJJoCR (2014) Lipid and polymer nanoparticles for drug delivery to bacterial biofilms. J Control Release 190:607–623
17. Lam SJ, Wong EH, Boyer C, Qiao GG (2018) Antimicrobial polymeric nanoparticles. Prog Polym Sci 76:40–64
18. Alexandra Muñoz-Bonilla MF-G (2012) Polymeric materials with antimicrobial activity. Prog Polym Sci 37(2):281–339
19. Nguyen TK, Lam SJ, Ho KK, Kumar N, Qiao GG, Egan S, Boyer C, Wong EH (2017) Rational design of single-chain polymeric nanoparticles that kill planktonic and biofilm bacteria. ACS Infect Dis 3(3):237–248
20. Li J, Zhang K, Ruan L, Chin SF, Wickramasinghe N, Liu H, Ravikumar V, Ren J, Duan H, Yang L (2018) Block copolymer nanoparticles remove biofilms of drug-resistant Gram-positive bacteria by nanoscale bacterial debridement. Nano Lett 18(7):4180–4187
21. Takahashi C, Ogawa N, Kawashima Y, Yamamoto HJM (2015) Observation of antibacterial effect of biodegradable polymeric nanoparticles on Staphylococcus epidermidis biofilm using FE-SEM with an ionic liquid. Microscopy 64(3):169–180
22. Gupta A, Landis RF, Li C-H, Schnurr M, Das R, Lee Y-W, Yazdani M, Liu Y, Kozlova A, Rotello VM (2018) Engineered polymer nanoparticles with unprecedented antimicrobial efficacy and therapeutic indices against multidrug-resistant bacteria and biofilms. J Am Chem Soc 140(38):12137–12143
23. Ajf D, Santos CG, Gündel SS, Roggia I, Raffin RP, Ourique AF, Rcv S, Gomes P (2017) Anti biofilm effect of dihydromyricetin-loaded nanocapsules on urinary catheter infected by Pseudomonas aeruginosa. Colloids Surf B Biointerfaces 156:282–291
24. Priyadarshini M, Antipina MN, Fawzy A (2018) Formulation and characterization of poly (lactic-co-glycolic acid) encapsulated clove oil nanoparticles for dental applications. IET Nanobiotechnol 12(3):311–317
25. Ivanova A, Ivanova K, Hoyo J, Heinze T, Sanchez-Gomez S, Tzanov T (2018) Layer-by-layer decorated nanoparticles with tunable antibacterial and antibiofilm properties against both gram-positive and Gram-negative bacteria. ACS Appl Mater Interfaces 10(4):3314–3323
26. Okshevsky M, Regina VR, Meyer RL (2015) Extracellular DNA as a target for biofilm control. Curr Opin Biotechnol 33:73–80
27. Baelo A, Levato R, Julián E, Crespo A, Astola J, Gavaldà J, Engel E, Mateos-Timoneda MA, Torrents E (2015) Disassembling bacterial extracellular matrix with DNase-coated nanoparticles to enhance antibiotic delivery in biofilm infections. J Control Release 209:150–158
28. Tan Y, Ma S, Leonhard M, Moser D, Haselmann GM, Wang J, Eder D, Schneider-Stickler B (2018) Enhancing antibiofilm activity with functional chitosan nanoparticles targeting biofilm cells and biofilm matrix. Carbohydr Polym 200:35–42
29. Xi W, Deng A, Cao W, Qiang L, Wang L, Jie Z, Hu B, Xing X (2018) Synthesis of chitosan/poly (ethylene glycol)-modified magnetic nanoparticles for antibiotic delivery and their enhanced anti-biofilm activity in the presence of magnetic field. J Mater Sci 53(9):1–17
30. Benjamin H, Klein MI, Geelsu H, Yong L, Dongyeop K, Hyun K, Benoit DSW (2015) pH-activated nanoparticles for controlled topical delivery of farnesol to disrupt oral biofilm virulence. ACS Nano 9(3):2390–2404

31. Mahmoud MY, Demuth DR, Steinbach-Rankins JM (2018) BAR-encapsulated nanoparticles for the inhibition and disruption of Porphyromonas gingivalis—Streptococcus gordonii biofilms. J Nanobiotechnol 16(1):69
32. Baek J-S, Tan CH, Ng NKJ, Yeo YP, Rice SA, Loo SCJ (2018) A programmable lipid-polymer hybrid nanoparticle system for localized, sustained antibiotic delivery to Gram-positive and Gram-negative bacterial biofilms. Nanoscale Horiz 3(3):305–311
33. Yong L, Mei HCVD, Zhao B, Yan Z, Cheng T, Li Y, Zhang Z, Busscher HJ, Ren Y, Shi L (2017) Eradication of multidrug-resistant *staphylococcal* infections by light-activatable micellar nanocarriers in a murine model. Adv Funct Mater 27(44):1701974
34. Liu Y, Busscher HJ, Zhao B, Li Y, Zhang Z, Hc VDM, Ren Y, Shi L (2016) Surface-adaptive, antimicrobially loaded, micellar nanocarriers with enhanced penetration and killing efficiency in staphylococcal biofilms. ACS Nano 10(4):4779–4789
35. Liu Y, Ren Y, Li Y, Su L, Zhang Y, Huang F, Liu J, Liu J, van Kooten TG, An Y (2018) Nanocarriers with conjugated antimicrobials to eradicate pathogenic biofilms evaluated in murine in vivo and human ex vivo infection models. Acta Biomater 79:331–343
36. Zhan YO, Shu JG, Yi YY (2013) Short synthetic β-sheet forming peptide amphiphiles as broad spectrum antimicrobials with antibiofilm and endotoxin neutralizing capabilities. Adv Funct Mater 23(29):3682–3692
37. Gao Y, Wang J, Hu D, Deng Y, Chen T, Jin Q, Ji J (2018) Bacteria-targeted supramolecular photosensitizer delivery vehicles for photodynamic ablation against biofilms. Macromol Rapid Commun 40:e1800763
38. Elzoghby AO, Samy WM, Elgindy NA (2012) Albumin-based nanoparticles as potential controlled release drug delivery systems. J Control Release 157(2):168–182
39. Goswami S, Thiyagarajan D, Das G, Ramesh A (2014) Biocompatible nanocarrier fortified with a dipyridinium-based amphiphile for eradication of biofilm. ACS Appl Mater Interfaces 6(18):16384–16394
40. Lillehoj H, Liu Y, Calsamiglia S, Fernandez-Miyakawa ME, Fang C, Cravens RL, Oh S, Gay CG (2018) Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Vet Res 49(1):76
41. Singh S, Verma D, Mirza MA, Das AK, Dudeja M, Anwer MK, Sultana Y, Talegaonkar S, Iqbal Z (2017) Development and optimization of ketoconazole loaded nano-transfersomal gel for vaginal delivery using box-Behnken design: in vitro, ex vivo characterization and antimicrobial evaluation. J Drug Delivery Sci Technol 39:95–103
42. Landis RF, Li CH, Gupta A, Lee YW, Yazdani M, Ngernyuang N, Altinbasak I, Mansoor S, Khichi MAS, Sanyal A (2018) Biodegradable nanocomposite antimicrobials for the eradication of multidrug-resistant bacterial biofilms without accumulated resistance. J Am Chem Soc 140(19):6176–6182
43. Li S, Dong S, Xu W, Tu S, Yan L, Zhao C, Ding J, Chen X (2018) Antibacterial hydrogels. Adv Sci 5(5):1700527
44. Markowska K, Grudniak AM, Wolska KI (2013) Silver nanoparticles as an alternative strategy against bacterial biofilms. Acta Biochim Pol 60(4):523–530
45. Martinez-Gutierrez F, Boegli L, Agostinho A, Sánchez EM, Bach H, Ruiz F, James G (2013) Anti-biofilm activity of silver nanoparticles against different microorganisms. Biofouling 29(6):651–660
46. Wu J, Hou S, Ren D, Mather PT (2009) Antimicrobial properties of nanostructured hydrogel webs containing silver. Biomacromolecules 10(9):2686–2693
47. Ferrer MCC, Dastgheyb S, Hickok NJ, Eckmann DM, Composto RJ (2014) Designing nanogel carriers for antibacterial applications. Acta Biomater 10(5):2105–2111
48. Mujahid M, Ahmad L, Ahmad M (2017) Synthesis of ZnO nanogel for the treatment of superficial skin microbial infections. J Drug Delivery Ther 7(2):58–61
49. Zhang Y, Zhang J, Chen M, Gong H, Thamphiwatana S, Eckmann L, Gao W, Zhang L (2016) A bioadhesive nanoparticle–hydrogel hybrid system for localized antimicrobial drug delivery. ACS Appl Mater Interfaces 8(28):18367–18374

50. Kłodzińska SN, Molchanova N, Franzyk H, Hansen PR, Damborg P, Nielsen HM (2018) Biopolymer nanogels improve antibacterial activity and safety profile of a novel lysine-based α-peptide/β-peptoid peptidomimetic. Eur J Pharm Biopharm 128:1–9
51. Abraham T, Mao M, Tan C (2018) Engineering approaches of smart, bio-inspired vesicles for biomedical applications. Phys Biol 15(6):061001
52. Lee JS, Feijen J (2012) Polymersomes for drug delivery: design, formation and characterization. J Control Release 161(2):473–483
53. Geilich BM, van de Ven AL, Singleton GL, Sepúlveda LJ, Sridhar S, Webster TJ (2015) Silver nanoparticle-embedded polymersome nanocarriers for the treatment of antibiotic-resistant infections. Nanoscale 7(8):3511–3519
54. Geilich BM, Gelfat I, Sridhar S, Ven ALVD, Webster TJ (2017) Superparamagnetic iron oxide-encapsulating polymersome nanocarriers for biofilm eradication. Biomaterials 119:78
55. Torchilin VP (2005) Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discov 4(2):145–160
56. Rukavina Z, Vanić Ž (2016) Current trends in development of liposomes for targeting bacterial biofilms. Pharmaceutics 8(2):18
57. Munaweera I, Shaikh S, Maples D, Nigatu AS, Sethuraman SN, Ranjan A, Greenberg DE, Chopra R (2018) Temperature-sensitive liposomal ciprofloxacin for the treatment of biofilm on infected metal implants using alternating magnetic fields. Int J Hyperth 34(2):189–200
58. Sun LM, Zhang CL, Li P (2012) Characterization, antibiofilm, and mechanism of action of novel PEG-stabilized lipid nanoparticles loaded with terpinen-4-ol. J Agric Food Chem 60(24):6150
59. Dong D, Thomas N, Thierry B, Vreugde S, Prestidge CA, Wormald PJ (2015) Distribution and inhibition of liposomes on Staphylococcus aureus and Pseudomonas aeruginosa biofilm. PLoS One 10(6):e0131806
60. Tucker N, Stanger J, Staiger M, Razzaq H, Hofman K (2012) The history of the science and technology of electrospinning from 1600 to 1995. J Eng Fibers Fabr 7:63–73
61. Vasantha VA, Rahim SZZ, Jayaraman S, Junyuan GH, Puniredd SR, Ramakrishna S, Teo SL-M, Parthiban A (2016) Antibacterial, electrospun nanofibers of novel poly (sulfobetaine) and poly (sulfabetaine) s. J Mater Chem B 4(15):2731–2738
62. Ahire JJ, Hattingh M, Neveling DP, Dicks LM (2016) Copper-containing anti-biofilm nanofiber scaffolds as a wound dressing material. PLoS One 11(3):e0152755
63. Alharbi HF, Luqman M, Fouad H, Khalil KA, Alharthi NH (2018) Viscoelastic behavior of core-shell structured nanofibers of PLA and PVA produced by coaxial electrospinning. Polym Test 67:136
64. Alharbi HF, Luqman M, Khan ST (2018) Antibiofilm activity of synthesized electrospun core-shell nanofiber composites of PLA and PVA with silver nanoparticles. Mater Res Express 5(9):095001
65. López-Esparza J, Espinosa-Cristóbal LnF, Donohue-Cornejo A, Reyes-López SnY (2016) Antimicrobial activity of silver nanoparticles in polycaprolactone nanofibers against gram-positive and gram-negative bacteria. Ind Eng Chem Res 55(49):12532–12538
66. Ahire JJ, Neveling DP, Hattingh M, Dicks LM (2015) Ciprofloxacin-eluting nanofibers inhibits biofilm formation by Pseudomonas aeruginosa and a methicillin-resistant Staphylococcus aureus. PLoS One 10(4):e0123648
67. Ashbaugh AG, Jiang X, Zheng J, Tsai AS, Kim W-S, Thompson JM, Miller RJ, Shahbazian JH, Wang Y, Dillen CA (2016) Polymeric nanofiber coating with tunable combinatorial antibiotic delivery prevents biofilm-associated infection in vivo. Proc Natl Acad Sci 113(45):E6919–E6928
68. Wolfmeier H, Pletzer D, Mansour SC, Rew H (2018) New perspectives in biofilm eradication. ACS Infect Dis 4(2):93–106
69. Álvarez-Paino M, Muñoz-Bonilla A, Fernández-García M (2017) Antimicrobial polymers in the nano-world. Nano 7(2):48

Chiral Stereochemical Strategy for Antimicrobial Adhesion

Zixu Xie, Guofeng Li, and Xing Wang

Abstract Chiral stereochemical strategy (CSS) is a universal strategy that utilizes the "chiral taste" of microbes against their adhesion on chiral stereochemical surfaces. For the issue of interaction between microorganisms and material surface, molecular chirality or chiral stereochemistry plays a crucial role on modulating microbial behavior. The CSS thus is a soft management and control of microbes. It would not artificially promote the evolution of microbes, potentially preventing the formation of resistant organisms. This mini-review summarizes recent research on borneol-based chiral antimicrobial materials. According to the composition of the materials, we classified the borneol-based materials into synthetic polymers, natural polymers, and organic–inorganic hybrids. Their antimicrobial adhesion performance and the potential for biomedical applications were discussed. Due to the new concept of managing and controlling microbes, instead of killing microbes blindly, this review may catch special interest and inspire new opportunities in many medical fields and disciplines.

Keywords Chirality · Stereochemistry · Borneol · Antimicrobial · Adhesion

Introduction

Infections are the main causes of considerable morbidity and mortality to patients [1, 2], which is one of the most threatening problems worldwide. For example, implant failure due to infection is a thorny problem, which usually results in the removal of the implant [3–5]. The patients suffer tremendous pain and cost. Otherwise reports showed that hospital-acquired bacterial infections are mainly

Z. Xie · G. Li · X. Wang (✉)
Beijing Laboratory of Biomedical Materials, College of Life Science and Technology, Beijing University of Chemical Technology, Beijing, PR China
e-mail: wangxing@mail.buct.edu.cn

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_19

attributed to the attachment of bacteria to surrounding objects, medical devices, and implants. Hospitals are not as safe as we think because of microbial distribution and contamination. Therefore, in order to reduce the risk of infections, researchers have been trying to explore efficient and safe antimicrobial materials. Until now, those developed antimicrobial materials have been fabricated mainly based on contact-killing mechanisms (such as quaternary ammoniums, quaternary phosphonium salts, and *N*-Halamines) [6–11] and release-killing mechanisms (such as antibiotic, silver or copper nanoparticles) [12–16]. However, the main problem now is the excessive use of antibiotics, which may lead to drug resistance of microbes [17–20]. Preventing bacterial resistance has become a hotspot for current antibacterial research. Thus, it is of great significance to develop new antimicrobial materials and strategies that are efficient, durable, and safe [21–23].

The chiral stereochemical strategy (CSS) is a novel strategy for antimicrobial studies, which is based on microbial recognition of the chiral stereochemical surface. A bacterium is a kind of microorganism who can distinguish different chiral surfaces [24] and have different adhesion and growth behavior on these surfaces [25]. According to studies, CSS for antimicrobial applications have been proposed [26, 27]. It is a broad-spectrum strategy that prevents bacterial and fungal cells from adhering to the material surfaces [26, 27], focusing on the initial stage of the microbial adhesion and contamination, allowing the microbes to autonomously leave the surface when they distinguish the chiral stereochemistry of the materials. The development of CSS has many potential advantages. First, it is a management and control of microbial behavior instead of killing the microbes, which does not artificially promote microbial evolution, prevent it from happening. Second, it is a broad-spectrum antimicrobial strategy against both bacteria (Gram-negative or Gram-positive) and fungi. Third, this strategy is applicable to a variety of materials, on the basis of versatile synthetic chemistry and surface modification. Finally, chiral units are mainly natural molecules, which are easily obtained in nature and cause little pollution to the natural environment. The chiral molecules are thus ideal candidates for antimicrobial materials.

In this review, we summarize recent studies about the applications of CSS in the field of synthetic polymers, natural polymers, and inorganic carbon materials. It is very exciting that all of these chiral stereochemical surfaces exhibit excellent antimicrobial adhesion activities.

Chiral Stereochemical Strategy

Chirality means that a molecule cannot overlap with its enantiomer, just like the left hand and the right hand mirrored each other cannot coincide. Chirality is ubiquitous in life. The interaction between biosystems and materials exhibits a high chiral preference. Therefore, CSS, based on the microbial recognition and follow-up response of the surface chiral stereochemistry, is a novel strategy for antimicrobial studies.

Chiral Effect on Cells

Selective interactions of cells on a chiral stereochemical surface was first found on calcium tartrate tetrahydrate crystals [28]. Epithelial A6 cells showed a strong preference of adhesion to the surfaces of (*R,R*) calcium tartrate tetrahydrate crystals, while few cells were found on (*S,S*) crystal surfaces (Fig. 1) [29]. The interaction between cells and the stereochemical structure of crystal surfaces indicated the specific chiral recognition of biosystems. From the macroscopic perspective, cells can distinguish chiral stereochemical signals of material surfaces. It is the first time we realized that this ability goes deep into the molecular level. This fact provided favorable evidence for the further study of cell behavior on chiral materials and interfaces.

Sun and his co-workers investigated the stereoselective behavior of immune cells on different *N*-Isobutyryl-L(D)-cysteine (NIBC) enantiomer-modified surfaces [30]. They used a mercapto self-assembled monolayer to simulate crystal surfaces. In a typical adhesion experiment, the number of macrophage cells adhering on the

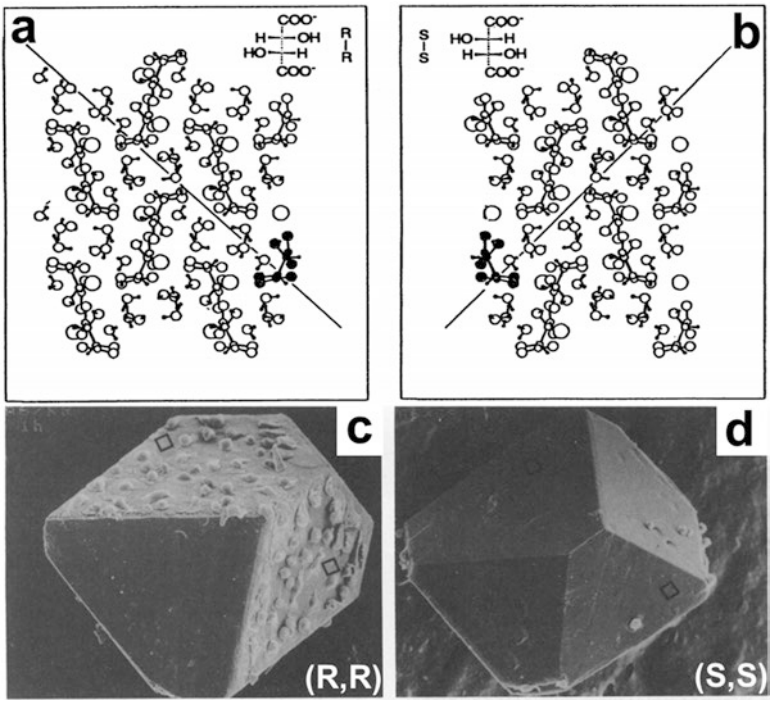


Fig. 1 (**a**, **b**) Computer graphic representation of the packing arrangement of (**a**) calcium *(R,R)*-tartrate tetrahydrate and (**b**) calcium *(S,S)*-tartrate tetrahydrate crystals viewed on the (100) plane. (**c**, **d**) Scanning electron micrographs of cultured Xenopus laevis kidney epithelial A6 cells plated on calcium tartrate tetrahydrate crystals. The short-term adhesive response (10 min) was shown for (**c**) the *(R,R)* form and (**d**) the *(S,S)* form [29]

L surface was much larger than that on the D surface. Moreover, macrophages on the L-NIBC surface showed a malformed morphology and highly spread status, whereas those on the D-NIBC surface remained a separate and round morphology. This phenomenon also happened in the adhesion experiment with neutrophils, further confirming the universality of cell behavior on chiral surfaces.

Besides, Luk et al. developed chiral antifouling materials inspired by the chiral recognition of biosystems [24, 31]. They found that mannitol terminated self-assembled monolayers (SAMs) decorated with various chiral enantiomer end-groups can oppose the conglutination of mammalian cells and bacteria on the surfaces. The adhesion of 3T3 fibroblast cells was limited on the D surface up to 19 days, while they were limited on the L surfaces for 13 days. Interestingly, the conglutination of cells on the surface that formed by the racemic mixture of the enantiomers was inhibited for 23 days, longer than either the L or D surface.

Compared to monolayer films, the polymer brush surface had a higher density of exposed chiral functional groups [32], which helped to enhance the chiral recognition of cells. Wang et al. investigated the adhesion of cells on the surface of chiral amino acid-based polymer brushes with different hydrophobic properties (Fig. 2). Cells were more willing to adhere to the hydrophobic surface; the adhesion number increased with increasing hydrophobicity of the polymer brushes. At the same time, they also found that chirality had the ability to regulate cell adhesion comparable to wettability properties. The concentration of cells on the L-poly(*N*-acryloyl-valine) (L-PV) film was evidently at a higher level than that on the D-poly(*N*-acryloyl-valine) (D-PV) film. For the quantitative analysis (Fig. 2a), the L-PV film also exhibited a much larger average area (*t*-test, $P < 0.01$) and higher I_f (fluorescent intensities) values (*t*-test, $P < 0.05$) than the D-PV film (Fig. 2b). These results emphasized the effect of chirality on cell adhesion [33]. More importantly, polymer brushes demonstrated attractive advantages such as the easy tailorability of chemical compositions, functions, and the precisely controlled surface properties with considerable bio-related applications.

Feng et al. discovered similar results for three-dimensional (3D) chiral nanofibers, which were prepared by 1,4-benzenedicarboxamide phenylalanine derivatives. It was revealed that left-handed helical nanofibers promoted cell adhesion and proliferation, while right-handed nanofibers inhibited these processes. These were attributed to the mediation of the stereospecific interaction between chiral nanofibers and fibronectin [35]. Furthermore, Liu et al. assembled chiral helical nanofibers by different chiral phenylalanine derivatives, and examined the effects of chiral molecules in these helical nanofibers on cells behavior. They found that there were clear-cut distinctions between the left- and right-handed nanofibers, even though both were formed by the same enantiomer phenylalanine derivatives. The results showed a rise in cell adhesion for the left-handed nanofibers formed by L-phenylalanine derivatives and a trifling impact on cell behavior on the corresponding right-handed nanofibers. By contrast, both left- and right-handed nanofibers formed by D-phenylalanine had minor and negative impacts on cell adhesion. The effects of single-handed molecules and helical nanofibers on cell adhesion are antagonistic [36]. This discovery indicated that the investigation on the chiral

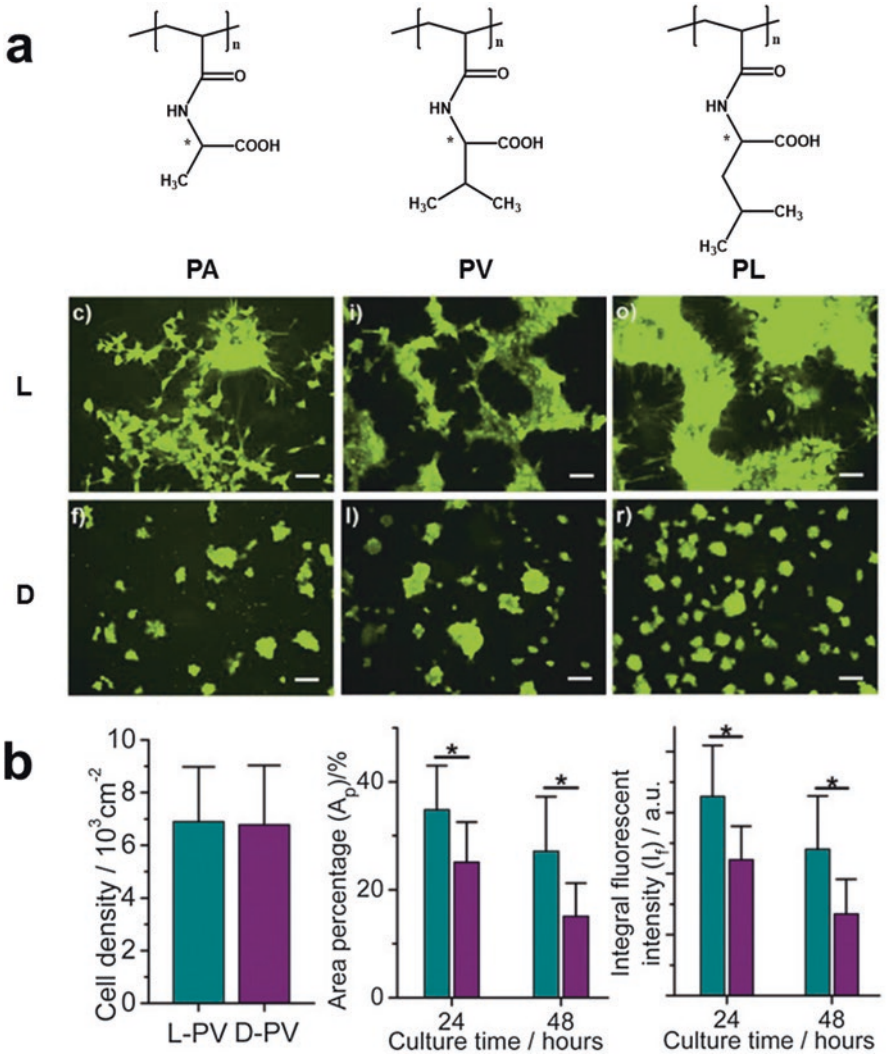


Fig. 2 (**a**) Structures of the chiral polymer and the corresponding typical fluorescent images of COS-7 cells incubated at different time periods on the chiral polymer brush films. (**b**) Cell counting results for 1 h of incubation (left); surface area ratios (Ar) occupied by cells after 24 and 48 h of incubation (middle); and integral fluorescent intensities (I_f, by arbitrary units (a.u.)) for the cell occupied areas on images after 24 and 48 h of incubation (right) [33, 34]

effect has gone deep into the relationship between designed molecular units and 3D assemblies.

Chirality adjusts not only the adhesion and growth of cells, but also their phagocytic behavior. Li et al. discovered that the chiral glutathione-coated CdTe quantum dots (GSH-QDs) exhibited differences in cytotoxicity, whereas L-GSH-QDs were

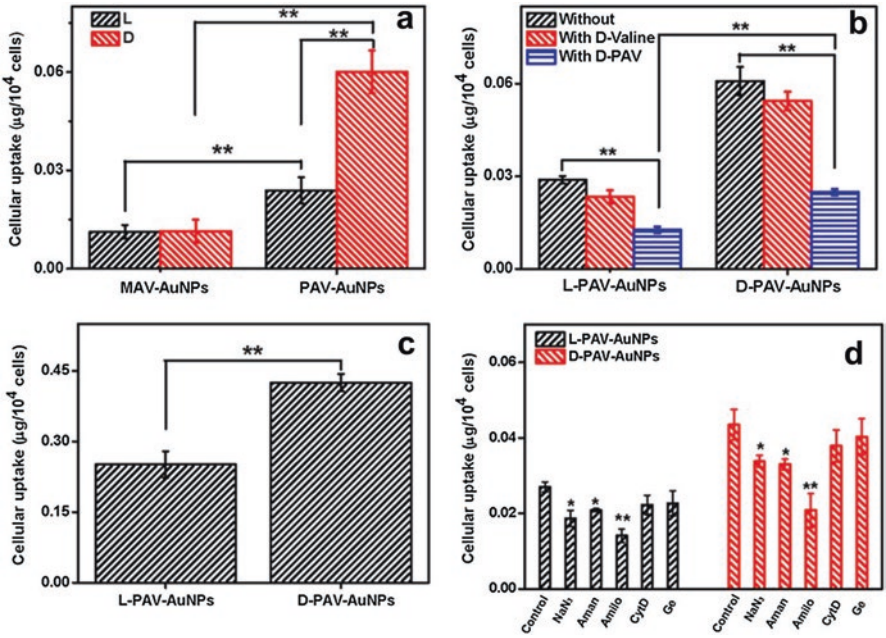


Fig. 3 Internalized amount by A549 cells at an Au concentration of 50 μg/mL. (**a**) L(D)-MAV-AuNPs and L(D)-PAV-AuNPs in 10% FBS/DMEM, (**b**) PAV-AuNPs pretreated with 1 mg/mL D-valine or D-PAV (M_w: 18,743 Da) in 10% FBS/DMEM, and (**c**) PAV-AuNPs in serum-free DMEM after co-incubation for 24 h. (**d**) Influence of pharmacological inhibitors on the uptake of L-PAV-AuNPs and D-PAV-AuNPs, respectively. The cells were cultured without or with pretreatment by amantadine-HCl (Aman, 1 mM, inhibitor of clathrin-mediated endocytosis), genistein (Ge, 100 μM, inhibitor of caveolae-mediated endocytosis), amiloride-HCl (Amilo, 2 mM, inhibitor of macropinocytosis), cytochalasin D (CytD, 10 μg/mL, inhibitor of cytoskeleton), NaN_3 (0.1% (w/v), inhibit energy-dependent process) for 1 h, and then cultured with PAV-AuNPs for another 4 h. ∗ and ∗∗ indicate significant difference at $p < 0.05$ and $p < 0.01$, respectively [38]

more cytotoxic than D-GSH-QDs due to the more cell uptake of L-GSH-QDs. Since the cytotoxicity of the QDs is associated with their ability to induce autophagy, this work exhibited a chiral dependent process [37].

After that, Gao et al. in 2016 found that the chirality of polymer-capped nanoparticles (NPs) could also affect cellular uptake. Interestingly, the internalization amount of D-poly(*N*-acryloyl-valine) gold nanoparticles (D-PAV-AuNPs) was significantly larger than that of the L-PAV-AuNPs (Fig. 3). This chirality-dependent cellular uptake might be ascribed to the stereoselective interaction between the cytomembrane and the chiral PAV molecule, which was deduced from the fact that L-phosphatidyl vesicles tend to interact with D-PAV molecules [38]. The chirality-dependent cellular uptake phenomenon was also improved by Kehr et al. They found that the cellular uptake amount of PMO-PL(D)L (Poly-L(D)-Lysine coated periodic mesoporous organosilica) varied by chirality [39]. Additionally, Gindi et al. found that C-6-glioma cells were able to adhere to the chiral penicillamine

functionalized zeolites, while the endothelial cells did not adhere at all. Therefore, they used an enantioselective L and D penicillamine functionalized zeolite (denoted as L-PEN-zeo and D-PEN-zeo) for the separation of C-6-glioma cells from primary endothelial cells [40].

All of these results demonstrated that the chiral recognition of cells on the chiral stereochemical surface is a universal phenomenon. Cells could distinguish chiral surfaces formed by small molecule modification, chiral polymer brushes, 3D chiral nanofibers, as well as chiral QDs and NPs. Cells can recognize different chiral interfaces for different responses, in which cells mainly showed a difference of adhesion at different chiral interfaces. Specifically, more cells preferred the L surfaces. This seems to be a ubiquitous phenomenon of cells.

Chiral Effect on Biomacromolecules

Biomacromolecules, such as proteins and DNA, were also found to recognize different chiral interfaces. In 2011, Wang et al. found that proteins could easily attach to the L surfaces of amino acid-based chiral polymer brushes [41]. Both bovine serum albumin (BSA, negatively charged) and gelatin (positively charged) were more inclined to adhere on the L-PV surface than on the D-PV surface (Fig. 4). Though electrostatic interactions affected the adhesion behavior of proteins on the surface to some extent, this electrostatic interaction was weaker compared with the stereoselective interaction between proteins and chiral surfaces. Some researchers also found that the chiral surface of NPs determined the adsorption orientation of proteins [42]. Chen et al. further confirmed this specific interaction by an adsorption test of BSA on the surface of chiral molecules-modified AuNPs. BSA adsorption orientation to chiral surfaces might result from the formation and location of salt bridges, which are affected greatly by the spatial distribution features of functional groups [43]. Moreover, the chirality of the surface could affect the morphology of proteins. For example, Aβ(1–40) preferred to form ring-like morphologies on

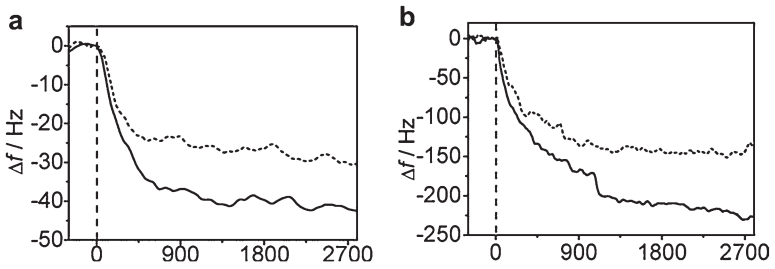


Fig. 4 Time-dependent curves of frequency change (Δf) in QCM experiments. (**a**) BSA adsorption and (**b**) gelatin adsorption on chiral polymer (L-PV and D-PV) brush films. Solid lines: L surfaces; dash lines: D surfaces [41]

L-*N*-isobutyryl cysteine (NIBC)-enantiomer-modified surfaces and rod-like morphologies on the D-NIBC-modified gold substrates [44].

Qing et al. studied the chiral effect at protein/graphene interfaces utilizing cysteine enantiomer-modified graphene oxide (Cys-GO) [45]. The result showed that the chirality of the Cys-GO surface greatly influenced protein folding, which would form amyloid aggregates. To be more specific, the R-Cys-GO inhibited the adsorption, nucleation, and fiber elongation processes of Aβ(1–40) and thus inhibited amyloid fibril formation on the surface to a large extent, while S-Cys-GO had the opposite effect. Surface chirality strongly influenced the conformational transition from an α-helix to β-sheet. More specifically, the S-Cys-GO accelerated this process, while the R-Cys-GO largely restrained this process. In addition, the adsorption of monomers and oligomers, and the subsequent fibrillation process were also influenced by the chirality of surfaces [45].

Gao et al. further proved that the chirality of NPs was one of the important factors, which affected the interaction of proteins and NPs. The adsorption of BSA on the L-PAV-AuNP surfaces was much higher than that on the D-PAV-AuNP surfaces, which was in consistence with the aforementioned conclusion. Moreover, when the BSA adhered on the L-PAV-AuNPs, it adopted an end-on configuration; however, when adsorbed on the D-PAV-AuNPs, the BSA displayed both side-on and end-on configurations (Fig. 5). The surface chiral modification of NPs could greatly influence the interaction between protein and NPs, and the subsequent protein adsorption and configuration on surfaces. Therefore, surface chirality could be an adjustable factor in the design of NPs for specific applications [46].

Chen et al. found that the L-Cys modified surfaces supported more serum protein adsorption than those on the D-Cys modified surfaces. More cells adhered on the

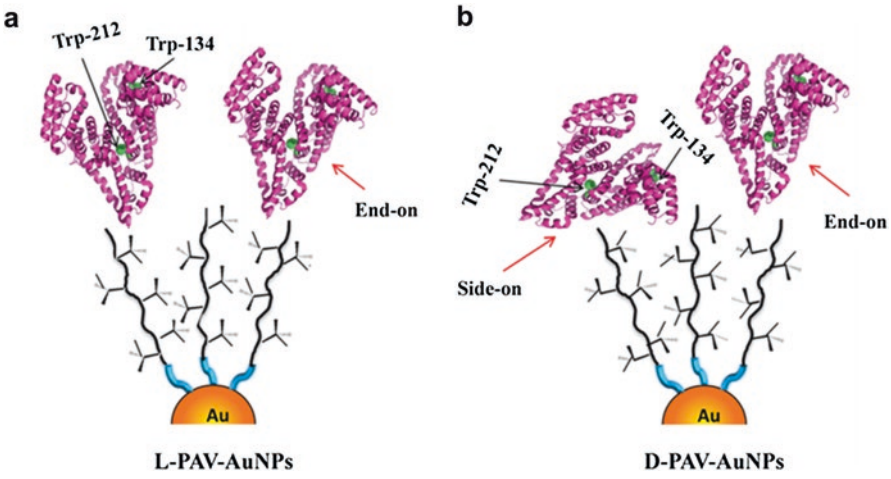


Fig. 5 Proposed binding geometries for BSA to (**a**) L-PAV-AuNPs and (**b**) D-PAV-AuNPs based on dynamic light scattering (DLS), fluorescence quenching, and isothermal titration calorimetry (ITC) measurements. NP and protein size are not drawn to scale

L-Cys modified surfaces under the serum-containing condition while under the serum-free condition, no significant differences were observed on both L and D surfaces. The results suggested that the differences of protein adsorption could be the reason for the preference of cells to attach to the L-Cys modified surfaces [47].

Tang et al. also found that DNA preferred to adhere on the surface modified with L-NIBC [48]. A full-sequence single-stranded DNA (ssDNA) from calf thymus tended to present a relaxed conformation on the L surfaces, while it folded on the D surface at a concentration of 50 μg/mL. When the concentration increased to 75 μg/mL, ssDNA preferred to exhibit a highly extended morphology (Fig 6). In addition, the quartz crystal microbalance (QCM) result showed that the quantity of the ssDNA on the L surface was much larger than that on the D surface (Fig. 6c, d). This stereoselective behavior of DNA was further confirmed by plasmid pcDNA3, which was a circular double-stranded DNA (ds-DNA). Similarly, the plasmid on the L surface exhibited a relaxed conformation, and its content was much larger than that on the D surface [49].

Proteins and DNA can recognize chiral systems and respond differently. This difference is not only reflected in the adhesion amount at different chiral interfaces, but also in the morphological conformation. Due to the effect of chirality, the conformation of protein could transfer from α-helix to β-sheet, while DNA could change from a relatively relaxed state to a rod-like folded state. These results provided clear evidence for the interaction between biomacromolecules and chiral systems, which may be an explanation of the difference of cell behavior on chiral surfaces.

To summarize, it is the nature of biosystems (cells, protein, DNA, etc.) that they can distinguish chiral stereochemistry structure and show different behavior. With this feature, chiral functional materials could be further designed and utilized, in particular, as antimicrobial materials in this review, where all of the abovementioned research can be regarded as the theoretical basis.

Antimicrobial Adhesion

Synthetic Polymers

Borneol molecules have been selected as ideal chiral units for antimicrobial applications. Borneol is a natural chiral drug that is presented in numerous medicinal plants. It has a hydrophobic molecular structure with four configurations, including endo-L-borneol, endo-D-borneol, and exo-isoborneol. They can be esterified into derivatives with increased activity compared to the parent borneol. Accordingly, our group has developed a series of chiral polyborneolacrylates (PBAs), including PLBA, PDBA, and PIBA depending on the three derivative enantiomers of BA [27].

In order to evaluate the antibacterial adhesion properties of PBAs, a “prison break” experiment was designed. The PBA rings were fixed into culture medium.

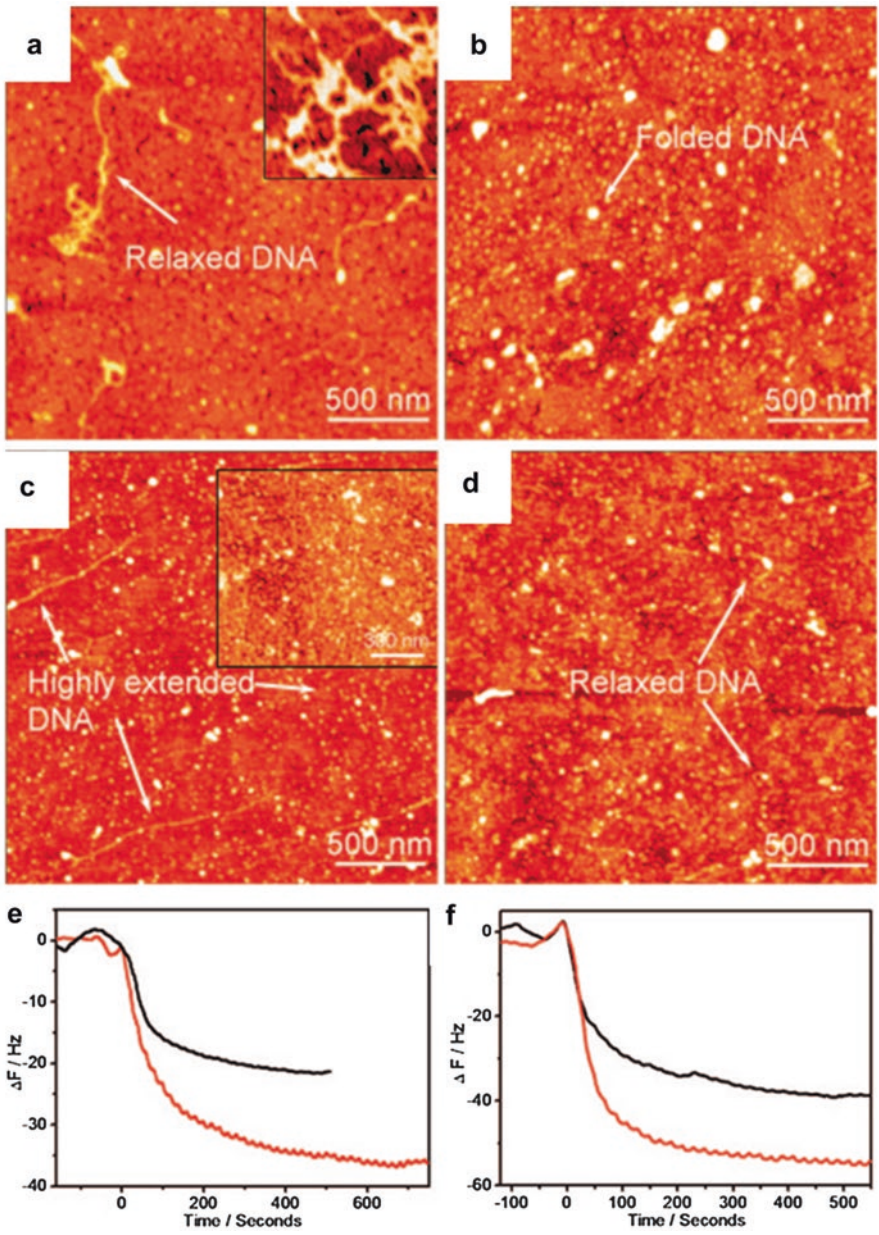


Fig. 6 Typical AFM (Atomic Force Microscope) images for DNA adsorption on L- (**a**, **c**) and D-NIBC (**b**, **d**) modified surface. DNA concentration: (**a** and **b**) 50 μg/mL; (**b** and **d**) 75 μg/mL. Inset in (**a**): Entangled DNA in another area. Inset in (**c**): DNA chains with another orientation and dense arrangement. (**e**, **f**) Time dependence of the QCM frequency shift of the D- (black) and L-NIBC (red) modified Au coated quartz-crystal resonator at DNA solutions with different concentrations. (**e**) 50 μg/mL; (**f**) 75 μg/mL. Experiment temperature: 25 °C [48]

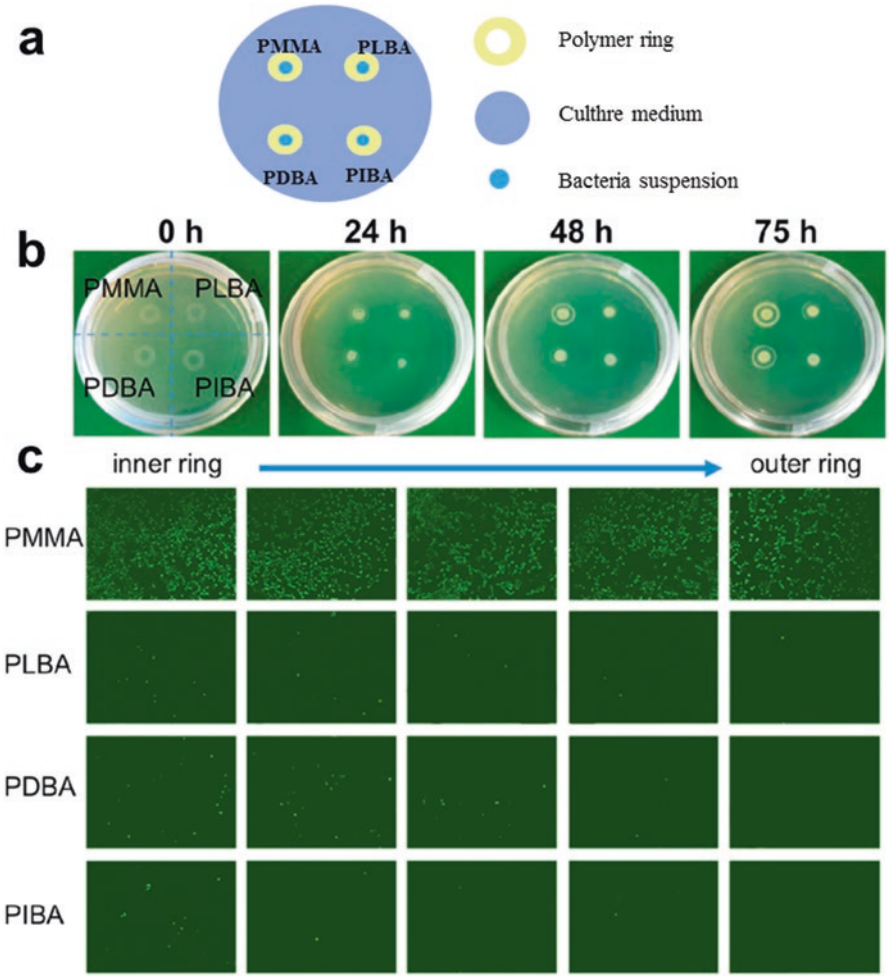


Fig. 7 (**a**) Schematic diagram of a "Prison Break" experiment for antibacterial adhesion assays of polymer films. (**b**) Effects on controlling the escape of *E. coli* from PMMA, PLBA, PDBA, and PIBA rings after different periods of incubation time. (**c**) Optical micrographs of *E. coli* adhered on the above polymers from the inner (left) to outer (right) edges of the rings after 60 h of incubation. The image size is approximately 897 μm^2 (34.5 μm × 26.0 μm) [27]

A bacterial suspension was placed onto the center of the circular ring and cultured for a period of time (Fig. 7).

A "Prison Break" experiment showed that bacteria easily broke the limitation of the poly(methyl methacrylate) (PMMA) control, while the PBAs showed excellent antibacterial adhesion properties. Scanning the polymer rings revealed that higher density of *E. coli* covered almost the entire surface of the PMMA from inside to outside. In contrary, only a few bacteria were found at the inner ring of the PBAs, while cells were barely observed on the outer ring of all the PBAs. These results

confirmed the antibacterial adhesion capability of the PBAs. The antibacterial capability of the PBA rings was caused by a biological surface recognition rather than the physical effect of blocking. Among the three enantiomers of the PBAs, the PLBA showed the best antibacterial adhesion property. Neither *E. coli* nor *S. aureus* could escape the PLBA ring after 75 h of incubation (Fig. 7b). This fact seems to be in violation of the previous finding, in which the L configuration surfaces may be good for the adhesion of biosystems. But, further investigation suggested that the camphane-type bicyclic structure of borneol had three chiral centers, located at C1, C2, and C4; the C2 chiral center in PLBA (*1S,2R,4S*-borneol pendants) corresponded to a D configuration, which usually provides surfaces with a cell or protein resistance capability. Thus, we envisioned that bacteria might mainly distinguish the chiral center at C2, rather than C1 or C4. Anyway, it was a successful antibacterial application of CSS.

Subsequently, the PLBA was used to modify the conventional biomedical polymer, PMMA, endowing PMMA with a surface stereochemistry property and enhancing its antibacterial adhesion capacity [50]. P(MMA-*co*-BA) was then synthesized via copolymerization of MMA and BA. By tuning the molar ratio of the MMA and BA, a series of P(MMA-*co*-BA)s were obtained. The modified "prison break" experiment (Fig. 8) showed that 10% of PBA (P_{10}) could give distinct antibacterial activity of the P(MMA-*co*-BA) copolymer. With increasing PBA content, the anti-adhesion activity of the P(MMA-*co*-BA) increased. P_{50} effectively prevented the adhesion and growth of *E. coli* on the copolymer surface and lasted for 120 h. In situ fluorescent live/dead staining showed that compared with P_0

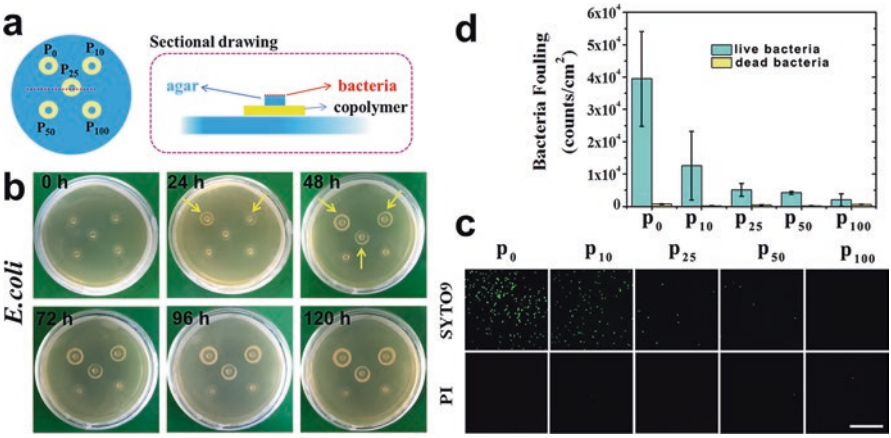


Fig. 8 (**a**) Schematic illustration of a modified "Prison Break" experiment for an antibacterial adhesion assay of polymer films. Controlling the escape of (**b**) *E. coli* and from *Px* films was recorded at the denoted periods (0, 24, 48, 72, 96, and 120 h). (**c**) Typical fluorescence microscopy images of attached *E. coli* from a suspension of 10^7 cells/mL after exposure to various films for 4 h. The live *E. coli* cells are stained green, while the dead cells are stained red. The scale bar in the image is 20 μm. (**d**) Quantitative results for bacterial adsorption on *Px* films. They were estimated using ImageJ software. Data values corresponded to mean ± SD ($n = 3$) [50]

(unmodified PMMA), the bacterial population reduced dramatically on the surfaces from P_{10} to P_{100}. Particularly, there was a 99.7% decrease in *E. coli* adhesion P_{100}. Meanwhile, only fragmentary dead bacteria could be found on all the surfaces, which was almost negligible. Thus, it not only testified that this kind of borneol-grafted copolymer had the capability of antibacterial adhesion but also confirmed that the activity was mainly due to initial sensing and subsequent selection of reversible bacterial attachment, relating interfacial stereochemistry rather than a normal mechanism of broken killing or virulence.

The PBAs showed excellent anti-adhesion ability against both Gram-positive and Gram-negative bacteria. Fungi, which are similar with mammalian cells, also have an inclination to different molecular chirality and tend to stay away from the stereochemical surface. Therefore, antifungal adhesion may be achieved on the PBA surfaces. To shed light on this issue, Xu et al. coated PBA polymers onto papers by a simple spraying method to impart the antifungal property of papers [51]. As shown in Fig. 9, the fungal growth started at the center of the plate, and then spread around to the edge of the material. After culturing for 8 days, fungal spores had covered almost the entire surface of the control paper, while the PBA-coated paper maintained a very clean surface, demonstrating that the PBA coating papers displayed an outstanding antifungal performance against *Aspergillus niger* (*A. niger*) and *Penicillium* sp. Unlike the traditional germicidal method, the PBA coating papers inhibited the attachment of fungal cells and the germination of fungal spores.

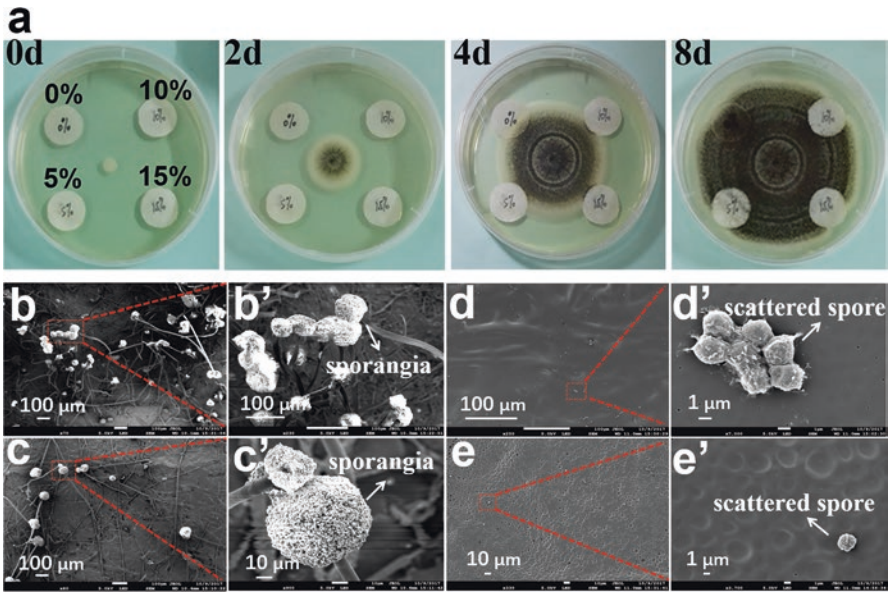


Fig. 9 (**a**) Antifungal effect of papers coated with 0 (left up), 5 (left down), 10 (right up), and 15% (right down) of PBA after incubating with *A. niger* for 8 days. (**b**–**e**) SEM images of *A. niger* cells on papers coated with 0 (**b**, **b**′), 5 (**c**, **c**′), 10 (**d**, **d**′) and 15% (**e**, **e**′) of PBA after incubating for 8 days. The images of (**b**′–**e**′) show zoomed-in views of areas in the corresponding images of (**b**–**e**) [51]

It was a remarkable breakthrough of CSS in the antifungal field, suggesting that CSS is a broad-spectrum antimicrobial strategy.

Utilizing the outstanding antibacterial adhesion ability of PBAs [27], Tan et al. introduced borneolacrylate into a diblock copolymer of poly[(N-3,4-dihydroxyphenethyl acrylamide)-*b*-(borneolacrylate)] (PDA-*b*-PBA) via reversible addition–fragmentation chain transfer (RAFT) polymerization. The PDA-*b*-PBA was endowed with both an excellent adhesive property and antibacterial ability. The PDA-*b*-PBA coating showed a good broad-spectrum antibacterial performance with inhibition rates of 92.7% and 81.3% for *E. coli* and *S. aureus*, respectively. In addition, the PDA-*b*-PBA coating could be applied in antibacterial textiles due to its easy fabrication on cotton fabrics and commercial gauze (see the plate count results in Fig. 10) [52]. Moreover, Wu et al. developed a waterborne polyurethane functionalized with IBA side group (IWPU). The introduction of IBA gave IWPU a unique chiral feature and good antibacterial adhesion activity against *E. coli* and *S. aureus*. The antibacterial activity was positively correlated with IBA side group content. When the content of the IBA side groups reached 25%, the IWPU exhibited a very effective resistance to bacterial adhesion [53].

Natural Polymers

Natural polymers, cellulose in particular, had been employed in textiles [54], food packaging [55, 56], the medical field [57–59], and the environmental domain [60, 61]. One of the main problems that hinder their applications is that these materials

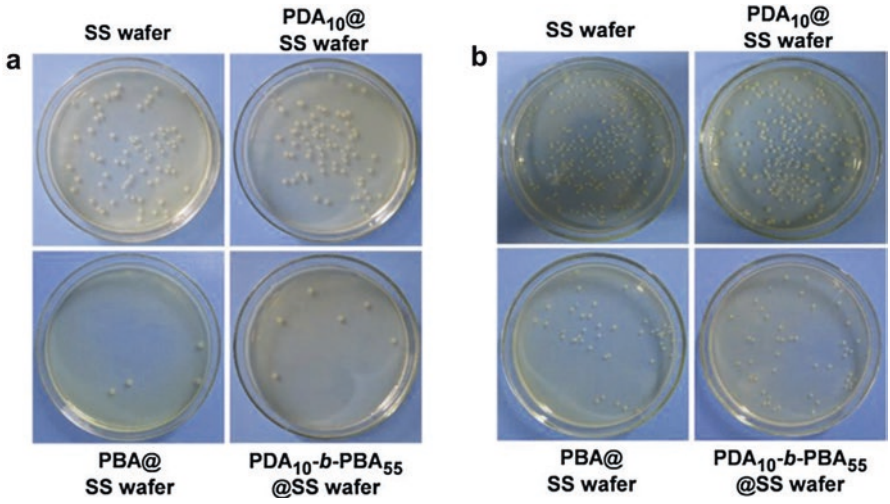


Fig. 10 The photos of (**a**) *E. coli* colonies and (**b**) *S. aureus* colonies in plate count experiments [52]

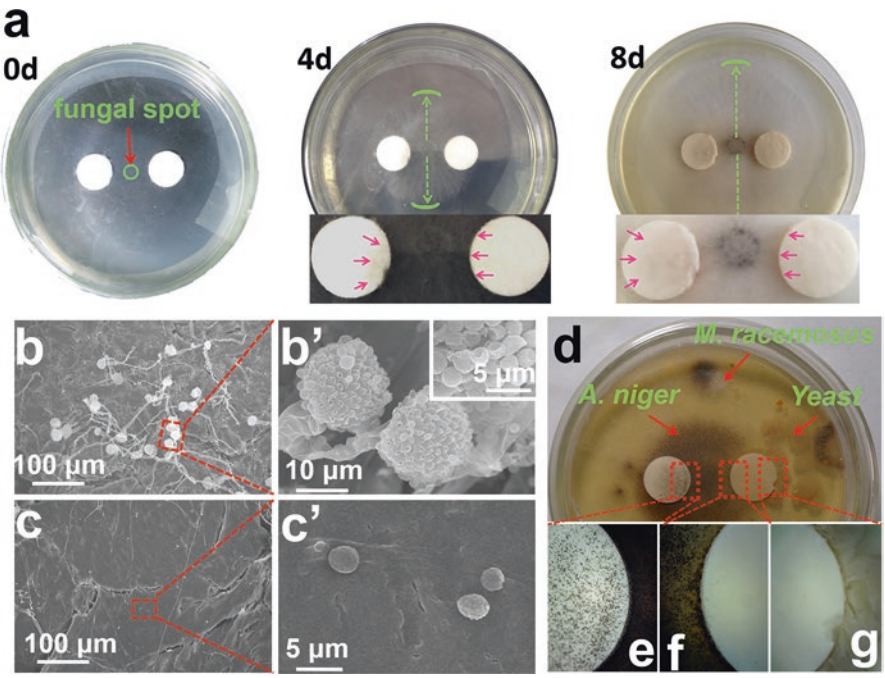


Fig. 11 (**a**) Study of the antifungal adhesion activity of cellulose (left) and BGC (right) pellets, by culturing *M. racemosus* in the central location of the solid medium, in the same plate for different periods of time. The results of 0, 4, and 8 days are shown here. The insets exhibit an enlarged image. Pink arrow: the growth frontier of *M. racemosus* on the pellets. Green circle: the range of the inoculated *M. racemosus* spot. Green arc: the growth range of *M. racemosus*; (**b**, **c**) SEM images of the antifungal adhesion results for the cellulose (**b**, **b**′) surface and the BGC (**c**, **c**′) surface. By comparison, distinct antifungal adhesion inhibition could be found after grafting borneol molecules onto cellulose; (**d**) antifungal adhesion activity of pellets of cellulose (left) and BGC (right) by culturing *A. niger* for 8 days. The operation was carried out in ambient conditions, thus, *M. racemosus* and yeast strains were also found. Optical micrographs showed that the fungal cells adhered to the cellulose pellet (inset **e**) and stopped adhering to the BGC pellet (inset **f** and **g**) [26]

do not have antimicrobial properties. Thus, to endow these natural materials with broad-spectrum antimicrobial properties, borneol was used to modify the surface of the materials and enabled them with antimicrobial properties.

Shi et al. synthesized borneol-grafted cellulose (BGC) by covalently tethering L-borneol to cellulose [26]. The BGC showed effective antifungal adhesion activity against *M. racemosus* and *A. niger*. Its surface kept a relative cleanliness even though the evaluation time was up to 8 days. By contrast, the control cellulose surface was almost covered with fungal cells (Fig. 11a, e). Scanning electron microscope (SEM) images showed that a large number of grown sporangia and hypha were found on the cellulose surface and lively germination of spores was exhibited therein. While on the BGC surface (Fig. 11a, f), only a few hyphae could be observed near the boundary and the serendipitous spores stayed near the

hypha presenting a whole sphericity, indicating growth inhibition of spores on the BGC surface. Then, a special antimicrobial evaluation was carried out in an open environment. A large number of *M. racemosus*, *A. niger*, and yeast grew in the medium and climbed on cellulose surface (Fig. 11g), while no cells tended to adhere on the surface of the BGC pellet, revealing its great application potential. Above all, these phenomena demonstrated the success of the cellulose modification and the high antifungal power of the grafted borneol molecules.

Xu et al. developed a borneol-decorated cotton textile (BDCT) through coupling of borneol 4-formylbenzoate molecules onto the amino-modified CT. The new functionalized CT exhibited prominent antifungal adhesion properties against *M. racemosus* and *A. niger* for more than 30 days (Fig. 12a). It also exhibited broad-spectrum antibacterial activities against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis* (Fig. 12c). The antimicrobial adhesion rates were all above 93%

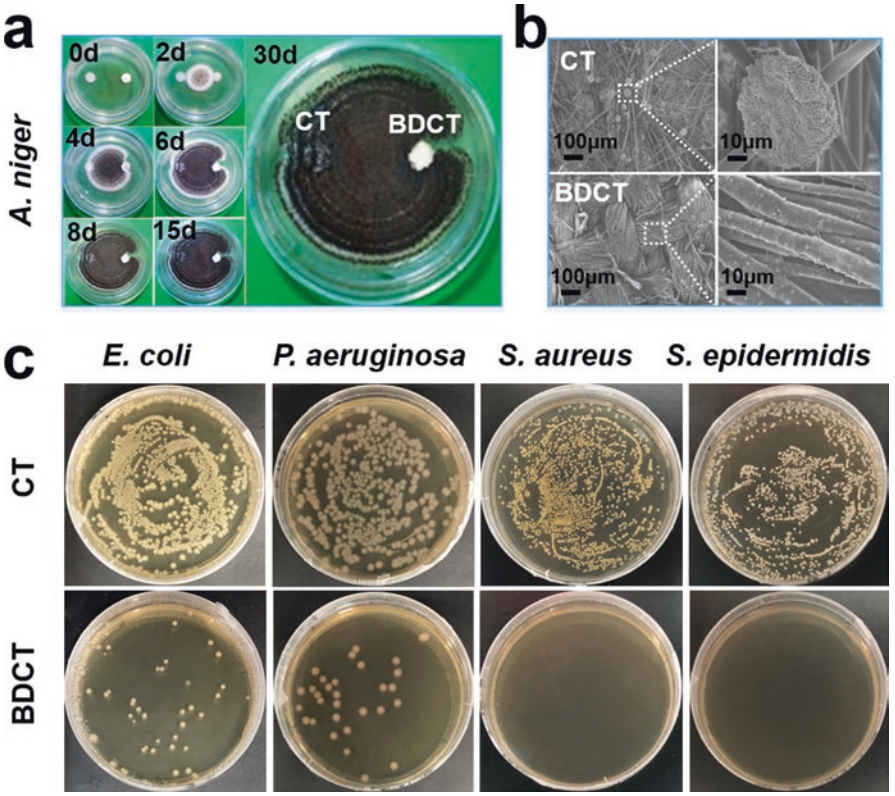


Fig. 12 (**a**) Effect of antifungal adhesion on raw CT (left), and BDCT (right) by culturing *A. niger* in the center location of solid medium, in the same plate for different time periods (0, 2, 4, 6, 8, 15, and 30 days). (**b**) SEM images of antifungal adhesion results on raw CT and BDCT surfaces. Left are images at low magnifications; right are images at high magnifications. (**c**) Antibacterial activities of raw control CT and BDCT against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. epidermidis* [62]

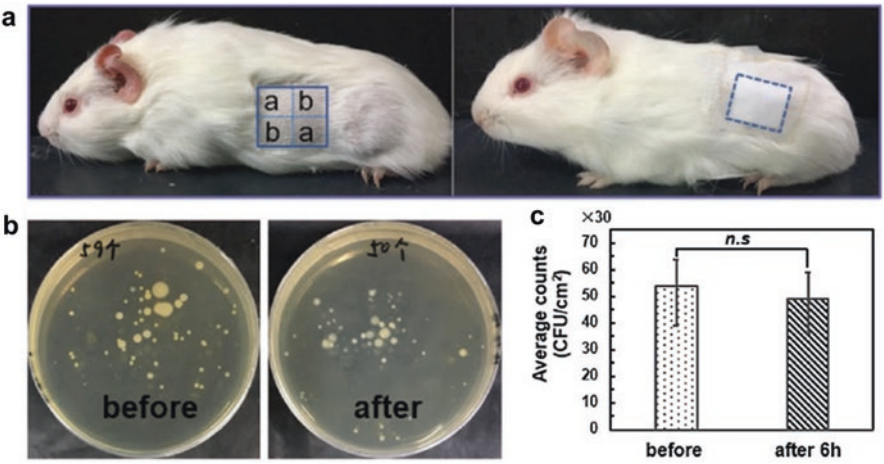


Fig. 13 (**a**) Model of skin flora test: Guinea pig in the left picture showed the application of the site to cover the sample (marked with blue line). The region "a" was the area bacteria were taken from before coating samples. The region "b" was the area bacteria were taken from after coating the samples. The right picture shows a guinea pig in contact with the BDCT. (**b**) The culturing results of bacterial flora from the guinea pig skin before and after applying BDCT. (**c**) Average of total germ count before and after the application of BDCT [62]

after 50 times of an accelerated laundering test. The antimicrobial mechanism was mainly due to the special stereochemistry of L-borneol instead of hydrophobicity. Therefore, it was different from a traditional bacterial-killing strategy, which was conducive to maintain skin microecological balance and did not damage the skin flora protection barrier. As shown in Fig. 13, the number and species of a guinea pig's skin flora remained almost the same after contacting with the BDCT for 6 h. Besides, it showed no skin stimulation due to no antibacterial release. For the perspective of application, the BDCT is meeting the frontier of antimicrobial CT, in which beneficial microbes should live in harmony with humans, as well as protecting us from potentially harmful microorganisms. Thus, the BDCT-like materials could be utilized in many industries such as clothing, medical, food packaging, as well as environmental domains to control the spread of infectious microorganisms.

Chitosan is another natural material that is derived from the deacetylation of chitin [63] and known as the only pseudonatural cationic polymer [64]. As a promising biomaterial, chitosan has the advantages of good biocompatibility, high safety, and excellent film-forming ability [65], and is widely used in pharmaceutical [66–68], food [69, 70], textile [71, 72], cosmetics industries [73, 74], etc. Furthermore, the broad-spectrum antibacterial property of chitosan [75–78] makes it an ideal antibacterial model. However, studies have shown that the good antibacterial properties of chitosan can only be acted in the aqueous solution state, while in the solid state, the antibacterial property of chitosan drops sharply [79]. Therefore, to solve this

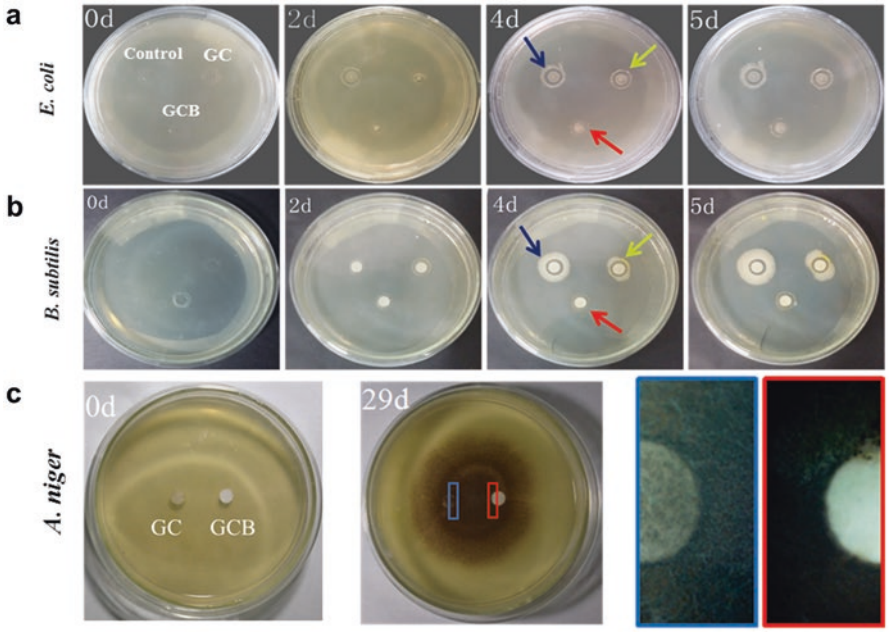


Fig. 14 (**a**, **b**) Effects on controlling the escape of *E. coli* (**a**) and *B. subtilis* (**b**) from control, GC, and GCB rings after different periods of incubation time. (**c**) Study of the antifungal adhesion activity of GC (left) and GCB (right) pellets, by culturing *A. niger* for 0 and 29 days. The red and blue boxes correspond to the adhesion of fungi on GC and GCB materials [85]

problem, the surface modification of chitosan is desirable. It mainly focuses on the introduction of silver [80, 81], quaternary ammonium salt [82, 83], antibacterial peptides [84], etc. to chitosan. However, all those efforts are traditional bacteria-killing strategies, either harmful pathogen or beneficial flora.

Therefore, our group prepared a glycol–chitosan/borneol (GCB) composite by a facile Schiff base reaction. The specific stereochemistry of the GCB impacted the microbial sensing system to achieve an antimicrobial purpose. The antibacterial "Prison Break" experiment showed that bacteria were confined within the GCB ring for at least 5 days (Fig. 14a, b). The antifungal experiment also confirmed that the GCB surface possessed long-term antifungal properties and was kept clean after 29 days of incubation, while the control tablet was covered with dense fungi (Fig. 14c). The skin flora evaluation was also carried out by the same animal model (Fig. 13a), where the silver NPs (AgNPs) modified material was used as a control group. On the skin of a healthy guinea pig, there was a lot of yellow flocculent *Kurthia* and white *Acinetobacter*, which were classified as cross-flora and symbiotic flora, respectively. After contacting with the GCB for 6 h in a preliminary test, the species and number of microorganisms were not reduced [85]. However, the number of microorganisms in that AgNPs material group decreased evidently. The results indicated that GCB was friendly to the intrinsic skin flora.

Inorganic Carbon Materials

Graphene and its derivatives have been widely used as bactericidal agents [86–88]. Their antibacterial mechanisms mainly include nanoknives, oxidative stress, and membrane wrapping or trapping. However, most of these mechanisms are available when the graphene-based materials (GMs) are dispersed in solution, while those are not feasible for on-surface graphene oxide (GO) or reduced GO (RGO). The solid graphene-based materials did not show any antifungal properties. Therefore, our group combined borneol and GO, endowed graphene-based material with antifungal performance, and further demonstrated its antifungal mechanism [89].

A GO–borneol (GOB) composite was synthesized by esterification of borneol with thiomalic acid-modified GO sheets. The landing test (Fig. 15a) showed that the GOB tablet was the only one that no *M. racemosus* cells adhered or grew on it, while the other tablets failed to resist the adhesion of *M. racemosus* (Fig. 15b). A large number of *M. racemosus* cells could be seen to gathering in the frontier of the tablets of GO, RGO, and GOC, and their edges were ambiguous (Fig. 15c). These results were in agreement with the previous study that neither solid GO nor RGO could resist fungal adhesion [90]. In contrast, the GOB exhibited outstanding antifungal activity. *M. racemosus* grew outside the GOB tablet. Only a few individual cells scattered in the edge of the GOB. Previous studies [87, 91] revealed that the needlelike nanostructure of GO (like a nanoknife) could pierce the bacteria cell membrane and led to the efflux of cell contents. This effect on fungi, however, was very weak because the fungal hyphae were too large to be damaged by these nanostructures of the solid GO (Fig. 15d). By contrast, the chiral borneol molecules in the GOB could be the key sensors to avoid the contamination of fungi. Therefore, this was a successful combination of inorganic material and the CSS.

Conclusions and Perspectives

In summary, biosystems have intrinsic chiral preference, in other words, the chiral taste is a nature of biosystems. Cells or microbes, proteins or DNA all exhibit significant differences in behavior on chiral stereochemical surfaces. With this feature, multi types of chiral functional materials could be designed and further tailored for a particular purpose. Until now, we know that it could be used for cell separation [40, 93], biosensing system [94, 95], pharmaceutical industries [96–98], and antimicrobial materials of course.

On the basis of this understanding, we utilized CSS to develop a series of borneol-based chiral stereochemical materials, including synthetic polymers, natural polymers, and organic–inorganic hybrids. The CSS is a ubiquitous antimicrobial adhesion strategy that is based on the reversible recognition and sensing effect of the microbes on a material interface, focusing on the initial stage of bacterial contamination, allowing the microbes to autonomously leave the surface when they

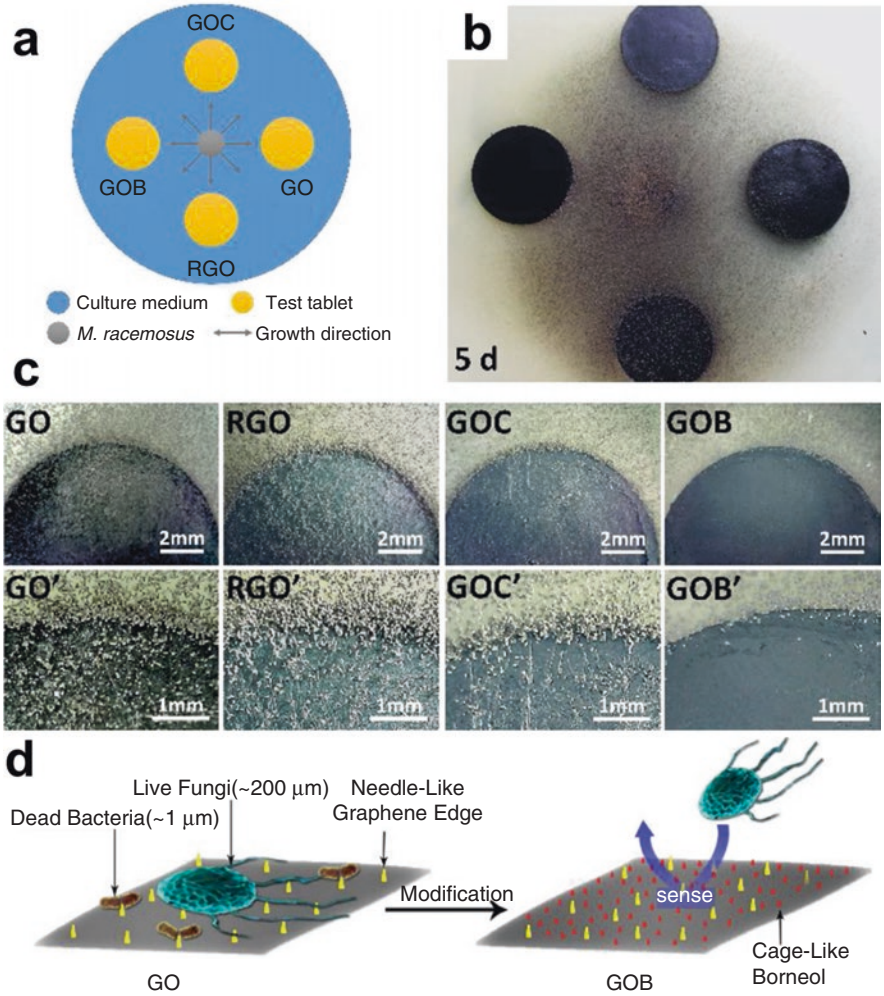


Fig. 15 Antifungal activity of GO, RGO, GOC, and GOB. (**a**) Schematic representation of the antifungal model. (**b**) Optical photograph of antifungal activity of the samples by culturing *M. racemosus* for 5 days. (**c**) Enlarged images of (**b**). (**d**) Schematic representation of the antifungal mechanism of the GOB. Fungi are large enough that they can weaken the damage caused by the needlelike nanostructure of the solid GO, while fungi avoid adhering on the surface of the GOB by sensing the carbon stereochemistry of the GOB [92]

distinguish the stereochemical signals of the materials. Therefore, the obtained borneol-based materials possessed broad-spectrum (Gram-negative bacteria, Gram-positive bacteria and various fungi), safe (antimicrobial adhesion other than killing or virulence) and long-term (lasting for more than 1 month) antimicrobial activities.

Although great achievements had been made in the application of CSS, the study is still in its infancy, and a lot of challenges remain to be solved. In our opinion,

the focus of future work will be tilted toward three aspects. The first is to find out the underlying antimicrobial adhesion mechanism since it has not been completely understood. Currently, it was confirmed that the antimicrobial activities of borneol-based materials were mainly due to initial sensing and subsequent selection of reversible microbial attachment, relating interfacial stereochemistry of borneol-based materials, rather than the normal mechanism of killing or virulence. However, it is still unclear what kind of bacterial sensory system is used to identify the chiral surface, and that will be the challenge for deeper research. The second aspect is finding a more flexible type of applicable chiral units. Although there is an infinity of possible chiral molecules, not all of them are capable for antimicrobial applications. In addition, CSS of the borneol-based materials is mainly a characteristic of antimicrobial adhesion, which does not artificially promote the evolution of microbes theoretically, but, detailed verification tests are still lacking. Therefore, the key work of the future is to determine if it will lead to the formation of resistance.

The CSS is, in fact, a management and control of microbial behavior. It will not take the initiative to attack microbes, which will be one trend of other antimicrobial material developed. Currently, this new antimicrobial concept can achieve a harmonic antimicrobial model, protecting us from potentially harmful microorganisms without harming the skin flora of human beings. This harmonic thinking is good agreement with the Chinese traditional military strategies and tactics, The Art of War, written by Sun Tzu: *Hence to fight and conquer in all your battles is not supreme excellence; supreme excellence consists in breaking the enemy's resistance without fighting. The highest form of generalship is to balk the enemy's plans; the next best is to prevent the junction of the enemy's forces; the next in order is to attack the enemy's army in the field, and the worst policy of all is to besiege walled cities.* Hence, a microbe's reversible adhesion on material surfaces in the initial stage can be seen as the microbe's plan; while the formed biofilm looks more like the walled city. Although there are limitations to kill microbes, antimicrobial adhesion is our option to some extent. What we will see in the future is, we are confident, a continuation of that harmonic trend, to solve a great variety of problems in many diverse scenarios.

Acknowledgments The authors thank the National Natural Science Foundation of China (21574008) and the Fundamental Research Funds for the Central Universities (BHYC1705B) for their financial support.

References

1. Rutledge-Taylor K, Matlow A, Gravel D, Embree J, Saux NL, Johnston L, Suh K, Embil J, Henderson E, Control, M. J. J. A. A. J. o. I (2012) A point prevalence survey of health care-associated infections in Canadian pediatric inpatients. Am J Infect Control 40(6):491–496
2. Allegranzi BJL (2011) Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. Lancet 377(9761):228–241

3. Zhao L, Chu PK, Zhang Y, Wu Z (2010) Antibacterial coatings on titanium implants. J Biomed Mater Res Pt B Appl Biomater 91B(1):470–480
4. Szaraniec B, Pielichowska K, Pac E, Menaszek E (2018) Multifunctional polymer coatings for titanium implants. Korean J Couns Psychother 93:950–957
5. Peng F, Wang D, Zhang D, Cao H, Liu X (2018) The prospect of layered double hydroxide as bone implants: a study of mechanical properties, cytocompatibility and antibacterial activity. Appl Clay Sci 165:179–187
6. Krishnamoorthy M, Hakobyan S, Ramstedt M, Gautrot JE (2014) Surface-initiated polymer brushes in the biomedical field: applications in membrane science, biosensing, cell culture, regenerative medicine and antibacterial coatings. Chem Rev 114(21):10976
7. Agnihotri S, Mukherji S, Mukherji S (2013) Immobilized silver nanoparticles enhance contact killing and show highest efficacy: elucidation of the mechanism of bactericidal action of silver. Nanoscale 5(16):7328–7340
8. Vincent M, Duval RE, Hartemann P, Engels-Deutsch M (2018) Contact killing and antimicrobial properties of copper. J Appl Microbiol 124(5):1032–1046
9. Dong A, Wang Y-J, Gao Y, Gao T, Gao G (2017) Chemical insights into antibacterial N-Halamines. Chem Rev 117(6):4806–4862
10. Makvandi P, Jamaledin R, Jabbari M, Nikfarjam N, Borzacchiello AJDM (2018) Antibacterial quaternary ammonium compounds in dental materials: a systematic review. Dent Mater 34(6):851–867
11. Kenawy ER, Abdel-Hay FI, El-Shanshoury AERR, El-Newehy MH (2002) Biologically active polymers. V. Synthesis and antimicrobial activity of modified poly(glycidyl methacrylate-co-2-hydroxyethyl methacrylate) derivatives with quaternary ammonium and phosphonium salts. J Polym Sci A Polym Chem 40(14):2384–2393
12. Li Z, Lee D, Sheng X, Cohen RE, Rubner MF (2006) Two-level antibacterial coating with both release-killing and contact-killing capabilities. Langmuir 22(24):9820–9823
13. Shekhar A, Soumyo M, Suparna MJN (2013) Immobilized silver nanoparticles enhance contact killing and show highest efficacy: elucidation of the mechanism of bactericidal action of silver. Nanoscale 5(16):7328–7340
14. Deng L, Deng Y, Xie K (2017) AgNPs-decorated 3D printed PEEK implant for infection control and bone repair. Colloids Surf B Biointerfaces 160:483–492
15. Stelzig SH, Menneking C, Hoffmann MS, Eisele K, Barcikowski S, Klapper M, Müllen KJEPJ (2011) Compatibilization of laser generated antibacterial Ag-and Cu-nanoparticles for perfluorinated implant materials. Eur Polym J 47(4):662–667
16. Bai H, Yuan H, Nie C, Wang B, Lv F, Liu L, Wang S (2015) A supramolecular antibiotic switch for antibacterial regulation. Angew Chem Int Ed 54(45):13208–13213
17. Walsh C (2000) Molecular mechanisms that confer antibacterial drug resistance. Nature 406(6797):775–781
18. Gold HS, Moellering RC Jr (1996) Antimicrobial-drug resistance. N Engl J Med 335(19):1445–1453
19. Stewart PS, Costerton JW (2001) Antibiotic resistance of bacteria in biofilms. Lancet 358(9276):135–138
20. Chandra J, Kuhn DM, Mukherjee PK, Hoyer LL, McCormick T, Ghannoum MA (2001) Biofilm formation by the fungal pathogen Candida albicans: development, architecture, and drug resistance. J Bacteriol 183(18):5385–5394
21. Coad BR, Kidd SE, Ellis DH, Griesser HJ (2014) Biomaterials surfaces capable of resisting fungal attachment and biofilm formation. Biotechnol Adv 32(2):296–307
22. Poverenov E, Shemesh M, Gulino A, Cristaldi DA, Zakin V, Yefremov T, Granit R (2013) Durable contact active antimicrobial materials formed by a one-step covalent modification of polyvinyl alcohol, cellulose and glass surfaces. Colloids Surf B Biointerfaces 112:356–361
23. Windler L, Height M, Nowack B (2013) Comparative evaluation of antimicrobials for textile applications. Environ Int 53:62–73
24. Bandyopadhyay D, Prashar D, Luk Y-Y (2011) Anti-fouling chemistry of chiral monolayers: enhancing biofilm resistance on racemic surface. Langmuir 27(10):6124–6131

25. Fu Q, Zhang K, Gao D, Wang L, Yang F, Liu Y, Xia Z (2017) Escherichia coli adhesive coating as a chiral stationary phase for open tubular capillary electrochromatography enantioseparation. Anal Chim Acta 969:63–71
26. Shi B, Luan D, Wang S, Zhao L, Tao L, Yuan Q, Wang X (2015) Borneol-grafted cellulose for antifungal adhesion and fungal growth inhibition. RSC Adv 5(64):51947–51952
27. Luo L, Li G, Luan D, Yuan Q, Wei Y, Wang X (2014) Antibacterial adhesion of borneol-based polymer via surface chiral stereochemistry. ACS Appl Mater Interfaces 6(21):19371–19377
28. Hanein D, Sabanay H, Addadi L, Geiger B (1993) Selective interactions of cells with crystal surfaces. Implications for the mechanism of cell adhesion. J Cell Sci 104(2):275–288
29. Hanein D, Geiger B, Addadi L (1994) Differential adhesion of cells to enantiomorphous crystal surfaces. Science 263(5152):1413–1416
30. Sun T, Han D, Rhemann K, Chi L, Fuchs H (2007) Stereospecific interaction between immune cells and chiral surfaces. J Am Chem Soc 129(6):1496–1497
31. Bandyopadhyay D, Prashar D, Luk Y-Y (2011) Stereochemical effects of chiral monolayers on enhancing the resistance to mammalian cell adhesion. Chem Commun 47(21):6165–6167
32. Ayres N (2010) Polymer brushes: Applications in biomaterials and nanotechnology. Polym Chem 1(6):769–777
33. Wang X, Gan H, Zhang M, Sun T (2012) Modulating cell behaviors on chiral polymer brush films with different hydrophobic side groups. Langmuir 28(5):2791–2798
34. Wang X, Gan H, Sun T, Su B, Fuchs H, Vestweber D, Butz S (2010) Stereochemistry triggered differential cell behaviours on chiral polymer surfaces. Soft Matter 6(16):3851–3855
35. Liu G, Zhang D, Feng C (2014) Control of three-dimensional cell adhesion by the chirality of nanofibers in hydrogels. Angew Chem Int Ed 53(30):7789–7793
36. Liu J, Yuan F, Ma X, Auphedeous D i Y, Zhao C, Liu C, Shen C, Feng C (2018) The cooperative effect of both molecular and supramolecular chirality on cell adhesion. Angew Chem Int Ed 130(22):6585–6589
37. Li Y, Zhou Y, Wang HY, Perrett S, Zhao Y, Tang Z, Nie G (2011) Chirality of glutathione surface coating affects the cytotoxicity of quantum dots. Angew Chem Int Ed 50(26):5860–5864
38. Deng J, Wu S, Yao M, Gao C (2016) Surface-anchored poly(acryloyl-L (D)-valine) with enhanced chirality-selective effect on cellular uptake of gold nanoparticles. Sci Rep 6:31595
39. Kehr NS, Jose J (2017) Chirality-dependent cellular uptake of chiral nanocarriers and intracellular delivery of different amounts of guest molecules. Appl Surf Sci 425:432–439
40. El-Gindi J, Benson K, De Cola L, Galla HJ, Seda Kehr N (2012) Cell adhesion behavior on enantiomerically functionalized zeolite L monolayers. Angew Chem Int Ed 51(15):3716–3720
41. Wang X, Gan H, Sun T (2011) Chiral design for polymeric biointerface: the influence of surface chirality on protein adsorption. Adv Funct Mater 21(17):3276–3281
42. Wang X, Wang M, Lei R, Zhu SF, Zhao Y, Chen C (2017) Chiral surface of nanoparticles determines the orientation of adsorbed transferrin and its interaction with receptors. ACS Nano 11(5):4606–4616
43. Wang X, Wang X, Wang M, Zhang D, Yang Q, Liu T, Lei R, Zhu S, Zhao Y, Chen C (2018) Probing adsorption behaviors of BSA onto chiral surfaces of nanoparticles. Small 14(16):1703982
44. Gao G, Zhang M, Lu P, Guo G, Wang D, Sun T (2015) Chirality-assisted ring-like aggregation of Aβ(1-40) at liquid-solid interfaces: a stereoselective two-step assembly process. Angew Chem Int Ed 54(7):2245–2250
45. Qing G, Zhao S, Xiong Y, Lv Z, Jiang F, Liu Y, Chen H, Zhang M, Sun T (2014) Chiral effect at protein/graphene interface: a bioinspired perspective to understand amyloid formation. J Am Chem Soc 136(30):10736–10742
46. Deng J, Li Z, Yao M, Gao C (2016) Influence of albumin configuration by the chiral polymer-grafted gold nanoparticles. Langmuir 32(22):5608–5616
47. Zhou F, Yuan L, Li D, Huang H, Sun T, Chen H (2012) Cell adhesion on chiral surface: the role of protein adsorption. Colloids Surf B Biointerfaces 90:97–101
48. Tang K, Gan H, Li Y, Chi L, Sun T, Fuchs H (2008) Stereoselective interaction between DNA and chiral surfaces. J Am Chem Soc 130(34):11284–11285

49. Gan H, Tang K, Sun T, Hirtz M, Li Y, Chi L, Butz S, Fuchs H (2009) Selective adsorption of DNA on chiral surfaces: supercoiled or relaxed conformation. Angew Chem Int Ed 48(29):5282–5286
50. Sun X, Qian Z, Luo L, Yuan Q, Guo X, Lei T, Wei Y, Xing W (2016) Antibacterial adhesion of polymethyl methacrylate modified by borneol acrylate. ACS Appl Mater Interfaces 8(42):28522–28528
51. Xu J, Bai Y, Wan M, Liu Y, Tao L, Wang X (2018) Antifungal paper based on a polyborneolacrylate coating. Polymers 10(4):448
52. Wang X, Jing S, Liu Y, Liu S, Tan Y (2017) Diblock copolymer containing bioinspired borneol and dopamine moieties: synthesis and antibacterial coating applications. Polymer 116:314–323
53. Wu J, Wang C, Mu C, Lin W (2018) A waterborne polyurethane coating functionalized by isobornyl with enhanced antibacterial adhesion and hydrophobic property. Eur Polym J 108:498–506
54. Krizova H, Wiener J (2013) Development of carboxymethyl cellulose/polyphenols gels for textile applications. Autex Res J 13(2):33–36
55. Tankhiwale R, Bajpai S (2009) Graft copolymerization onto cellulose-based filter paper and its further development as silver nanoparticles loaded antibacterial food-packaging material. Colloids Surf B Biointerfaces 69(2):164–168
56. Gemili S, Yemenicioğlu A, Altınkaya SA (2009) Development of cellulose acetate based antimicrobial food packaging materials for controlled release of lysozyme. J Food Eng 90(4):453–462
57. Petersen N, Gatenholm P (2011) Bacterial cellulose-based materials and medical devices: current state and perspectives. Appl Microbiol Biotechnol 91(5):1277
58. Cherian BM, Leão AL, de Souza SF, Costa LMM, de Olyveira GM, Kottaisamy M, Nagarajan E, Thomas S (2011) Cellulose nanocomposites with nanofibres isolated from pineapple leaf fibers for medical applications. Carbohydr Polym 86(4):1790–1798
59. Dautzenberg H, Schuldt U, Grasnick G, Karle P, MÜller P, LÖhr M, Pelegrin M, Piechaczyk M, Rombs KV, GÜnzburg WH (1999) Development of cellulose sulfate-based polyelectrolyte complex microcapsules for medical applications. Ann N Y Acad Sci 875(1):46–63
60. Ude S, Arnold DL, Moon CD, Timms-Wilson T, Spiers AJ (2006) Biofilm formation and cellulose expression among diverse environmental Pseudomonas isolates. Environ Microbiol 8(11):1997–2011
61. Feese E, Sadeghifar H, Gracz HS, Argyropoulos DS, Ghiladi RA (2011) Photobactericidal porphyrin-cellulose nanocrystals: synthesis, characterization, and antimicrobial properties. Biomacromolecules 12(10):3528–3539
62. Xu J, Zhao H, Xie Z, Ruppel S, Zhou X, Chen S, Liang JF, Wang X (2019) Stereochemical strategy advances microbially antiadhesive cotton textile in safeguarding skin flora. Adv Healthc Mater 8(12):1900232
63. Kumar MNR (2000) A review of chitin and chitosan applications. React Funct Polym 46(1):1–27
64. Rinaudo M (2006) Chitin and chitosan: properties and applications. Prog Polym Sci 31(7):603–632
65. Elsabee MZ, Abdou ES (2013) Chitosan based edible films and coatings: a review. Mater Sci Eng C 33(4):1819–1841
66. Ilium L (1998) Chitosan and its use as a pharmaceutical excipient. Pharm Res 15(9):1326–1331
67. Kumar MR, Muzzarelli RA, Muzzarelli C, Sashiwa H, Domb A (2004) Chitosan chemistry and pharmaceutical perspectives. Chem Rev 104(12):6017–6084
68. Fu YN, Li Y, Li G, Yang L, Yuan Q, Tao L, Wang X (2017) Adaptive chitosan hollow microspheres as efficient drug carrier. Biomacromolecules 18(7):2195–2204
69. Shahidi F, Arachchi JKV, Jeon Y-J (1999) Food applications of chitin and chitosans. Trends Food Sci Technol 10(2):37–51
70. Wang H, Qian J, Ding F (2018) Emerging chitosan-based films for food packaging applications. J Agric Food Chem 66(2):395–413

71. Naz F, Zuber M, Zia KM, Salman M, Chakraborty J, Nath I, Verpoort F (2018) Synthesis and characterization of chitosan-based waterborne polyurethane for textile finishes. Carbohydr Polym 200:54–62
72. Dutta P, Tripathi S, Mehrotra G, Dutta J (2009) Perspectives for chitosan based antimicrobial films in food applications. Food Chem 114(4):1173–1182
73. Lang G, Clausen T (1989) The use of chitosan in cosmetics. Chitin and chitosan. Sources, Chemistry, Biochemistry. Physical Properties and Applications, vol 139. Elsevier Applied Science, London and New York, p 147
74. Jimtaisong A, Saewan N (2014) Utilization of carboxymethyl chitosan in cosmetics. Int J Cosmet Sci 36(1):12–21
75. Liu N, Chen X-G, Park H-J, Liu C-G, Liu C-S, Meng X-H, Yu L-J (2006) Effect of MW and concentration of chitosan on antibacterial activity of Escherichia coli. Carbohydr Polym 64(1):60–65
76. Sudarshan N, Hoover D, Knorr D (1992) Antibacterial action of chitosan. Food Biotechnol 6(3):257–272
77. Kong M, Chen XG, Xing K, Park HJ (2010) Antimicrobial properties of chitosan and mode of action: a state of the art review. Int J Food Microbiol 144(1):51–63
78. Vaz JM, Pezzoli D, Chevallier P, Campelo CS, Candiani G, Mantovani D (2018) Antibacterial coatings based on chitosan for pharmaceutical and biomedical applications. Curr Pharm Des 24(8):866–885
79. Foster LJR, Butt J (2011) Chitosan films are NOT antimicrobial. Biotechnol Lett 33(2):417–421
80. Pinto RJ, Fernandes SC, Freire CS, Sadocco P, Causio J, Neto CP, Trindade T (2012) Antibacterial activity of optically transparent nanocomposite films based on chitosan or its derivatives and silver nanoparticles. Carbohydr Res 348:77–83
81. Fu J, Ji J, Fan D, Shen J (2006) Construction of antibacterial multilayer films containing nanosilver via layer-by-layer assembly of heparin and chitosan-silver ions complex. J Biomed Mater Res A 79(3):665–674
82. Jia Z, Xu W (2001) Synthesis and antibacterial activities of quaternary ammonium salt of chitosan. Carbohydr Res 333(1):1–6
83. Vallapa N, Wiarachai O, Thongchul N, Pan J, Tangpasuthadol V, Kiatkamjornwong S, Hoven VP (2011) Enhancing antibacterial activity of chitosan surface by heterogeneous quaternization. Carbohydr Polym 83(2):868–875
84. Costa F, Carvalho IF, Montelaro RC, Gomes P, Martins MCL (2011) Covalent immobilization of antimicrobial peptides (AMPs) onto biomaterial surfaces. Acta Biomater 7(4):1431–1440
85. Xin Y, Zhao H, Xu J, Xie Z, Li G, Gan Z, Wang X (2019) Borneol-modified chitosan: antimicrobial adhesion properties and application in skin flora protection. Carbohydr Polym 228:115378
86. Hegab HM, Elmekawy A, Zou L, Mulcahy D, Saint CP, Ginic-Markovic M (2016) The controversial antibacterial activity of graphene-based materials. Carbon 105:362–376
87. Zou X, Zhang L, Wang Z, Luo Y (2016) Mechanisms of the antimicrobial activities of graphene materials. J Am Chem Soc 138(7):2064–2077
88. Zhu J, Wang J, Hou J, Zhang Y, Liu J, Bruggen BV (2017) Graphene-based antimicrobial polymeric membranes: a review. J Mater Chem A 5(15):6776–6793
89. Li G, Zhao H, Hong J, Quan K, Yuan Q, Wang X (2017) Antifungal graphene oxide-borneol composite. Colloids Surf B Biointerfaces 160:220–227
90. Dai X, Guo Q, Zhao Y, Zhang P, Zhang T, Zhang X, Li C (2016) Functional silver nanoparticle as a benign antimicrobial agent that eradicates antibiotic-resistant bacteria and promotes wound healing. ACS Appl Mater Interfaces 8(39):25798–25807
91. Jia Z, Shi Y, Xiong P, Zhou W, Cheng Y, Zheng Y, Xi T, Wei S (2016) From solution to biointerface: graphene self-assemblies of varying lateral sizes and surface properties for biofilm control and osteo-differentiation. ACS Appl Mater Interfaces 8(27):17151–17165
92. Li G, Quan K, Xu C, Deng B, Wang X (2018) Synergy in thrombin-graphene sponge for improved hemostatic efficacy and facile utilization. Colloids Surf B Biointerfaces 161:27–34

93. Motealleh A, Hermes H, Jose J, Kehr NS (2018) Chirality-dependent cell adhesion and enrichment in Janus nanocomposite hydrogels. Nanomed Nanotechnol Biol Med 14(2):247–256
94. Guo Y, Yao R, Wang Z, Zhang Y, Cui M, Zhao Q, Wang H (2018) Novel potential type electrochemical chiral recognition biosensor for amino acid. J Solid State Electrochem 22(1):41–49
95. Feng L, Xu B, Ren J, Zhao C, Qu X (2012) A human telomeric DNA-based chiral biosensor. Chem Commun 48(72):9068–9070
96. Bommarius AS, Schwarm M, Drauz K (1998) Biocatalysis to amino acid-based chiral pharmaceuticals—examples and perspectives. J Mol Catal B: Enzym 5(1-4):1–11
97. Wistuba D, Schurig V (2000) Enantiomer separation of chiral pharmaceuticals by capillary electrochromatography. J Chromatogr A 875(1-2):255–276
98. Evans SE, Davies P, Lubben A, Kasprzyk-Hordern B (2015) Determination of chiral pharmaceuticals and illicit drugs in wastewater and sludge using microwave assisted extraction, solid-phase extraction and chiral liquid chromatography coupled with tandem mass spectrometry. Anal Chim Acta 882:112–126

Bioinspired Interfaces for the Management of Skin Infections

Sandra L. Arias, Joshua Devorkin, Ana Civantos, Camilo Jaramillo, and Jean Paul Allain

Abstract The percutaneous invasion of microorganisms through damaged skin layers can lead to the onset of infections with potentially life-threatening complications, especially in vulnerable populations like newborns, elderly, and diabetic patients. With the emergence of superbugs that are resistant to almost all the available antibiotics and the unfruitful discovery of new antimicrobial compounds in the last few decades, there is a demand for novel engineering strategies to approach skin and soft tissue infections associated with the used of biomaterials. Naturally occurring anti-biofouling and antimicrobial interfaces based on spatial structure offer an unprecedented opportunity for biomaterial design, as they do not contribute to bacterial resistance, do not pollute the environment, and can be easily implemented in a variety of biomaterial interfaces. In this article, we review the complications caused by biomaterials in contact with the skin, especially those that compromise medical adhesives, sutures, and wound dressing materials. Then, we introduced bioinspired designs that can be implemented in those materials based on nano- and microscale topographies.

Keywords Skin microbiome · Skin and soft tissue infections · Bioinspired · Biofilms · Bactericidal nanostructures · Microtopography · Nanotopography · Nanofabrication · Medical adhesives · Bandages · Tapes · Sutures · Wound dressing

S. L. Arias (✉)
Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY, USA
e-mail: ariassu2@illinois.edu

J. Devorkin
Pritzker School of Law, Northwestern University, Chicago, IL, USA

A. Civantos · C. Jaramillo · J. P. Allain
Ken and Mary Alice Lindquist Department of Nuclear Engineering, Pennsylvania State University, State College, PA, USA

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_20

Introduction

The skin is the largest organ in the human body and serves several functions including thermoregulation, sensation, maintenance of internal homeostasis, and protection against harsh environmental aggressors, such as harmful substances or pathogens [1]. The skin hosts numerous species of fungus, viruses, and about 1000 different species of bacteria, that constitute together the skin microbiome. The distribution and composition of these microbial flora is driven by many factors, including the skin topography (e.g., thickness and density of glands and hair follicles), the host physiology (e.g., age and sex), the environment (e.g., climate), and the immune system [1, 2]. Those factors together affect the type of microorganisms that can colonize the epithelial surface, which can further act competitively to exclude one another or synergistically for mutual benefit [3]. For example, commensal strains such as *Staphylococcus epidermidis* have been shown to cooperate with the host's immune system to prevent the colonization of invasive microorganisms, ultimately reinforcing the epithelial barrier function [4]. It is now widely accepted that a broad community of protective bacteria normally habits the healthy skin and provides a host defense against pathogens. Indeed, alterations in the microbiome composition have been associated with the delay in the healing process of cutaneous injuries and the onset of some disease states [5].

The skin barrier can be disrupted because of cutaneous puncturing during routine medical procedures (e.g., the insertion of invasive devices like intravenous catheters), in chronic wounds like burns and other cutaneous lesions. It can also be broken as a consequence of an imbalance between commensal and invasive microbes in diseases such as atopic dermatitis. Similarly, continuous skin stripping associated with the removal of dressing materials and adhesives can also affect the epithelial layers beneath the biomaterial, particularly in chronically ill patients and vulnerable groups like newborns and elderly, where the skin is immature and susceptible to trauma [6]. The percutaneous invasion of microorganisms through damaged skin layers especially in diabetic, immunocompromised, and vulnerable populations, can lead to the onset of infections with potentially life-threatening complications, including bacterial dissemination to other body sites and bacteremia [7]. A retrospective study performed in California between 2005 and 2011 for patients with a primary diagnosis of skin and soft tissue infections (SSTIs) found that in both ambulatory and health care settings the incidence of SSTIs were almost twice that of urinary tract infections and tenfold of that of pneumonia [8]. SSTIs are commonly associated with exudates, ulcerations, fluid collection, or abscesses; whereas the bacterial strains often isolated from complicated SSTIs include *Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus* spp., *Escherichia coli*, and other Enterobacteriaceae [9, 10]. In chronic wounds, for example, the nutritional composition of the wound exudate is ideal for the growth of bacteria (e.g., *S. aureus*), yeast (e.g., *Candida albicans*), and fungus (e.g., *Tinea* species) [7, 11]. Moreover, foreign materials brought into contact with broken epithelial layers can further exacerbate the foci of infection, because the material's surface can serve as a substrate and

shelter for bacterial colonization and growth [7]. The likelihood of skin erosion and inflammation can also be increased by occlusive adhesive materials, which can trap moisture and contribute to fungal and bacterial proliferation [12].

A key issue in the treatment of skin infections and restoration of skin integrity is the presence of bacterial biofilms [10]. Biofilms are microbial communities encased by an extracellular polymeric substance that provide microbes with increased resistance to harsh environments such as a high concentration of antibiotics and desiccation. Biofilms challenge the treatment of wound infections either by conventional administration of antibiotics or by topic application of antimicrobial ointments and are one of the significant causes of the emergence and dissemination of antibiotic-resistant strains. Indeed, the spread of multidrug-resistant organisms have increased over the past 50 years, and there is little evidence that bacterial resistance to antibiotics will go away [13, 14]. Multidrug-resistant pathogens not only threaten our ability to control infections with antibiotics, but also lead to clinical and economic adverse outcomes, encompassing treatment failure with potential patient death, and increased health care costs linked to disease management, respectively [13]. In the context of skin infections associated with the use of biomaterials, conventional treatments rely on platforms that elude antibiotics, nanoparticles (NPs), or biocides agents at the infected site. Clinical treatments usually lack specificity against the infection-causing microorganisms, and thus, eradicating commensal bacteria that is necessary for the fitness of the skin, and contributing to the selection of increasingly resistant strains. Among the antimicrobial agents, NPs have gained attention due to their easy fabrication and broad-spectrum activity; however, the long-term effects associated with the use of NPs in human health and the environment remain to be seen. Preliminary reports have indicated that NPs can travel and gain access to organ systems and affect the biological function at the tissue, cellular, subcellular, and proteins levels [15].

In the last few decades, due to the emergence of superbugs that are resistant to almost all of the available antibiotics and the unfruitful discovery of new antimicrobial compounds [14], have led to the search of novel engineering strategies to treat skin and soft tissue infections. From the material science perspective, naturally occurring anti-biofouling and antimicrobial interfaces as well as the dynamic interactions between microbial communities and their niches, offer inspiration for the design of material interfaces that can target pathogenic bacteria while protecting the beneficial microbiome of the skin. Bioinspired anti-biofouling interfaces compromise physical and chemical barriers, and the symbiotic interactions between microbe–host and microbe–microbe (Fig. 1). Physical barriers consist of the natural components of the immune system capable of disrupting biofilm formation, killing bacteria by contact, or preventing the adhesion of microbes to the underlying surface by physical means. On the contrary, chemical barriers endure antimicrobial agents including Q-sensing quenchers, peptides, and enzymes, which are secreted by the host. A better understanding of the ecological interactions between microorganisms and their niches (e.g., abiotic surfaces, host, and interactions between microbes) can also lead to the discovery of new routes to treat lesions on the skin caused by pathogenic microorganisms.

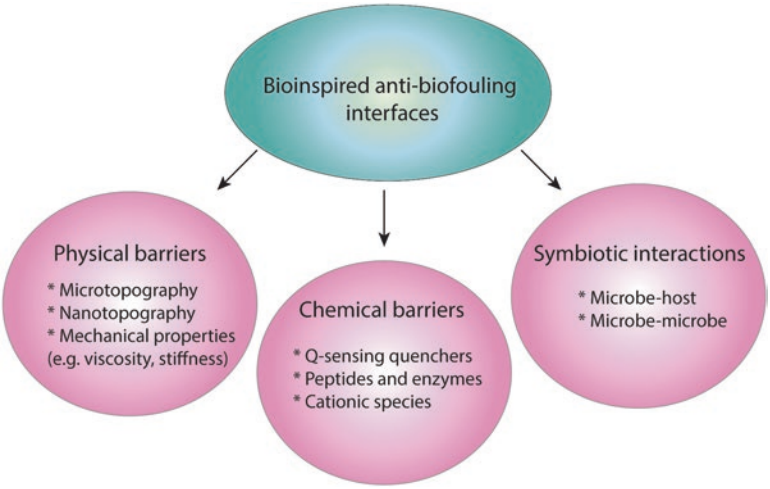


Fig. 1 Bioinspired anti-biofouling interfaces can be divided into three main subcategories: physical and chemical barriers and symbiotic interactions. (i) Physical barriers are commonly found in the skin of animals, insects, and plants in the form of micro and nanoscale structures, as well as viscous mucus layers covering the epithelium. (ii) Chemical barriers compromise antimicrobial compounds excreted by the host. (iii) Symbiotic interactions favor the retention of favorable bacteria in the niche while limiting the resources available for pathogenic microbes

The advantage of physical barriers over chemical ones is that the former does not contribute to bacterial resistance, do not pollute the environment, and can be easily implemented in a variety of materials of clinical importance. In many ecological niches, physical and chemical barriers and symbiotic associations exist together as the first aid of defense against invasive pathogens. One example of this is the structure and composition of the mucus layer that covers mucosal tissues like the gastrointestinal, respiratory, reproductive, and urinary tracts. Mucin glycoproteins, the major constituent of the mucus layer, are responsible for the viscous and gel-like appearance of the mucus layer, which serves as a reservoir for a variety of antimicrobial molecules produced by the host [16]. The viscosity of the mucus glycoproteins has been implicated in preventing the adhesion of the bacteria to the underlying epithelium by promoting the motility in their planktonic state and inhibiting their aggregation into biofilms [17]. Similarly, the array of microbial agents embedded in the mucus layer favor the retention of commensal bacteria, while limiting the niche available for invasive microorganisms [16].

In this chapter, the state of the art on skin infections associated with the use of medical adhesives, sutures and wound dressing materials are briefly introduced. Bioinspired strategies based on antimicrobial and anti-biofouling topographies that can be implemented on those materials is critically reviewed here. Whenever possible, we place emphasis on anti-biofouling and antimicrobial properties of physical barriers found in the innate immune system of animals, insects, or plants.

Skin Lesions Associated with Biomaterials in Contact with the Skin

Medical Adhesives and Surgical Sutures

In recent years, tremendous advances have been taking place in the design of health monitoring and wearable medical devices that incorporate flexible and lightweight polymeric substrates. Those can conform to the surface of the skin while providing a noise-free, sensitive, and accurate monitoring of body signals such as temperature, heart rate, respiration rate, and blood pressure [18]. Medical adhesives are one integral part of such diagnostic devices and exist in the form of patches, bandages, or tapes that are used to fix to the skin electrodes and sensors for noninvasive monitoring of vital signals. Medical adhesives are also used to fix intravenous catheters and other dwelling devices. While a variety of fabrication techniques and materials have been investigated to provide a strong adhesion and noise-free signal at the interface between skin/material [19], there is a lack of studies focusing on strategies that mitigate medical adhesive-related skin injuries. Those lesions include the removal of superficial epithelial layers and undesirable growth of microorganisms underneath medical adhesives (e.g., fungus and bacteria), which are common complications that negatively affect vulnerable populations such as newborns, elderly, and critically ill patients, and that are often referred to as "forgotten wounds" [6, 20]. For example, conventional tapes applied to secure intravenous access have been reported to traumatize the skin and favor the colonization of *Aspergillus Fumigatus* conidia in immunocompromised infants, causing acute fungal infections [21]. Similarly, the application of skin protectants (such as Stomadhesive®) was implicated in a 5-year outbreak of *S. aureus* in a neonatal unit in the UK. In this study, in vitro Staphylococcal desiccation experiments indicated that the hydrated niche provided by Stomadhesive® on the skin plus the high content of sweat and other secretions absorbed by the material prevented bacterial desiccation and allowed the survival of *S. aureus* for up to 71 days [22]. In another study performed in patients aged 65 or older for 8 weeks, results indicated that the application of medical adhesive tapes resulted in contact dermatitis in 70.6 % of the cases, while trauma and infection had an incidence of the 20.6% and 8.8%, respectively. Because skin injuries in elderly groups take longer to heal, secondary infections have the potential to develop as a complication [23].

In neonate health care settings, skin lesions promoted by medical adhesives and other materials are well documented and include contact dermatitis, disruption of the epithelial layers as result of adhesive removal and skin infections (Fig. 2) [24, 25]. Moreover, it has been reported that neonates are at a higher risk of percutaneous invasion of microbes through broken skin layers, especially when they are kept inside incubators, which offer a moist and warm habitat for microbial growth and survival. The consequences of infection in neonates include secondary infections, additional days of hospitalization, increased risk of mortality, and a higher prevalence of a poor neurodevelopmental outcome [25, 26].

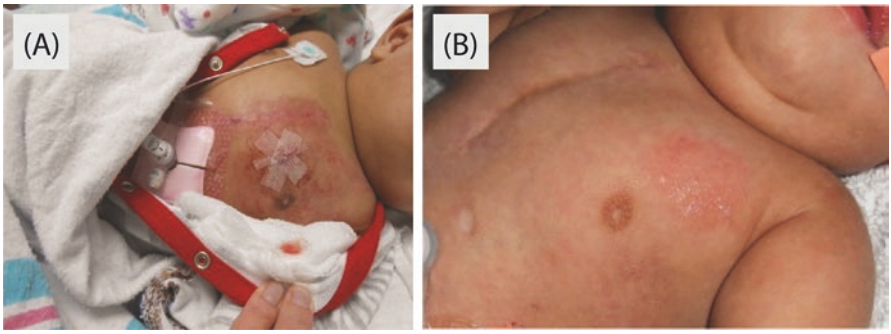


Fig. 2 Contact dermatitis caused by medical adhesives in two infants. (**A**) Contact dermatitis reaction caused by transparent adhesive dressing, (**B**) hydrogel in EKG electrodes. (Image source: [27])

Besides the fixation of biomedical devices to the skin, medical adhesives can also be used for wound closure; however, in some scenarios, the medical adhesive does not support the required strength to facilitate wound closure. In this case, the surgical incision may require the use of sutures (for internal skin layers) or the combination with medical adhesives to ensure proper wound healing. Sutures are stranded materials used to join tissue edges and help in the closure and healing process of broad and deep wound incisions, as well as subcuticular openings [28, 29]. The use of linen as a suture material date more than 4000 years ago [30], along with a variety of other types of materials including silver, gold, iron, silk, cotton, steel wires, and even animal gut and hair [31]. It is estimated that about 234 million surgical operations are performed worldwide every year including surgical incisions, which requires the use of sutures. However, sutures are commonly associated with surgical site infections (SSI). The prevalence of SSI is around 5% and are responsible for increased patient morbidity, secondary infections, and more extended days in the hospital [32, 33]. According to the American Medical Association, between 2006 and 2009, 1.9% of surgical procedures were complicated by SSI in the USA alone [34]. In 2005 and 2002, the estimated cost of SSI was calculated in $10,443 to $25,546 US dollars each year, respectively. Indeed, this cost was superior when resistant microorganisms to antibiotics were present at the incision site, or if prosthetic joint implants were involved [35].

SSI has been classified into three different categories: superficial incisional, deep incisional, and intracavitary. Depending on the type of SSI, various procedures may be used to solve or reduce the infection such as reopening, draining, adjuvant antibiotic treatment, or even a new surgical intervention [36]. It has been suggested that an ideal suture should accomplish multiple requirements: suitable bending strength and mechanical properties, easy sterilization and manipulation, good tissue biocompatibility (lack of allergies, nontoxic leakages, noncarcinogenic), and proper biodegradability. Moreover, suture materials should avoid the adhesion of bacteria to reduce the risk of infections and skin complications [29]. To prevent bacterial infections, two main strategies have been performed on suture materials: (1) the use of cationic polymers that modify the surface chemistry, called passive coatings, and

(2) the functionalization with drugs and molecules, which are released from the suture. However, surface modification by introducing micro- or nanotopography features has received increased attention because several studies have reported that nano- and microscale topographies can impair bacterial attachment [37].

Wound Dressing Materials

In the USA alone, chronic wounds are estimated to affect 1–2% of the whole population, estimated at about 25 billion dollars per year [38, 39]. The physical barrier and sensory system of the skin's functional control includes a limiting capability to prevent microbial infections and cellular regeneration. An abrasion to the skin due to physical or chemical means, rupturing the epidermis, is defined as a wound. For the structure and sensory functions to be regained, a novel wound dressing, "artificial skin," should provide a barrier to the external environment ultimately leading to accelerated tissue regeneration that in parallel limits bacterial infections. Initially, the first stage of infection is dominated by Gram-positive organisms (*S. aureus, Streptococcus pyogenes*); however, as the wound progresses, Gram-negative bacteria (*E. coli, P. aeruginosa*) are found when the wound becomes chronic [40]. The skin's physical barrier protects from the external stimuli by activating the natural immunity system. After an abrasion to the skin, the migration of microbial organisms compromises the body's natural self-defense system, limiting the function of the fibroblasts and collagen production. The tissue scaffold from the previous wound area begins to form about 3 days after the initial wound due to fibroblasts and production of collagen and ground substance [41]. Despite the advancements and progress in wound dressing applications, current dressing technologies are limited by their susceptibility to bacterial infections while promoting healthy cellular regeneration.

Different research has highlighted the possibility of wounds developing from different materials, natural or synthetic, with various physical forms (films, foams, hydrogels, hydrocolloids). For example, cotton gauze is used primarily to treat shallow wounds and are favorable due to the low cost, availability, and easy use. However, gauze only provides the wound with limited antimicrobial and poor wetting properties. The inflammatory response and reepithelization are poorly affected by dry gauze due to the oxygen environment under the gauze [42]. Limited by bacterial growth and contamination, cotton gauze was coated with chitosan-Ag-ZnO to increase the antimicrobial properties [43]. The coated gauze was able to retain water, showed an increase in drying time and antibacterial efficiency. Foam or sponges provide an alternative to gauze characterized by their absorbance of massive wound exudate suitable for deep wounds and minor burns [44]. Hydrogels are characterized by their three-dimensional networks, maintain moisture levels in the wound environment, high absorbance, and tailorable mechanical properties. The macromolecular networks produced by chemical or physical crosslinking of suitable polymers give rise to biocompatible dressings sensitive to the physiological

environments, wettability, and flexibility. However, hydrogels have been reported to display weak mechanical properties requiring a secondary dressing or a surface modification necessary to make it a suitable wound dressing [45].

Nanotechnology-based wound dressings offer an approach to target the rising complexity of the cellular regeneration process as well as provide solutions to microbial infections. Several nanotechnology approaches for the formation of wound dressings have been tested including electrospinning, self-assembly, and phase separation techniques that allows for polymeric scaffolds to mimic properties of the extracellular matrix (ECM), including their fibrous nature and unique nanoscale features [46]. Current research has highlighted electrospinning as a suitable technique for nanofiber fabrication due to the ability to produce well-defined porous networks analogous to those of the ECM. Electrospinning, a voltage driven process, uses an applied electrical force where droplets or fibers are extruded from the soluble polymer that can produce mechanically strong pours networks with high aspect ratios. Electrospun PLGA/silk fibroin to create scaffolds with improved biocompatibility that showed improved attachment and proliferation of fibroblasts for a fast and healthy regeneration process of the skin [47]. Electrospinning thus provides the required nanotopography for a suitable cellular regeneration process through the production of porous networks.

Bioinspired Physical Barriers

High Aspect Ratio Bactericidal Nanostructures

Nature has been making high aspect ratio (HAR) nanostructures with bactericidal properties for millions of years. Those topographies can be found in the cuticles of certain insects like the dragonfly and cicada wings, and the skin of animals such as a gecko (Fig. 4a). The topography of the naturally occurring bactericidal surfaces has been replicated into a variety of materials such as silicon [48], poly(methyl methacrylate) (PMMA) [49, 50], and diamond [51], and all of them have shown a bactericidal activity similar to that of their native natural counterparts. Thus, and although the data are still incomplete, various lines of evidence are consistent with the hypothesis that bactericidal properties of HAR nanostructures are due to a contact killing mechanism that involves the physical disruption of the bacterial cell envelope, and that is independent of the surface composition. However, how envelope damage occurs, and how the membrane layer (s) are affected remains unclear. In Gram-negative bacteria, the cell envelope is formed by a thin peptidoglycan wall sandwiched between the plasma and outer membrane, whereas in Gram-positive bacteria, the cell envelope is made of a thick peptidoglycan layer and a plasma membrane only. It has been long believed that the peptidoglycan wall is the major responsible for the mechanical properties of the bacterial cell envelope; however, this concept has recently challenged by showing that the outer membrane in Gram-negative bacteria can be stiffer than the peptidoglycan wall [52].

At least three mechanisms have been proposed to explain the bactericidal activity of HAR nanostructures based on experimental observations (Fig. 3b, c). Using yeast cells (i.e., *Saccharomyces cerevisiae*) seeded on the wings of cicada and dragonfly species, Nowlin et al. [53] showed that the strength of the cell–substrate interaction and the nanostructure geometry were critical for the reduction of cell viability, and

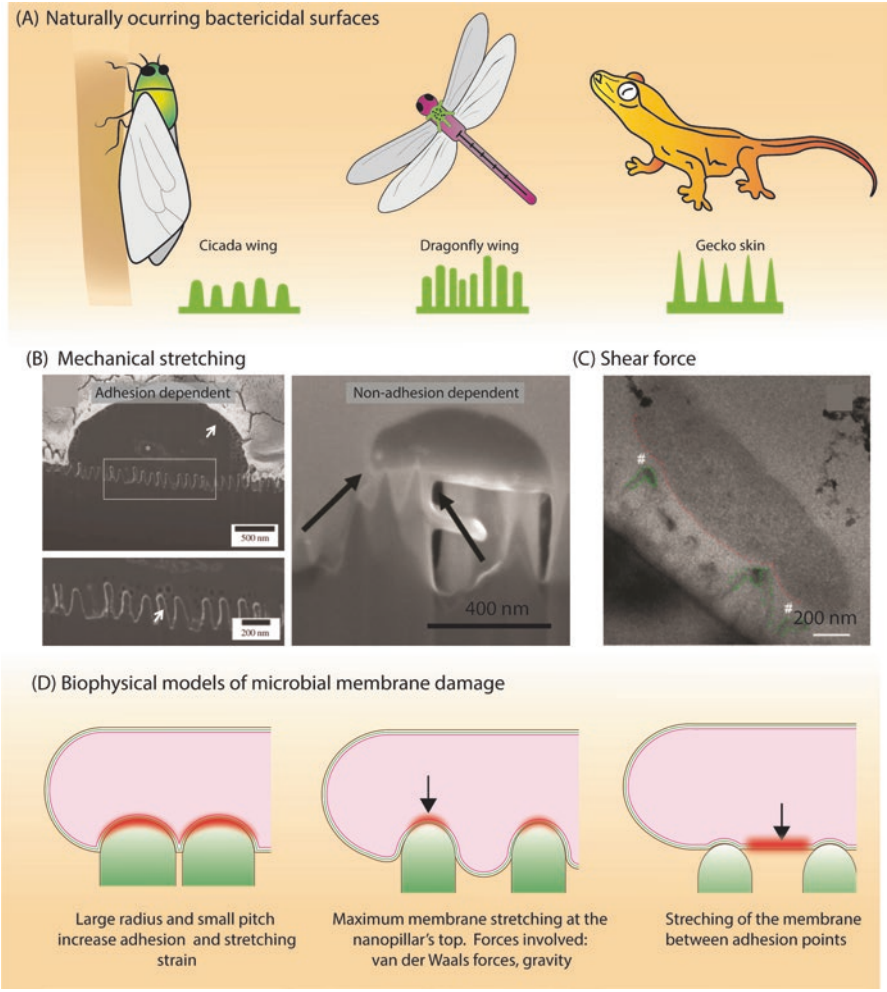


Fig. 3 Naturally occurring bactericidal surfaces are characterized by high aspect ratio nanostructures. (**A**) High aspect ratio (HAR) bactericidal nanostructures can be found in the cuticles and skin of some insects and animals. Based on experimental observations, at least three mechanisms are proposed for the observed bactericidal activity of HAR nanostructures: (**B**) mechanical stretching of the membrane with or without cell adhesion [48, 53], and (**C**) shear forces that tear apart the outer membrane [55]. (**D**) Proposed biophysical models of microbial membrane damage depicting the cell envelope in Gram-negative bacteria

that stronger adhesion forces induced greater physical damage of the wall membrane in *S. cerevisiae*. Micrographs, showing the interface between yeast cells and the dragonfly wing surface, clearly depicted the extension of the yeast wall membrane into the underlying topography, as indicated by the presence of the vacuoles on the space separating the nanopillars (Fig. 3b). Thus, cell lysis was believed to occur as a result of the mechanical stretching induced by the cell/substrate adhesion strength at the top of the nanostructures. In another set of experiments, Ivanova et al. [54] prepared hydrophilic nanocones of variable height on silicon by increasing the exposure time of the material to plasma etching. This etched silicon was shown to be hydrophilic. Thus, a set of nanostructured silicon was further modified with a thin layer of silane to yield hydrophobic substrates. Both hydrophilic and hydrophobic nanostructured silicon were then tested against *S. aureus* and *P. aeruginosa.* Their results indicated that the adhesion affinity of bacterial cells to the nanostructured surface did not play a pivotal role in the mechanistic killing of these bacterial cells (Fig. 3b). They proposed a mechanism also based on the mechanical stretching of the microbial wall membrane; however, in this case, cell adhesion to the underlying topography was neglected. The rupture of the cell wall was proposed to occur at the area suspending between attachment points, as mechanical stress overcomes the elasticity of the wall [48]. Moreover, based on the same mechanism, these authors have proposed that Gram-negative bacteria are more susceptible to cell lysis compared to Gram-positive species due to differences in the cell wall thickness, the latter being thicker.

Besides mechanical stretching of the microbial wall, other authors have suggested that shear forces are a key factor involved in the bactericidal mechanisms of HAR nanostructures. Bandara et al. [55] by studying the interaction between *E. coli* and dragonfly wing nanopillars, indicated that wall damage in *E. coli* occurred without direct contact with the topography (Fig. 3c). The nanopillars were proposed to tear the outer membrane as the bacteria attempted to move away from the unfavorable surface. The authors also suggested that under this condition, *E. coli* could secrete higher amounts of extracellular polymeric substance (EPS), which in turn increased the adhesion strength to the underlying topography [55]. However, bacterial lysis can occur only when the cell wall is compromised; therefore, cell integrity should be preserved even after outer membrane tearing. The production of EPS can indeed be an emergent behavior upon mechanical stress and has also been reported for *S. aureus* growing on nanopillared-Si surfaces [56]. Diu et al. [57] have also proposed that shear forces may be associated with the stronger bactericidal effects on nanowire titania surfaces observed against motile bacteria [57]. All of these experimental observations indicate that more than one mechanism involved in the disruption of the bacterial cell envelope on HAR nanostructures may exist. For example, simple differences in wall thickness do not explain the biocidal activity observed in a variety of microorganisms with marked differences in envelope composition, like those of eukaryotic microorganisms (e.g., yeast) and Gram-negative and Gram-positive bacterial cells when subject to the topographies such as those of dragonfly wings. A complete list of biocidal nanotopographies found in nature and obtained via synthetic means has been recently summarized in excellent reviews by other authors (see [58, 59]).

Like the mechanisms based on experimental observations, biophysical models have been proposed aiming to shed light on the bactericidal mechanism observed experimentally on HAR nanostructures. Li and Chen [50] have proposed a thermodynamic model based on the balance between the adhesion energy and deformation energy of the cell membrane. According to this model, nanopillars with a large radius and small spacing induce more substantial stretching degree on the cell envelope compared to those with a radius smaller than a critical value (Fig. 3c). It is proposed that thick nanopillars generate a drastic increase in the contact adhesion per unit of horizontal area, which is accompanied by an increase in the stretching strain of the wall [50]. However, experimental observations made on cicada wing surfaces have shown that sharper and higher number of nanopillars have enhanced killing activity against *Pseudomonas fluorescens* [60]. An alternative mathematical model using a classical elasticity theory framework has been proposed by Xue et al. [61]. In this model, only gravity force and geometrical parameters of the surface topography are considered and predicts that the maximum stretching of the bacterial envelope happens at the top of the nanopillars, thus smaller and sharper nanopillars generate greater stretching responses to bacteria resting on them [61]. Moreover, authors indicate that the physical interactions at the interface between the bacterial cell and the nanostructure, cannot provide enough energy to permit full cell adhesion. Instead, gravity and nonspecific forces such as van der Waals interactions play a crucial role in cell damage. However, this model does not account for the deformation of the membrane in *S. cerevisiae* observed by Nowlin et al. [53] in dragonfly wing surfaces.

Finally, another thermodynamic model based on the experimental observations made on *P. aeruginosa* in contact with cicada wing topographies has been proposed [62]. This model also suggests mechanical disruption of the microbial envelope, being the wall rigidity a key determinant in the bacterial sensitivity to the bactericidal nature of the cicada wing topographies. As a result, less rigid bacterial walls will be more affected by nanopillars as in the case of Gram-negative species, in which a single peptidoglycan layer composes the cell envelope compared to the much thicker wall in Gram-positive bacteria. It is suggested that when the bacterium absorbs onto the nanopillar structures, the cell wall breaks in the regions suspended between the pillars (Fig. 3d). In this model, the bending of the membrane between adhesion points is neglected (i.e., the stretching degree of the cell wall at the vertices of the nanopillars and suspending between the them is assumed to be the same), and no specific biological interactions play a significant role in cell lysis [62]. Despite the lack of clarity on the exact killing contact mechanism, all the biophysical models proposed till now agree upon the idea that bactericidal nanopillars do not pierce the microbial envelope, but instead, it involves some mechanical stretching. Indeed, both superhydrophobic and hydrophilic HAR nanostructures have been observed to induce bacterial lysis by mechanical rupture of the cell wall, pointing out that the surface morphology rather than an alteration in the interfacial energy is the major responsible for the observed bactericidal activity [54]. However, a common observation is that both superhydrophobic and hydrophilic HAR nanostructures yield lower bacterial adhesion compared to smooth substrates of similar chemical composition, probably due to a reduction in surface area available for bacterial attachment [63].

As mentioned above, the topographies of the dragonfly and cicada wings have been replicated in polymers such as PMMA or acrylic. However, high aspect ratio bactericidal nanostructures have not been yet achieved in softer polymers and hydrogels of clinical relevance, because HAR nanostructures tend to ground collapse in aqueous environments due to adhesion and capillary forces acting on them [64]. For example, HAR nanopillars fabricated on polydimethylsiloxane (PDMS) obtained by replica molding, collapse when the dimension of the nanopillars exceed an aspect ratio higher than six [64]. Even though HAR nanostructures have been fabricated in polyurethane (PU), there have yet been any reports regarding their mechano-bactericidal activity. Topographies inspired by the shark's skin has been reproduced in PDMS [65], but its effect on the bacterial viability and physiology is different from that evoked by HAR nanostructures (see section "Biofilm Control via Surface Micro and Nanotexture"). Like polymers with a low Young's modulus (few KPa), the fabrication HAR topographies in hydrogels at the nanoscale with precise control of the size and shape distribution capable of resisting capillary forces have remained challenging [64, 66]. Hydrogels possess mechanical characteristics like those of soft tissues, which make them a desirable interface with the skin. Plasma modification can address the needs for unique nanotopography in hydrogels and clinically relevant polymers by altering the surface morphology and chemical composition. Unlike chemical modifications, plasma treatment eliminates the high cost of chemicals and toxic by-products. For a successful wound dressing, for instance, there is a need for plasma surface modification to alter the properties of the material to improve the biocompatibility and increase the antimicrobial properties, leaving the bulk material unaffected. The bulk properties of the material can be a composite of materials that are known to have antimicrobial properties or other desirable properties. By manipulating the surface properties, the surface can be used to control the attachment and colonization of bacteria, maintain the wettability and tissue-like structure to improve compatibility and offer the shortest healing time.

In recent studies, we have employed plasma modification for nanostructure growth in a variety of natural polymers including bacterial cellulose, chitosan, and silk (Fig. 4a). Bacterial cellulose (BC) is a hydrogel produced at the interface liquid/air by *Acetobacter xylinum* and is commonly used as a wound dressing material for the treatment of burns and other skin lesions. BC has also been employed as a substitute for small blood vessels and as a platform for the design of magnetic hydrogels for endovascular reconstruction [67–69]. By treating bacterial cellulose with low-energy argon ions, we have observed the growth of nanocones at the surface of the material, which can resist heat and aqueous immersion. Preliminary results using *E. coli* and *Klebsiella pneumoniae* cultured on these nanostructured BC have been shown to kill these bacterial strains effectively via a contact killing mechanism similar to that of natural bactericidal nanostructures. We have observed maximum envelope stretching at the top of the nanocones, which may be linked to cell lysis and the observed bacterial death.

Similar to BC, chitosan has shown a remarkable response to plasma irradiation (Fig. 4b). Chitosan is a deacetylated derivative of chitin and one of the most abundant natural polymers. Chitosan is known for its biocompatibility, biodegradability,

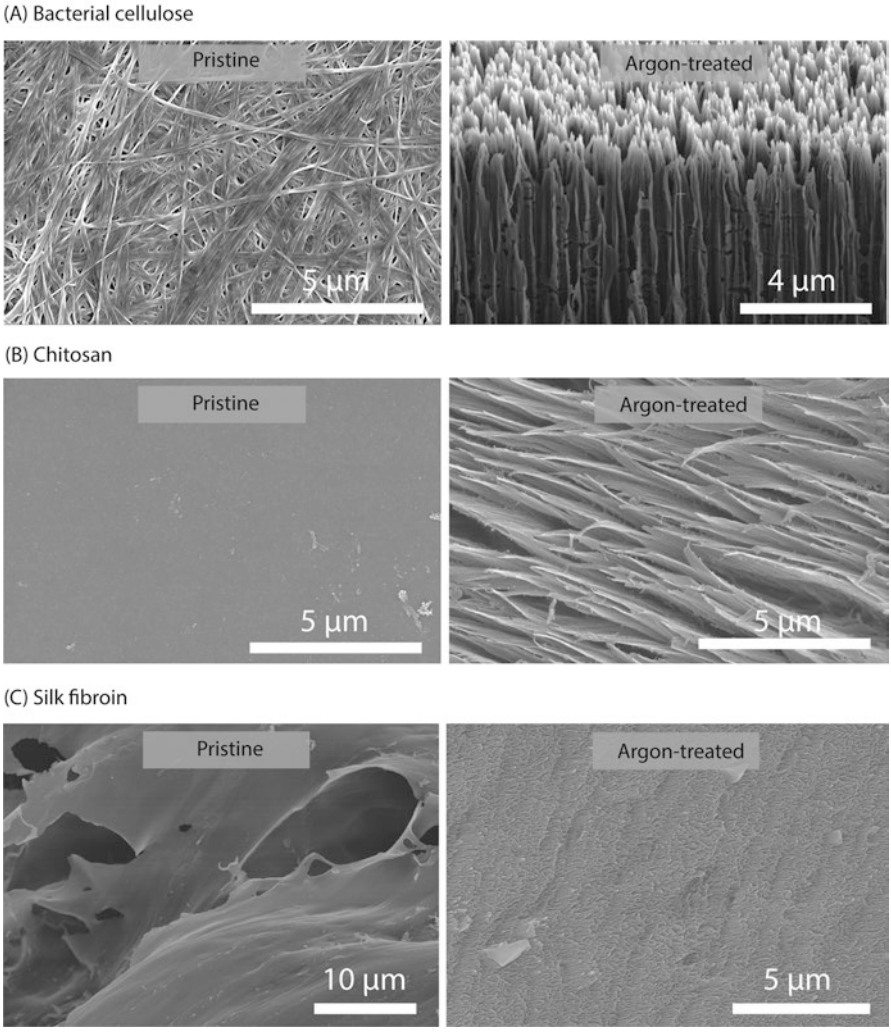


Fig. 4 Argon plasma irradiation induces nanopillar growth in bacterial cellulose and chitosan. (**A**) Pristine or unmodified bacterial cellulose (BC) is composed of interlaced fibers. After treatment with argon plasma, nanocones evolved in the surface of the material. (**B**) Chitosan is characterized by a relatively smooth surface. Similar to BC, it develops high aspect nanostructures whose orientation and height depend on the irradiation parameters. (**C**) Pristine silk reveals a heterogeneous surface at the microscale. When silk is irradiated with argon plasma, it develops oriented nanopillars capable of impairing bacteria adhesion. All the panels correspond to scanning electron micrographs

non-toxicity, antimicrobial properties, and biofunctionality. Its applications include coatings for bone implant materials [70, 71], wound dressings [72] and carrier for drug delivery [73]. We have observed the formation of nanofeatures on chitosan upon plasma irradiation. The type and dimensions of these features have been

shown to be dependent on the irradiation parameters. The activity of cultured bacteria on these structures was studied. Surfaces with high aspect ratio nanopillars, triggered by high fluence (i.e., number of ions impacting the surface per unit area) irradiations, showed prominent bactericidal behavior. Such antibacterial activity was observed against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria. Observations on these results showed a reduced presence of bacteria and increased death on the surface with increasing HAR nanopillars (unpublished data by the authors).

The formation of HAR nanostructures is not unique to polysaccharides such as BC and chitosan, but instead can also be fabricated on natural proteins such as silk (Fig. 4c). Silk fibroin, which is produced by silkworms (e.g., *Bombyx mori*) and spiders (e.g., *Nephila clavipes*), is a biocompatible and biodegradable protein, with tailorable mechanical properties. Thanks to these properties, silk has been used in sutures, scaffolds for tissue engineering, and the regeneration of bone, tendon, skin, and in peripheral nerve restoration [74, 75]. Because surface properties modulate the immune response of biomaterials and can control microbial infections, we have been introducing nanopillars into silk by plasma modification to enhance the cellular response and the bactericidal capabilities of this material.

Biofilm Control via Surface Micro- and Nanotexture

The manifestation of microbial association into biofilms either as a free-floating or surface-bound community is abundant in nature, as we see the formation of biofilms in almost every habitat on earth ranging from the plaque that grows on the surface of our teeth to the biofilms that form in the bottom of the ocean. The key benefits of microbial aggregation compared to a planktonic lifestyle lie in that biofilms provide to microbial communities increased ecological stability, cooperative and collective social behaviors, and protection against environmental challenges [76]. Bacterial attachment to surfaces is believed to occur in two main stages. During the first stage, bacterial cells are loosely attached or close to the surface. Bacteria can easily spin, vibrate, and return to the planktonic state. The primary forces dominating this reversible attachment are van der Waals forces, electrostatic forces, and hydrophobic interactions. The second stage is characterized by an irreversible attachment of the bacteria to the substratum, which is assisted by exopolysaccharides, and by adhesins such as pili and flagella [77].

One of the most outstanding puzzles in studies of microbial/surface interactions has been to understand how surface properties affect microbial life. This has been difficult given the complexity and variations in the composition of the outer membrane among microbial species, the impact of external factors such as the medium composition and pH in the microbial and surface properties, and the difficulty in isolating the surface parameters in material interfaces. Many studies, for example, have investigated the low adhesion and anti-biofouling capabilities of a variety of leaves, including those of the rice, taro, and lotus plants based on their low-adhesion

and hydrophobic nature, but these features seem to be more critical on water immersed environments than on aerial habitats [59]. The consensus is that microorganisms can colonize any surface sooner or later and that even hydrophobic surfaces experienced microbial growth.

However, the investigation on microbial communities in a variety of ecological niches has indicated that the spatial structure of the microenvironment has essential implications on bacterial growth and biofilm structure. For example, the spatial variation and heterogeneity of the leaf surface in plants such as composition, thickness, permeability, and topography drives the nonrandom microscale spatial distribution of microorganisms and its interaction with other microbial colonizers [78]. This compartmentalized microbial distribution is not unique to the leaves in plants, but instead is a common feature in a variety of ecological niches. Regional differences in skin anatomy, for example, contribute to the selection of a unique set of microorganisms adapted to inhabit specific body sites [1].

In micropatterned arrays, bacteria have been observed to organize spontaneously and follow the symmetry of the underlying topography independently of the surface composition, especially when the characteristic dimensions of the topographical features become comparable to those of the bacterial cells. A variety of geometries have been considered, and all of them support the idea that bacteria tend to maximize the adhesion area when presented with features at the micro- and nanoscale. Bacterial arrangement in some of those geometries is exemplified in Fig. 5. For example, by using a periodic array with variable dimensional parameters such as post diameter, height, pitch, and array symmetry, Hochbaum and Aizenberg [79] showed that *P. aeruginosa* oriented normal to the substrate and along the nanoposts when the spacing between adjacent features approached that of the characteristic size of the cell. In contrast, when the nearest neighbor post spacing was more significant than the length of the cell, the adhesion of *P. aeruginosa* to the substrate was random, as depicted in Fig. 5. Inspired by the nonfouling properties of the skin of echinoderms and other marine organisms, the same authors employed static and dynamic microscale wrinkle topographies on polydimethylsiloxane (PDMS) to reduce and

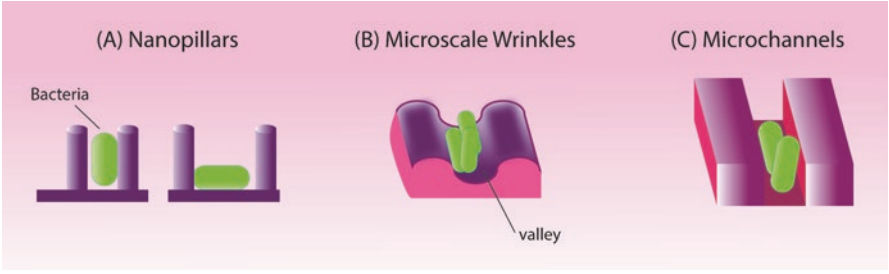


Fig. 5 Bacteria self-organization in nano- and microscale topographies. Bacteria attached perpendicular to a substrate with nanopillar arrays when the spacing between posts is comparable to the bacterium size. The same effect is observed in microscale wrinkles, channels, and ridges with similar dimensions. Bacteria adopt those configurations to maximize the contact area with the substratum

control bacterial biofilm attachment. In the static configuration, bacteria patterned spontaneously on the PDMS wrinkles following the spacing and orientation of the trough [80]. By applying a dynamic uniaxial mechanical strain to the PDMS, those authors showed up to 80% of *P. aeruginosa* biofilm reduction for a specific set of strain parameters. In our laboratory, we also have fabricated wrinkles in PDMS by using argon plasma irradiation for bacteria/surface interaction studies, but at dimensions much smaller than those used by Epstein et al. [80]. We have observed that besides the preferential settlement of *E. coli* on the valley of the wrinkles, bacterial deformation also occurs as a result of the tortuosity imposed by the topography.

Microtopograhies inspired by the sharkskin have also been demonstrated to limit bacterial communication and alter biofilm formation. For example, Chung et al. [65] used a topography inspired on the sharkskin (Sharklet AF™), which was fabricated in PDMS with 2 μm width and spacing and 3 μm height and cultured with *S. aureus* for 14 days. At the end of the incubation period, the Sharklet AF™ topography did not show evidence of biofilm development, whereas, on flat PDMS, biofilm formation was evident at day 7 [65]. The authors suggested that the height of the features contributed to colony isolation. In a similar study, Sakamoto et al. [81], using the sharkskin micropatterned surface, determined whether the height of the patterns in the Sharklet AF™ topography was a determinant for biofilm disruption. Those authors found that the depth of the groove was not crucial for the antibacterial effect, but instead, the tortuosity due to the variety of surface topography was vital for the decrease in biofilm formation and swarming motility [81].

Future Perspectives

A key issue in the treatment of skin infections is the presence of bacterial biofilms, which are particularly difficult to manage when they are associated with the surface of biomaterials. Interfaces implementing nano- and microscale topographies offers an unprecedented opportunity to prevent the microbial colonization and biofilm formation in common biomaterial products used in contact with the skin, such as medical adhesives, sutures, and wound dressing materials, which usually serve as the origin of infection. One of the major advantages that anti-biofouling and antimicrobial physical barriers provide compared to other methods is that they do not contribute to bacterial resistance, do not pollute the environment, and are easy to implement. Moreover, the potential application of those nano- and microscale interfaces are not limited to the design of materials for the treatment of infections but also provide a tool for studying how variations in the structural space of natural environments modulate the interactions between microbial communities and the host.

Acknowledgments Material characterization was partially carried out in the Materials Research Laboratory Central Facilities at the University of Illinois at Urbana-Champaign. Authors would like to thank Zachariah Koyn for his technical support on performing part of the material irradiations. Sandra L. Arias would like to thank Monika Makurath and Roshni Bano for their helpful discussions on microbial physiology.

References

1. Grice EA, Segre JA (2011) The skin microbiome. Nat Rev Microbiol 9(4):244
2. Byrd AL, Belkaid Y, Segre JA (2018) The human skin microbiome. Nat Rev Microbiol 16(3):143
3. Williams MR, Gallo RL (2015) The role of the skin microbiome in atopic dermatitis. Curr Allergy Asthma Rep 15(11):65
4. Naik S, Bouladoux N, Linehan JL et al (2015) Commensal–dendritic-cell interaction specifies a unique protective skin immune signature. Nature 520(7545):104
5. Nakatsuji T, Chen TH, Narala S et al (2017) Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis. Sci Transl Med 9(378):eaah4680
6. McNichol L, Lund C, Rosen T, Gray M (2013) Medical adhesives and patient safety: state of the science consensus statements for the assessment, prevention, and treatment of adhesive-related skin injuries. J Dermatol Nurses Assoc 5(6):323–338
7. Qin Y (2019) Antimicrobial textile dressings to manage wound infection. In: Rajendran S (ed) Advanced textiles for wound care. Woodhead Publishing, Cambridge
8. Miller LG, Eisenberg DF, Liu H et al (2015) Incidence of skin and soft tissue infections in ambulatory and inpatient settings, 2005–2010. BMC Infect Dis 15(1):362
9. Fung HB, Chang JY, Kuczynskf S (2003) A practical guide to the treatment of complicated skin and soft tissue infections. Drugs 63(14):1459–1480
10. Serra R, Grande R, Butrico L et al (2015) Chronic wound infections: the role of Pseudomonas aeruginosa and Staphylococcus aureus. Expert Rev Anti Infect Ther 13(5):605–613
11. Lawton S, Langøen A (2009) Assessing and managing vulnerable periwound skin. World Wide Wounds. http://www.worldwidewounds.com/2009/October/Lawton-Langoen/vulnerable-skin-2.html. Accessed 30 January 2019
12. Gray M, Black JM, Baharestani MM et al (2011) Moisture-associated skin damage: overview and pathophysiology. J Wound Ostomy Continence Nurs 38(3):233–241
13. Friedman ND, Temkin E, Carmeli Y (2016) The negative impact of antibiotic resistance. Clin Microbiol Infect 22(5):416–422
14. Projan SJ (2003) Why is big pharma getting out of antibacterial drug discovery? Curr Opin Microbiol 6(5):427–430
15. Schrand AM, Rahman MF, Hussain SM, Schlager JJ, Smith DA, Syed AF (2010) Metal-based nanoparticles and their toxicity assessment. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2(5):544–568
16. Linden SK, Sutton P, Karlsson NG, Korolik V, McGuckin MA (2008) Mucins in the mucosal barrier to infection. Mucosal Immunol 1(3):183
17. Caldara M, Friedlander RS, Kavanaugh NL, Aizenberg J, Foster KR, Ribbeck K (2012) Mucin biopolymers prevent bacterial aggregation by retaining cells in the free-swimming state. Curr Biol 22(24):2325–2330
18. Khan Y, Ostfeld AE, Lochner CM, Pierre A, Arias AC (2016) Monitoring of vital signs with flexible and wearable medical devices. Adv Mater 28(22):4373–4395
19. Kwak MK, Jeong HE, Suh KY (2011) Rational design and enhanced biocompatibility of a dry adhesive medical skin patch. Adv Mater 23(34):3949–3953
20. LeBlanc K, Baranoski S (2014) Skin tears: the forgotten wound. Nurs Manage 45(12):36–46
21. Anderson A, Foster RS, Brand R, Blyth CC, Kotecha RS (2014) Acute onset of pustules at the site of tape placement in an immunocompromised infant with acute myeloid leukemia. Pediatr Dermatol 31(5):609–610
22. Wilcox MH, Fitzgerald P, Freeman J et al (2000) A five year outbreak of methicillin-susceptible Staphylococcus aureus phage type 53, 85 in a regional neonatal unit. Epidemiol Infect 124(1):37–45
23. Konya C, Sanada H, Sugama J, Okuwa M, Kamatani Y, Nakagami G, Sakaki K (2010) Skin injuries caused by medical adhesive tape in older people and associated factors. J Clin Nurs 19(9-10):1236–1242

24. de Goffau MC, Bergman KA, de Vries HJ, Meessen NE, Degener JE, van Dijl JM, Harmsen HJ (2011) Cold spots in neonatal incubators are hot spots for microbial contamination. Appl Environ Microbiol 77(24):8568–8572
25. Ness MJ, Davis DM, Carey WA (2013) Neonatal skin care: a concise review. Int J Dermatol 52(1):14–22
26. Hornik CP, Fort P, Clark RH, Watt K, Benjamin DK Jr, Smith PB, Manzoni P, Jacqz-Aigrain E, Kaguelidou F, Cohen-Wolkowiez M (2012) Early and late onset sepsis in very-low-birth-weight infants from a large group of neonatal intensive care units. Early Hum Dev 88:S69–S74
27. Lund C (2014) Medical adhesives in the NICU. Newborn Infant Nurs Rev 14(4):160–165
28. Anjum S, Gupta A, Sharma D et al (2017) Antimicrobial nature and healing behavior of plasma functionalized polyester sutures. J Bioact Compat Pol 32(3):263–279
29. Kondrup JD, Qayyum AM (2018) Closing the gap: novel abdominal wound closure techniques. Surg Technol Int 32:25–31
30. Greenberg JA, Clark RM (2009) Advances in suture material for obstetric and gynecologic surgery. Rev Obstet Gynecol 2(3):146
31. Dennis C, Sethu S, Nayak S, Mohan L, Morsi Y, Manivasagam G (2016) Suture materials—current and emerging trends. J Biomed Mater Res A 104(6):1544–1559
32. Altman GH, Diaz F, Jakuba C, Calabro T et al (2003) Silk-based biomaterials. Biomaterials 24(3):401–416
33. Walter CJ, Dumville JC, Sharp CA, Page T (2012) Systematic review and meta-analysis of wound dressings in the prevention of surgical-site infections in surgical wounds healing by primary intention. Br J Surg 99(9):1185–1194
34. Mu Y, Edwards JR, Horan TC, Berrios-Torres SI, Fridkin SK (2011) Improving risk-adjusted measures of surgical site infection for the National Healthcare Safely Network. Infect Control Hosp Epidemiol 32(10):970–986
35. Berrios-Torres SI, Umscheid CA, Bratzler DW et al (2017) Centers for disease control and prevention guideline for the prevention of surgical site infection. JAMA Surg 152(8):784–791
36. Kathju S, Nistico L, Hall-Stoodley L, Post JC, Ehrlich GD, Stoodley P (2009) Chronic surgical site infection due to suture-associated polymicrobial biofilm. Surg Infect (Larchmt) 10(5):457–461
37. Serrano C, García-Fernández L, Fernández-Blázquez JP et al (2015) Nanostructured medical sutures with antibacterial properties. Biomaterials 52:291–300
38. Crovetti G, Martinelli G, Issi M, Barone M et al (2004) Platelet gel for healing cutaneous chronic wounds. Transfus Apher Sci 30(2):145–151
39. Gottrup F (2004) A specialized wound-healing center concept: importance of a multidisciplinary department structure and surgical treatment facilities in the treatment of chronic wounds. Am J Surg 187(5):S38–S43
40. Simões D, Miguel SP, Ribeiro MP, Coutinho P, Mendonça AG, Correia IJ (2018) Recent advances on antimicrobial wound dressing: a review. Eur J Pharm Biopharm 127:130–141
41. Cardona AF, Wilson SE (2015) Skin and soft-tissue infections: a critical review and the role of telavancin in their treatment. Clin Infect Dis 61(suppl_2):S69–S78
42. Han G, Ceilley R (2017) Chronic wound healing: a review of current management and treatments. Adv Ther 34(3):599–610
43. Abbasipour M, Mirjalili M, Khajavi R, Majidi MM (2014) Coated cotton gauze with Ag/ZnO/chitosan nanocomposite as a modern wound dressing. J Eng Fiber Fabr 9(1):155892501400900114
44. Brown MS, Ashley B, Koh A (2018) Wearable technology for chronic wound monitoring: current dressings, advancements, and future prospects. Front Bioeng Biotechnol 6:47
45. Kamoun EA, Kenawy ERS, Chen X (2017) A review on polymeric hydrogel membranes for wound dressing applications: PVA-based hydrogel dressings. J Adv Res 8(3):217–233
46. Hamdan S, Pastar I, Drakulich S, Dikici E, Tomic-Canic M, Deo S, Daunert S (2017) Nanotechnology-driven therapeutic interventions in wound healing: Potential uses and applications. ACS Cent Sci 3(3):163–175

47. Shahverdi S, Hajimiri M, Esfandiari MA, Larijani B, Atyabi F, Rajabiani A, Dehpour AR, Gharehaghaji AA, Dinarvand R (2014) Fabrication and structure analysis of poly(lactide-co-glycolic acid)/silk fibroin hybrid scaffold for wound dressing applications. Int J Pharm 473(1):345–355
48. Linklater DP, Juodkazis S, Rubanov S, Ivanova EP (2017) Comment on "Bactericidal Effects of Natural Nanotopography of Dragonfly Wing on Escherichia coli". ACS Appl Mater Interfaces 9(35):29387–29393
49. Dickson MN, Liang EI, Rodriguez LA, Vollereaux N, Yee AF (2015) Nanopatterned polymer surfaces with bactericidal properties. Biointerphases 10(2):021010
50. Li X, Chen T (2016) Enhancement and suppression effects of a nanopatterned surface on bacterial adhesion. Phys Rev E 93(5):052419
51. Fisher LE, Yang Y, Yuen MF, Zhang W, Nobbs AH, Su B (2016) Bactericidal activity of biomimetic diamond nanocone surfaces. Biointerphases 11(1):011014
52. Rojas ER, Billings G, Odermatt PD, Auer GK, Zhu L, Miguel A, Chang F, Weibel DB, Theriot JA, Huang KC (2018) The outer membrane is an essential load-bearing element in Gram-negative bacteria. Nature 559(7715):617
53. Nowlin K, Boseman A, Covell A, LaJeunesse D (2015) Adhesion-dependent rupturing of Saccharomyces cerevisiae on biological antimicrobial nanostructured surfaces. J R Soc Interface 12(102):20140999
54. Ivanova EP, Hasan J, Webb HK et al (2013) Bactericidal activity of black silicon. Nat Commun 4:2838
55. Bandara CD, Singh S, Afara IO, Wolff A, Tesfamichael T, Ostrikov K, Oloyede A (2017) Bactericidal effects of natural nanotopography of dragonfly wing on Escherichia coli. ACS Appl Mater Interfaces 9(8):6746–6760
56. Hizal F, Choi CH, Busscher HJ, van der Mei HC (2016) Staphylococcal adhesion, detachment and transmission on nanopillared Si surfaces. ACS Appl Mater Interfaces 8(44):30430–30439
57. Diu T, Faruqui N, Sjöström T, Lamarre B, Jenkinson HF, Su B, Ryadnov MG (2014) Cicada-inspired cell-instructive nanopatterned arrays. Sci Rep 4:7122
58. Lin N, Berton P, Moraes C, Rogers RD, Tufenkji N (2018) Nanodarts, nanoblades, and nanospikes: mechano-bactericidal nanostructures and where to find them. Adv Colloid Interface Sci 252:55–68
59. Tripathy A, Sen P, Su B, Briscoe WH (2017) Natural and bioinspired nanostructured bactericidal surfaces. Adv Colloid Interface Sci 248:85–104
60. Kelleher SM, Habimana O, Lawler J, O'reilly B, Daniels S, Casey E, Cowley A (2015) Cicada wing surface topography: an investigation into the bactericidal properties of nanostructural features. ACS Appl Mater Interfaces 8(24):14966–14974
61. Xue F, Liu J, Guo L, Zhang L, Li Q (2015) Theoretical study on the bactericidal nature of nanopatterned surfaces. J Theor Biol 385:1–7
62. Pogodin S, Hasan J, Baulin VA et al (2013) Biophysical model of bacterial cell interactions with nanopatterned cicada wing surfaces. Biophys J 104(4):835–840
63. Luan Y, Liu S, Pihl M, van der Mei HC, Liu J, Hizal F, Choi CH, Chen H, Ren Y, Busscher HJ (2018) Bacterial interactions with nanostructured surfaces. Curr Opin Colloid Interface Sci 38:170–189
64. Zhang Y, Lo CW, Taylor JA, Yang S (2006) Replica molding of high-aspect-ratio polymeric nanopillar arrays with high fidelity. Langmuir 22(20):8595–8601
65. Chung KK, Schumacher JF, Sampson EM, Burne RA, Antonelli PJ, Brennan AB (2007) Impact of engineered surface microtopography on biofilm formation of Staphylococcus aureus. Biointerphases 2(2):89–94
66. Ma S, Yu B, Pei X, Zhou F (2016) Structural hydrogels. Polymer 98:516–535
67. Arias SL, Shetty A, Devorkin J, Allain JP (2018) Magnetic targeting of smooth muscle cells in vitro using a magnetic bacterial cellulose to improve cell retention in tissue-engineering vascular grafts. Acta Biomater *77*:172–181

68. Arias SL, Shetty AR, Senpan A, Echeverry-Rendón M, Reece LM, Allain JP (2016) Fabrication of a functionalized magnetic bacterial nanocellulose with iron oxide nanoparticles. J Vis Exp (JoVE):111
69. Echeverry-Rendon M, Reece LM, Pastrana F, Arias SL, Shetty AR, Pavón JJ, Allain JP (2017) Bacterial nanocellulose magnetically functionalized for neuro-endovascular treatment. Macromol Biosci 17(6):1600382
70. Civantos A, Martinez-Campos E, Ramos V, Elvira C, Gallardo A, Abarrategi A (2017) Titanium coatings and surface modifications: toward clinically useful bioactive implants. ACS Biomater Sci Eng 3(7):1245–1261
71. Douglas TE, Kumari S, Dziadek K et al (2017) Titanium surface functionalization with coatings of chitosan and polyphenol-rich plant extracts. Mater Lett 196:213–216
72. Ribeiro MP, Espiga A, Silva D, Baptista P, Henriques J, Ferreira C, Silva JC, Borges JP, Pires E, Chaves P, Correia IJ (2009) Development of a new chitosan hydrogel for wound dressing. Wound Repair Regen 17(6):817–824
73. Abarrategi A, Civantos A, Ramos V, Sanz Casado JV, López-Lacomba JL (2007) Chitosan film as rhBMP2 carrier: delivery properties for bone tissue application. Biomacromolecules 9(2):711–718
74. Lopez-Cebral R, Silva-Correia J, Reis RL, Silva TH, Oliveira JM (2017) Peripheral nerve injury: current challenges, conventional treatment approaches, and new trends in biomaterials-based regenerative strategies. ACS Biomater Sci Eng 3(12):3098–3122
75. Vepari C, Kaplan DL (2007) Silk as a biomaterial. Prog Polym Sci 32(8-9):991–1007
76. Nadell CD, Drescher K, Foster KR (2016) Spatial structure, cooperation and competition in biofilms. Nat Rev Microbiol 14(9):589
77. Petrova OE, Sauer K (2012) Sticky situations: key components that control bacterial surface attachment. J Bacteriol 194(10):2413–2425
78. Leveau JH (2015) Life of microbes on aerial plant parts. In: Lugtenberg B (ed) Principles of plant-microbe interactions. Springer, Cham
79. Hochbaum AI, Aizenberg J (2010) Bacteria pattern spontaneously on periodic nanostructure arrays. Nano Lett 10(9):3717–3721
80. Epstein AK, Hong D, Kim P, Aizenberg J (2013) Biofilm attachment reduction on bioinspired, dynamic, micro-wrinkling surfaces. New J Phys 15(9):095018
81. Sakamoto A, Terui Y, Horie C et al (2014) Antibacterial effects of protruding and recessed shark skin micropatterned surfaces of polyacrylate plate with a shallow groove. FEMS Microbiol Lett 361(1):10–16

Local Delivery of Anti-biofilm Therapeutics

Zoe Harrison, Leslie Pace, Rukhsana Awais, and J. Amber Jennings

Abstract Biofilm infections are incredibly resistant to the immune response and antibiotic treatments, and often must be removed mechanically through tissue debridement or lavage. Current therapeutic methods for prevention and treatment include large doses of antibiotics and mechanical removal methods, which can lead to increased hospital time, costs, and trauma for patients. To combat these issues, therapies with emphasis on biofilm prevention and removal have been the subject of much current research. Methods in development include peptides and amino acids, fatty acids and lipids, enzymes, small molecules, metabolites, nanoparticles, and living cells. Each method uniquely targets the altered phenotypic state of bacteria within biofilm to either prevent biofilm formation entirely or treat existing biofilm infections, proving successful through the in vitro, in vivo, and clinical studies discussed in this chapter.

Keywords Biofilm · Infection · *Staphylococcus* · Exopolymeric substance · Diffusible signaling factor · Antimicrobial peptide · Antibiotic · Dispersal · Tissue engineering · Enzymes · Nanoparticles · Bacteriophage

Introduction

The attachment of bacteria to implant surfaces or necrotic tissue to form biofilm poses a particular risk for infection. Biofilm-associated bacteria are extremely resistant to both antimicrobial therapy and immune cell clearance [1]. Biofilm formation can lead to infection in various sites and can affect any tissue, with orthopedic injuries being especially vulnerable. Repair of orthopedic defects or restoration of function often necessitates the use of biomaterial implants for use as bone fixation

Z. Harrison · L. Pace · R. Awais · J. A. Jennings (✉)
Department of Biomedical Engineering, University of Memphis, Memphis, TN, USA
e-mail: jjnnings@memphis.edu

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_21

hardware, periprosthetic joint replacements, or scaffolds for bone regeneration [2]. Infection in orthopedics leads to osteomyelitis [3, 4], for which treatment can be difficult and costly [5–7]. The most common pathogens in osteomyelitis also have a tendency to form biofilms [8–10]. Staphylococcal microorganisms are the most prevalent pathogens, though other microorganisms including Gram-negative *Enterobacteriaceae* and *Pseudomonas*, as well as polymicrobial contamination have been observed [7, 11].

The recalcitrance of biofilms to treatment is multifactorial and complex, owing in part to an altered metabolic state, secretion of exopolymeric substances (EPS), and manipulation of immune cells [12–15]. Small populations of cells within a biofilm become dormant persister cell phenotypes, limiting the action of antibiotics that interfere with metabolic processes [16, 17]. Biofilm bacteria secrete exopolymeric substances that enhance agglomeration, resist mechanical stresses, facilitate nutrient transport, and may limit the diffusion of antimicrobials or immune cell penetration [18, 19]. Recent research also suggests that while immune cells like neutrophils and macrophages can penetrate biofilm EPS, biofilm-associated bacteria can influence their ability to kill and clear infecting microorganisms [14, 20–22].

Current therapies for biofilm-associated infection include high dose systemic and local antibiotics, mechanical removal of a biofilm through debridement and lavage, and in many cases removal of implanted devices. As an alternative or adjunct to mechanical removal methods [23, 24], the development of advanced therapeutic strategies that specifically target biofilm modes of bacterial infection may increase the efficacy of treatment and reduce treatment time and cost. In this chapter, we review the strategies using specific biofilm-targeting molecules or cells with specific emphasis on biomaterial delivery, co-delivery with antimicrobials, and in vivo studies.

Peptides and Amino Acids

Peptides are formed by amino acids, and amino acids have been shown to be produced by bacteria prior to disassembly of biofilms, particularly **D-Leucine**, **D-Methionine, D-Tryptophan**, and **D-Tyrosine** [25, 26]. These D-Amino Acids have been shown to be hydrophobic biofilm dispersal agents that are proposed to function through disruption of bacterial cell walls that can inhibit formation of a biofilm [26]. These agents also have added benefits of having minimal toxicity to mammalian cells and broad-spectrum effectiveness against various bacterial strains [25, 27]. Sanchez et al. fabricated polyurethane scaffolds as a delivery system for D-amino acids and tested the efficacy of these scaffolds in an in vivo rat femoral segmental defect model. They found that while the loaded scaffolds significantly reduced biofilm in the UAMS-1 (Methicillin sensitive *Staphylococcus aureus*) bac-

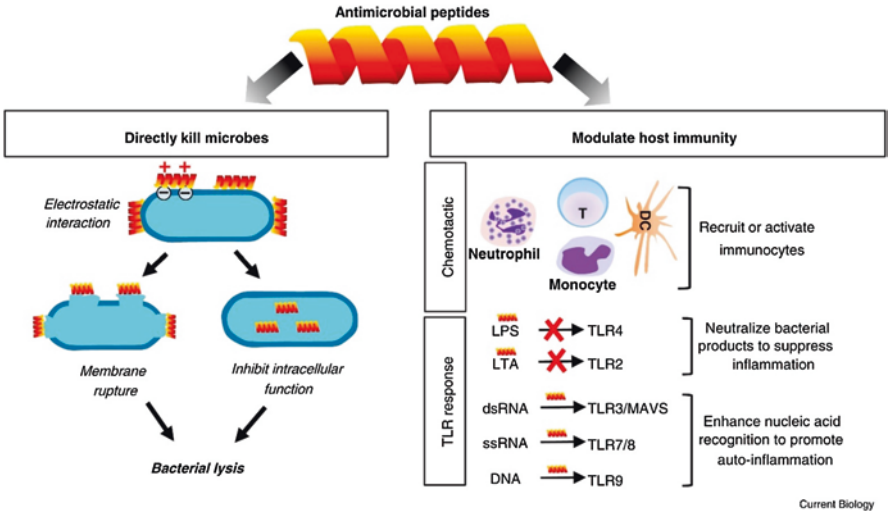


Fig. 1 Biological function of antimicrobial peptides. AMPs bind to bacterial membranes through electrostatic interactions either to disrupt the membrane or to enter the bacterium to inhibit intracellular function. Some AMPs also modulate host immunity by recruiting/activating immunocytes or by influencing Toll-like receptor (TLR) recognition of microbial products and nucleic acids released upon tissue damage. *DC* dendritic cell, *LPS* lipopolysaccharide, *LTA* lipoteichoic acid, *MAVS* mitochondrial antiviral signaling protein. (Reprinted with permission from Current Biology 2016 26(1): R14–R19. Copyright 2016 Elsevier Ltd)

terial strain, it did not have an effect on Xen36 strain [28]. D-amino acids have also been delivered through bone cements in an ovine model [29]. While inhibition occurred for both methicillin-susceptible and methicillin-resistant strains of *Staphylococcus aureus* (*S. aureus*) in vitro and the bone grafts did not inhibit osteoclast or osteoblast differentiation in vivo, an infected animal model would be required for validation of efficacy of this delivery system [30].

Antimicrobial peptides (AMPs) are ubiquitous proteins that are typically harvested from natural sources like insects and animals, because they are a component of many organisms' innate immune responses (Fig. 1); they have also been developed synthetically to tailor their properties to increase effectiveness. These molecules function to mediate the inflammatory response to increase cytokine release and facilitate wound healing [31]. AMPs typically consist of anywhere between 10 and 50 amino acid residues, and are generally classified as either beta sheet, alpha helical, loop, or extended peptides; despite these differences, they are all typically cationic and adopt amphipathic structures [32]. Beta sheet and alpha helical AMPs are commonly found in nature, and thus have been studied most extensively [33]. While the exact mechanism of action for AMPs remains inconclusive, it is known that they generally function by disrupting the membranes of bacterial cells;

however, their specific functions differ based on their varying structural properties [33]. These structural differences also explain the stability of various AMPs in harsh conditions such as extreme temperatures and pH conditions [34]. If delivered adequately to sites of biofilm infection, these molecules show significant potential to disable both planktonic and persister cell types [35], work synergistically with antibiotics [36], and disrupt biofilm during various stages of formation [32]. These characteristics make AMPs advantageous agents for biofilm dispersal and prevention of biofilm formation.

Of the 221 currently identified AMPs, there are a select few that have been tested extensively and have begun testing in clinical trials with human patients. **Omiganan** is one AMP that has been tested in human trials to treat and prevent catheter related infections. This peptide is derived from bovine neutrophils, and is being developed as a chemically synthesized, aqueous gel for topical application. Results of related studies showed that the omiganan gel inhibited formation of a biofilm by major Gram-negative species at concentrations well below the 1% gel concentration (10,000 μg/mL) [37]. Further clinical testing has been planned to determine this peptide's efficacy against dermal infections such as dermatitis, acne, and rosacea [37]. The AMP **pexiganan** has also proceeded to clinical trials to treat diabetic foot ulcer infections. This molecule is developed synthetically as an analog of the peptide magainin II, which can be isolated from the skin of frogs [38]. **Lytixar** is another synthetic AMP that has proceeded to clinical trials to treat Gram-positive skin infections (specifically *S. aureus*). Like omiganan, lytixar is also delivered as a topical hydrogel [39]. Derived from the N terminus of human lactoferrin, **hLF1-11** is another AMP tested in clinical trials, which is delivered intravenously to immunocompromised patients experiencing bacterial infections following hematopoietic stem cell transplantations [40]. This AMP is unique because it is delivered systemically rather than locally like most other AMP treatments**. Ribonucleic acid III Inhibiting peptides (RIP)** inhibit translation of proteins from expressed genes and the application of RIP in combination with an antibacterial agent to an implant was shown to increase the antibiotic efficacy in clearing normally resilient biofilms formed by *S. aureus* in a rat model [41]. Sterile, 1 cm^2 collagen-sealed Dacron vascular grafts were soaked in RIP 20 mg/L for 20 min and intraperitoneal injection of RIP (10 mg/kg) was given. Activity of RIP was also demonstrated for other in vivo applications, including ureteral stents [42] and poly(methyl methacrylate) bone cement beads [43]. **OP-145**, a derivative of the human peptide cathelicidin (a component of the innate immune response) has been tested as a liquid, ear-drop treatment to combat middle ear bacterial infections [44] (Table 1).

Despite the success of AMPs in both in vitro and in vivo trials, commercial difficulties with partnering pharmaceutical companies has made the progression of these molecules into clinical therapies very slow and riddled with setbacks. Continuing research and the promise of future licensing show hope for widespread approval of the entirety of these molecules, rather than just a select few [32].

Table 1 Antimicrobial peptides delivered for biofilm targeting

AMP	Obtained from	Delivery system	Application	
Arenicins	Lugworm secretions	Still in preclinical trials	Urinary tract infections	[45]
Brilacidin	Defensin mimetic	Intravenously administered	Skin infections	[46]
C16G2	Synthetic	Oral solution	Tooth infections	[47]
CZEN-002	Dimeric octamer derived from alpha MSH	Hydrogel	Vaginal candidiasis-	[32]
hLF1-11	Human lactoferrin	Intravenously administered, calcium phosphate cement	Infections following hematopoietic stem cell transplantations	[40, 48]
Iseganan	Porcine leukocytes	Oral solution	Oral mucositis following radiation and chemotherapy	[49]
LL-37	Human cathelicidin	Polyvinyl alcohol solution	Leg ulcers	[50]
Lytixar	Synthetic	Hydrogel	Skin infections	[39]
Novexatin	Human defensins	Brush on treatment	Nail fungus	[51]
Omiganan	Bovine neutrophils	Hydrogel	Catheter infections	[52]
OP-145	Derivative of LL-37	Ear drops	Middle ear infections	[44]
PAC-113	Human saliva	Oral solution	Oral candidiasis	[53]
Pexiganan	Frog skin	Cream	Diabetic foot ulcers	[38]
PXL01	Human lactoferrin	Hyaluronic acid hydrogel	Treatment following hand surgery	[54]
Ribonucleic acid III inhibiting peptides (RIP	*S. xylosus*; synthetic	Poly(methyl methacrylate), injection or coating	Orthopedic implants, ureteral stents, catheter	[41–43]

Fatty Acids and Lipids

Various fatty acids have been shown to disperse preformed biofilms or inhibit biofilm formation. These specific medium chain fatty acids are members of a family of diffusible signaling factors (DSFs) in bacteria. DSFs are molecules secreted by bacteria to function as a cell-to-cell communication mechanism, called quorum sensing [55]. It has been observed that some of these fatty acid signaling factors revert persister cells to a metabolically active state, which in combination with antimicrobials could decrease bacterial viability [56]. Moreover, these compounds act to inhibit and disperse biofilms formed by multiple types of microorganisms, meaning they have cross-kingdom efficacy [57]. Debate continues regarding whether these DSFs are independently antimicrobial, or if they require combinational therapy with conventional antibiotics [58]. Furthermore, delivery of these molecules is somewhat limited by their hydrophobic nature.

One well-studied DSF molecule is **cis-2-decenoic acid** (C2DA), which has been shown to inhibit biofilm formation of *Pseudomonas aeruginosa* (*P. aeruginosa*) and

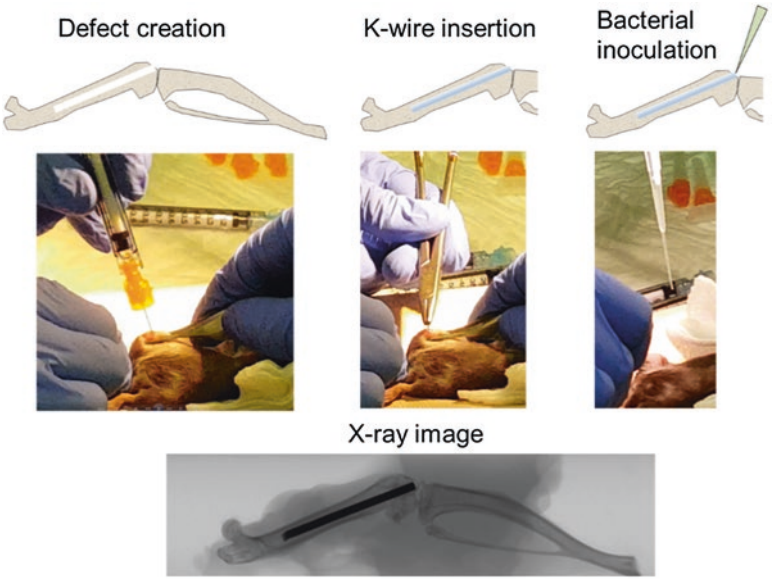


Fig. 2 Schematic and images of surgical procedure for murine model of biofilm-associated infection, including reaming defect, inserting k-wire, and inoculating with bacteria. Bottom image shows X ray confirmation of placement of a k-wire in the femur

disperse established biofilms of multiple strains [59]. While mechanisms of action remain unclear [60], recent work shows that the cis-conformation also increases membrane permeability and could let more small molecule antibiotics into the cells [61]. C2DA was also shown to inhibit *S. aureus* growth and biofilm formation entirely, with concentrations that were not cytotoxic to fibroblasts [60]. Due to the challenge in delivering such hydrophobic fatty acid molecules, in vitro studies have used ethanol as a carrier to improve solubility. However, organic solvents have limited applicability in clinical delivery systems. Local delivery mechanisms showed a successful release of C2DA from chitosan sponges using a poly(ethylene glycol) (PEG) carrier as solvent [60]. Furthermore, Harris et al. performed a pilot study examining C2DA loaded into manually applied phosphatidylcholine coatings in a murine biofilm-based infection model (Fig. 2), which showed complete inhibition of *S. aureus* on titanium pins and bone, both with and without antibiotics [62]. However, this application requires further development to determine efficacy of this treatment for use in preventing periprosthetic joint infections. This delivery strategy could be used as an intraoperative coating for implants or wound sites for infection prevention [60]. C2DA has also been deemed useful in the eradication of catheter-associated biofilms; in vitro studies showed that when combined with antibiotics, C2DA induced dispersal of both *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) biofilms formed on catheters [63].

Similarly, biosurfactants released by bacteria also play a role in bacterial adhesion and biofilm development. One biosurfactant class, called **rhamnolipids**, are vital during many stages of biofilm development, and also mediate the eventual

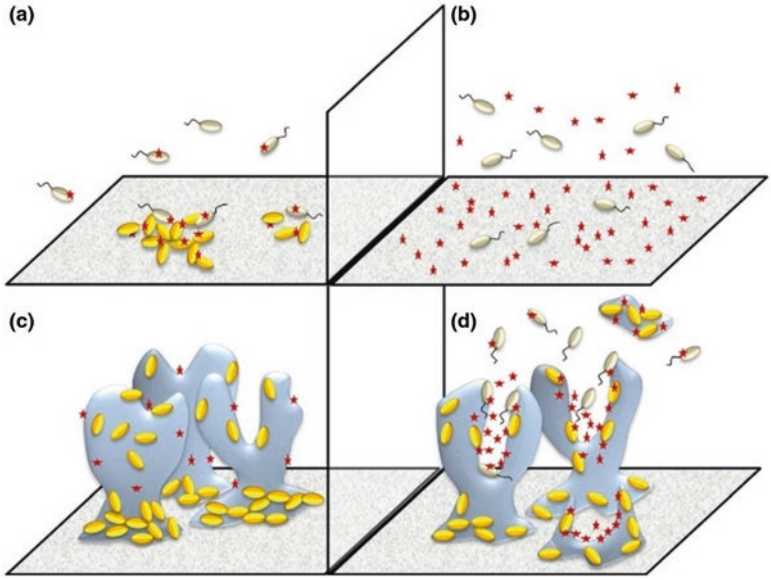


Fig. 3 Representation of rhamnolipid implication in different stages of *P. aeruginosa* biofilm development. (**a**) Low concentration of rhamnolipids increase affinity of cells for initial adherence to surfaces through increasing a cell's surface hydrophobicity; (**b**) Presence of high concentrations of rhamnolipids in the surrounding medium prevents attachment of cells and further microcolony formation; (**c**) At the proliferation stage, rhamnolipids are actively involved in the maintenance of the complex-differentiated architecture of the biofilm; (**d**) At late stages of biofilm development, rhamnolipids promote the seeding dispersal of motile cells. The red stars represent rhamnolipids. (Reprinted with permission from Letters in Applied Microbiology, 2014 58(3): 447–453. Copyright 2014 The Society for Applied Microbiology)

dispersal of biofilms they produce (Fig. 3). Thus, like C2DA, rhamnolipids have begun to be utilized as a potential dispersal agent against the bacterial strains that produce them (*P. aeruginosa*) and various others (*Bordetella bronchiseptica, Streptococcus salivarius, Candida tropicalis,* etc.) [64]. Rhamnolipids have shown anti-adhesive properties when soaked onto silicone rubber material, as used in voice prostheses [65]. Rhamnolipid soaking showed similar results when tested on hydrophilic and hydrophobic glasses [66], as well as when tested against marine biofilm species [67]. Rhamnolipids showed some amount of anti-adhesive capabilities against fungal biofilms on polystyrene surfaces [68], but required much higher concentrations than that used against bacterial biofilms.

Enzymes

Biofilm dispersion can be achieved at different stages of biofilm formation. Early stage inhibition of biofilm formation could be achieved by targeting exopolymeric substance (EPS) production and/or cell division. Strategies for the disruption of

mature biofilms include degradation of the EPS matrix, eliminating dormant persister cells, inhibiting the pathogenic environment, and targeting social interaction through signal molecules. There are various dispersal mechanisms which can disaggregate bacteria from biofilm. The following discussion is related to the role of extracellular enzymes in the degradation of the EPS matrix and the release of bacteria in their planktonic form from biofilms. The combination of antimicrobials and matrix degrading enzymes can lead to elimination of the established biofilms in infections.

Glycoside Hydrolases

EPS varies in composition, with the majority of the components being polyanionic macromolecules due to the presence of uronic acids (D-glucuronic acid and D-mannuronic acid) and ketal-linked pyruvates [69]. Three different exopolysaccharides that are common in biofilm EPS are Pel, Psl, and alginate. Pel is a cationic polysaccharide composed of N-acetyl galactosamine and N-acetyl glucosamine, and Psl contains pentasaccharide-repeating units of D-mannose, D-glucose and L-rhamnose [70]. **Glycoside hydrolases** (GH) are a group of enzymes that catalyze the glycolytic cleavage of O-glyosidic bonds. This enzymatic treatment can cause biofilm dispersion and reduction in biomass thereby releasing bacteria back into planktonic form. Fleming et al. showed in a mouse model of wound infections, dispersal of biofilms formed by *S. aureus* and *P. aeruginosa* when treated with 10% (w/v) GH dissolved in phosphate buffered saline. However, in the absence of an antibiotic this dispersal event resulted in significant septicemia within 15 h [71]. In this study, dispersal-mediated septicemia depended on the swimming motility and on wound size. Combining 10% GH in combination with topical (3 mg/mL) or systemic (300 mg/mL) meropenem was investigated for infection treatment using bioluminescent *P. aeruginosa* monitored by an in vivo imaging system (IVIS) for systemic spread (Fig. 4). Enzyme solutions were compared to heat-inactivated controls, revealing that infection clearance occurred significantly faster when treated with the GH plus meropenem group.

Studies by Yu et al. [72] demonstrated that PslG, a GH, could eradicate *P. aeruginosa* biofilms in the mouse peritoneum when combined with locally delivered tobramycin (50 nM PslG: 50 mg/kg Tobramycin in 0.9% NaCl). PslG acts as an endoglycosidase and targets the Psl matrix resulting in biofilm disassembly. A synergistic effect between the enzyme and antibiotic was observed in the significant reduction of biofilm on an implant compared to treatment with either enzyme or antibiotic alone [72]. Enzymes specific to Pel and Psl, $PelA_h$, and $PslG_h$ were investigated in a study by Baker et al., showing disruption of Pel dependent *P. aeruginosa* biofilms. IMR-90 human fibroblast cells treated with concentrations of 1 mg/mL of either enzyme suggested that the enzymes do not interfere with cell morphology and viability. Prophylactic treatment with $PelA_h$ or $PslG_h$ before treatment with colistin (50 μg/mL) showed compatibility with antibiotic therapy and resulted in a 2.5-log reduction in bacterial colony-forming units [73].

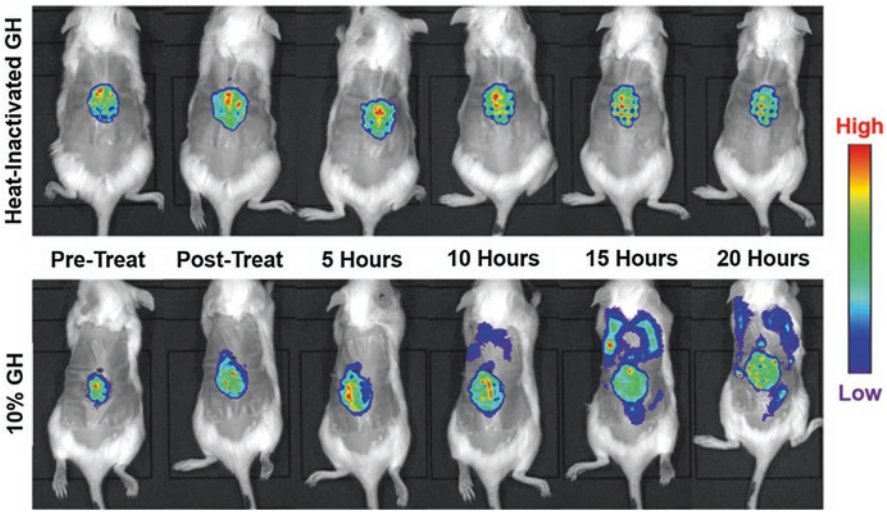


Fig. 4 IVIS imaging (in vivo imaging system) of in vivo dispersal triggered by glycoside hydrolase therapy. Treatment of 48-h-old mouse chronic wounds, infected with bioluminescent *P. aeruginosa*, with 10% α-amylase and cellulase (1:1; GH), or a heat-inactivated control, resulted in dispersal and systemic spread of the infection. Clear localization of bacteria in the organs can be seen in the treated group. A representative animal from the treatment and control groups at each time point are shown. (Figure reprinted from Scientific Reports, 2018, 8, Article number: 10738 [71]. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/)

Alginate is an exopolysaccharide synthesized by *P. aeruginosa* in biofilm development and under environmental stresses. It is made of guluronic acid, mannuronic acid with modification of O-acetyl groups. The gel-forming and water-binding capacity of the alginate polymer increases biofilm attachment to the surface [74]. Alginate lyase A1-III–His has been shown to reduce extracellular matrix in biofilms formed by *P. aeruginosa* in comparison to untreated and tobramycin-treated biofilms [75].

Protease

The EPS matrix that promotes biofilm stability and regulation is mostly composed of polysaccharides, surface proteins, and eDNA. Proteases like Proteinase K and trypsin cleave the peptide bonds in surface proteins leading to biofilm disassembly, hence causing the degradation of surface structures. Iwase et al. showed in vivo that serine protease Esp secreted by *Staphylococcus epidermidis* (*S. epidermidis)* inhibited biofilm formation and nasal colonization by *S. aureus* [76]. An extracellular cysteine protease, SpeB, dispersed staphylococcal biofilms and inhibitors of this protease reduced cutaneous lesion formation in an in vivo mouse model [77]. SpeB activates host proteins by cleaving both host and bacterial proteins and thus is capable of dispersion of Group A Streptococcus, a common cause of skin and

pharyngeal infections in humans. SpeB was also shown to cause apoptosis in phagocytic immune cells which could cause increased inflammation and tissue damage. Loughran et al. demonstrated that the extracellular proteases aureolysin, ScpA, SspA and SspB limited the formation of a biofilm in both LAC (Methicillin-resistant *S. aureus*) and UAMS-1 in vitro and in vivo [78].

Nucleases

Nucleases cleave the phosphodiester bonds in monomers of nucleic acids, with substrate specificities: DNA specific (DNase) and RNA specific (RNase). *S. aureus* nucleases promote resistance against neutrophil extracellular traps-mediated killing by neutrophils, hence impairing host immune defense mechanisms. Kaplan et al. in their studies showed that human DNase I at 10 mg/L significantly inhibited biofilm formed by *S. aureus*. Furthermore, recombinant human deoxyribonuclease (rhDNase) combined with tobramycin showed an increased survival of *Caenorhabditis elegans* (*C. elegans*) nematodes infected with *S. aureus* compared to the group treated with tobramycin alone [79]. Weiss et al. demonstrated that the mutation of either or both nuclease genes (nuc 1 and nuc2) limits biofilm formation in a murine model of catheter linked biofilm formation and resulted in enhanced susceptibility to daptomycin [80]. Hymes et al. investigated a murine vaginal colonization model for *Gardneralla vaginalis* and demonstrated that DNase exhibited >tenfold inhibition of colonization (Fig. 5) [81]. Low concentrations of DNase and metronidazole led to greater biofilm dispersion compared to either agent alone.

Dispersin B

Dispersin B, a poly-N-acetyl glucosamine degrading enzyme produced by *Aggregatibacter actinomycetemcomitans* showed synergistic effects in combination with an antibiotic, cefamandole nafate, by exerting hydrolytic activity against the EPS matrix produced *S. epidermidis* [82]. Chaignon et al. suggested that Dispersin B followed by Proteinase K or trypsin can eradicate biofilms formed by staphylococcal strains in orthopedic implant-related infections [83]. Hogan et al. showed that Dispersin B at concentrations of 1 μg/mL used in combination with vancomycin or rifampicin significantly reduced the viability of methicillin-susceptible *S. aureus* [84]. Dispersin B combined with broad spectrum antimicrobial peptide KSL-W as a wound gel dispersed and inhibited biofilm formation in vitro against pathogens found in chronic wound infections, such as diabetic foot ulcers [85]. In vivo efficacy of Dispersin B and triclosan coated catheters was compared with chlorhexidine–silver coated catheters [86]. The triclosan + Dispersin B combination was synergistic and reduced bacterial colonization compared to chlorhexidine. DNase1 and Dispersin B were shown to inhibit skin colonization as well as to detach *S. epidermis* and *S. aureus* biofilms in a pig model in vivo. Further, it increased susceptibility to killing by povidone iodine [87].

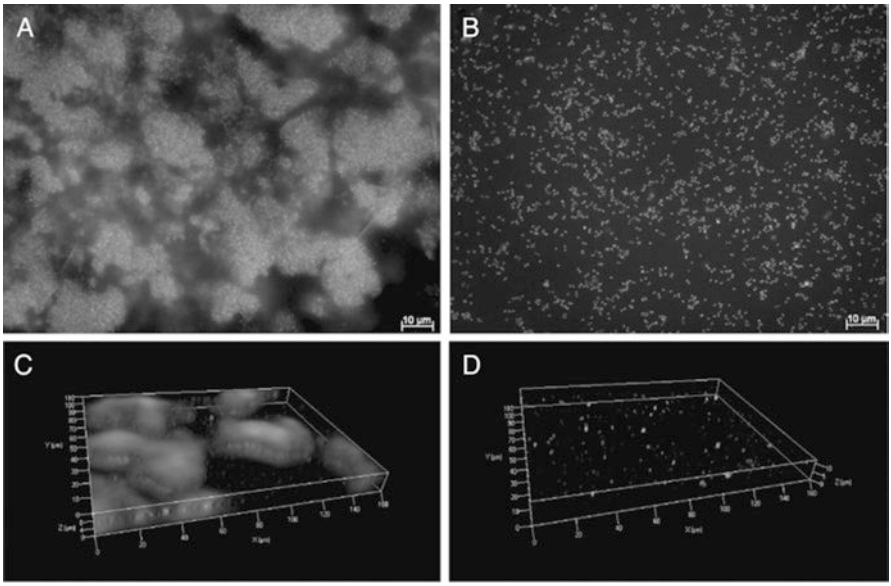


Fig. 5 *Gardnerella vaginalis* forms three-dimensional biofilms that are inhibited by DNase. *G. vaginalis* strain 49-145 was grown on glass supports for 24 h and treated with vehicle control (**A** and **C**) or with DNase (**B** and **D**). Two-dimensional images of DAPI-stained biofilms (**A** and **B**) or 3-dimensional reconstruction from serial z-stack images or propidium iodide-stained biofilms (**C** and **D**) demonstrate both bacteria-associated and extracellular DNA. Similar results were obtained with *G. vaginalis* strain ARG3 (data not shown). (Reprinted with permission from Journal of Infectious Diseases, 2013; 207(10): 1491–1497. DOI: 10.1093/infdis/jit047. Copyright 2013 Oxford University Press)

Lysostaphin

Lysostaphin is a commercially available proteolytic enzyme, produced by *Staphylococcus simulans*, shown to have antimicrobial properties specific to other *Staphylococcus* species [88]. The enzyme has two distinct domains, a cell wall-targeting domain, responsible for the specificity of staphylococcal species, and a lytic domain, responsible for cleaving the pentaglycine cross-bridges present in the cell wall [89]. Lysostaphin has been shown to exhibit activity toward antibiotic-resistant *S. aureus* strains, including methicillin-resistant *S. aureus* (MRSA), vancomycin-intermediate S. *aureus*, vancomycin-resistant *S. aureus*, and *S. epidermidis* [90–93]. Unlike most antibiotics, lysostaphin does not require metabolic activity and has been shown to kill both planktonic and quiescent bacteria, as well as cells growing in a biofilm [94].

In order for lysostaphin to be considered a clinically relevant enzybiotic, an effective mode of delivery should be established to ensure that activity, stability, and dosage conditions are maintained. Delivery systems for lysostaphin have included injectable hydrogels, bone cement, wound dressings, fibers, and implant coatings. Lysostaphin itself is able to bind to polymeric materials used in catheters and could

provide a facile loading system for rinsing to reduce the incidence of *S. aureus* related infections [95]. Using polydopamine to covalently attach lysostaphin, Yeroslavsky et al. demonstrated the creation of *S. aureus* resistant ceramic and polymer surfaces [96]. Lysostaphin was loaded into a cross-linked polyethylene glycol (PEG) hydrogel with cell adhesive peptides, and demonstrated diffusive and proteolytic release over time [97]. When delivered through the gel, lysostaphin inhibited *S. aureus* biofilms in vitro and supported bone repair in a model of infected fracture healing. Another hydrogel approach using a chitosan–collagen hydrogel incorporated with lysostaphin by Cui et al. was tested in vitro and in vivo with a rabbit infected burn model, both with MRSA, showing a reduction in bacterial viability [98]. A protease degradable hydrogel platform for the in situ delivery of lysostaphin was created by Singh and outperformed prophylactic antibiotic treatment in clearing bacteria from infected fractures in vivo, while also facilitating fracture healing and restoring a sterile inflammatory environment in a mouse model [99]. Nithya et al. prepared a chitosan gel with lysostaphin and tested it in vitro against established biofilms on catheters and pig ears to show a reduction in *S. aureus* biofilm potentiation. Additionally, in vitro tests for biocompatibility were performed with erythrocytes and mouse macrophages (RAW 264.67) showing nontoxicity of the delivery system [100]. Xue et al. developed a lysostaphin-loaded hydroxyapatite/chitosan composite bone cement capable of inhibiting *S. aureus* growth for up to 9 days when tested in vitro [101]. Biocompatibility was assessed against MC 3T3-E1 cells and in subcutaneous tissues of mice, but a major concern with this material is the 96 min set time as it may not have utility for mixing in the operating room [101]. Several studies have placed lysostaphin directly into wound dressings in order to reduce or treat biofilm formation in skin and soft tissue infections. Hathway et al. used poly-(N- isopropylacrylamide) (PNIPAM), a thermoresponsive polymer, molded into nanoparticles for the controlled release of the bacteriophage CHAPk, which has previously demonstrated anti-staphylococcal activity [102]. PNIPAM nanoparticles were then anchored to non-woven polypropylene to simulate a wound dressing, using plasma deposition, and loaded with lysostaphin via soaking. Together, the CHAPk and lysostaphin delivery had synergistic effects on the viability of MRSA252, when at physiologically relevant temperatures of 37 °C. Lysostaphin encapsulated in poly(D,L)-lactide coatings on titanium effectively reduced osteomyelitic progression in an implant-associated infection model [103]. It was concluded that this approach was capable of reducing infection, while subsequently promoting fracture healing and bridging of bone.

Overall, lysostaphin has been shown to substantially reduce *S. aureus*-related infections in several clinically applicable situations including skin-related infections, osteomyelitis, implant-associated infections, and catheter-associated infections. Despite the evidence of lysostaphin as a viable and highly selective treatment option for *S. aureus* infections, with a reduced likelihood of any tolerance or resistance occurring, there are no current lysostaphin delivery devices commercially available for clinical use. Determining the best mode for delivering pharmacologically active lysostaphin is still a pressing issue, with a need for more in vivo tests and human clinical trials in order to effectively implement the use of lysostaphin for biofilm prevention and treatment.

Nitric Oxide

Nitric oxide (NO) is an endogenously produced diatomic free radical, holding a central role in the body's wound healing process and immune response to infection [104–106]. The interaction of NO with reactive oxygen and nitrogen forms reactive antimicrobial derivatives including peroxynitirite, nitrogen dioxide, dinitrogen trioxide, dinitrogen tetroxide, and S-nitrosothiols [107]. These derivatives interfere with DNA, resulting in deamination and oxidative damage containing strand breaks, abasic sites, and other DNA alterations [108]. NO has also been shown to inhibit bacterial adhesion by destroying bacterial membrane adhesion proteins, due to the interaction of nitrogen intermediates with reactive thiols, iron sulfur clusters, heme groups, amines, phenol or aromatic amino acid residues of proteins [109]. Finally, NO has also been shown to inactivate metabolic enzymes by releasing iron from metalloenzymes, inhibiting critical metalloproteins in bacterial respiratory reactions [104]. One of the limitations of NO, for clinical applications, is its short in vivo half-life, which can be mitigated by local delivery with the additional advantage of reducing risks of systemic toxic effects [107].

Recent studies addressing the potential of NO to act as an anti-biofilm agent have been mostly limited to in vitro feasibility studies, though a wide range of in vivo studies have demonstrated the potential of NO to promote wound healing [106, 110]. NO has been delivered from alkyl chain modified poly(amidoamine) dendrimers, silica nanoparticles, polymeric coatings, polymeric films, poly(DL-lactic-co-glycolic acid) (PLGA) nanoparticles, xerogels, and S-nitrosothiol-modified chitosan oligosaccharides (Fig. 6) [111–113]. Brisbois et al. used Tecoflex SG-80-A

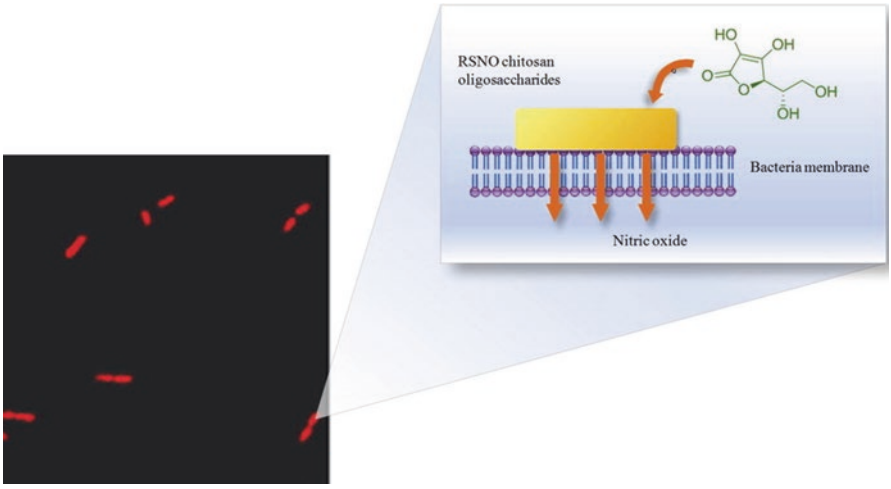


Fig. 6 RITC-labeled NO-releasing chitosan oligosaccharides association with planktonic *P. aeruginosa*, with a diagram of the mechanism of released nitric oxide across the bacterial membrane. (Reprinted with permission from Acta Biomaterialia, 2015; 12: 62–69. DOI: 10.1016/j.actbio.2014.10.028, Copyright 2014 Acta Materialia Inc)

polyurethane film with added diazeniumdiolated dibutylhexanediamine/N_2O_2 and PLGA to create a NO-releasing film [111]. In a mouse burn model, this NO-releasing film resulted in a ~4 log reduction in *Acinetobacter baumanni*, a common bacterial pathogen in burn victims [111]. Recent studies from Schoenfisch et al. have highlighted several NO delivery systems and their effectiveness at reducing *P. aeruginosa* and *S. aureus* biofilms, as well as their cytocompatibility when tested with murine fibroblasts (L929), although to date no in vivo studies have been reported [112, 114–117]. The topical delivery of NO-releasing molecules was demonstrated by Hoang et al. with the creation of an injectable gelatin hydrogel with S-nitrosothiolated gelatin [118]. Others have directed their approach to coating clinically used indwelling medical devices. Fleming et al. created a NO-releasing polymer coating using aminosilane precursors which decreased *P. aeruginosa* growth over 24 h [119]. While NO-releasing systems show promise, there is limited evidence supporting its efficacy in vivo to prevent biofilm formation or to disrupt already established biofilms. Most of these preclinical investigations of NO-releasing biomaterials evaluated NO release without adjunct antibiotic therapy; combinations with antibiotics may reveal additive or synergistic effects that could add to the clinical utility of these therapies.

Metabolites

Persister cells are transient subpopulations of slow-growing or growth-arrested bacterial cells, which often can result in recalcitrance and relapse of infections. Persisters are able to resume growth after exposure to otherwise lethal stresses and have been linked to an increase in risk for the emergence of antibiotic resistance during treatment [16, 120–122]. Due to their decreased metabolic state, researchers have shown that metabolic stimulation can increase the efficacy of antibiotics, particularly aminoglycosides [123]. Allison et al. demonstrated that aminoglycosides in combination with specific metabolites can be used to treat *E. coli* and *S. aureus* biofilms and improve the treatment of chronic infection in a mouse urinary tract infection model [123]. Mannitol and fructose were among the metabolites used to establish a metabolic-based approach for eradication of persister cells [123]. It has been proposed that adding metabolites generates a proton motive force, which increases the uptake of internally acting aminoglycoside antibiotics [123]. Barraud et al. demonstrated the ability of mannitol to increase the efficacy of tobramycin against *P. aeruginosa* biofilms in a concentration-dependent manner [124]. The delivery of mannitol by inhalation has been approved by regulatory agencies for the purpose of an osmotic agent for improved lung function in patients with cystic fibrosis [125, 126].

Another microbial metabolite studied for biofilm effects is erythritol, a naturally occurring **sugar alcohol**, similar in structure to mannitol, and recently shown to have an inhibitory effect on oral biofilm formation [127]. Mechanisms underlying erythritol's effects are hypothesized to be suppression of bacterial growth, decrease in expression of endopeptidase, glucosyltransferase, and fructosyltransferase genes, and the enhanced penetration of antibiotics into the mature biofilm, though this

particular mechanism remains unclear [128–132]. As erythritol diffuses into biofilm, it may weaken the cohesion of the intermolecular forces that cause aggregation of bacteria and EPS within a biofilm [133]. Lim et al. recently described a model to increase the efficacy of erythritol in weakening the cohesion of mature biofilms by the formation of a complex composed of erythritol and the zwitterionic molecule, betaine [133]. This complex successfully showed an increase in spontaneous biofilm detachment of *Streptococcus mutans* (*S. mutans*)biofilms in vitro [133]. For dental applications, oral rinses could be used to deliver erythritol and antimicrobials, as erythritol and other polyalcohols are approved for use as artificial sweeteners. Ammons et al. showed a reduction in biofilm viability with a lactoferrin/xylitol wound hydrogel used in combination with a silver wound dressing [134]. The combination of lactoferrin, an iron-binding glycoprotein, and xylitol had a synergistic effect against established *P. aeruginosa*, *S. aureus*, and MRSA biofilms [134].

The antimicrobial and anti-biofilm properties of honeys may also be mediated by sugar metabolites. Manuka and Ulmo honeys contain methylglyoxal as well as hydrogen peroxide, which are thought to drive their antimicrobial activities [135]. Majtan et al. evaluated the wound pathogens *Proteus mirabilis* and *Enterobacter cloacae* in the presence of different types of honey, finding that Manuka honey had the highest anti-biofilm activity and that methylglyoxal was responsible for biofilm eradication [136]. Lu et al. showed that the effects of methylglyoxal on the inhibition and eradication of *S. aureus* biofilms were mediated by glucose, fructose, and sucrose components of honey [137]. While honey affects the viability of biofilms for common orthopedic pathogens, including *S. aureus* and *P. aeruginosa* [138], it has no effect on some pathogenic biofilms such as *Enterococcus faecalis* (*E. faecalis)* [139]. There have been conflicting reports on the cytotoxicity of methylglyoxal and Manuka honey on cells [140–144]. Honey has been applied to tissues such as the ear and nasal sinuses undiluted or diluted to concentrations below cytotoxic thresholds through rinses. Biomaterial platforms for honey release for antimicrobial and tissue engineering applications, including electrospun membranes, cryogels, and hydrogels are reviewed by Minden-Birkenmaier et al. [145].

Phenols are naturally obtained plant metabolites that have various roles throughout the plant kingdom, including regulation of growth, structure, communication, and many aspects of their relationships with microorganisms [146–148]. These phenolic compounds have been investigated for their potential ability to protect against cardiovascular disease and cancer [146]. Because phenols are instrumental in protecting plants from microorganisms, they have also been investigated for their ability to treat infection and prevent biofilm formation [148]. Plyuta et al. tested the effects of eight different plant phenolic compounds on *P. aeruginosa* biofilm formation in vitro, and found that each compound could provide both stimulating or inhibiting effects depending on the concentration applied; when tested at concentrations lower than the MIC, all compounds enhanced bacterial growth, whereas above the MIC each compound suppressed bacterial growth and thus inhibited biofilm formation [148]. LaPlante et al. extracted phenolic compounds from cranberries to determine the ability of these extracts to prevent urinary tract- and catheter-related biofilm infections. While the cranberry phenols proved inactive against *E. coli*, they found that the extracts both halted bacterial growth and prevented biofilm formation

for each tested Gram-positive species in their in vitro studies [149]. Another phenolic compound with antimicrobial activity is tyrosol, which is produced by *Candida albicans* as a quorum sensing molecule and is also a component of olive oils [150, 151]. Recent studies have also shown that it has activity against *S. aureus* [152] as well as mixed species biofilms modeling oral cavity microorganism communities [153]. Including this phenolic compound in oral rinses or delivered through biomaterial systems could reduce biofilm formation clinically, though expanded preclinical studies are needed to determine an optimal dose and safety.

A well-studied sesquiterpene alcohol, farnesol, is released by *Candida albicans* to regulate biofilm formation in yeast, and is also a component of many essential oils. It has also been shown to inhibit and eradicate biofilms for bacterial strains, including pathogenic *S. aureus* [154–157]. While coating titanium alloy disks in a farnesol solution resulted in 3 days of protection from biofilms, dried coatings also had negative effects on pre-osteoblast cells [158]. In a rat model, farnesol proved to significantly decrease the occurrence of *S. mutans* biofilms in dental carries, especially on smoother surfaces, even when applied at low concentrations [147]. Horev et al. recognized that despite the beneficial anti-biofilm properties demonstrated by farnesol, it became much less active in acidic environments. Because of the low pH (about 4.5) of dental plaque, they sought to optimize a pH-activated nanoparticle delivery system to allow for the higher activity of farnesol in these environments. They found that these nanoparticles (fabricated from diblock copolymers composed of 2-(dimethylamino) ethylmethacrylate (DMAEMA), butyl methacrylate (BMA), and 2-propylacrylic acid (PAA) (p(DMAEMA)-b-p(DMAEMA-co-BMA-co-PAA)) successfully demonstrated the pH-responsive release of farnesol, and thus led to a higher anti-biofilm activity than in previous studies (Fig. 7) [159].

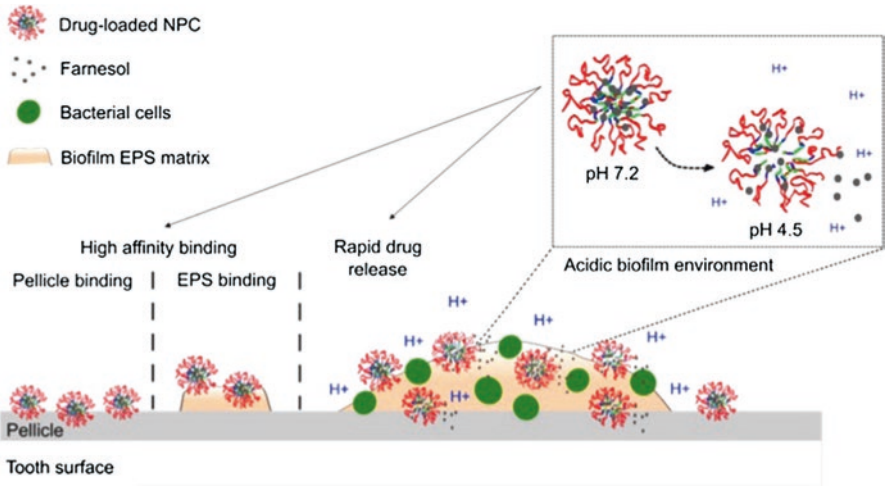


Fig. 7 Schematic of nanoparticle carriers for farnesol interacting with a tooth pellicle, biofilm EPS, and pH responsive drug release in the low pH environment of biofilm infection. (Reprinted with permission from ACS Nano, 2015, 9(3): 2390–2404, DOI: 10.1021/nn507170s. Copyright 2015 American Chemical Society)

Looking forward, while preliminary studies demonstrate a proof of principle, there are limited in vivo studies demonstrating the efficacy of metabolite-based therapies. Further, most of the studies on metabolites have not explored whether local or systemic delivery systems are needed for biofilm reduction. While the mechanisms of action for polyacohol metabolites have not been fully elucidated, co-delivery with an antibiotic or antimicrobial molecule is required for this strategy. Sugar alcohols, phenols, and other phytoceuticals show a promising avenue to eradicate biofilms trhough both a cellular and physical approach, and demonstration of in vivo efficacy and expansion into different tissue systems, such as chronic wounds or osteomyelitis, would be next steps in using metabolites as anti-infective therapies.

Nanoparticles

The use of nanotechnology in therapeutics has been studied extensively in the context of cancer therapies, for which many of the principles of treatment can be applied to wound biofilm infections [160–162]. Nanomaterials possess increased reactivity, attributed to their large surface area to volume ratio, as well as the flexibility in fabrication to control their chemical and physical properties [162]. Small nanoparticles can penetrate biofilms and accumulate within the EPS [163]. The local delivery of antibiotics has been explored within the context of many different types of nanoparticles, including with liposomal and polymeric nanoparticles [162]. Li et al. were able to encapsulate daptomycin in liposomes, which were effective at inhibiting *S. aureus* biofilm growth in a mouse model of subcutaneous infection [164].) PLGA nanoparticles were also used to encapsulate rifampicin and levofloxacin to effectively inhibit biofilm formation of *P. aeruginosa*, *S. aureus*, and *E. coli* [165–167]. These studies have successfully supported the idea of using nanoparticles for the effective targeted delivery of therapeutically relevant levels of antibiotics directly to a biofilm. Drawbacks to liposomal and polymeric nanoparticle delivery systems include cost of fabrication, inefficient drug loading, premature drug release, and toxicity concerns. Advances in this field could include methods to achieve site-specific release of antibiotics once these nanoparticles have reached their target [162]. New technologies targeting biofilms have focused on developing intrinsically antimicrobial nanoparticles, targeting drug or antimicrobial molecule delivery, or providing a construct for biophysical approaches, such as photothermal or alternating magnetic field (AMF) induced stimulation [162].

Metal nanoparticles, such as silver, copper, gold, titanium, and zinc have exhibited antimicrobial activity against biofilm. Among these, silver nanoparticles have been investigated extensively for biomedical applications [162]. Silver-based nanoparticles can penetrate biofilms and release antimicrobial silver ions. Silver ions released from nanoparticles can generate reactive oxygen species and interact with sulfhydryl groups, leading to the decline in bacterial cell membrane integrity, respiration, enzyme activities, and cell proliferation [168, 169]. Silver nanoparticles

have been shown to reduce the formation of biofilms from both Gram-positive and Gram-negative bacteria, including *P. aeruginosa* and *S. epidermidis* [170, 171]. The clinical applications of silver nanoparticles are limited by diminished therapeutic effect after prolonged treatment as well as cytotoxicity concerns [172]. Loo et al. combined both silver nanoparticles and curcumin, an inherently antimicrobial phenolic plant extract, to show an additive effect for the inhibition of *S. aureus* biofilm formation compared to silver nanoparticles alone [173]. Zinc oxide (ZnO) nanoparticles have also been shown to have inherent antimicrobial properties, which may be beneficial for the treatment of wound infections [174]. Kumar et al. were able to show improved wound healing and a decrease in bacterial growth in a rat model of a skin wound infection, using the combination of a B-chitin dressing in combination with ZnO nanoparticles [175].

Recently, the idea of using nanoparticles for energy conduits from outside sources, such as lasers or AMFs, has gained momentum. By applying an external energy source such as laser, increasing local temperatures of these particles induce irreversible thermal damage to bacterial cells [162]. Meeker et al. used spherical gold nanoconstructs conjugated to different antibodies toward bacterial membrane proteins and loaded them with antibiotics to show a successful reduction in *S. aureus* and *P. aeruginosa* biofilms [176]. Pulsed laser irradiation was used to achieve a photothermal effect accompanied by the release of desired antibiotics directly to established biofilm [176]. This technique supports the tunability to load several different antibiotics as well as different antibodies conjugated to the "nanocages" (Fig. 8) [176]. Superparamagnetic iron oxide nanoparticles can induce local hyperthermia in magnetic fields that can eradicate biofilm. Hyperthermia triggered by magnetic nanoparticles placed in an AMF reduced *P. aeruginosa* biofilms by more than 4-logs in vitro [177]. Kim et al. successfully validated the ability of MNP heating to effectively disrupt *S. aureus* biofilms using both in vitro and in vivo assays of a cutaneous wound infection mouse model [178]. The combination of magnetic nanoparticles with D-amino acids eradicated biofilms after exposure to an alternating magnetic field [179]. Magnetic nanoparticles were loaded within a glycol chitin hydrogel that formed a stable complex at body temperature. Magnetic nanoparticles modified with poly(ethylene glycol) and chitosan were shown to have increased biofilm penetration and higher eradication potential in the presence of magnetic fields [180]. However, a major concern for the translation of these technologies is the potential for tissue damage associated with elevated temperatures. Along with the risk of harming adjacent tissues from a rapid temperature increase, high intensity AMF on any conductive biological medium can induce Eddy currents, which can result in no n-specific inductive heating in the body [162].

Overall, the use of nanotechnologies to successfully eradicate a biofilm in the context of chronic infections seems promising. In vivo studies are limited and few nanoparticle therapies have been approved for clinical use, and thus limits exist on understanding how nanoparticles interact with biofilms in vivo, the interaction between nanoparticles and the host immune system, and the ultimate fate of the nanoparticles as they remain in the wound or migrate throughout the body.

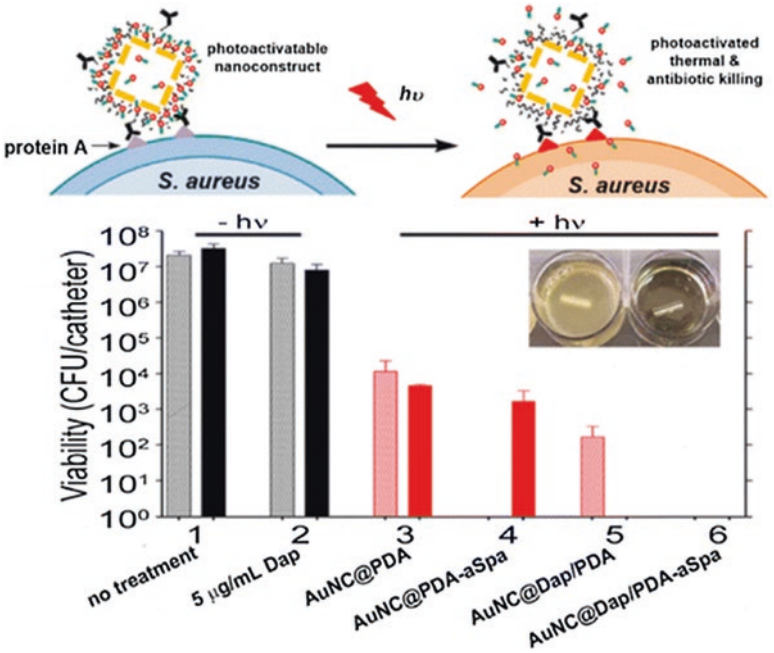


Fig. 8 Schematic of gold nanocages with an antibody targeting of a *S. aureus* protein activated by laser irradiation to kill a biofilm and release antibiotics. Bacterial cell killing using a biofilm model. Experimental groups are (1) no treatment, (2) 5 μg/mL daptomycin, and irradiation plus (3) AuNC@PDA, (4) AuNC@PDA−aSpa, (5) AuNC@DapHi/PDA, and (6) AuNC@DapHi/PDA−aSpa. Killing was assessed at 0 h (striped bars) and 24 h (solid bars) after treatment. Black bars indicate nonirradiated groups, and red bars indicate irradiated groups. (Reprinted with permission from ACS Infect Dis, 2016; 2(4): 241–250, DOI: 10.1021/acsinfecdis.5b00117. https://pubs.acs.org/doi/abs/10.1021/acsinfecdis.5b00117, further permissions related to the material excerpted should be directed to the ACS. Copyright 2016 American Chemical Society)

Delivery of Living Cells

With the advent of biomaterial tissue engineering strategies, proof of principle studies have suggested technologies to engineer biofilm dispersal by delivering living cells. Studies by Thurlow et al. have suggested that evasion of immune cell clearance by *S. aureus* biofilm is in part due to the influence of biofilm on immune cells, skewing macrophage activation to anti-inflammatory and pro-fibrotic pathways [14]. Macrophages polarized toward the M2 phenotype by biofilm-secreted proteins [181] or those deficient in IL-1b-triggered activation [182] lack the phagocytic response necessary to clear biofilm. Hanke et al. demonstrated that delivery of macrophages activated toward the M1 proinflammatory phenotype were able to attenuate biofilm formation in vitro and in vivo [183]. In this study, delivery of a proinflammatory peptide EP67 and administration of M1 activated macrophages successfully reduced the occurrence of early infections by *S. aureus* in a mouse

catheter-associated biofilm model. In the treatment of established catheter infections, delivery of activated macrophages, but not the proinflammatory peptide, was able to achieve a reduction of a biofilm on catheters. Labeled macrophages delivered locally in saline were shown to be present at the sites of injection for up to 5 days. Administration of macrophages for treatment in this study occurred in four separate local injections delivered in three doses timed at 12, 24, and 48 h to improve contact with biofilm-infected surfaces and tissue. Cell delivery through biodegradable polymer scaffolds may improve efficacy of immune cell therapy for biofilm treatment and improve cell retention in the tissue and biomaterial.

Lytic bacteriophages are viruses that infect bacterial cells, replicate inside them, and rupture bacterial membranes to release more virus. An advantage of bacteriophage therapy is that these viruses do not infect host cells, and show no adverse effects in animal and clinical trials [184, 185]. Recent studies have indicated that bacteriophages could have anti-biofilm activity and could increase antibiotic efficacy against biofilms (Fig. 9) [186–188], particularly when combined with antibiotic therapy [189]. One limitation of bacteriophage therapy is that strain specificity often requires a cocktail of multiple types of bacteriophages for bacterial clearance [190–192]. A potential impediment to phage therapy for biofilms is the ability of phage to penetrate into the EPS [193], though some bacteriophages also produce enzymes such as polysaccharide dehydrolases, alginate lyases, and glucoside hydrolases that degrade EPS and could promote the penetration of phages into biofilms [194–196]. Resch et al. demonstrated that a phage cocktail delivered in saline could clear catheters of *Pseudomonas* infection in endocarditis models when combined with the antibiotic ciprofloxacin [197]. In the first clinical trials of bacteriophage therapy for otitis media, bacteriophages applied to the ear in a saline glycerol carrier successfully improved indicators of clinical outcomes [185]. The delivery routes of phage therapy vary by application including parenteral administration, oral ingestion, and direct delivery to tissue [198]. An advantage of phage therapy is that it can be administered systemically with little adverse effects, though high concentrations necessary for therapeutic effect may be achieved more readily with a delivery system. Carson et al. showed that immersing hydrogel coated catheters directly in bacteriophage culture removed biofilms on Foley catheters [199]. Hydrogel delivery systems for phage therapies have been commercialized in some countries, designed for the treatment of skin wound infections [200]. Milo et al. investigated the pH-sensitive polymer EUDRAGIT®S 100 to form a stimuli-triggered release of phages in the presence of *P. mirabilis* urinary tract infections, which increase the pH of urine [201]. Vinner et al. also evaluated the EUDRAGIT polymer as an intestinal delivery system for phages specific to *Clostridium difficile*, making use of the pH responsiveness to control release [202]. Hathaway et al. formulated nanosphere carriers for phage release from thermoreversible poly(N-isopropylacrylamide) copolymerized with allylamine from woven wound dressing materials upon exposure to elevated temperatures associated with wound infections [203]. The combination of bacteriophages with lysostaphin delivered from thermoresponsive nanospheres had a synergistic effect specific to *S. aureus* [102]. A versatile fibrin glue biomaterial delivery system showed a sustained release of bacteriophage, but have not been evaluated in vivo [204]. Encapsulation and delivery methods for phages should maintain the activity of phages, and the effects of different encapsulation strategies

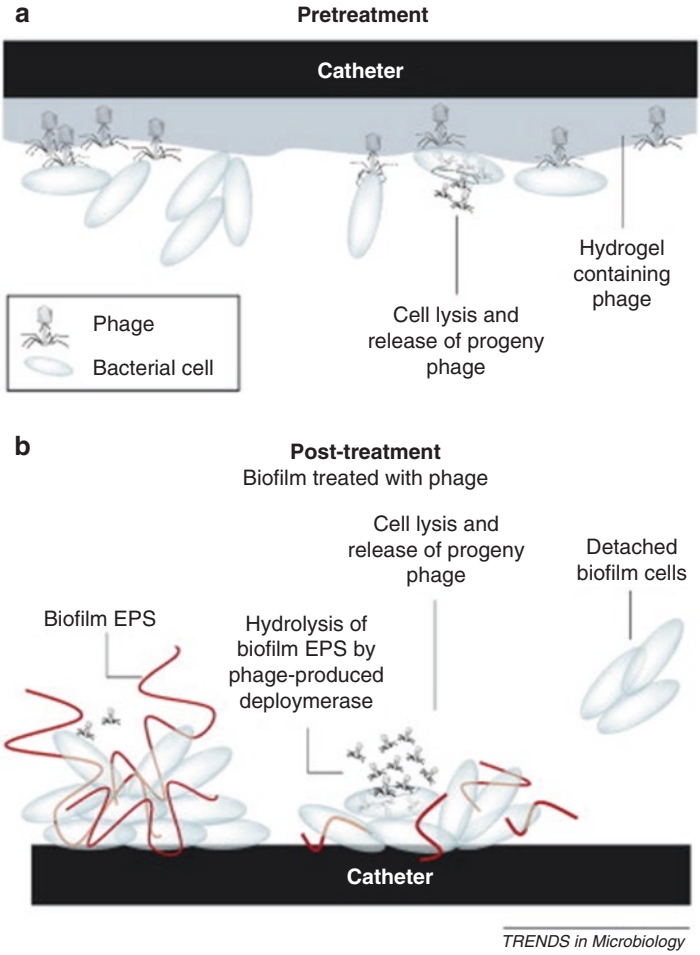


Fig. 9 Potential applications for using phages to treat biofilms. **(a)** Application of phage pretreatment of an indwelling catheter for the prevention of bacterial colonization and biofilm formation on catheter surfaces. Phages embedded in a hydrogel on the catheter are able to infect and lyse bacterial cells. **(b)** Application of phage for the treatment of an existing biofilm on an indwelling catheter. Phages are added to a catheter with an existing bacterial biofilm leading to hydrolysis of the EPS and detachment of the biofilm bacteria from the catheter. Phages can also infect and lyse bacterial cells. (Reprinted with permission from Trends in Microbiol, 2009; 17(2): 66–72. Copyright 2009 Elsevier Ltd)

have been reviewed extensively by Malik et al. [205]. Another potential limitation of phage therapy for biofilm eradication is the CRISPR-Cas9 system of bacteria to overcome viral infection to acquire immunity from bacteriophages, though the inclusion of anti-CRISPR phages within cocktails could overcome this limitation [206]. Phage therapy is a promising strategy with growing evidence of safety and efficacy to treat problematic infections. Further research on biomaterial delivery routes and controlled release could advance this therapy for specific applications, such as osteomyelitis.

Conclusions

Recent advances in the study of biofilms have led to novel therapeutic approaches, including molecular, cellular, and nanotechnology approaches to target biofilms (Table 2). Several of these advances effectively inhibit biofilms, though eradicating

Table 2 Summary of anti-biofilm therapeutic treatment

Anti-biofilm therapeutic	Type of studies	Key contributions	References
Peptides and amino acids			
D-amino acids	In vitro	Disassembly and inhibition of biofilm in *B. subtilis. Staphylococcus*, and *Pseudomonas*	[25–27]
	In vivo	Efficacy of D-amino acids in animal models of orthopedic infection	[28, 29, 179]
Antimicrobial peptides	In vitro	Structure and stability of AMPs; synergism with antimicrobials	[31, 34–36, 38]
	In vivo	Safety and efficacy of AMPs	[44]
	Clinical		[37, 40]
Fatty acids and lipids			
Diffusible signaling factors and cis-2-decenoic acid	In vitro	Cis-2-decenoic activity in inhibiting, dispersing biofilms Broad-spectrum activity in dispersal Reverses persister cell state Synergism with antibiotics and antimicrobials	[56, 59–61, 63]
	In vivo	Cis-2-decenoic acid within coatings prevent biofilm on orthopedic implants	[207]
Rhamnolipids	In vitro		[65–68]
Enzymes			
Glycoside Hydrolase and alginate lyase	In vitro	Activity of PslG and PelA in triggering disassembly of biofilm	[72, 73, 75]
	In vivo	Dispersal of wound biofilms could lead to septicemia if antibiotics are not co-delivered	[71]
Protease	In vitro	Esp and SpeB disperse and inhibit Staphylococcal biofilms	[76, 78]
	In vivo	SpeB disperses biofilm in murine model but	[77]
	Clinical	Protease from S. epidermidis inhibits nasal colonization with *S. aureus*	[76]
Nuclease	In vitro	Decrease biofilm and increase antibiotic susceptibility	[79]
	In vivo	Prevent biofilm formation in animal models of vaginosis; Mutants with mutations of nuclease are less susceptible to biofilm catheter infection	[81, 80]
Dispersin B	In vitro	Hydrolyzes matrix components Could eradicate biofilm on orthopedic implants Combination with antibiotics increases susceptibility	[82–85]
	In vivo		[86, 87]

(continued)

Table 2 (continued)

Lysostaphin	In vitro	Anti-biofilm activity; ability to coat catheters and covalently attach to surfaces; loading with calcium/chitosan bone cement; delivery with bacteriophage through thermal-responsive polymer	[91, 93–96, 101, 102]
	In vivo	Prevention of implant biofilm; supports bone healing and wound healing	[90, 97–100, 103]
Nitric oxide	In vitro	Derivatives that release NO; silica nanoparticles; superhydrophobic xerogels; chitosan gels, combination with silver, Poly(amidoamine) Dendrimers, peroxynitrite loaded hydrogels	[108, 112–119]
	In vivo	Efficacy in burn wound treatment	[111]
Metabolites			
Mannitol and erythritol	In vitro	Reversion of persister cells, increased susceptibility to antimicrobials, anti-cariogenic uses, fungicidal combinations	[122–125, 127–133]
	In vivo	Urinary tract infection treatment	[123]
Honey	In vitro	Identification of active components; cell activity stimulated; biofilm eradication; efficacy against *S. aureus* and *P. aeruginosa*, not effective against *E. faecalis*	[135–139, 144]
	In vivo	Otological safety; effective treatment for chronic rhinosinusitis; safety in treating chronic otitis	[140, 141, 143]
Phenols	In vitro	Biofilm inhibition properties of tyrosol, essential oils	[148–150, 152, 153]
Farnesol	In vitro	Biofilm inhibition, combination with antimicrobials, osteoblast compatibility, delivery through pH responsive polymers	[154–158]
	In vivo	Anticariogenic application, pH Responsive release	[147, 159]
Nanoparticles	In vitro	Interactions of nanoparticles with biofilms, liposomal daptomycin, antibiotic-loaded PLGA nanoparticles, silver nanoparticles, zinc oxide particles, targeted gold nanocages for antibiotics	[163, 164, 166–168, 170–177, 179, 180]
	In vivo	Hyperthermia in magnetic nanoparticles in cutaneous infection model	[178]
Living cells			
Macrophages	In vitro	Biofilm secretes factors that inhibit macrophage migration and phagocytosis	[14, 20–22, 181]
	In vivo	M1 activated macrophages attenuate biofilm infection; Deficiency in IL-1beta hinders clearance of *S. aureus* biofilm; M2 polarized macrophages lack phagocytic response toward biofilm	[14, 181–183]
Bacteriophages	In vitro	Biofilm dispersal and clearance by macrophages; combination with antibiotic therapy; phages with enzymatic degradation of EPS; delivery of phage through polymeric and fibrin glue systems	[186–189, 191, 193–195, 198, 199, 201, 203–205]
	In vivo	Clearance of endocarditis, treatment of chronic osteomyelitis	[190, 197, 202]
	Clinical	Otitis media, skin wound infections	[185, 200]

mature biofilms remains a challenge. Due to these challenges as well as the risk of dispersed biofilm leading to hematogenous spread of bacteria and septicemia, these approaches are often combined with traditional antibiotic approaches. Further, investigations on the therapeutic approaches are in varying stages of the translational pipeline, ranging from in vitro preclinical to clinical studies. Opportunities for advanced delivery strategies for controlled or targeted release and activity are highlighted and can be tailored to the molecule delivered and therapeutic application.

Acknowledgments JAJ, ZH, and LP are supported by and the National Institute of Arthritis and Musculoskeletal and Skin Disease of the National Institutes of Health (NIH) under Award Number R01AR066050. RA was supported by the University of Memphis First Generation PhD Fellowship, with additional student support from the Herff College of Engineering.

References

1. Fux C, Costerton JW, Stewart PS, Stoodley P (2005) Survival strategies of infectious biofilms. Trends Microbiol 13(1):34–40
2. Stoodley P, Hall-Stoodley L, Costerton B, DeMeo P, Shirtliff M, Gawalt E, Kathju S (2013) Biofilms, biomaterials, and device-related infections. Appl Med Med Devices 2013:77–102
3. Brady RA, Leid JG, Calhoun JH, Costerton JW, Shirtliff ME (2008) Osteomyelitis and the role of biofilms in chronic infection. FEMS Immunol Med Microbiol 52(1):13–22. https://doi.org/10.1111/j.1574-695X.2007.00357.x
4. Stoodley P, Ehrlich GD, Sedghizadeh PP, Hall-Stoodley L, Baratz ME, Altman DT et al (2011) Orthopaedic biofilm infections. Curr Orthopaed Pract 22(6):558–563. https://doi.org/10.1097/BCO.0b013e318230efcf
5. Bezstarosti H, Van Lieshout E, Voskamp L, Kortram K, Obremskey W, McNally M et al (2018) Insights into treatment and outcome of fracture-related infection: a systematic literature review. Arch Orthop Trauma Surg 139:61–72
6. Geraghty T, LaPorta G (2019) Current health and economic burden of chronic diabetic osteomyelitis. Exp Rev Pharmacoecon Outcomes Res (just-accepted)
7. Geurts J, Vranken T, Gabriels F, Arts J, Moh P (2018) Contemporary treatment of chronic osteomyelitis: implementation in low-and middle-income countries. SA Orthopaed J 17(2):40–43
8. Akers KS, Mende K, Cheatle KA, Zera WC, Yu X, Beckius ML et al (2014) Biofilms and persistent wound infections in United States military trauma patients: a case–control analysis. BMC Infect Dis 14(1):1
9. Barsoumian AE, Mende K, Sanchez CJ, Beckius ML, Wenke JC, Murray CK, Akers KS (2015) Clinical infectious outcomes associated with biofilm-related bacterial infections: a retrospective chart review. BMC Infect Dis 15(1):223
10. Kord M, Ardebili A, Jamalan M, Jahanbakhsh R, Behnampour N, Ghaemi EA (2018) Evaluation of biofilm formation and presence of Ica genes in Staphylococcus epidermidis clinical isolates. Osong Publ Health Res Perspect 9(4):160
11. Rodríguez-Pardo D, Pigrau C, Lora-Tamayo J, Soriano A, Del Toro M, Cobo J et al (2014) Gram-negative prosthetic joint infection: outcome of a debridement, antibiotics and implant retention approach. A large multicentre study. Clin Microbiol Infect 20(11):O911–O919
12. Brauner A, Fridman O, Gefen O, Balaban NQ (2016) Distinguishing between resistance, tolerance and persistence to antibiotic treatment. Nat Rev Microbiol 14(5):320–330. https://doi.org/10.1038/nrmicro.2016.34
13. Flemming H-C, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S (2016) Biofilms: an emergent form of bacterial life. Nat Rev Microbiol 14(9):563

14. Thurlow LR, Hanke ML, Fritz T, Angle A, Aldrich A, Williams SH et al (2011) Staphylococcus aureus biofilms prevent macrophage phagocytosis and attenuate inflammation in vivo. J Immunol 186(11):6585–6596. https://doi.org/10.4049/jimmunol.1002794
15. Walters MC, Roe F, Bugnicourt A, Franklin MJ, Stewart PS (2003) Contributions of antibiotic penetration, oxygen limitation, and low metabolic activity to tolerance of Pseudomonas aeruginosa biofilms to ciprofloxacin and tobramycin. Antimicrob Agents Chemother 47(1):317–323
16. Fisher RA, Gollan B, Helaine S (2017) Persistent bacterial infections and persister cells. Nat Rev Microbiol 15(8):453
17. Rowe SE, Conlon BP, Keren I, Lewis K (2016) Persisters: methods for isolation and identifying contributing factors—a review. In: Bacterial persistence. Springer, Berlin, pp 17–28
18. Flemming H-C (2016) EPS—then and now. Microorganisms 4(4):41
19. Gupta P, Sarkar S, Das B, Bhattacharjee S, Tribedi P (2016) Biofilm, pathogenesis and prevention—a journey to break the wall: a review. Arch Microbiol 198(1):1–15
20. Bhattacharya M, Berends ET, Chan R, Schwab E, Roy S, Sen CK et al (2018) Staphylococcus aureus biofilms release leukocidins to elicit extracellular trap formation and evade neutrophil-mediated killing. Proc Natl Acad Sci 115(28):7416–7421
21. Ebersole JL, Peyyala R, Gonzalez OA (2019) Biofilm-induced profiles of immune response gene expression by oral epithelial cells. Mol Oral Microbiol 34(1)
22. Le KY, Park MD, Otto M (2018) Immune evasion mechanisms of Staphylococcus epidermidis biofilm infection. Front Microbiol 9:359
23. Fabbri S, Stoodley P (2016) Mechanical properties of biofilms. In: Flemming H-C, Wingender J, Neu T (eds) The perfect slime—microbial extracellular polymeric substances, pp 153–178
24. Leary JT, Werger MM, Broach WH, Shaw LN, Santoni BG, Bernasek TL, Lyons ST (2017) Complete eradication of biofilm from orthopedic materials. J Arthroplasty 32(8):2513–2518. https://doi.org/10.1016/j.arth.2017.03.050
25. Hochbaum AI, Kolodkin-Gal I, Foulston L, Kolter R, Aizenberg J, Losick R (2011) Inhibitory effects of D-amino acids on Staphylococcus aureus biofilm development. J Bacteriol 193:5616–5622. https://doi.org/10.1128/jb.05534-11
26. Kolodkin-Gal I, Romero D, Cao S, Clardy J, Kolter R, Losick R (2010) D-amino acids trigger biofilm disassembly. Science 328(5978):627–629. https://doi.org/10.1126/science.1188628
27. Leiman SA, May JM, Lebar MD, Kahne D, Kolter R, Losick R (2013) D-amino acids indirectly inhibit biofilm formation in Bacillus subtilis by interfering with protein synthesis. J Bacteriol 195:5391–5395
28. Sanchez CJ Jr, Akers KS, Romano DR, Woodbury RL, Hardy SK, Murray CK, Wenke JC (2014) D-Amino Acids Enhance the Activity of Antimicrobials Against Biofilms of Clinical Wound Isolates of Staphylococcus aureus and Pseudomonas aeruginosa. Antimicrob Agents Chemother 58:4353–4361. https://doi.org/10.1128/AAC.02468-14
29. Harmata AJ, Ma Y, Sanchez CJ, Zienkiewicz KJ, Elefteriou F, Wenke JC, Guelcher SA (2015) D-amino acid inhibits biofilm but not new bone formation in an ovine model. Clin Orthop Relat Res 473(12):3951–3961. https://doi.org/10.1007/s11999-015-4465-9
30. Jennings J (2015) CORR insights1: D-amino acid inhibits biofilm but not new bone formation in an ovine Model. Clin Orthop Relat Res 473:3962–3964
31. Fjell CD, Hiss JA, Hancock RE, Schneider G (2012) Designing antimicrobial peptides: form follows function. Nat Rev Drug Discov 11(1):37
32. Mahlapuu M, Håkansson J, Ringstad L, Björn C (2016) Antimicrobial peptides: an emerging category of therapeutic agents. Front Cell Infect Microbiol 6:194
33. Zhang LJ, Gallo RL (2016) Antimicrobial peptides. Curr Biol 26(1):R14–R19. https://doi.org/10.1016/j.cub.2015.11.017
34. Malik E, Dennison S, Harris F, Phoenix D (2016) pH dependent antimicrobial peptides and proteins, their mechanisms of action and potential as therapeutic agents. Pharmaceuticals 9(4):67
35. Batoni G, Maisetta G, Esin S (2016) Antimicrobial peptides and their interaction with biofilms of medically relevant bacteria. Biochim Biophys Acta 1858(5):1044–1060. https://doi.org/10.1016/j.bbamem.2015.10.013

36. Chung PY, Khanum R (2017) Antimicrobial peptides as potential anti-biofilm agents against multidrug-resistant bacteria. J Microbiol Immunol Infect 50(4):405–410. https://doi.org/10.1016/j.jmii.2016.12.005
37. Fritsche TR, Rhomberg P, Jones RN (2008) Activity of Omiganan, a novel peptide, tested against contemporary gram-negative pathogens: results from an international surveillance program. In: Paper presented at the Interscience Conference of Antimicrobial Agents and Chemotherapy Washington, DC
38. Flamm RK, Rhomberg PR, Simpson KM, Farrell DJ, Sader HS, Jones RN (2015) In vitro spectrum of pexiganan activity when tested against pathogens from diabetic foot infections and with selected resistance mechanisms. Antimicrob Agents Chemother 59:1751–1754
39. Gomes A, Teixeira C, Ferraz R, Prudêncio C, Gomes P (2017) Wound-healing peptides for treatment of chronic diabetic foot ulcers and other infected skin injuries. Molecules 22(10):1743
40. van der Velden WJ, van Iersel TM, Blijlevens NM, Donnelly JP (2009) Safety and tolerability of the antimicrobial peptide human lactoferrin 1-11 (hLF1-11). BMC Med 7(1):44
41. Balaban N, Stoodley P, Fux CA, Wilson S, Costerton JW, Dell'Acqua G (2005) Prevention of staphylococcal biofilm-associated infections by the quorum sensing inhibitor RIP. Clin Orthop Relat Res 437:48–54
42. Cirioni O, Ghiselli R, Minardi D, Orlando F, Mocchegiani F, Silvestri C et al (2007) RNAIII-inhibiting peptide affects biofilm formation in a rat model of staphylococcal ureteral stent infection. Antimicrob Agents Chemother 51(12):4518–4520
43. Anguita-Alonso P, Giacometti A, Cirioni O, Ghiselli R, Orlando F, Saba V et al (2007) RNAIII-inhibiting-peptide-loaded polymethylmethacrylate prevents in vivo Staphylococcus aureus biofilm formation. Antimicrob Agents Chemother 51(7):2594–2596
44. de Breij A, Riool M, Kwakman PH, de Boer L, Cordfunke RA, Drijfhout JW et al (2016) Prevention of Staphylococcus aureus biomaterial-associated infections using a polymer-lipid coating containing the antimicrobial peptide OP-145. J Control Release 222:1–8
45. Andrä J, Jakovkin I, Grötzinger J, Hecht O, Krasnosdembskaya AD, Goldmann T et al (2008) Structure and mode of action of the antimicrobial peptide arenicin. Biochem J 410(1):113–122
46. Kowalski RP, Romanowski EG, Yates KA, Mah FS (2016) An independent evaluation of a novel peptide mimetic, brilacidin (PMX30063), for ocular anti-infective. J Ocul Pharmacol Ther 32(1):23–27
47. Kaplan CW, Sim JH, Shah KR, Kolesnikova A, Shi W, Eckert R (2011) Selective membrane disruption: the mode of action of C16G2, a specifically-targeted antimicrobial peptide. Antimicrob Agents Chemother 55:3446–3452
48. Stallmann HP, Faber C, Bronckers AL, Nieuw Amerongen AV, Wuisman PI (2004) Osteomyelitis prevention in rabbits using antimicrobial peptide hLF1-11-or gentamicin-containing calcium phosphate cement. J Antimicrob Chemother 54(2):472–476
49. Trotti A, Garden A, Warde P, Symonds P, Langer C, Redman R et al (2004) A multinational, randomized phase III trial of iseganan HCl oral solution for reducing the severity of oral mucositis in patients receiving radiotherapy for head-and-neck malignancy. Int J Radiat Oncol Biol Phys 58(3):674–681
50. Grönberg A, Mahlapuu M, Ståhle M, Whately-Smith C, Rollman O (2014) Treatment with LL-37 is safe and effective in enhancing healing of hard-to-heal venous leg ulcers: a randomized, placebo-controlled clinical trial. Wound Repair Regen 22(5):613–621
51. O'Neil D, Mercer D, Stewart C (2013) Method of establishing a fungal nail infection. In: Google Patents
52. Li P, Li X, Saravanan R, Li CM, Leong SSJ (2012) Antimicrobial macromolecules: synthesis methods and future applications. RSC Adv 2(10):4031–4044
53. Greber K, Dawgul M (2017) Antimicrobial peptides under clinical trials. Curr Top Med Chem 17(5):620–628
54. Wiig M, Olmarker K, Håkansson J, Ekström L, Nilsson E, Mahlapuu M (2011) A lactoferrin-derived peptide (PXL01) for the reduction of adhesion formation in flexor tendon surgery: an experimental study in rabbits. J Hand Surg Eur Vol 36(8):656–662

55. Solano C, Echeverz M, Lasa I (2014) Biofilm dispersion and quorum sensing. Curr Opin Microbiol 18:96–104
56. Marques CN, Morozov A, Planzos P, Zelaya HM (2014) The fatty acid signaling molecule cis-2-decenoic acid increases metabolic activity and reverts persister cells to an antimicrobial-susceptible state. Appl Environ Microbiol 80(22):6976–6991. https://doi.org/10.1128/AEM.01576-14
57. Landini P, Antoniani D, Burgess JG, Nijland R (2010) Molecular mechanisms of compounds affecting bacterial biofilm formation and dispersal. Appl Microbiol Biotechnol 86(3):813–823. https://doi.org/10.1007/s00253-010-2468-8
58. Estrela AB, Abraham W-R (2010) Combining biofilm-controlling compounds and antibiotics as a promising new way to control biofilm infections. Pharmaceuticals 3(5):1374–1393. https://doi.org/10.3390/ph3051374
59. Davies DG, Marques CN (2009) A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. J Bacteriol 191(5):1393–1403. https://doi.org/10.1128/JB.01214-08
60. Jennings JA, Courtney HS, Haggard WO (2012) Cis-2-decenoic acid inhibits S. aureus growth and biofilm in vitro: a pilot study. Clin Orthop Relat Res 470(10):2663–2670. https://doi.org/10.1007/s11999-012-2388-2
61. Masters E, Harris M, Jennings J (2016) Cis-2-Decenoic acid interacts with bacterial cell membranes to potentiate additive and synergistic responses against biofilm. J Bacteriol Mycol 3(3):1–8
62. Harris M, Ahmed H, Barr B, LeVine D, Pace L, Mohapatra A et al (2017a) Magnetic stimuli-responsive chitosan-based drug delivery biocomposite for multiple triggered release. Int J Biol Macromol 104:1407–1414
63. Rahmani-Badi A, Sepehr S, Mohammadi P, Soudi MR, Babaie-Naiej H, Fallahi H (2014) A combination of cis-2-decenoic acid and antibiotics eradicates pre-established catheter-associated biofilms. J Med Microbiol 63(Pt 11):1509–1516. https://doi.org/10.1099/jmm.0.075374-0
64. Nickzad A, Déziel E (2014) The involvement of rhamnolipids in microbial cell adhesion and biofilm development—an approach for control? Lett Appl Microbiol 58(5):447–453
65. Rodrigues L, Banat IM, Van der Mei H, Teixeira J, Oliveira R (2006) Interference in adhesion of bacteria and yeasts isolated from explanted voice prostheses to silicone rubber by rhamnolipid biosurfactants. J Appl Microbiol 100(3):470–480
66. Sodagari M, Wang H, Newby B-m Z, Ju L-K (2013) Effect of rhamnolipids on initial attachment of bacteria on glass and octadecyltrichlorosilane-modified glass. Colloids Surf B Biointerfaces 103:121–128
67. Dusane DH, Nancharaiah YV, Zinjarde SS, Venugopalan VP (2010) Rhamnolipid mediated disruption of marine Bacillus pumilus biofilms. Colloids Surf B Biointerfaces 81(1):242–248
68. De Araujo LV, Abreu F, Lins U, Santa Anna LMDM, Nitschke M, Freire DMG (2011) Rhamnolipid and surfactin inhibit Listeria monocytogenes adhesion. Food Res Int 44(1):481–488
69. Sutherland IW (2001) Biofilm exopolysaccharides: a strong and sticky framework. Microbiology 147(1):3–9
70. Jennings LK, Storek KM, Ledvina HE, Coulon C, Marmont LS, Sadovskaya I et al (2015) Pel is a cationic exopolysaccharide that cross-links extracellular DNA in the Pseudomonas aeruginosa biofilm matrix. Proc Natl Acad Sci 112(36):11353–11358
71. Fleming D, Rumbaugh K (2018) The consequences of biofilm dispersal on the host. Sci Rep 8(1):10738
72. Yu S, Su T, Wu H, Liu S, Wang D, Zhao T et al (2015) PslG, a self-produced glycosyl hydrolase, triggers biofilm disassembly by disrupting exopolysaccharide matrix. Cell Res 25(12):1352
73. Baker P, Hill PJ, Snarr BD, Alnabelseya N, Pestrak MJ, Lee MJ et al (2016) Exopolysaccharide biosynthetic glycoside hydrolases can be utilized to disrupt and prevent Pseudomonas aeruginosa biofilms. Sci Adv 2(5):e1501632

74. Cotton LA, Graham RJ, Lee RJ (2009) The role of alginate in P. aeruginosa PAO1 biofilm structural resistance to gentamicin and ciprofloxacin. J Exp Microbiol Immunol 13:58–62
75. Lamppa JW, Griswold KE (2013) Alginate lyase exhibits catalysis-independent biofilm dispersion and antibiotic synergy. Antimicrob Agents Chemother 57(1):137–145
76. Iwase T, Uehara Y, Shinji H, Tajima A, Seo H, Takada K et al (2010) Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization. Nature 465(7296):346
77. Connolly KL, Roberts AL, Holder RC, Reid SD (2011) Dispersal of Group A streptococcal biofilms by the cysteine protease SpeB leads to increased disease severity in a murine model. PLoS One 6(4):e18984
78. Loughran AJ, Atwood DN, Anthony AC, Harik NS, Spencer HJ, Beenken KE, Smeltzer MS (2014) Impact of individual extracellular proteases on Staphylococcus aureus biofilm formation in diverse clinical isolates and their isogenic sarA mutants. Microbiology Open 3(6):897–909
79. Kaplan JB, LoVetri K, Cardona ST, Madhyastha S, Sadovskaya I, Jabbouri S, Izano EA (2012) Recombinant human DNase I decreases biofilm and increases antimicrobial susceptibility in staphylococci. J Antibiot 65(2):73
80. Weiss EC, Zielinska A, Beenken KE, Spencer HJ, Daily SJ, Smeltzer MS (2009) Impact of sarA on daptomycin susceptibility of Staphylococcus aureus biofilms in vivo. Antimicrob Agents Chemother 53(10):4096–4102. https://doi.org/10.1128/AAC.00484-09
81. Hymes SR, Randis TM, Sun TY, Ratner AJ (2013) DNase inhibits Gardnerella vaginalis biofilms in vitro and in vivo. J Infect Dis 207(10):1491–1497
82. Donelli G, Francolini I, Romoli D, Guaglianone E, Piozzi A, Ragunath C, Kaplan J (2007) Synergistic activity of dispersin B and cefamandole nafate in inhibition of staphylococcal biofilm growth on polyurethanes. Antimicrob Agents Chemother 51(8):2733–2740
83. Chaignon P, Sadovskaya I, Ragunah C, Ramasubbu N, Kaplan J, Jabbouri S (2007) Susceptibility of staphylococcal biofilms to enzymatic treatments depends on their chemical composition. Appl Microbiol Biotechnol 75(1):125–132
84. Hogan S, Zapotoczna M, Stevens N, Humphreys H, O'Gara J, O'Neill E (2017) Potential use of targeted enzymatic agents in the treatment of Staphylococcus aureus biofilm-related infections. J Hosp Infect 96(2):177–182
85. Gawande PV, Leung KP, Madhyastha S (2014) Antibiofilm and antimicrobial efficacy of DispersinB®-KSL-W peptide-based wound gel against chronic wound infection associated bacteria. Curr Microbiol 68(5):635–641
86. Darouiche RO, Mansouri MD, Gawande PV, Madhyastha S (2009) Antimicrobial and antibiofilm efficacy of triclosan and DispersinB® combination. J Antimicrob Chemother 64(1):88–93
87. Kaplan JB, Mlynek KD, Hettiarachchi H, Alamneh YA, Biggemann L, Zurawski DV et al (2018) Extracellular polymeric substance (EPS)-degrading enzymes reduce staphylococcal surface attachment and biocide resistance on pig skin in vivo. PLoS One 13(10):e0205526
88. Schindler CA, Schuhardt VT (1964) Lysostaphin: a new bacteriolytic agent for the Staphylococcus. Proc Natl Acad Sci U S A 51:414–421
89. Baba T, Schneewind O (1996) Target cell specificity of a bacteriocin molecule: a C-terminal signal directs lysostaphin to the cell wall of Staphylococcus aureus. EMBO J 15(18):4789–4797
90. Climo MW, Patron RL, Goldstein BP, Archer GL (1998) Lysostaphin treatment of experimental methicillin-resistant Staphylococcus aureus aortic valve endocarditis. Antimicrob Agents Chemother 42(6):1355–1360
91. Kiri N, Archer G, Climo MW (2002) Combinations of lysostaphin with beta-lactams are synergistic against oxacillin-resistant Staphylococcus epidermidis. Antimicrob Agents Chemother 46(6):2017–2020
92. Mohamed MF, Hamed MI, Panitch A, Seleem MN (2014) Targeting methicillin-resistant Staphylococcus aureus with short salt-resistant synthetic peptides. Antimicrob Agents Chemother 58(7):4113–4122. https://doi.org/10.1128/AAC.02578-14

93. Patron RL, Climo MW, Goldstein BP, Archer GL (1999) Lysostaphin treatment of experimental aortic valve endocarditis caused by a Staphylococcus aureus isolate with reduced susceptibility to vancomycin. Antimicrob Agents Chemother 43(7):1754–1755
94. Wu JA, Kusuma C, Mond JJ, Kokai-Kun JF (2003) Lysostaphin disrupts Staphylococcus aureus and Staphylococcus epidermidis biofilms on artificial surfaces. Antimicrob Agents Chemother 47(11):3407–3414
95. Shah A, Mond J, Walsh S (2004) Lysostaphin-coated catheters eradicate Staphylococccus aureus challenge and block surface colonization. Antimicrob Agents Chemother 48(7):2704–2707. https://doi.org/10.1128/AAC.48.7.2704-2707.2004
96. Yeroslavsky G, Girshevitz O, Foster-Frey J, Donovan DM, Rahimipour S (2015) Antibacterial and antibiofilm surfaces through polydopamine-assisted immobilization of lysostaphin as an antibacterial enzyme. Langmuir 31(3):1064–1073. https://doi.org/10.1021/la503911m
97. Johnson CT, Wroe JA, Agarwal R, Martin KE, Guldberg RE, Donlan RM et al (2018) Hydrogel delivery of lysostaphin eliminates orthopedic implant infection by Staphylococcus aureus and supports fracture healing. Proc Natl Acad Sci U S A 115(22):E4960–E4969. https://doi.org/10.1073/pnas.1801013115
98. Cui F, Li G, Huang J, Zhang J, Lu M, Lu W et al (2011) Development of chitosan-collagen hydrogel incorporated with lysostaphin (CCHL) burn dressing with anti-methicillin-resistant Staphylococcus aureus and promotion wound healing properties. Drug Deliv 18(3):173–180. https://doi.org/10.3109/10717544.2010.509363
99. Singh A (2018) Bactericide hydrogel prevents orthopedic implant infections. Sci Transl Med 10(443):eaau0463
100. Nithya S, Nimal TR, Baranwal G, Suresh MK, C P A, Anil Kumar V et al (2018) Preparation, characterization and efficacy of lysostaphin-chitosan gel against Staphylococcus aureus. Int J Biol Macromol 110:157–166. https://doi.org/10.1016/j.ijbiomac.2018.01.083
101. Xue B, Zhang C, Wang Y, Wang J, Zhang J, Lu M et al (2014) A novel controlled-release system for antibacterial enzyme lysostaphin delivery using hydroxyapatite/chitosan composite bone cement. PLoS One 9(12):e113797. https://doi.org/10.1371/journal.pone.0113797
102. Hathaway H, Ajuebor J, Stephens L, Coffey A, Potter U, Sutton JM, Jenkins AT (2017) Thermally triggered release of the bacteriophage endolysin CHAPK and the bacteriocin lysostaphin for the control of methicillin resistant Staphylococcus aureus (MRSA). J Control Release 245:108–115. https://doi.org/10.1016/j.jconrel.2016.11.030
103. Windolf CD, Logters T, Scholz M, Windolf J, Flohe S (2014) Lysostaphin-coated titan-implants preventing localized osteitis by Staphylococcus aureus in a mouse model. PLoS One 9(12):e115940. https://doi.org/10.1371/journal.pone.0115940
104. Fang FC (1997) Perspectives series: host/pathogen interactions. Mechanisms of nitric oxide-related antimicrobial activity. J Clin Investig 99(12):2818–2825. https://doi.org/10.1172/JCI119473
105. Fang FC (2004) Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. Nat Rev Microbiol 2(10):820–832. https://doi.org/10.1038/nrmicro1004
106. Witte MB, Barbul A (2002) Role of nitric oxide in wound repair. Am J Surg 183(4):406–412
107. Yang Y, Qi PK, Yang ZL, Huang N (2015) Nitric oxide based strategies for applications of biomedical devices. Biosurf Biotribol 1:177–201
108. Carpenter AW, Schoenfisch MH (2012) Nitric oxide release: part II. Therapeutic applications. Chem Soc Rev 41(10):3742–3752. https://doi.org/10.1039/c2cs15273h
109. Ischiropoulos H, al-Mehdi AB (1995) Peroxynitrite-mediated oxidative protein modifications. FEBS Lett 364(3):279–282
110. Soneja A, Drews M, Malinski T (2005) Role of nitric oxide, nitroxidative and oxidative stress in wound healing. Pharmacol Rep 57:108
111. Brisbois EJ, Bayliss J, Wu J, Major TC, Xi C, Wang SC et al (2014) Optimized polymeric film-based nitric oxide delivery inhibits bacterial growth in a mouse burn wound model. Acta Biomater 10(10):4136–4142. https://doi.org/10.1016/j.actbio.2014.06.032
112. Hetrick EM, Shin JH, Paul HS, Schoenfisch MH (2009) Anti-biofilm efficacy of nitric oxide-releasing silica nanoparticles. Biomaterials 30(14):2782–2789. https://doi.org/10.1016/j.biomaterials.2009.01.052

113. Worley BV, Schilly KM, Schoenfisch MH (2015) Anti-biofilm efficacy of dual-action nitric oxide-releasing alkyl chain modified poly(amidoamine) dendrimers. Mol Pharm 12(5):1573–1583. https://doi.org/10.1021/acs.molpharmaceut.5b00006
114. Backlund CJ, Worley BV, Schoenfisch MH (2016) Anti-biofilm action of nitric oxide-releasing alkyl-modified poly(amidoamine) dendrimers against Streptococcus mutans. Acta Biomater 29:198–205. https://doi.org/10.1016/j.actbio.2015.10.021
115. Lu Y, Shah A, Hunter RA, Soto RJ, Schoenfisch MH (2015) S-Nitrosothiol-modified nitric oxide-releasing chitosan oligosaccharides as antibacterial agents. Acta Biomater 12:62–69. https://doi.org/10.1016/j.actbio.2014.10.028
116. Storm WL, Johnson JA, Worley BV, Slomberg DL, Schoenfisch MH (2015) Dual action antimicrobial surfaces via combined nitric oxide and silver release. J Biomed Mater Res A 103(6):1974–1984. https://doi.org/10.1002/jbm.a.35331
117. Storm WL, Youn J, Reighard KP, Worley BV, Lodaya HM, Shin JH, Schoenfisch MH (2014) Superhydrophobic nitric oxide-releasing xerogels. Acta Biomater 10(8):3442–3448. https://doi.org/10.1016/j.actbio.2014.04.029
118. Hoang Thi TT, Lee Y, Le Thi P, Park KD (2018) Nitric oxide-releasing injectable hydrogels with high antibacterial activity through in situ formation of peroxynitrite. Acta Biomater 67:66–78. https://doi.org/10.1016/j.actbio.2017.12.005
119. Fleming G, Aveyard J, Fothergill JL, McBride F, Raval R, D'Sa RA (2017) Nitric oxide releasing polymeric coatings for the prevention of biofilm formation. Polymers 9(11):601
120. Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S (2004) Bacterial persistence as a phenotypic switch. Science 305(5690):1622–1625
121. Lewis K (2007) Persister cells, dormancy and infectious disease. Nat Rev Microbiol 5(1):48
122. Vega NM, Allison KR, Khalil AS, Collins JJ (2012) Signaling-mediated bacterial persister formation. Nat Chem Biol 8(5):431–433. https://doi.org/10.1038/nchembio.915
123. Allison KR, Brynildsen MP, Collins JJ (2011) Metabolite-enabled eradication of bacterial persisters by aminoglycosides. Nature 473(7346):216–220. https://doi.org/10.1038/nature10069
124. Barraud N, Buson A, Jarolimek W, Rice SA (2013) Mannitol enhances antibiotic sensitivity of persister bacteria in Pseudomonas aeruginosa biofilms. PLoS One 8(12):e84220. https://doi.org/10.1371/journal.pone.0084220
125. Loo CY, Lee WH, Lauretani G, Scalia S, Cipolla D, Traini D et al (2018) Sweetening inhaled antibiotic treatment for eradication of chronic respiratory biofilm infection. Pharm Res 35(3):50. https://doi.org/10.1007/s11095-018-2350-4
126. Nevitt SJ, Thornton J, Murray CS, Dwyer T (2018) Inhaled mannitol for cystic fibrosis. Cochrane Database Syst Rev 2:CD008649
127. Saran S, Mukherjee S, Dalal J, Saxena RK (2015) High production of erythritol from Candida sorbosivorans SSE-24 and its inhibitory effect on biofilm formation of Streptococcus mutans. Bioresour Technol 198:31–38
128. Drago L, Bortolin M, Taschieri S, De Vecchi E, Agrappi S, Del Fabbro M et al (2017) Erythritol/chlorhexidine combination reduces microbial biofilm and prevents its formation on titanium surfaces in vitro. J Oral Pathol Med 46(8):625–631
129. Hashino E, Kuboniwa M, Alghamdi SA, Yamaguchi M, Yamamoto R, Cho H, Amano A (2013) Erythritol alters microstructure and metabolomic profiles of biofilm composed of Streptococcus gordonii and Porphyromonas gingivalis. Mol Oral Microbiol 28(6):435–451
130. Ichikawa T, Yano Y, Fujita Y, Kashiwabara T, Nagao K (2008) The enhancement effect of three sugar alcohols on the fungicidal effect of benzethonium chloride toward Candida albicans. J Dent 36(11):965–968
131. Park Y-N, Jeong S-S, Zeng J, Kim S-H, Hong S-J, Ohk S-H, Choi C-H (2014) Anticariogenic effects of erythritol on growth and adhesion of Streptococcus mutans. Food Sci Biotechnol 23(5):1587–1591
132. Yao J, Zhang J, Wu Y, Lu Z (2009) Contrasting study of erythritol and xylitol on Streptococcus mutans. West China J Stomatol 27(6):603–605

133. Lim JH, Song S-H, Park H-S, Lee JR, Lee S-M (2017) Spontaneous detachment of Streptococcus mutans biofilm by synergistic effect between zwitterion and sugar alcohol. Sci Rep 7(1):8107
134. Ammons MCB, Ward LS, James GA (2011) Anti-biofilm efficacy of a lactoferrin/xylitol wound hydrogel used in combination with silver wound dressings. Int Wound J 8(3):268–273
135. Sherlock O, Dolan A, Athman R, Power A, Gethin G, Cowman S, Humphreys H (2010) Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. BMC Complement Altern Med 10:47
136. Majtan J, Bohova J, Horniackova M, Klaudiny J, Majtan V (2014) Anti-biofilm effects of honey against wound pathogens Proteus mirabilis and Enterobacter cloacae. Phytother Res 28(1):69–75. https://doi.org/10.1002/ptr.4957
137. Lu J, Turnbull L, Burke CM, Liu M, Carter DA, Schlothauer RC et al (2014) Manuka-type honeys can eradicate biofilms produced by Staphylococcus aureus strains with different biofilm-forming abilities. Peer J 2:e326
138. Alandejani T, Marsan J, Ferris W, Slinger R, Chan F (2009) Effectiveness of honey on Staphylococcus aureus and Pseudomonas aeruginosa biofilms. Otolaryngol Head Neck Surg 141(1):114–118. https://doi.org/10.1016/j.otohns.2009.01.005
139. Sojka M, Valachova I, Bucekova M, Majtan J (2016) Antibiofilm efficacy of honey and bee-derived defensin-1 on multispecies wound biofilm. J Med Microbiol 65:337–344. https://doi.org/10.1099/jmm.0.000227
140. Aron M, Akinpelu OV, Gasbarrino K, Daniel SJ (2015) Safety of transtympanic application of 4 % manuka honey in a chinchilla animal model. Eur Arch Otorhinolaryngol 272(3):537–542. https://doi.org/10.1007/s00405-013-2842-0
141. Aron M, Victoria Akinpelu O, Dorion D, Daniel S (2012) Otologic safety of manuka honey. J Otolaryngol Head Neck Surg 41(Suppl 1):S21–S30
142. Hirahara I, Kusano E, Yanagiba S, Miyata Y, Ando Y, Muto S, Asano Y (2006) Peritoneal injury by methylglyoxal in peritoneal dialysis. Perit Dial Int 26(3):380–392
143. Paramasivan S, Drilling AJ, Jardeleza C, Jervis-Bardy J, Vreugde S, Wormald PJ (2014) Methylglyoxal-augmented manuka honey as a topical anti-Staphylococcus aureus biofilm agent: safety and efficacy in an in vivo model. Int Forum Allergy Rhinol 4(3):187–195. https://doi.org/10.1002/alr.21264
144. Sell SA, Wolfe PS, Spence AJ, Rodriguez IA, McCool JM, Petrella RL et al (2012) A preliminary study on the potential of manuka honey and platelet-rich plasma in wound healing. Int J Biomater 2012:313781. https://doi.org/10.1155/2012/313781
145. Minden-Birkenmaier BA, Bowlin GL (2018) Honey-based templates in wound healing and tissue engineering. Bioengineering (Basel) 5(2):E46. https://doi.org/10.3390/bioengineering5020046
146. Hollman PCH (2001) Evidence for health benefits of plant phenols: local or systemic effects? J Sci Food Agric 81(9):842–852
147. Koo H, Pearson S, Scott-Anne K, Abranches J, Cury J, Rosalen P et al (2002) Effects of apigenin and tt-farnesol on glucosyltransferase activity, biofilm viability and caries development in rats. Oral Microbiol Immunol 17(6):337–343
148. Plyuta V, Zaitseva J, Lobakova E, Zagoskina N, Kuznetsov A, Khmel I (2013) Effect of plant phenolic compounds on biofilm formation by Pseudomonas aeruginosa. APMIS 121(11):1073–1081. https://doi.org/10.1111/apm.12083
149. LaPlante KL, Sarkisian SA, Woodmansee S, Rowley DC, Seeram NP (2012) Effects of cranberry extracts on growth and biofilm production of Escherichia coli and Staphylococcus species. Phytother Res 26(9):1371–1374
150. Alem MA, Oteef MD, Flowers TH, Douglas LJ (2006) Production of tyrosol by Candida albicans biofilms and its role in quorum sensing and biofilm development. Eukaryot Cell 5(10):1770–1779. https://doi.org/10.1128/EC.00219-06
151. Giovannini C, Straface E, Modesti D, Coni E, Cantafora A, De Vincenzi M et al (1999) Tyrosol, the major olive oil biophenol, protects against oxidized-LDL-induced injury in Caco-2 cells. J Nutr 129(7):1269–1277. https://doi.org/10.1093/jn/129.7.1269

152. Abdel-Rhman SH, Rizk DE (2016) Effect of tyrosol on Staphylococcus aureus antimicrobial susceptibility, biofilm formation and virulence factors. Afr J Microbiol Res 10(20):687–693
153. Arias LS, Delbem AC, Fernandes RA, Barbosa DB, Monteiro DR (2016) Activity of tyrosol against single and mixed-species oral biofilms. J Appl Microbiol 120(5):1240–1249. https://doi.org/10.1111/jam.13070
154. Gomes F, Teixeira P, Cerca N, Azeredo J, Oliveira R (2011) Effect of farnesol on structure and composition of Staphylococcus epidermidis biofilm matrix. Curr Microbiol 63(4):354–359. https://doi.org/10.1007/s00284-011-9984-3
155. Inoue Y, Togashi N, Hamashima H (2016) Farnesol-induced disruption of the staphylococcus aureus cytoplasmic membrane. Biol Pharm Bull 39(5):653–656. https://doi.org/10.1248/bpb.b15-00416
156. Jabra-Rizk MA, Meiller TF, James CE, Shirtliff ME (2006) Effect of farnesol on Staphylococcus aureus biofilm formation and antimicrobial susceptibility. Antimicrob Agents Chemother 50(4):1463–1469. https://doi.org/10.1128/AAC.50.4.1463-1469.2006
157. Kong EF, Tsui C, Kucharikova S, Van Dijck P, Jabra-Rizk MA (2017) Modulation of Staphylococcus aureus response to antimicrobials by the candida albicans quorum sensing molecule farnesol. Antimicrob Agents Chemother 61(12):e01573–e01517. https://doi.org/10.1128/AAC.01573-17
158. Unnanuntana A, Bonsignore L, Shirtliff ME, Greenfield EM (2009) The effects of farnesol on Staphylococcus aureus biofilms and osteoblasts. An in vitro study. J Bone Joint Surg Am Vol 91(11):2683–2692. https://doi.org/10.2106/JBJS.H.01699
159. Horev B, Klein MI, Hwang G, Li Y, Kim D, Koo H, Benoit DS (2015) pH-activated nanoparticles for controlled topical delivery of farnesol to disrupt oral biofilm virulence. ACS Nano 9(3):2390–2404. https://doi.org/10.1021/nn507170s
160. Chowdhury MR, Schumann C, Bhakta-Guha D, Guha G (2016) Cancer nanotheranostics: strategies, promises and impediments. Biomed Pharmacother 84:291–304
161. Jo SD, Ku SH, Won YY, Kim SH, Kwon IC (2016) Targeted nanotheranostics for future personalized medicine: recent progress in cancer therapy. Theranostics 6(9):1362–1377. https://doi.org/10.7150/thno.15335
162. Kim MH (2016) Nanoparticle-based therapies for wound biofilm infection: opportunities and challenges. IEEE Trans Nanobioscience 15(3):294–304. https://doi.org/10.1109/TNB.2016.2527600
163. Ikuma K, Decho AW, Lau BL (2015) When nanoparticles meet biofilms—interactions guiding the environmental fate and accumulation of nanoparticles. Front Microbiol 6:591
164. Li C, Zhang X, Huang X, Wang X, Liao G, Chen Z (2013) Preparation and characterization of flexible nanoliposomes loaded with daptomycin, a novel antibiotic, for topical skin therapy. Int J Nanomedicine 8:1285
165. Bala I, Hariharan S, Kumar MR (2004) PLGA nanoparticles in drug delivery: the state of the art. Crit Rev Ther Drug Carrier Syst 21(5):387–422
166. Cheow WS, Chang MW, Hadinoto K (2010) Antibacterial efficacy of inhalable levofloxacin-loaded polymeric nanoparticles against E. coli biofilm cells: the effect of antibiotic release profile. Pharm Res 27(8):1597–1609
167. Esmaeili F, Hosseini-Nasr M, Rad-Malekshahi M, Samadi N, Atyabi F, Dinarvand R (2007) Preparation and antibacterial activity evaluation of rifampicin-loaded poly lactide-co-glycolide nanoparticles. Nanomed Nanotechnol Biol Med 3(2):161–167
168. Brayner R, Ferrari-Iliou R, Brivois N, Djediat S, Benedetti MF, Fiévet F (2006) Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Lett 6(4):866–870
169. Lansdown AB (2002) Silver I: its antibacterial properties and mechanism of action. J Wound Care 11(4):125–130
170. Huang Z, Jiang X, Guo D, Gu N (2011) Controllable synthesis and biomedical applications of silver nanomaterials. J Nanosci Nanotechnol 11(11):9395–9408
171. Kalishwaralal K, BarathManiKanth S, Pandian SRK, Deepak V, Gurunathan S (2010) Silver nanoparticles impede the biofilm formation by Pseudomonas aeruginosa and Staphylococcus epidermidis. Colloids Surf B Biointerfaces 79(2):340–344

172. Kostenko V, Lyczak J, Turner K, Martinuzzi RJ (2010) Impact of silver-containing wound dressings on bacterial biofilm viability and susceptibility to antibiotics during prolonged treatment. Antimicrob Agents Chemother 54(12):5120–5131
173. Loo CY, Rohanizadeh R, Young PM, Traini D, Cavaliere R, Whitchurch CB, Lee WH (2016) Combination of silver nanoparticles and curcumin nanoparticles for enhanced anti-biofilm activities. J Agric Food Chem 64(12):2513–2522. https://doi.org/10.1021/acs.jafc.5b04559
174. Dizaj SM, Lotfipour F, Barzegar-Jalali M, Zarrintan MH, Adibkia K (2014) Antimicrobial activity of the metals and metal oxide nanoparticles. Korean J Couns Psychother 44:278–284. https://doi.org/10.1016/j.msec.2014.08.031
175. Kumar S, Lakshmanan VK, Raj M, Biswas R, Hiroshi T, Nair SV, Jayakumar R (2013) Evaluation of wound healing potential of beta-chitin hydrogel/nano zinc oxide composite bandage. Pharm Res 30(2):523–537. https://doi.org/10.1007/s11095-012-0898-y
176. Meeker DG, Wang T, Harrington WN, Zharov VP, Johnson SA, Jenkins SV et al (2018) Versatility of targeted antibiotic-loaded gold nanoconstructs for the treatment of biofilm-associated bacterial infections. Int J Hyperthermia 34(2):209–219. https://doi.org/10.1080/02656736.2017.1392047
177. Park H, Park H-J, Kim JA, Lee SH, Kim JH, Yoon J, Park TH (2011) Inactivation of Pseudomonas aeruginosa PA01 biofilms by hyperthermia using superparamagnetic nanoparticles. J Microbiol Methods 84(1):41–45
178. Kim MH, Yamayoshi I, Mathew S, Lin H, Nayfach J, Simon SI (2013) Magnetic nanoparticle targeted hyperthermia of cutaneous Staphylococcus aureus infection. Ann Biomed Eng 41(3):598–609. https://doi.org/10.1007/s10439-012-0698-x
179. Abenojar EC, Wickramasinghe S, Ju M, Uppaluri S, Klika A, George J et al (2018) Magnetic glycol chitin-based hydrogel nanocomposite for combined thermal and d-amino-acid-assisted biofilm disruption. ACS Infect Dis 4(8):1246–1256. https://doi.org/10.1021/acsinfecdis.8b00076
180. Wang X, Deng A, Cao W, Li Q, Wang L, Zhou J et al (2018) Synthesis of chitosan/poly (ethylene glycol)-modified magnetic nanoparticles for antibiotic delivery and their enhanced anti-biofilm activity in the presence of magnetic field. J Mater Sci 53(9):6433–6449
181. Scherr TD, Hanke ML, Huang O, James DB, Horswill AR, Bayles KW et al (2015) Staphylococcus aureus biofilms induce macrophage dysfunction through leukocidin AB and alpha-toxin. MBio 6(4):e01021–e01015. https://doi.org/10.1128/mBio.01021-15
182. Bernthal NM, Pribaz JR, Stavrakis AI, Billi F, Cho JS, Ramos RI et al (2011) Protective role of IL-1β against post-arthroplasty Staphylococcus aureus infection. J Orthop Res 29(10):1621–1626
183. Hanke ML, Heim CE, Angle A, Sanderson SD, Kielian T (2013) Targeting macrophage activation for the prevention and treatment of Staphylococcus aureus biofilm infections. J Immunol 190(5):2159–2168. https://doi.org/10.4049/jimmunol.1202348
184. Akanda ZZ, Taha M, Abdelbary H (2018) Current review—the rise of bacteriophage as a unique therapeutic platform in treating peri-prosthetic joint infections. J Orthop Res 36(4):1051–1060
185. Wright A, Hawkins CH, Anggard EE, Harper DR (2009) A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant Pseudomonas aeruginosa; a preliminary report of efficacy. Clin Otolaryngol 34(4):349–357. https://doi.org/10.1111/j.1749-4486.2009.01973.x
186. Fu W, Forster T, Mayer O, Curtin JJ, Lehman SM, Donlan RM (2010) Bacteriophage cocktail for the prevention of biofilm formation by Pseudomonas aeruginosa on catheters in an in vitro model system. Antimicrob Agents Chemother 54(1):397–404
187. Lu TK, Collins JJ (2007) Dispersing biofilms with engineered enzymatic bacteriophage. Proc Natl Acad Sci 104(27):11197–11202
188. Sass P, Bierbaum G (2007) Lytic activity of recombinant bacteriophage φ11 and φ12 endolysins on whole cells and biofilms of Staphylococcus aureus. Appl Environ Microbiol 73(1):347–352
189. Donlan RM (2009) Preventing biofilms of clinically relevant organisms using bacteriophage. Trends Microbiol 17(2):66–72

190. Kishor C, Mishra RR, Saraf SK, Kumar M, Srivastav AK, Nath G (2016) Phage therapy of staphylococcal chronic osteomyelitis in experimental animal model. Indian J Med Res 143:87–94. https://doi.org/10.4103/0971-5916.178615
191. Melo LD, Veiga P, Cerca N, Kropinski AM, Almeida C, Azeredo J, Sillankorva S (2016) Development of a phage cocktail to control Proteus mirabilis catheter-associated urinary tract infections. Front Microbiol 7:1024
192. Pires DP, Melo LD, Boas DV, Sillankorva S, Azeredo J (2017) Phage therapy as an alternative or complementary strategy to prevent and control biofilm-related infections. Curr Opin Microbiol 39:48–56
193. Doolittle MM, Cooney JJ, Caldwell DE (1996) Tracing the interaction of bacteriophage with bacterial biofilms using fluorescent and chromogenic probes. J Ind Microbiol 16(6):331–341. https://doi.org/10.1007/Bf01570111
194. Hughes KA, Sutherland IW, Clark J, Jones MV (1999) Bacteriophage and associated polysaccharide depolymerases—novel tools for study of bacterial biofilms (vol 85, pg 583, 1998). J Appl Microbiol 86(2):359–359
195. Parasion S, Kwiatek M, Gryko R, Mizak L, Malm A (2014) Bacteriophages as an alternative strategy for fighting biofilm development. Pol J Microbiol 63(2):137–145
196. Yan J, Mao J, Xie J (2014) Bacteriophage polysaccharide depolymerases and biomedical applications. BioDrugs 28(3):265–274
197. Resch G, Entenza JM, Moreillon P, Piccardi P, Mancini S, Oechslin F et al (2016) Synergistic interaction between phage therapy and antibiotics clears Pseudomonas aeruginosa infection in endocarditis and reduces virulence. J Infect Dis 215(5):703–712. https://doi.org/10.1093/infdis/jiw632
198. Ryan EM, Gorman SP, Donnelly RF, Gilmore BF (2011) Recent advances in bacteriophage therapy: how delivery routes, formulation, concentration and timing influence the success of phage therapy. J Pharm Pharmacol 63(10):1253–1264. https://doi.org/10.1111/j.2042-7158.2011.01324.x
199. Carson L, Gorman SP, Gilmore BF (2010) The use of lytic bacteriophages in the prevention and eradication of biofilms of Proteus mirabilis and Escherichia coli. FEMS Immunol Med Microbiol 59(3):447–455
200. Kutateladze á, Adamia R (2008) Phage therapy experience at the Eliava Institute. Med Mal Infect 38(8):426–430
201. Milo S, Hathaway H, Nzakizwanayo J, Alves DR, Esteban PP, Jones BV, Jenkins ATA (2017) Prevention of encrustation and blockage of urinary catheters by Proteus mirabilis via pH-triggered release of bacteriophage. J Mater Chem B 5(27):5403–5411
202. Vinner GK, Vladisavljević GT, Clokie MR, Malik DJ (2017) Microencapsulation of Clostridium difficile specific bacteriophages using microfluidic glass capillary devices for colon delivery using pH triggered release. PLoS One 12(10):e0186239
203. Hathaway H, Alves DR, Bean J, Esteban PP, Ouadi K, Sutton JM, Jenkins AT (2015) Poly(N-isopropylacrylamide-co-allylamine) (PNIPAM-co-ALA) nanospheres for the thermally triggered release of Bacteriophage K. Eur J Pharm Biopharm 96:437–441. https://doi.org/10.1016/j.ejpb.2015.09.013
204. Rubalskii E, Ruemke S, Salmoukas C, Aleshkin A, Bochkareva S, Modin E et al (2019) Fibrin glue as a local drug-delivery system for bacteriophage PA5. Sci Rep 9(1):2091
205. Malik DJ, Sokolov IJ, Vinner GK, Mancuso F, Cinquerrui S, Vladisavljevic GT et al (2017) Formulation, stabilisation and encapsulation of bacteriophage for phage therapy. Adv Colloid Interface Sci 249:100–133
206. Landsberger M, Gandon S, Meaden S, Rollie C, Chevallereau A, Chabas H et al (2018) Anti-CRISPR phages cooperate to overcome CRISPR-Cas immunity. Cell 174(4):908–916. e912
207. Harris MA, Beenken KE, Smeltzer MS, Haggard WO, Jennings JA (2017b) Phosphatidylcholine coatings deliver local antimicrobials and reduce infection in a murine model: a preliminary study. Clin Orthop Relat Res 475:1847–1853. https://doi.org/10.1007/s11999-016-5211-7

Antimicrobial Hydrogels: Key Considerations and Engineering Strategies for Biomedical Applications

Kasturi Joshi Navare, Loek J. Eggermont, Zachary J. Rogers, Halimatu S. Mohammed, Thibault Colombani, and Sidi A. Bencherif

Abstract Current healthcare practices often involve insertion of foreign devices into the body. Infections related to such devices or procedures pose a major threat to patient safety. As a result, there is an urgent need to develop technologies that better control healthcare-associated infections. In this regard, the latest innovations in hydrogel fabrication address some of these biomedical challenges. Particularly, hydrogels with antimicrobial characteristics have the remarkable potential to prevent or combat microbial infections. In this chapter, we review a number of promising strategies in the development of hydrogels with biocidal properties. First, common approaches are described for designing antimicrobial hydrogels. These techniques include using antimicrobic components such as bacteria-fighting polymers as well as encapsulating or conjugating agents that have antimicrobial functions with the gel. Next, various strategies and key factors relevant to the performance of antimicrobial hydrogels are detailed. Lastly, a comprehensive account of specific applications of antimicrobial hydrogels is provided, including implant coatings, contact lenses, wound dressings, and scaffolds for tissue reconstruction. The modular nature of antimicrobial hydrogel fabrication underscores their ability to address several biomedical challenges.

All authors contributed critically to the chapter and gave final approval for publication. Kasturi Joshi Navare and Loek J. Eggermont contributed equally to this work.

K. Joshi Navare · L. J. Eggermont · Z. J. Rogers · H. S. Mohammed · T. Colombani
Department of Chemical Engineering, Northeastern University, Boston, MA, USA

S. A. Bencherif (✉)
Department of Chemical Engineering, Northeastern University, Boston, MA, USA

Department of Bioengineering, Northeastern University, Boston, MA, USA

Harvard John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, USA

Sorbonne University, UTC CNRS UMR 7338, Biomechanics and Bioengineering, University of Technology of Compiègne, Compiègne, France
e-mail: s.bencherif@northeastern.edu

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_22

Keywords Antimicrobial · Hydrogels · Tissue regeneration · Implant · Coating · Wound healing · Drug delivery

Introduction

Current medical treatments often involve insertion of implants or devices into the body which are susceptible to contamination and colonization by harmful microbes. Infections related to such devices or procedures are called healthcare-associated infections (HAIs). HAIs are of serious concern as they can cause high morbidity and mortality rates [1, 2]. In 2018, the cost caused by HAIs was estimated to be nearly $33 billion in the USA. Furthermore, the incidence rate of HAIs was reported to be three times higher in developing countries when compared to Europe or North America [3]. Common pathogens for HAIs include *Clostridium difficile*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterococcus* spp., *Pseudomonas aeruginosa*, coagulase-negative *Staphylococci*, *Enterobacter* spp., *Acinetobacter baumannii*, and *Klebsiella oxytoca* [1, 4]. The five most common HAIs are central line bloodstream infections, ventilator-related pneumonia, surgical site infections, *Clostridium difficile* infections, and catheter-associated urinary tract infections. Moreover, 33.7% of HAI-related expenses were attributed to surgical site infections [3–5]. These challenges underpin the need for advanced antimicrobial technologies.

Antibiotics, the standard of care for managing HAIs, have been revolutionary in medicine. However, soon after the use of antibiotics became widespread, antibiotic-resistant strains of pathogens appeared [5, 6]. Bacteria acquire antibiotic resistance from spontaneous mutations or horizontal gene transfer, which results from conjugation, phage-mediated transduction, or transformation of mobile genetic elements (i.e., plasmids) [7]. Exposure of bacteria to sub-inhibitory concentrations of antibiotics leads to the emergence of resistant forms [6]. Antibiotics are prevalent in the environment due to inappropriate prescriptions and extensive use in animal feed. As a result, antibiotic-resistant bacteria have become a global health threat as infections are becoming more difficult to treat [8]. For instance, the high mortality rates associated with blood stream infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and third-generation cephalosporin-resistant *E. coli* are at par with the mortality rates observed for HIV/AIDS [9].

Biofilm formation also contributes to antibiotic resistance. Biofilms are microbial consortia attached to biotic or abiotic surfaces. They are formed on a broad range of surfaces, including polymeric medical implants or living tissues, where they cause HAIs. Therefore, biofilms are of utmost concern while considering HAIs. Biofilms typically contain extracellular polymeric substances which consist of microbial polysaccharides, proteins, and extracellular DNA. These substances act as a shield to protect microorganisms within them by resisting the penetration of some antimicrobials [10–13]. Additionally, bacteria within biofilms are often dormant which can confer them with antibiotic resistance [13, 14]. Biofilm structure also favors the exchange of antibiotic resistance genes within the bacterial community [15]. Given these points,

bacteria protected within biofilms can be 1000 times more resistant to antibiotics compared to their solitary forms [16]. As a result of this elevated antibiotic resistance, biofilms are hard to eradicate, which often leads to chronic infections. In these situations, removal of the implant remains the only option. To avoid these complications, an efficient strategy is to effectively prevent biofilm formation early on [13]. Therefore, it has become imperative that implant materials contain or are coated with antimicrobial agents that will prevent attachment and colonization of microorganisms [17]. To this end, a wide variety of antimicrobial hydrogels have been developed to address HAIs as described in this chapter.

Overview of Hydrogels

Hydrogels are defined as three-dimensional (3D) networks of cross-linked hydrophilic polymers with high water content. Hydrogels can be synthesized from a vast range of natural or synthetic polymers [18, 19]. For instance, they have been fabricated using extracellular matrix (ECM)-mimetic polymers that allow cell–material interactions at the molecular level. Particularly, this allows efficient cell adhesion, migration, and multicellular morphogenesis, making them suitable for tissue engineering applications [18].

Hydrogels are classified based on various criteria (Fig. 1) [20]. Depending upon the nature of their building blocks, hydrogels can be categorized as natural, synthetic, or hybrid. In general, naturally derived hydrogels exhibit superior biocompatibility and favor biological processes, such as cell attachment and tissue healing, while purely synthetic hydrogels have more consistent biochemical and mechanical attributes. Naturally derived hydrogels can be fabricated using precursors belonging to different structural classes of biopolymers, namely polysaccharides (e.g., alginate, hyaluronic acid) and peptides/proteins (e.g., collagen, gelatin, fibrin). Hydrogels may be grouped based on how they are cross-linked (chemically or physically) [21].

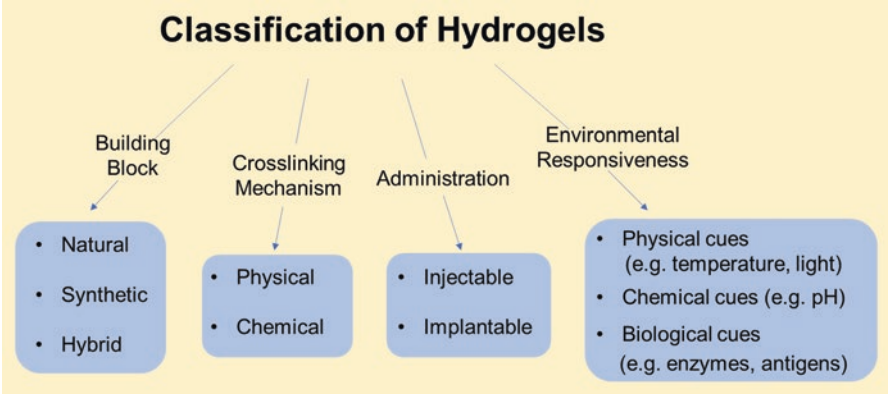


Fig. 1 Classification of hydrogels. Different criteria used for classification of hydrogels. Variants belonging to each category are listed

Chemically cross-linked hydrogels are made of permanent covalent bonds mainly through chain growth, addition and condensation polymerization. Physically cross-linked hydrogels are made of reversible bonds formed through ionic interactions, crystallization, stereocomplex formation, hydrophobized polysaccharides, protein interaction and hydrogen bonds. For patient administration, hydrogels are either implanted or injected. Injectable hydrogels are either preformed before injection or formed in situ. Furthermore, hydrogels can be designed to be responsive to environmental cues such as pH, temperature, light, antigens, or enzymes. Since hydrogels can be fine-tuned to achieve specific properties, they can be engineered to fit a number of diverse applications [22].

Due to their physical properties, hydrogels can serve as tissue repair scaffolds [23], drug delivery systems [23, 24], biological adhesives [18], implant coatings [25], medical implants, wound dressings [26], and fillers [27–29]. As hydrogels inherently absorb large amounts of water, they mimic the flexibility of native soft tissues [18]. A high water content is also beneficial for tissue restructuring and accelerates wound healing processes [30]. However, bacteria thrive in water-rich environments, making hydrogels susceptible to bacterial adhesion and colonization. Implantable hydrogels are particularly at risk for infection since they require invasive surgery. Imparting hydrogels with antimicrobial properties can address this limitation. To enhance their potential for clinical applications, a number of researchers have synthesized antimicrobial hydrogels with sophisticated strategies, including incorporation of polymers with intrinsic or acquired antimicrobial properties [2, 31, 32], antibiotics [33], antimicrobial peptides [34, 35], chemical biocides [6, 36], microbe-fighting nanoparticles (NPs) [33], and combinations thereof (Fig. 2). In the next sections of this chapter, we discuss various engineering strategies and biomedical applications of antimicrobial hydrogels in detail.

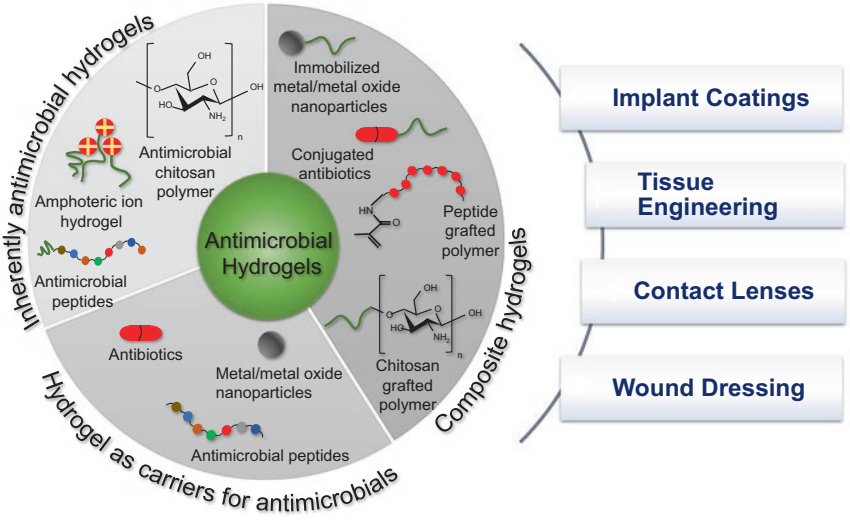


Fig. 2 Strategies for the fabrication of antimicrobial hydrogels. The three main approaches employed for developing antimicrobial hydrogels are depicted along with representative examples and applications

Engineering Antimicrobial Hydrogels

There has been a great interest in the development of antimicrobial hydrogels to control HAIs. This section focuses on the latest advances in the development of such hydrogels (Table 1) and how their antimicrobial properties have been evaluated.

Inherently Antimicrobial Hydrogels

Natural Polymers

Several positively charged natural polymers possess antimicrobial properties, making them excellent candidates as building blocks for the fabrication of microbe-fighting hydrogels [37, 38]. For instance, the inherent antimicrobial properties of chitosan have been well-documented [29, 39]. Chitosan is a natural cationic polymer derived mostly from shrimp shell wastes. It has become the biopolymer of choice for the development of naturally derived antimicrobial hydrogels due to its attractive properties including biodegradability, biocompatibility, positive charge and ability to cross-link quickly [40, 41]. The positively charged amines of chitosan interact with bacterial cell membranes leading to cell lysis [42, 43]. Due to its unique features, this biopolymer has been extensively explored for its antimicrobial activity in plant and food preservation [44–47]. Chitosan has also been investigated as an antimicrobial agent [48, 49] or as an antibacterial delivery system [44, 50–52].

Table 1 Advances in the fabrication of antimicrobial hydrogels

Inherently antimicrobial hydrogels	
Natural polymers	Chitosan, dextran, alginate
Synthetic polymers	Poly(acrylic acid) (PAA), Poly(vinyl alcohol) (PVA) with incorporation of antibacterial component such as poly([2(methacryloyloxy)-ethyl] trimethylammonium iodide) (PMETA)
Peptide-based hydrogels	EPL (ε poly-L-lysine)
Amphoteric ion hydrogels	Poly(norbornene), poly(acrylate), poly(methacrylic acid), poly(vinylpyrrolidone), poly(carbonate)
Composite antimicrobial hydrogels	
Hydrogels containing immobilized antimicrobial agents	Metal nanoparticles (AgNPs), antibiotics (aminoglycosides), AMPs (inverso-CysHH10)
Incorporation of antimicrobial polysaccharides to existing hydrogels	Chitosan
Peptide-hybridized hydrogels	EPL, Ala5-Tritrp7, ABU-CHRG01, Temporin-A, Cecropin A, and Thanatin
Incorporation of antifouling agents	AgNPs, Zwitterionic polymers, poly(N-hydroxyethyl acrylamide) (PHEAA)
Hydrogels as carrier of antimicrobials	
Metal and metal oxide nanoparticles, antibiotics, AMPs, synthetic antimicrobials, biological extracts	

In their work, Helander et al. investigated the antimicrobial potential of chitosan [49]. Treatment of *E. coli* and *Salmonella* sp. with chitosan showed that the polymer interacted with the bacterial outer cell membrane, mitigating its ability to act as a barrier [49]. Similarly, Szymanska et al. prepared chitosan-based hydrogels and studied the effects of structural modification of hydrogels on their antifungal activity against three different Candida strains [50]. Modification of chitosan with β-glycerophosphate or clotrimazole resulted in decreased antifungal activity, which was attributed to the weakening of the polycationic nature of chitosan [50]. In another study, Badawy et al. varied the degree of substitution of *N*-(6-carboxyl cyclohex-3-ene carbonyl) to alter the structure of *N*-(maleoyl) chitosan. Increasing the degree of substitution enhanced antibacterial properties, antifungal properties, and water solubility [53].

Other polysaccharides such as dextran and alginate have also been explored in the treatment of infections [30]. Overall, polysaccharide-based hydrogels are widely used in the fabrication of biomaterials for a number of biomedical applications due to their biocompatibility, cytocompatibility, antimicrobial activity [54–56]. In their studies, Aziz et al. employed dextran in conjunction with chitosan for endoscopic sinus surgery [57]. These hydrogels were found to be highly potent against various bacterial strains. Using scanning electron microscopy, it was determined that chitosan–dextran hydrogels bind to bacterial cell wall proteins, leading to cell lysis and ultimately bacterial cell death [57].

Synthetic Polymers

Many antimicrobial hydrogels are made of synthetic polymers in combination with antimicrobial factors or functional groups such as quaternary ammonium. These microbe-fighting components are typically covalently cross-linked or incorporated into the polymer backbone. A number of synthetic polymers have been considered in the fabrication of antimicrobial polymeric hydrogels, including PAA, poly(acrylamide) (PAM), poly(vinyl acetate) (PVAc), and poly(ethylene glycol) (PEG) [58, 59]. Synthetic polymers can be easily fine-tuned to achieve antimicrobial properties that are tailored to a specific application [60]. In one study, Li et al. fabricated an injectable hydrogel in which a PMETA central block was sandwiched between two PEG chains. The hydrogel exhibited a potent antibacterial action, which was attributed to the cationic functional groups [32].

Peptide-Based Hydrogels

Another class of antimicrobial hydrogels consists of antimicrobial peptides (AMPs) incorporated within a polymer matrix. All multicellular organisms are equipped with AMPs as “first line of defense” molecules [61, 62]. AMPs kill a wide spectrum of microorganisms including viruses, gram-negative bacteria, and fungi. Although the mechanisms of action by which AMPs kill microorganisms are poorly understood,

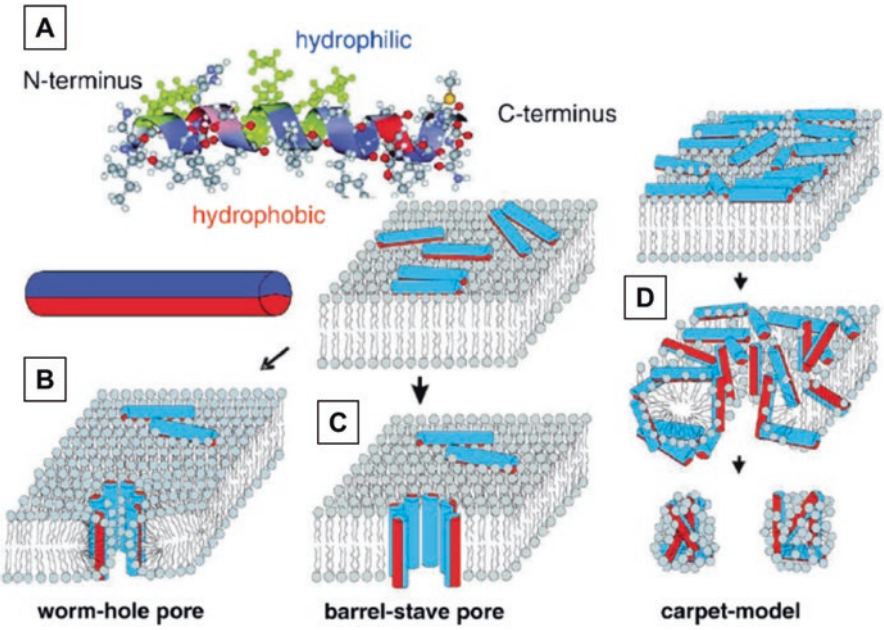


Fig. 3 Various mechanisms of interaction of AMPs with the lipid bilayer of cell membranes. (**A**) AMPs can fold into amphiphilic α-helices that contain hydrophilic and hydrophobic sides. The hydrophobic side of the cylinder can form pores in the cell membrane in three different ways: (**B**) worm-hole pore, (**C**) barrel-stave pore, and (**D**) carpet model. (Reprinted with permission from Springer nature [63])

two mechanisms of action have been proposed. According to the widely accepted first mechanism, the AMP directly interacts with the bacterial cell membrane via electrostatic interaction, which causes the cell membrane to rupture leading to cell death (Fig. 3) [63]. The second proposed mechanism attributes microbial killing to the recruitment and activation of host immune cells by AMPs [62, 64, 65].

Applications of AMPs are hindered by their rapid degradation in the body. However, their incorporation into hydrogels overcomes this challenge. A number of researchers have strategically introduced shorter active recombinant peptides, or specific structural features into the design of hydrogels to improve the overall antimicrobial activity [59, 66–68]. Evidence suggests that among polymers containing peptides as building blocks, those with amino side chains have a superior antimicrobial activity when compared to those with guanidine side chains [69]. In one study, AMPs were employed into epsilon-poly-L-lysine graft-methacrylamide (EPL-MAm)-based hydrogels to serve as a coating for medical devices [70]. In this particular hydrogel, EPL and MAm promote two mechanisms of interaction with the bacterial cell membrane, electrostatic and hydrophobic, respectively. The resulting AMP-based hydrogels showed a wide spectrum of antimicrobial activity against various strains of bacteria and fungi.

Amphoteric Ion Hydrogels

Amphoteric ion hydrogels are functionally similar to AMP-based hydrogels but made of synthetic polymers carrying both acidic and basic groups. These hydrogels bind to the negatively charged bacterial cell membrane through electrostatic interactions, leading to cell rupture [59, 71]. For instance, poly(norbornene) is a cationic polymer that has been extensively investigated for its antimicrobial properties [72, 73]. The antibacterial activity was found to be enhanced when increasing the hydrophobicity of the monomer repeat unit of the resulting polymer. Overall, fine-tuning monomer composition (hydrophobic to hydrophilic ratio and molecular weight) led to improved selectivity (>100 fold) for disruptive interactions with bacteria versus human red blood cells (hRBCs) [74]. Subsequently, Al-Badri et al. confirmed that structural tuning of cationic properties indeed affects the hemolytic action of poly(norbornene). This is especially important since hydrogels should prevent any hemolytic activity while enabling antibacterial activity [75]. Other antimicrobial cationic polymers that have been reported include PAA, poly(methacrylic acid), poly(vinylpyrrolidone) and poly(carbonate). As an example, Kozlovskaya et al. introduced an amphoteric pH-responsive hydrogel made of hydrogen-bonded poly(methacrylic acid) and ethylene diamine [76]. This system was found to be dependent on both pH and ionic strength and represents a new class of amphoteric hydrogels. Furthermore, multiple strategies have been investigated to incorporate antimicrobial agents within these hydrogels in order to reinforce their overall antimicrobial activity.

Composite Antimicrobial Hydrogels

Composite antimicrobial hydrogels are based on the incorporation of antimicrobial components, covalently or physically, into standard hydrogels. Strategies include incorporating antibiotics, NPs and AMPs. The antimicrobial characteristics of the resulting hydrogels can be altered by changing the monomer composition or the cross-linker.

Hydrogels Containing Immobilized Antimicrobial Agents

Hydrogels loaded with antimicrobials have been widely explored to control implant-associated infections. These hydrogels release antimicrobials as a result of passive diffusion or gel degradation [59, 77]. However, this approach is not optimal for long-term applications, as drug diffusion creates dose gradients around the hydrogel matrix, leading to drug-resistant bacteria [78]. To overcome these challenges, Cleophas et al. immobilized a highly active AMP, namely inverso-CysHHC10, onto hydrogels using thiol–ene chemistry. These gels displayed sustained bactericidal activity against *S. aureus* and *S. epidermidis* [79]. Hu et al. used amikacin, an amino-

glycoside antibiotic, as a cross-linker to form hydrogels with oxidized polysaccharides via an acid-labile Schiff base linkage. The gels exhibited on-demand controlled release of the antibiotic when exposed to acid-producing bacteria, resulting in remarkable inhibitory activity against gram-positive and gram-negative bacteria. This approach prevented adverse effects and limited the risk of bacterial resistance to antibiotics. The same strategy can be applied for the other antibiotics belonging to the class of aminoglycosides [77]. Dai et al. reported the fabrication of nanocomposite hydrogels co-immobilizing cationic dendrimers and silver NPs in dextran hydrogels. These advanced gels had potent and durable antibacterial properties [33]. Agnihotri et al. employed a chitosan–PVA hydrogel as a matrix for in situ synthesis of silver NPs. The process resulted in uniform immobilization of monodispersed silver NPs with potent antibacterial attributes [80].

Incorporation of Antimicrobial Polysaccharides to Existing Hydrogels

As described previously, chitosan is the most widely studied naturally derived antimicrobial polymer. To obtain chitosan-based gels with more desirable properties, chitosan is typically combined with synthetic polymers. For instance, Noppakundilograt et al. reported the development of chitosan grafted with poly(acrylic acid-*co*-hydroxyethyl methacrylate) with enhanced antibacterial activity against *S. aureus* [81]. Furthermore, El-Salmawi et al. fabricated photopolymerized hydrogels made of PVA and chitosan. They investigated the effects of polymer composition on their mechanical and antimicrobial properties. Their findings suggested that increasing PVA concentration improved gel mechanical properties while retaining sufficient antimicrobial activity [82]. Straccia et al. developed a hybrid antimicrobial hydrogel by coating chitosan onto alginate hydrogels to improve their antimicrobial characteristics. This hybrid scaffold showed effective antibacterial activity against *E. coli* [83]. In another report, Liu et al. coated chitosan onto poly(*N*-isopropylacrylamide-*co*-urethane) hydrogels to make temperature-sensitive antibacterial fabrics. Their antibacterial activity was successfully tested against *S. aureus* and *E. coli* [84].

Peptide-Hybridized Hydrogels

In peptide-hybridized hydrogels, peptide moieties are typically covalently anchored to polymer backbones to generate more effective antimicrobial hydrogels. A number of reports have described attempts to conjugate AMPs, including Ala5-Tritrp7, ABU-CHRG01, Temporin-A, to poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogels to create a new class of materials with improved antimicrobial properties. In line with this strategy, AMPs have also been conjugated to PEG maleate citrate-*co*-poly(ethylene glycol diacrylate), leading to in situ forming biodegradable hydrogels (iFBH). The various AMPs mentioned above were conjugated to iFBH and tested for their ability to prevent infections. Functionalized iFBH offered

effective antimicrobial activity by inhibiting bacterial growth, but also promoted wound healing [85]. Another interesting hybrid antimicrobial hydrogel was introduced by Liu et al. The hybrid antimicrobial peptides cecropin A and thanatin were found to be potent antimicrobial agents against most gram-positive and gram-negative bacteria [86].

Incorporation of Antifouling Agents

Biofouling is the deposition of microbes on the surface of biomedical devices, which is typically mediated by proteins. Antifouling refers to treatments that prevent biofouling. In the case of hydrogels this can be achieved by the incorporation of bactericidal agents or hydrophilic moieties that repel proteins [87]. Although the use of bactericidal agents is more efficient, this strategy can be impaired due to deposition of dead microorganisms on the surface. This limitation highlights the need to repel proteins and bacteria [87, 88]. For instance, Ghavami Nejad et al. incorporated silver NPs into a zwitterionic hydrogel, which resulted in a gel system with efficient antibacterial as well as antifouling properties [89]. Zwitterionic polymers are known to have effective protein resistance because of their high hydrophilicity [87]. Combining antifouling and antimicrobial properties is usually challenging as the accumulation of dead bacteria on these hydrogels limits their antifouling capacity. Mi et al. fabricated a zwitterionic hydrogel in which antibacterial salicylate anions were first released. Next, salicylate anions were substituted with carboxylate ions to maintain the gel antifouling properties [88]. Similarly, poly(*N*-hydroxyethyl acrylamide)-*co*-poly(salicylate) (PHEAA-*co*-PSAL) hydrogels were also developed with built-in antifouling and antimicrobial features. In these gels, antifouling properties were granted by the strong hydration layer around PHEAA [90]. To test their antifouling capability, these hydrogels were exposed to proteins and bacteria, while the antimicrobial activity was assessed against *E. coli* RP437 and *S. epidermidis*. Overall, these gels demonstrated potent antibacterial activity with high surface resistance to protein adsorption and bacterial colonization [90].

Hydrogels as a Delivery Vehicle for the Controlled Release of Antimicrobial Agents

To overcome current challenges associated with standard methods of drug administration, including high dosages, repeated administration, and patient toxicity [91], hydrogels have been employed as drug delivery systems. Hydrogels can serve as a carrier for a number of antimicrobial agents including metal/metal-oxide NPs, antibiotics, synthetic antimicrobial substances, AMPs and biological extracts [59].

Due to the high water content and relatively large pore size of hydrogels, drug release is typically fast [92]. To overcome this challenge, several approaches have been investigated to better control the release of antimicrobial agents in order to

enhance their biocidal activity while reducing their toxicity. The latest strategies include (a) physically incorporating NPs into hydrogels, (b) integrating enzyme cleavage sites into hydrogels designed to release antimicrobial agents, (c) optimizing hydrogel properties, and (d) engineering bacteria-responsive hydrogels.

Nanoparticle-Mediated Antimicrobial Release

Several strategies employing NPs have been utilized to control antimicrobial release, including formation of metal NPs [93, 94], NP-stabilized liposomes [95], and antibiotic-loaded NPs [96]. For instance, to target skin pathogens that thrive in acidic infection sites, pH-responsive hydrogels containing gold NP-stabilized liposomes (AuC-liposomes) were synthesized and subsequently incorporated into PAM hydrogels [95]. AuC-liposome release from the gels can be effectively slowed down by increasing the cross-linker concentration. As a result, AuC-liposome-loaded hydrogels exhibited potent pH-dependent antibacterial activity against *S. aureus* at pH 4.5 [95].

To design hydrogels capable of sustaining high shear stress from biological fluid flow while simultaneously controlling antimicrobial release, Zhang et al. combined ciprofloxacin-loaded poly(lactid-*co*-glycolide) NPs with dopamine methacrylamide. Dopamine-containing hydrogels exhibit high adhesive strength in wet environments (Fig. 4) [96]. The bioadhesive hydrogels exhibited 92% higher retention of ciprofloxacin-loaded NPs as compared to their non-adhesive counterparts. In another study, NP-loaded gels showed a gradual release of ciprofloxacin (40% within 12 h) whereas NP-free gels released 94% within 12 h [96]. In another example, Posadowska et al. introduced an injectable hydrogel for the treatment of bone-related infections. This hydrogel consisted of gentamicin-loaded poly(lactid-*co*-glycolide) NPs incorporated into a gellan gum hydrogel. In comparison to free gentamicin, the resulting composite hydrogel was found to be an effective antimicrobial against *Staphylococcus saprophyticus* without inhibiting bone-forming cells [97]. Overall, these data suggest that loading antimicrobial agents into NPs holds great promise for a better control over drug pharmacokinetics.

Enzyme/Nanozyme-Mediated Antimicrobial Release

Passive drug diffusion often exhibits an initial burst release followed by short-lived release of sub-inhibitory drug concentrations [98]. Controlled drug delivery can be achieved by enzymatic degradation of hydrogels, in which degrading enzymes trigger drug release. Based on this principle, a chitosan-based hydrogel for wound healing was fabricated in which the antibiotic cefuroxime was released in the presence of esterases, a class of enzymes found abundantly at wound sites [98]. Similarly, Islan et al. developed antibacterial alginate-based hydrogels containing levofloxacin and alginate lyase. The drug release was associated with pH dependent activation of alginate lyase leading to gel degradation [99]. As a potential wound dressing material,

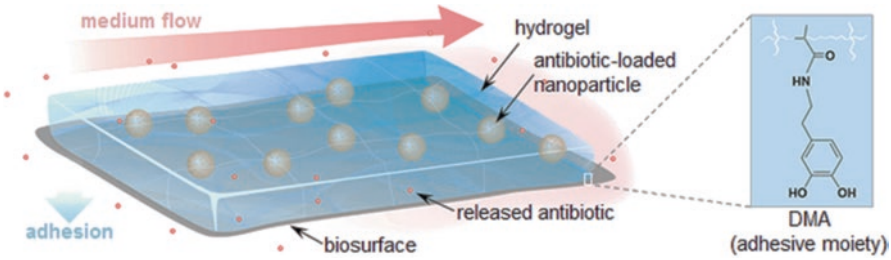


Fig. 4 Bioadhesive NP gels are strongly adhesive due to DMA, and can withstand fluid flows experienced in vivo. The ciprofloxacin NPs are slowly released from the DMA hydrogel. The adhesive layer (blue) can withstand medium flow, resulting in stable release of antibiotics (pink). (Reprinted with permission from [96]. Copyright 2016 American Chemical Society)

cellobiose dehydrogenase (CDH) was immobilised on succinyl chitosan/carboxymethyl cellulose (SC/CMC). These enzymatically active hydrogels release hydrogen peroxide (H_2O_2), a precursor of reactive oxygen species known to exert antimicrobial activity [100]. Another enzyme, a cellulase from *Trichoderma longibrachiatum* (*Trl*Cel), was also incorporated into these hydrogels to hydrolyze CMC as a feedstock for CDH, which converts CMC into hydrogen peroxide in a continuous manner. CDH/CMC hydrogels maintained concentrations of ~35 μM of H_2O_2 for 20 h, above the minimum inhibitory concentration (10 μM). A zone of inhibition assay confirmed the antibacterial activity of these hydrogels (*E. coli*, *S. aureus*), whereas *Trl*Cel -free hydrogels did not inhibit the growth of either bacterial species [100].

Modifying Hydrogel Properties to Control Antimicrobial Release

Antimicrobial properties of hydrogels can be modulated through a number of parameters including overall charge [101], polymerization method [102], monomer composition [103, 104], cross-linker concentration [77, 95] and antimicrobial concentration [77]. To improve the retention of antibiotic vancomycin (VAN) within hydrogels, oligo(poly(ethylene glycol)fumarate) sodium methacrylate hydrogel films were fabricated. This polymer bears negative charge which interacts with the positively charged VAN, thereby extending its release to 4 days [101]. This strategy resulted in efficient loading of VAN without affecting its potency and can be regarded as first step to overcome issues associated with VAN administration.

Although aminoglycosides are effective in bacterial infection treatment, they can cause adverse effects by inhibiting ribosomes [105]. To prevent side effects, Hu et al. fabricated oxidized polysaccharide hydrogels (dextran, carboxymethyl cellulose, alginate, and chondroitin sulfate) using aminoglycosides as cross-linkers [77]. These gels were responsive to bacteria, releasing aminoglycoside via degradation rather than diffusion. To evaluate this erosion-based strategy, aminoglycoside

release kinetics of oxidized dextran with aminoglycoside cross-linker was compared to a calcium cross-linked alginate hydrogel encapsulating aminoglycoside. The alginate hydrogel released nearly 100% of encapsulated aminoglycoside within 8 h, whereas the dextran-based hydrogel released only 7% at this time point. Hu et al. demonstrated that release kinetics can be controlled by changing the concentration of aminoglycoside linker: gels with 0.71% wt/v amikacin released most of the drug within 1 day, while the gel with 2.14% wt/v amikacin delivered only 36% of the drug after a month. Next, hydrogels with varying amikacin concentrations were compared to commercially available hydrogels (e.g., Nano-Ag, Achromycin gel) for the mitigation of bacterial growth (*E. coli*, *S. epidermidis*, *S. aureus*, *P. aeruginosa*). All hydrogels with a concentration of 1.43% wt/v of amikacin were more effective in neutralizing the four bacterial strains than the commercial gels [77]. Others employed PVA and PAM in their hydrogel design for the slow delivery of antibacterial sterculia to wounds. Sterculia gum, derived from the trees belonging to the genus Sterculia is known to have some antibacterial properties [106]. Specifically, nanosized hydrogels consisting of either PVA cross-linked with sterculia or made from PVA and PAM cross-linked with sterculia were developed in order to slow down the release of sterculia [107].

Bacteria-Responsive Antimicrobial Release

Hydrogels can be engineered to change their properties in response to external stimuli including temperature [108, 109], pH [110, 111], light [112, 113], electricity [111, 114], and bacteria [36, 102, 115, 116]. Here, we describe hydrogels that release their payloads upon detection of bacteria. The release is triggered by external stimuli, such as the acidic environment created by bacteria as described by Hu et al. [77]. Alternatively, a pathogen-specific trigger can initiate release as described by Zhou et al. They developed a wound dressing that is capable of distinguishing pathogenic bacteria. Gelatin methacryloyl hydrogel was embedded with 10,12-tricosadiynoic acid vesicles containing antimicrobials. Rupture of vesicles was selectively triggered by pore forming toxins secreted by *P. aeruginosa* or *S. aureus*, while vesicles remained intact in presence of nonpathogenic *E. coli* [116]. To target MRSA, Zhang et al. developed nanogels, coated with red blood cell (RBC) membranes, capable of neutralizing MRSA virulence. Additionally, these nanogels were designed to preferentially deliver VAN within MRSA-infected phagocytes [115] since MRSA is known to persist within host phagocytes [117]. RBC toxin neutralization facilitates phagocytic uptake, whereas controlled release within phagocyte endosomes was achieved by employing a redox-responsive cross-linker, cystine dimethacrylate. It was also shown that RBC-nanogels decreased intracellular bacterial burden when incubated with MRSA-containing macrophages [115].

Tian et al. designed hyaluronic acid (HA) hydrogels capable of releasing Fe^{3+} when colonized with bacteria [36]. Bacteria take up Fe^{3+} and reduce it into Fe^{2+}.

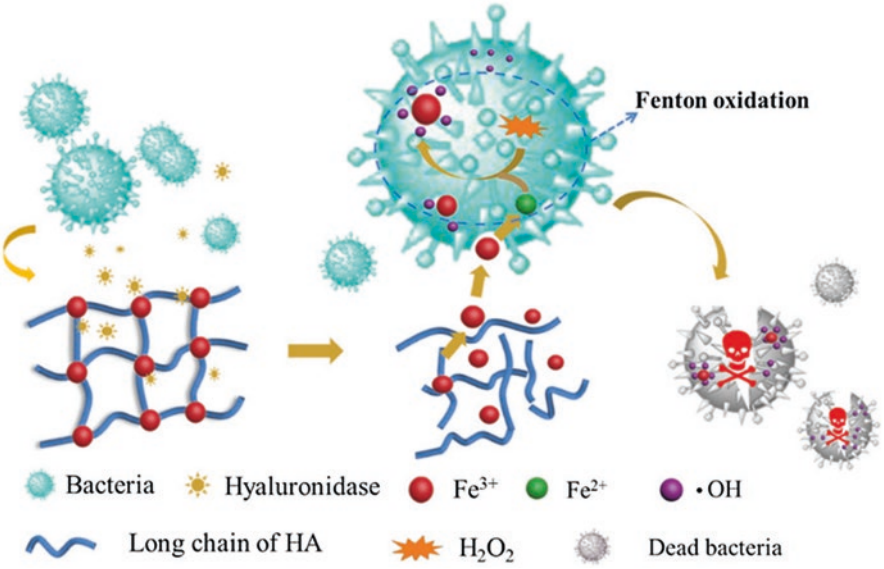


Fig. 5 Illustration of bacteria-responsive, iron-releasing (Fe^{3+}) HA hydrogels. Bacteria (turquoise) secrete hyaluronidase, which releases Fe^{3+} from the hydrogel matrix. Fe^{3+} is absorbed by the bacteria and reduced intracellularly to Fe^{2+}. Fe^{2+} reacts with H_2O_2 to form a hydroxyl radical, killing the bacterial cell (grey). (Reproduced with permission from [36]. Inquiries requiring reproduction of this figure should be directed to ACS)

Subsequently, Fe^{2+} reacts with H_2O_2 to form hydroxyl radicals leading to bacterial cell death (Fig. 5). Hydrogels were fabricated by self-assembly of precoordinated Fe^{3+}-EDTA complexes and HA through metal-ligand interactions. To evaluate antimicrobial activity in vivo, HA-Fe-EDTA hydrogel patches were placed on mice wounds contaminated with bacteria. Compared to untreated controls, HA-Fe-EDTA patches increased the wound healing rate (~85% compared to ~40% after 10 days), indicating the antimicrobial effectiveness of released Fe^{3+} [36].

Potential Biomedical Applications of Antimicrobial Hydrogels

Over the last decades, progress of antimicrobial hydrogels has been tremendous, and their utilization has revolutionized several biomedical applications (Fig. 6). In the next section, we will discuss the strategies using microbe-fighting hydrogels to confer antimicrobial properties to a number of biomedical materials. Their utilization as antimicrobial tissue engineering hydrogels for wound healing and tissue regeneration is also reviewed.

Fig. 6 Various biomedical applications of antimicrobial hydrogels. The main biomedical applications of antimicrobial hydrogels are depicted along with strategies to impart antimicrobial character to hydrogels

Antimicrobial Hydrogels for Biomedical Devices

Despite advances in sterilization techniques, the colonization of biomedical materials by bacteria and subsequent formation of biofilms represent the main cause of infections [118]. In the United States alone, device-associated infections account for 25.6% of HAIs [1] which can lead to implant failure, increased healthcare expenditure, and more importantly, severe complications, hospitalization, and even death [119]. Systemic treatments using antibiotics or small bioactive molecules have been investigated, but the impenetrable mechanical barrier formed by biofilms [120, 121] and the development of antibiotic resistances [122] have spurred the need to develop alternative strategies. Antimicrobial hydrogels have recently emerged as a promising strategy to prevent both biofilm formation and biomaterial-associated infection [123, 124]. Their applications to prevent implant, catheter, and contact lens-related infections are discussed.

Implants

Many hydrogel-based coatings have been developed to confer antimicrobial properties to medical implants. However, current technologies are still hampered by unreliable antibacterial effects or safety concerns. To overcome these limitations, several strategies have focused on using biodegradable coatings for local delivery of antibiotics. Gollwitzer et al. developed a coating based on poly (D,L-lactic acid) (PDLLA) encapsulating gentamicin or teicoplanin, allowing their release over a period of 96 h. This extended release led to a reduction of bacterial adhesion and viability [125]. Challenging the current paradigm consisting of developing long-term or permanent antimicrobial coating, Drago et al. investigated the use of fast-resorbable hydrogels for a complete release of antibacterial compounds within 96 h [25]. They showed that Defensive Antibacterial Coating (DAC) hydrogels loaded with standard antibiotics or antibiofilm agents minimized or prevented biofilm formation. More strikingly, clinical trials using antibiotic-loaded DAC confirmed their potential to reduce post-surgical infections, without any noticeable side effects [126, 127]. Finally, to reduce coating thickness, De Giglio et al. investigated the electrosynthesis of PHEMA or poly(ethylene glycol diacrylate)-*co*-poly(acrylic acid) (PEGDA-*co*-PAA) loaded with ciprofloxacin at the surface of titanium implants [128]. These hydrogels demonstrated a potent ciprofloxacin release when compared to PHEMA hydrogels. Moreover, PEGDA-*co*-PAA hydrogels efficiently inhibited MRSA growth, while showing cytocompatibility with MG63 osteoblast-like cells.

The emergence of pathogens that are resistant to antibiotics stimulated the development of alternative coating strategies for biomedical applications. With recent advances in nanotechnology to eradicate antibiotic-resistant bacteria, De Giglio et al. developed PEGDA-*co*-PAA hydrogels loaded with silver NPs, a broad-spectrum antimicrobial agent. These antimicrobial hybrid hydrogels were used to coat titanium implants [129]. This coating showed effective antibacterial activity against *S. aureus*, *P. aeruginosa*, and *E. coli*. Other strategies rely on coating medical implants with hydrogels with inherent antimicrobial properties. This approach aims to address both the potential side effects of soluble molecules on surrounding tissues, but also the loss of activity over time [130]. Similarly, Li et al. proposed to develop an antimicrobial coating using cationic chitosan polymer [2]. They fabricated dimethyl decylammonium chitosan (with high quaternization)-graft-PEG methacrylate (DMDC-Q-*g*-PEGMA) hydrogels with superior antimicrobial activity against relevant hospital organisms. DMDC-Q-*g*-PEGMA hydrogels have also been used to form thin and uniform coating on the surface of substrates without the alteration of their antimicrobial properties. Additionally, these gels proved to be biocompatible following implantation in a rabbit model. Although several approaches to engineer antimicrobial implants have been investigated, only a handful of them have reached clinical trials. This further highlights the critical need to develop more efficient and reliable strategies.

Catheters

In the United States, urinary and intravascular catheters represent the most commonly inserted biomedical devices, but they also are the main causes of bloodstream nosocomial infections [131]. Despite efficient terminal sterilization, catheters become coated with several biomolecules following insertion, including fibrin, fibronectin, electrolytes, and proteins, which promote microbial attachment [132, 133]. Therefore, efforts have focused on developing hydrogel-based coatings to give antifouling properties to catheters. Using hydrophilic polymers to make antibacterial surfaces, Mc Coy et al. co-polymerized chemically modified poloxamer 188 with PHEMA to form antifouling hydrogels [134]. The developed coatings were able to significantly reduce over 90% of *E.coli* adhesion. More elaborated strategies using zwitterionic materials have also been investigated to create hydration shells that provide antifouling properties. For example, Lee et al. have reported a new zwitterionic sulfobetaine methacrylate hydrogel coating on polyurethane (PU). This strategy demonstrated the ability of this coating to decrease fibronectin adsorption and human dermal fibroblast adhesion by 80% compared to untreated PU substrate [135].

Although antifouling coatings are promising, persistent bacteria can still attach on coated catheters. Therefore, there is a need to develop more effective antimicrobial coatings. In several studies, metal NPs have proven their effectiveness at eradicating microbes. For instance, Fisher et al. combined antimicrobial silver NPs with anticoagulant PEG-*co*-Heparin to develop a long-term antimicrobial coating for catheters [136]. The resulting multilayered coating demonstrated sustained antiseptic activities against *E. coli* and *S. aureus* over 5 days, as well as hemocompatibility with fresh human whole blood. However, due to concerns with the toxicity of silver, Lim et al. have proposed a dual coating of silicone catheters. Anhydrous polycaprolactone was impregnated with HHC36, a potent AMP and subsequently covered with a thin film of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine to control diffusion [137]. This strategy showed a sustained release of the antimicrobial HHC36 for 14 days, outperforming commercially available silver-based catheters [137]. Moreover, the dual coating demonstrated a high degree of cytocompatibility when exposed to hRBCs and uroepithelial cells.

Another investigated strategy consists of combining both antifouling and antimicrobial properties for the design of advanced catheter coatings. For example, Vaterrodt et al. fabricated a multilayer zwitterionic hydrogel coating for silicone surfaces [138]. They combined an antifouling copolymer, poly(2-(dimethylamino) ethyl methacrylate)-*co*-poly(sulfobetaine methacrylate), with antimicrobial cellobiose dehydrogenase enzymes and poly(styrenesulfonate) polycations. In addition to remaining stable for 10 days in water and urine, this coating allowed up to a 60% reduction in bacterial adhesion. Furthermore, this formulation exhibited antimicrobial potency against adherent bacteria. In another study, Yang et al. investigated a four-step surface coating to create a thin smooth layer on segmented PU catheters [139]. Using chitosan/PVA hydrogels, they demonstrated their capability to signifi-

cantly reduce protein adsorption at the catheter surface while exhibiting effective antimicrobial properties. Nonetheless, despite all of these emerging strategies, most of them have failed in the clinic due to microbial resistance, lack of long-term efficacy, or discomfort to patients [140]. Therefore, careful attention to optimal catheter selection should be pursued to prevent catheter-related infections.

Contact Lenses

Soft hydrogel lenses are broadly used to correct vision. Despite major advances to address adverse side effects as a result of contact lens wear, bacterial infection remains a serious concern leading to various ophthalmic pathologies [141]. One solution proposed by Thissen et al. is based on providing antifouling properties to silicone hydrogel lenses. This is achieved by coating contact lenses with allylamine plasma polymer and PEG dialdehyde [142]. A clinical study confirmed their biocompatibility as well as a reduction of microbial contaminations during normal lens use [142].

In addition to vision correction, hydrogel lenses have been used as a drug delivery device. For instance, several strategies have been considered to fight antimicrobial infections locally and overcome limitations associated with topical delivery of drugs such as fast clearance and systemic absorption [143, 144]. Garty et al. proposed to coat PHEMA hydrogel lenses with norfloxacin, a quinolone antibiotic [145]. These contact lenses exhibited a sustained release of norfloxacin at a therapeutic level for several weeks. More strikingly, norfloxacin-coated PHEMA hydrogels were effective in treating intraocular infection in a rabbit model following cataract surgery.

To optimize antibiotic loading and delivery, Shi et al. fabricated PHEMA-*co*-poly(methacrylic acid) hydrogel lenses with efficient antibiotic loading. They further demonstrated that these lenses could accomplish a slow and sustained release of gatifloxacin. Animal studies in a rat model of bacterial keratitis showed potent antimicrobial activity, low cytotoxicity and decrease in stromal ulceration [146]. In another study, Huang et al. engineered hybrid hydrogel lenses, composed of quaternized chitosan, silver NPs, and graphene oxide [147]. These materials exhibited good antimicrobial properties and cytocompatibility. Furthermore, these hybrid lenses were loaded with voriconazole, allowing for sustained antifungal activity in a mouse model. More advanced techniques such as imprinting have also been investigated to better control antibiotic loading and delivery. Malakooti et al. developed imprinted polymyxin B-loaded PHEMA hydrogels for the controlled release of antimicrobial peptides [148]. Preliminary studies showed their ability to be efficiently loaded with antibiotics. For instance, polymyxin was released in a controlled fashion without compromising cytocompatibility. Additionally, they further demonstrated that these imprinted gels could effectively neutralize *P. aeruginosa*. Similarly, Hui et al. used imprinting techniques to modify ciprofloxacin release from silicone-based hydrogel lenses [149]. They showed a sustained release of ciprofloxacin and highlighted their potential in the treatment of *P. keratitis* infections in a rabbit model.

Although promising, more animal and human studies are needed prior to translating these antimicrobial lenses to the clinic. It is critical to confirm and validate their long-term effectiveness and safety [150].

Antimicrobial Hydrogels for Wound Healing and Tissue Regeneration

Hydrogels have been extensively applied for tissue engineering and controlled delivery of cells and drugs, due to their high-water content and inherent tissue-like composition. By mimicking the natural ECM, hydrogel scaffolds can provide physical and biochemical cues needed to control cellular differentiation and tissue regeneration [151, 152]. However, as described earlier, the moist environment provided by hydrogels is also a niche for microorganism growth and invasion. These microorganisms can hamper gel integration within the host tissue, and their expansion can lead to life-threatening infections. In order to address these concerns, a number of antimicrobial scaffolds have been developed and extensively applied in tissue engineering to minimize the risk of infection [30]. As discussed in this section, antimicrobial hydrogels have been mostly applied to wound healing but some researchers have also investigated their use for bone and cartilage regeneration.

Wound Healing

In severely injured patients, wound infections are common and are a leading cause of morbidity and mortality. Therefore, open wounds need to be healed quickly in a microbe-free environment. One strategy is to apply antimicrobial wound dressings at the site of injury to promote tissue regeneration while reducing the risk of infection [153]. In this effort, a number of antimicrobial hydrogels have been developed over the last decades [30]. Among them, the most extensively tested are hydrogels made out of natural polymers or loaded with antibiotics and NPs.

Antibiotic-Loaded Antimicrobial Hydrogels

Many antibiotic-containing hydrogels have been designed for wound healing. Although most wound dressings were not translated from bench to bedside [154–156], several formulations showed great promise in preclinical studies. For example, ciprofloxacin-loaded keratin hydrogels effectively inhibited *S. aureus* and *P. aeruginosa* infection while enhancing skin regeneration in a porcine burn model [157]. Furthermore, the simultaneous co-delivery of two antibiotics can potentially enhance antimicrobial activity. Mebert et al. showed that the delivery of two antibiotics, gentamicin sulfate and sodium rifamycin from collagen–silica nanocomposite hydrogels significantly minimized the risk of infection in a rabbit wound model [158].

Similarly, Kim et al. indicated that the co-delivery of two antibiotics, clindamycin and nitrofurazone, from PVA/alginate-based hydrogels promoted wound healing in an infection-free rat model [159, 160]. However, these hydrogels did not outperform commercially available Medifoam, an antibiotic-free PU foam-based wound dressing. The same group later developed minocycline-releasing PVA/chitosan hydrogels. When applied in a rat wound model, these hydrogels actually induced better healing and reduced inflammation when compared to Medifoam [161]. In the context of wound healing, other groups have investigated the antimicrobial activity of chitosan-based hydrogels when combined with antibiotics [162]. For example, full-thickness wound healing in rats was significantly enhanced when norfloxacin was released from collagen/chitosan gels [163]. Another sophisticated approach is based on using the properties of biomimetic antimicrobial hydrogels to boost wound healing. Hu et al. developed collagen–carboxymethyl chitosan scaffolds capable of releasing ciprofloxacin HCL and gentamicin sulfate in a sustained fashion. In a rat wound model, these gels were able to promote reepithelialization, collagen deposition, and angiogenesis, while preventing wound infection [164].

In humans, hydrogels that release conventional antibiotics are not widely explored for wound healing. Clinical use is focused on gels loaded with metals, iodine or antimicrobial polymers such as polyhexamethylene biguanide [165]. The release of low molecular weight antibiotics from hydrogels may increase the risk of bacterial resistance. This is a concern for prolonged antibiotic release below inhibitory concentrations [166]. Therefore, clinical application of hydrogels that circumvent the utilization of standard antibiotics is preferred as described next.

Hydrogels Containing Metal-Based NPs

Over the past few years, metal ions have been very popular for wound healing applications. For instance, silver (e.g., silver sulfadiazine) has been widely used in topical burn care treatment to prevent infection. In particular, a nanostructured version of these metal ions (e.g. NPs) may circumvent antibiotic resistance mechanisms in bacteria and inhibit biofilm formation. As a result, composite NP-loaded gel dressings have been extensively used for wound healing [30]. Various formulations of hydrogels hybridized with silver, zinc oxide and titanium dioxide NPs have been tested in wound healing models. Among these materials, silver NPs have been extensively investigated as silver is known to stimulate wound healing while being inherently antimicrobial. For instance, Neibert et al. confirmed that alginate hydrogels loaded with silver NPs efficiently reduced inflammation and promoted tissue repair in a mouse wound model [167]. Silver NPs were also applied in dopamine-containing methacrylamide hydrogels, which promoted wound healing in rats [168]. Mekkawy et al. demonstrated that PEG-coated silver NPs were effective against both gram-positive and gram-negative bacteria. In a rat model, carboxymethyl chitosan hydrogels hybridized with these NPs resulted in an improved wound dressing with better antibacterial and healing properties when compared to a silver sulfadiazine cream [169]. These antimicrobial gels were also combined with therapeutics to

further encourage wound healing. In mice, with infected diabetic wounds, He et al. showed that the healing was most effective and more rapid when the dressing released both silver NPs and anti-inflammatory fibroblast-stimulating drugs [170].

Hydrogels containing zinc oxide NPs alone or in combination with silver NPs have also shown to stimulate skin regeneration and reduce infection in a rat model of wound healing [171]. However, concerns associated with the biocompatibility of zinc oxide and silver have limited their applications [112]. Nonetheless, several off-the-shelf topical silver-containing hydrogels are available for patients, including ReliaMed, Acticoat, Gentell Silver hydrogel, Silvermed, Silver-Sept, SilvaSorb, silver genesis colloidal Solver hydrogel, and DermaSyn/Ag [156]. Many of these antimicrobial topical gels proved to be effective against a broad spectrum of pathogenic bacteria and fungi, suggesting their great potential. However, long-term studies are needed to evaluate their potential adverse effects [172, 173].

Hydrogels Made of Natural Antimicrobial Polymers

Hydrogels can be made from antimicrobial polymers. This approach could prevent utilization of harmful agents (e.g., metal NPs) and limit risks of bacterial resistance. Antimicrobial natural substances, including honey, and biopolymers, such as chitosan, have extensively been used for wound healing. In a diabetic mouse model, honey-containing hydrogels promoted wound healing and tissue formation [174]. Furthermore, in a rabbit burn model, hydrogel sheets made of honey, chitosan, and gelatin exhibited a substantial antimicrobial activity. Additionally, these multilayer gels enhanced wound contraction when compared to a standard antimicrobial ointment (e.g., moist-exposed burn ointment) [175].

As previously highlighted, chitosan hydrogels have been combined with antibiotics and/or metal NPs. Beside its unique antimicrobial property, chitosan is known to promote wound healing by recruiting construction workers such as neutrophils and macrophages [176]. Other studies have confirmed the remarkable wound healing capacity of chitosan hydrogels [177, 178]. However, the antimicrobial properties of chitosan hydrogels can be enhanced by increasing cationic charges along the polymer backbone [2]. For instance, quaternized chitosan hydrogels containing tertiary amino groups along the backbone significantly reduced risk of infection while fostering tissue repair [179]. Other groups have investigated similar approaches and tested them successfully in rodent models [176, 180, 181]. Overall, chitosan is becoming the polymer of choice for wound healing and a number of chitosan-based wound dressings are commercially available such as HidroKi, Celox, ChiGel, and Kytocel [182].

Evidence suggests that honey has natural healing properties [183]. To capitalize on honey's intrinsic biological properties, several gel products containing honey are available in the market. The wound healing capacity of Medihoney, an alginate-based hydrogel sheet containing Manuka honey, was assessed in several clinical trials. Medihoney was reported to reduce pain and infections of surgical wounds and ulcers. However, its capacity to stimulate wound healing was less evident [184].

Bone and Cartilage Tissue Regeneration

Bone fracture often requires surgery which may cause infection and complications. To safely promote bone healing, various antimicrobial hydrogels have been engineered. However, only a small number of these gels were actually tested in vivo. Ter Boo et al. designed injectable thermo-responsive gentamicin-loaded HA hydrogels for surgical fixation of open fractures. In a rabbit bone fracture model contaminated with *S. aureus*, these bioactive hydrogels effectively prevented bacterial growth [185]. Although these gels did not impair fracture healing, they failed to enhance bone regeneration [186]. To repair critical-sized bone defects, Dhivya et al. developed chitosan-based hydrogels that were reinforced with nanohydroxyapatite, a natural bone component known to facilitate bone formation. These gels were loaded with zinc to further improve their antimicrobial activity. Preclinical studies have shown that these composite hydrogels effectively enhanced bone formation in rat tibial fractures [187]. Similarly, HA/elastin-like polypeptide hydrogels containing zinc oxide NPs have been utilized as antimicrobial hydrogels for cartilage tissue engineering. Incorporating zinc oxide NPs efficiently inhibited MRSA infection in a rat model. Furthermore, these scaffolds degraded slowly over time leaving some space for autologous cartilage tissue formation [188].

Conclusion and Perspectives

In this chapter, a number of recent strategies to design antimicrobial hydrogels have been covered. Due to their high-water content and physical properties, hydrogels mimic human soft tissues and are implemented in a variety of biomedical applications. These applications include antimicrobial coatings, wound healing materials, drug delivery vehicles, and tissue engineering scaffolds.

With the emergence of drug resistant superbugs, the problem of HAIs has progressively intensified. The high mortality and morbidity of HAIs highlight the critical need to design antimicrobial biomaterials. Fortunately, hydrogels stand as a material with great potential to address and control HAIs. As described previously, hydrogels can be fabricated using polymers with inherent antimicrobial properties. Chitosan, a naturally derived polysaccharide, is the most widely explored polymer. Hydrogels can also serve as a drug delivery device for a variety of antimicrobial agents such as antibiotics, metal NPs and AMPs. Alternatively, these antimicrobial agents can be conjugated to the hydrogel backbones for a prolonged or stimuli-responsive drug release.

These antimicrobial components and strategies come with their pros and cons. For example, the use of antibiotics can be associated with the risk of bacterial resistance while the use of NPs brings in the risk of toxicity. In this context, more research is needed towards the development of composite antimicrobial hydrogels to tilt the balance in their favor. This would alleviate the risk of drug resistance and may achieve a better management of hospital-associated infections. The design of

smart, stimuli-responsive hydrogels has opened a promising avenue for on-demand antibiotic release in order to reduce the risk of toxicity and drug resistance. Furthermore, these risks could be further reduced using antifouling hydrogels that resist the deposition of biological molecules or bacterial cells.

For clinical success, antimicrobial hydrogels must achieve bactericidal drug levels while preserving sustained antimicrobial action. Furthermore, they should be cell-friendly and at the same time, fight the most virulent superbugs. If hydrogels are applied for tissue engineering, they should act as a physical scaffold for tissue reconstruction. All these challenges explain the gap between the volume of ongoing research and the number of products reaching the market. Many promising antimicrobial hydrogels have shown encouraging in vitro results but lack convincing preclinical investigations. High costs of advanced hydrogels are another factor that hinders their commercialization. Among commercially viable antimicrobial hydrogels, there are a notable number of silver NP-based and chitosan-based hydrogels. Honey-containing hydrogels have also demonstrated some clinical success in the wound healing arena. Successive coatings capitalizing on the synergistic action of various antimicrobial agents have also shown some success.

To develop more clinically viable antimicrobial hydrogels, more sophisticated gels with a wide action spectrum and a low risk of bacterial resistance should be investigated. Therefore, constant integration of research on hydrogel engineering and antimicrobial therapies will enable the development of potential solutions to efficiently alleviate or prevent HAIs.

Acknowledgements This project was funded by the Northeastern University (Boston, MA, USA) Seed Grant/Proof of Concept Tier 1 Research Grant and startup funds provided by the Department of Chemical Engineering. Awards from the Burroughs Wellcome Fund (1018798), Thomas Jefferson/ Face foundation, DFCI/NU Joint Program Grant, and NSF CAREER (DMR 1847843) are also acknowledged.

References

1. Magill SS, Edwards JR, Bamberg W, Beldavs ZG, Dumyati G, Kainer MA, Lynfield R, Maloney M, McAllister-Hollod L, Nadle J et al (2014) Multistate point-prevalence survey of health care-associated infections. N Engl J Med 370(13):1198–1208
2. Li P, Poon YF, Li W, Zhu HY, Yeap SH, Cao Y, Qi X, Zhou C, Lamrani M, Beuerman RW et al (2011) A polycationic antimicrobial and biocompatible hydrogel with microbe membrane suctioning ability. Nat Mater 10(2):149–156
3. Allegranzi B, Nejad SB, Combescure C, Graafmans W, Attar H, Donaldson L, Pittet D (2011) Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. Lancet 377(9761):228–241
4. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, Fridkin SK, National Healthcare Safety Network T, Participating National Healthcare Safety Network F (2008) NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infect Control Hosp Epidemiol 29(11):996–1011

5. Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 74(3):417–433
6. Priya SG, Gupta A, Jain E, Sarkar J, Damania A, Jagdale PR, Chaudhari BP, Gupta KC, Kumar A (2016) Bilayer cryogel wound dressing and skin regeneration grafts for the treatment of acute skin wounds. ACS Appl Mater Interfaces 8(24):15145–15159
7. Andersson DI, Hughes D (2010) Antibiotic resistance and its cost: is it possible to reverse resistance? Nat Rev Microbiol 8(4):260–271
8. D'Agata EM, Dupont-Rouzeyrol M, Magal P, Olivier D, Ruan S (2008) The impact of different antibiotic regimens on the emergence of antimicrobial-resistant bacteria. PLoS One 3(12):e4036
9. de Kraker ME, Davey PG, Grundmann H, Group BS (2011) Mortality and hospital stay associated with resistant Staphylococcus aureus and Escherichia coli bacteremia: estimating the burden of antibiotic resistance in Europe. PLoS Med 8(10):e1001104
10. Delphine Dufour VLCML (2012) Bacterial biofilm structure, function and antimicrobial resistance. Endod Top 22:2–16
11. Percival SL, Suleman L, Vuotto C, Donelli G (2015) Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. J Med Microbiol 64(Pt 4):323–334
12. Donlan R (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8(9):881–890
13. Hoiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O (2010) Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 35(4):322–332
14. Thien-Fah C, Mah GAOT (2001) Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 9(1):34–39
15. Hall CW, Mah TF (2017) Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. FEMS Microbiol Rev 41(3):276–301
16. Potera C (2010) Biofilm dispersing agent rejuvenates older antibiotics. Environ Health Perspect 118(7):A288
17. Anjum S, Arora A, Alam MS, Gupta B (2016) Development of antimicrobial and scar preventive chitosan hydrogel wound dressings. Int J Pharm 508(1–2):92–101
18. Seliktar D (2012) Designing cell-compatible hydrogels for biomedical applications. Science 336(6085):1124–1128
19. Kisiday MJ, Kurz B (2002) Self assembling peptide hydrogel fosters chondrocyte ECM production cell division. PNAS 99(15):9996–10001
20. Ullah F, Othman MB, Javed F, Ahmad Z, Md Akil H (2015) Classification, processing and application of hydrogels: a review. Mater Sci Eng C Mater Biol Appl 57:414–433
21. Gonzalez-Heriquez CM, Sarabia-Vallejos M, Rodriguez-Hernandez J (2017) Advances in the fabrication of antimicrobial hydrogels for biomedical applications. Materials 10(3):1–23
22. Blache U, Ehrbar M (2018) Inspired by nature: hydrogels as versatile tools for vascular engineering. Adv Wound Care (New Rochelle) 7(7):232–246
23. Chung HJ, Park TG (2009) Self-assembled and nanostructured hydrogels for drug delivery and tissue engineering. Nano Today 4(5):429–437
24. Censi R, Di Martino P, Vermonden T, Hennink WE (2012) Hydrogels for protein delivery in tissue engineering. J Control Release 161(2):680–692
25. Drago L, Boot W, Dimas K, Malizos K, Hänsch GM, Stuyck J, Gawlitta D, Romanò CL (2014) Does implant coating with antibacterial-loaded hydrogel reduce bacterial colonization and biofilm formation in vitro? Clin Orthop Relat Res 472(11):3311–3323
26. Jayakumar R, Prabaharan M, Sudheesh Kumar PT, Nair SV, Tamura H (2011) Biomaterials based on chitin and chitosan in wound dressing applications. Biotechnol Adv 29(3):322–337
27. Luo Y, Shoichet MS (2004) A photolabile hydrogel for guided three-dimensional cell growth and migration. Nat Mater 3(4):249–253
28. Warren DS, Sutherland SPH, Kao JY, Weal GR, Mackay SM (2017) The preparation and simple analysis of a clay nanoparticle composite hydrogel. J Chem Educ 94(11):1772–1779
29. Peppas NA, Hilt JZ, Khademhosseini A, Langer R (2006) Hydrogels in biology and medicine: from molecular principles to bionanotechnology. Adv Mater 18(11):1345–1360

30. Veiga AS, Schneider JP (2013) Antimicrobial hydrogels for the treatment of infection. Biopolymers 100(6):637–644
31. Giano MC, Ibrahim Z, Medina SH, Sarhane KA, Christensen JM, Yamada Y, Brandacher G, Schneider JP (2014) Injectable bioadhesive hydrogels with innate antibacterial properties. Nat Commun 5:4095
32. Li L, Yan B, Yang J, Huang W, Chen L, Zeng H (2017) Injectable self-healing hydrogel with antimicrobial and antifouling properties. ACS Appl Mater Interfaces 9(11):9221–9225
33. Dai T, Wang C, Wang Y, Xu W, Hu J, Cheng Y (2018) A nanocomposite hydrogel with potent and broad-spectrum antibacterial activity. ACS Appl Mater Interfaces 10(17):15163–15173
34. Cheng H, Yue K, Kazemzadeh-Narbat M, Liu Y, Khalilpour A, Li B, Zhang YS, Annabi N, Khademhosseini A (2017) Mussel-inspired multifunctional hydrogel coating for prevention of infections and enhanced osteogenesis. ACS Appl Mater Interfaces 9(13):11428–11439
35. Kazemzadeh-Narbat M, Lai BF, Ding C, Kizhakkedathu JN, Hancock RE, Wang R (2013) Multilayered coating on titanium for controlled release of antimicrobial peptides for the prevention of implant-associated infections. Biomaterials 34(24):5969–5977
36. Tian R, Qiu X, Yuan P, Lei K, Wang L, Bai Y, Liu S, Chen X (2018) Fabrication of self-healing hydrogels with on-demand antimicrobial activity and sustained biomolecule release for infected skin regeneration. ACS Appl Mater Interfaces 10(20):17018–17027
37. Ferreira MOG, Leite LLR, de Lima IS, Barreto HM, Nunes LCC, Ribeiro AB, Osajima JA, da Silva Filho EC (2016) Chitosan hydrogel in combination with nerolidol for healing wounds. Carbohydr Polym 152:409–418
38. Allan CR, Hadwiger LA (1979) The fungicidal effect of chitosan on fungi of varying cell wall composition. Exp Mycol 3:285–287
39. Kong M, Chen XG, Xing K, Park HJ (2010) Antimicrobial properties of chitosan and mode of action: a state of the art review. Microbiology 144(1):51–63
40. Dash M, Chiellini F, Ottenbrite R, Chiellini E (2011) Chitosan—a versatile semi-synthetic polymer in biomedical applications. Prog Polym Sci 36:981–1014
41. Wahid F, Zhong C, Wang HS, Hu XH, Chu LQ (2017) Recent advances in antimicrobial hydrogels containing metal ions and metals/metal oxide nanoparticles. Polymers 9(636):1–27
42. El Hadrami A, Adam LR, El Hadrami I, Daayf F (2010) Chitosan in plant protection. Mar Drugs 8(4):968–987
43. Goy RC, De Britto D, Assis OBG (2009) A review of the antimicrobial activity of chitosan. Polimeros 19(3):241–247
44. Badawy MEI, Rabea EI (2011) A biopolymer chitosan and its derivatives as promising antimicrobial agents against plant pathogens and their applications in crop protection. Int J Carbohydr Chem 2011:460381
45. Badawy MEaR EI (2018) Current applications in food preservation based on marine biopolymers. In: Gutierrez T (ed) Polymers for food applications. Springer, Cham, pp 609–650
46. Badawy MEI, Rabea EI (2016) Chitosan and its derivatives as active ingredients against plant pests and diseases. In: Press A (ed) Chitosan in the preservation of agricultural commodities. Elsevier BV, Press, Academic, pp 179–219
47. Romanazzi G, Feliziani E, Bautista-Baòos S, Sivakumar D (2017) Shelf life extension of fresh fruit and vegetables by chitosan treatment. Crit Rev Food Sci Nutr 57:579–601
48. Dutta PK, Tripathi S, Mehrotra GK, Dutta J (2009) Perspectives for chitosan based antimicrobial films in food applications. Food Chem 114(4):1173–1182
49. Helader IM, Nurmiaho-Lassila EL, Ahvenainen R, Rhoades J, Roller S (2001) Chitosan disrupts the barrier properties of outer membrane of gram-negative bacteria. Int J Food Microbiol 71:235–244
50. Szymanska E, Winnicka K, Wieczorek P, Sacha PT, Tryniszewska EA (2014) Influence of unmodified and β-glycerophosphate cross-linked chitosan on anti-Candida activity of clotrimazole in semi-solid delivery systems. Int J Mol Sci 15(10):17765–17777
51. Dai T, Tanala M, Huang YY, Hamblin MR (2011) Chitosan preparation for wounds and burns: antimicrobial and wound-healing effects. Expert Rev Antiinfect Therap 9(7):857–879

52. Ong SY, Wu J, Moochhala SM, Tan MH, Lu J (2008) Development of a chitosan-based wound dressing with improved hemostatic and antimicrobial properties. Biomaterials 29:4323–4332
53. Badawy MEI, Rabea EI (2017) Synthesis and characterization of N-(maleoyl) chitosan at different degrees of substitution with antibacterial activity. J Polym Mater 34(1):249–259
54. Bellich B, D'Agostino I, Semeraro S, Gamini A, Cesaro A (2016) "The good, the bad and the ugly" of chitosans. Mar Drugs 14(5):99
55. Cascone MGaM S (1999) Hydrogels based on chitosan and dextran as potential drug delivery systems. J Mater Sci Mater Med 10(5):301–307
56. Janciauskaite U, Rakutyte V, Miskinis J, Makuska R (2008) Synthesis and properties of chitosan-N-dextran graft copolymers. React Funct Polym 68(3):787–796
57. Aziz MA, Cabral JD, Brooks HJL, Moratti SC, Hanton LR (2012) Antimicrobial properties of a chitosan dextran-based hydrogel for surgical use. Antimicrob Agents Chemother 56(1):280–287
58. Li S, Dong S, Xu W, Tu S, Yan L, Zhao C, Ding J, Chen X (2018) Antibacterial hydrogels. Adv Sci 5:1–17
59. Yang K, Han Q, Chen B, Zheng Y, Zhang K, Li Q, Wang J (2018) Antimicrobial hydrogels: promising materials for medical application. Int J Nanomedicine 13:2217–2263
60. Smithers G (2008) Whey and whey proteins from "gutter-to-gold". Int Dairy J 18:695–704
61. Zasloff M (2002) antimicrobial peptides of multicellular organisms. Nature 415(24):389–395
62. Zhang L-JaG RL (2016) Antimicrobial peptides. Curr Biol 26(1):R14–R19
63. Giuliani A, Pirri G, Bozzi A, Di Giulio A, Aschi M, Rinaldi AC (2008) Antimicrobial peptides: natural templates for synthetic membrane-active compounds. Cell Mol Life Sci 65(16):2450–2460
64. Fjell CD, Hiss JA, Hancock RE, Schneider G (2011) Designing antimicrobial peptides: form follows function. Nat Rev Drug Discov 11(1):37–51
65. Lakshmaiah NJCJY (2015) Antimicrobial peptides: possible anti-infective agents. Peptides 72:88–95
66. Zhao X, Pan F, Xu H, Yaseen M, Shan H, Hauser CAE, Zhang S, Lu JR (2010) Molecular self-assembly and applications of designer peptide amphiphiles. Chem Soc Rev 39(9):3480–3498
67. Midura-Nowaczek K, Markowska A (2014) Antimicrobial peptides and their analogs: searching for new potential therapeutics. Perspect Med Chem 6:73–80
68. Mitra RN, Shome A, Paul P, Das PK (2009) Antimicrobial activity, biocompatibility and hydrogelation ability of dipeptide-based amphiphiles. Org Biomol Chem 7(1):94–102
69. Venkatesh M, Barathi VA, Goh ET, Anggara R, Fazil MH, Ng AJ, Harini S, Aung TT, Fox SJ, Liu S, Yang L (2017) Antimicrobial activity and cell selectivity of synthetic and biosynthetic cationic polymers. Antimicrob Agents Chemother 61(10):e00469–e00417
70. Zhou C, Li P, Qi X, Sharif AR, Poon YF, Cao Y, Chag MW, Leong SS, Chan-Park MB (2011) Photopolymerized antimicrobial hydrogel coating derived from epsilon-poly-L-lysine. Biomaterials 32(11):2704–2712
71. Liu L, Huang Y, Riduan SN, Gao S, Yang Y, Fan W, Zhang Y (2012) Main-chain imidazolium oligomer material as a selective biomimetic antimicrobial agent. Biomaterials 33(33):8625–8863
72. Colak S, Nelson CF, Nüsslein K, Tew GN (2009) Hydrophilic modifications of an amphiphilic polynorbornene and the effects on its hemolytic and antibacterial activity. Biomacromolecules 10(2):353–359
73. Konai MM, Bhattacharjee B, Ghosh S, Haldar J (2018) Recent progress in polymer research to tackle infections and antimicrobial resistance. Biomacromolecules 19(6):1888–1917
74. Ilker MF, Nüsslein K, Tew GN, Coughlin EB (2004) Tuning the hemolytic and antibacterial activities of amphiphilic polynorbornene derivatives. J Am Chem Soc 16(48):15870–15875
75. AL-Badri ZM, Som A, Lyon S, Nelson CF, Nüsslein K, Tew GN (2008) Investigating the effect of increasing charge density on the hemolytic activity of synthetic antimicrobial polymers. Biomacromolecules 9(10):2805–2810
76. Kozlovskaya V, Sukhishvili A (2006) Amphoteric hydrogel capsules: multiple encapsulation and release routes. Macromolecules 39(18):6191–6199

77. Hu J, Quan Y, Lai Y, Zheng Z, Hu Z, Wang X, Dai T, Zhang Q, Cheng Y (2017) A smart aminoglycoside hydrogel with tunable gel degradation, on-demand drug release, and high antibacterial activity. J Control Release 247:145–152
78. Neut D (2001) Biomaterial associated infection of gentamicin loaded PMMA beads in orthopedic revision surgery. Antimicrob Chemother 47:885–891
79. Cleophas RT, Sjollema J, Busscher HJ, Kruijtzer JA, Liskamp RM (2014) Characterization and activity of an immobilized antimicrobial peptide containing bactericidal PEG-hydrogel. Biomacromolecules 15(9):3390–3395
80. Agnihotri S, Mukherji S, Mukherji S (2012) Antimicrobial chitosan–PVA hydrogel as a nanoreactor and immobilizing matrix for silver nanoparticles. Appl Nanosci 2(3):179–188
81. Noppakundilograt S, Sonjaipanich K, Thongchul N, Kiatkamjornwong S (2013) Syntheses, characterization, and antibacterial activity of chitosan grafted hydrogels and associated mica-containing nanocomposite hydrogels. J Appl Polym Sci 127(6):4927–4938
82. El-Salmawi KM (2007) Gamma radiation-induced crosslinked PVA/chitosan blends for wound dressing. J Macromol Sci Part A Pure Appl Chem 44:541–545
83. Straccia MC, d'Ayala GG, Romano I, Oliva A, Laurienzo P (2015) Alginate hydrogels coated with chitosan for wound dressing. Mar Drugs 13(5):2890–2908
84. Liu B, Hu J, Meng Q (2009) Nonwoven supported temperature-sensitive poly(N-isopropylacrylamide)/polyurethane copolymer hydrogel with antibacterial activity. J Biomed Mater Res B Appl Biomater 89(1):1–8
85. Xie Z, Aphale NV, Kadapure TD, Wadajkar AS, Orr S, Gyawali D, Qian G, Nguyen KT, Yang J (2015) Design of antimicrobial peptides conjugated biodegradable citric acid derived hydrogels for wound healing. J Biomed Mater Res Part A 103A:3907–3918
86. Liu Z, Zhu M, Chen X, Yang G, Yang T, Yu L, Hui L, Wang X (2018) Expression and antibacterial activity of hybrid antimicrobial peptide cecropinA-thanatin in Pichia pastoris. Front Lab Med 2(1):23–29
87. Damodaran VB, Murthy NS (2016) Bio-inspired strategies for designing antifouling biomaterials. Biomater Res 20:18
88. Mi L, Jiang S (2012) Synchronizing nonfouling and antimicrobial properties in a zwitterionic hydrogel. Biomaterials 33(35):8928–8933
89. GhavamiNejad A, Park CH, Kim CS (2016) In situ synthesis of antimicrobial silver nanoparticles with antifouling zwitterionic hydrogels by catecholic redox chemistry for wound healing application. Biomacromolecules 17(3):1213–1223
90. Zhao C, Li X, Li L, Cheng G, Gong X, Zheng J (2013) Dual functionality of antimicrobial and antifouling of poly(N-hydroxyethylacrylamide)/salicylate hydrogels. Langmuir 29(5):1517–1524
91. Li J, Mooney DJ (2016) Designing hydrogels for controlled drug delivery. Nat Rev Mater 1(12):16071
92. Hoare TR, Kohane DS (2008) Hydrogels in drug delivery: progress and challenges. Polymer 49(8):1993–2007
93. Raghavendra GM, Jayaramudu T, Varaprasad K, Sadiku R, Ray SS, Mohana Raju K (2013) Cellulose–polymer–Ag nanocomposite fibers for antibacterial fabrics/skin scaffolds. Carbohydr Polym 93(2):553–560
94. Jayaramudu T, Raghavendra GM, Varaprasad K, Sadiku R, Raju KM (2013) Development of novel biodegradable Au nanocomposite hydrogels based on wheat: for inactivation of bacteria. Carbohydr Polym 92(2):2193–2200
95. Gao W, Vecchio D, Li J, Zhu J, Zhang Q, Fu V, Li J, Thamphiwatana S, Lu D, Zhang L (2014) Hydrogel containing nanoparticle-stabilized liposomes for topical antimicrobial delivery. ACS Nano 8(3):2900–2907
96. Zhang Y, Zhang J, Chen M, Gong H, Thamphiwatana S, Eckmann L, Gao W, Zhang L (2016) A bioadhesive nanoparticle-hydrogel hybrid system for localized antimicrobial drug delivery. ACS Appl Mater Interfaces 8(28):18367–18374
97. Posadowska U, Brzychczy-Włoch M, Drożdż A, Krok-Borkowicz M, Włodarczyk-Biegun M, Dobrzyński P, Chrzanowski W, Pamuła E (2016) Injectable hybrid delivery system com-

posed of gellan gum, nanoparticles and gentamicin for the localized treatment of bone infections. Expert Opin Drug Deliv 13(5):613–620
98. Pawar V, Dhanka M, Srivastava R (2019) Cefuroxime conjugated chitosan hydrogel for treatment of wound infections. Colloids Surf B Biointerfaces 173:776–787
99. Islan GA, Dini C, Bartel LC, Bolzan AD, Castro GR (2015) Characterization of smart autodegradative hydrogel matrix containing alginate lyase to enhance levofloxacin delivery against bacterial biofilms. Int J Pharm 496(2):953–964
100. Huber D, Tegl G, Mensah A, Beer B, Baumann M, Borth N, Sygmund C, Ludwig R, Guebitz GM (2017) A dual-enzyme hydrogen peroxide generation machinery in hydrogels supports antimicrobial wound treatment. ACS Appl Mater Interfaces 9(18):15307–15316
101. Gustafson CT, Boakye-Agyeman F, Brinkman CL, Reid JM, Patel R, Bajzer Z, Dadsetan M, Yaszemski MJ (2016) Controlled delivery of vancomycin via charged hydrogels. PLoS One 11(1):e0146401
102. Zhuk I, Jariwala F, Attygalle AB, Wu Y, Libera MR, Sukhishvili SA (2014) Self-defensive layer-by-layer films with bacteria-triggered antibiotic release. ACS Nano 8(8):7733–7745
103. Zhao Y, Zhang X, Wang Y, Wu Z, An J, Lu Z, Mei L, Li C (2014) In situ cross-linked polysaccharide hydrogel as extracellular matrix mimics for antibiotics delivery. Carbohydr Polym 105:63–69
104. Jiang D, Zhang Y, Zhang F, Liu Z, Han J, Wu X (2016) Antimicrobial and antifouling nanocomposite hydrogels containing polythioether dendron: high-loading silver nanoparticles and controlled particle release. Colloid Polym Sci 294(12):2021–2028
105. Perez-Fernandez D, Shcherbakov D, Matt T, Leong NC, Kudyba I, Duscha S, Boukari H, Patak R, Dubbaka SR, Lang K et al (2014) 4'-O-substitutions determine selectivity of aminoglycoside antibiotics. Nat Commun 5:3112
106. Padil VV, Senan C, Cernik M (2015) Dodecenylsuccinic anhydride derivatives of gum karaya (Sterculia urens): preparation, characterization, and their antibacterial properties. J Agric Food Chem 63(14):3757–3765
107. Singh B, Pal L (2012) Sterculia crosslinked PVA and PVA-poly(AAm) hydrogel wound dressings for slow drug delivery: mechanical, mucoadhesive, biocompatible and permeability properties. J Mech Behav Biomed Mater 9:9–21
108. Pakzad Y, Ganji F (2015) Thermosensitive hydrogel for periodontal application: in vitro drug release, antibacterial activity and toxicity evaluation. J Biomater Appl 30(7):919–929
109. Casadidio C, Butini ME, Trampuz A, Di Luca M, Censi R, Di Martino P (2018) Daptomycin-loaded biodegradable thermosensitive hydrogels enhance drug stability and foster bactericidal activity against Staphylococcus aureus. Eur J Pharm Biopharm 130:260–271
110. Pavlukhina S, Lu Y, Patimetha A, Libera M, Sukhishvili S (2010) Polymer multilayers with pH-triggered release of antibacterial agents. Biomacromolecules 11(12):3448–3456
111. Qu J, Zhao X, Ma P, Guo B (2018) Injectable antibacterial conductive hydrogels with dual response to an electric field and pH for localized "smart" drug release. Acta Biomater 72:55–69
112. Mao C, Xiang Y, Liu X, Cui Z, Yang X, Yeung KWK, Pan H, Wang X, Chu PK, Wu S (2017) Photo-inspired antibacterial activity and wound healing acceleration by hydrogel embedded with Ag/Ag@AgCl/ZnO nanostructures. ACS Nano 11(9):9010–9021
113. Shi Y, Truong VX, Kulkarni K, Qu Y, Simon GP, Boyd RL, Perlmutter P, Lithgow T, Forsythe JS (2015) Light-triggered release of ciprofloxacin from an in situ forming click hydrogel for antibacterial wound dressings. J Mater Chem B 3(45):8771–8774
114. Cirillo G, Curcio M, Spizzirri UG, Vittorio O, Tucci P, Picci N, Iemma F, Hampel S, Nicoletta FP (2017) Carbon nanotubes hybrid hydrogels for electrically tunable release of Curcumin. Eur Polym J 90:1–12
115. Zhang Y, Zhang J, Chen W, Angsantikul P, Spiekermann KA, Fang RH, Gao W, Zhang L (2017) Erythrocyte membrane-coated nanogel for combinatorial antivirulence and responsive antimicrobial delivery against Staphylococcus aureus infection. J Control Release 263:185–191
116. Zhou J, Yao D, Qian Z, Hou S, Li L, Jenkins ATA, Fan Y (2018) Bacteria-responsive intelligent wound dressing: simultaneous in situ detection and inhibition of bacterial infection for accelerated wound healing. Biomaterials 161:11–23

117. Lehar SM, Pillow T, Xu M, Staben L, Kajihara KK, Vandlen R, DePalatis L, Raab H, Hazenbos WL, Hiroshi Morisaki J et al (2015) Novel antibody–antibiotic conjugate eliminates intracellular S. aureus. Nature 527:323
118. Arciola CR, Campoccia D, Montanaro L (2018) Implant infections: adhesion, biofilm formation and immune evasion. Nat Rev Microbiol 16(7):397–409
119. Qiu Y, Zhang N, An YH, Wen X (2007) Biomaterial strategies to reduce implant-associated infections. Int J Artif Organs 30(9):828–841
120. Lowy FD (1998) Staphylococcus aureus infections. N Engl J Med 339(8):520–532
121. Zimmerli W, Moser C (2012) Pathogenesis and treatment concepts of orthopaedic biofilm infections. FEMS Immunol Med Microbiol 65(2):158–168
122. Li B, Webster TJ (2018) Bacteria antibiotic resistance: new challenges and opportunities for implant-associated orthopedic infections. J Orthop Res 36(1):22–32
123. Salwiczek M, Qu Y, Gardiner J, Strugnell RA, Lithgow T, McLean KM, Thissen H (2014) Emerging rules for effective antimicrobial coatings. Trends Biotechnol 32(2):82–90
124. Swartjes JJ, Sharma PK, van Kooten TG, van der Mei HC, Mahmoudi M, Busscher HJ, Rochford ET (2015) Current developments in antimicrobial surface coatings for biomedical applications. Curr Med Chem 22(18):2116–2129
125. Gollwitzer H, Ibrahim K, Meyer H, Mittelmeier W, Busch R, Stemberger A (2003) Antibacterial poly(D,L-lactic acid) coating of medical implants using a biodegradable drug delivery technology. J Antimicrob Chemother 51(3):585–591
126. Malizos K, Blauth M, Danita A, Capuano N, Mezzoprete R, Logoluso N, Drago L, Romano CL (2017) Fast-resorbable antibiotic-loaded hydrogel coating to reduce post-surgical infection after internal osteosynthesis: a multicenter randomized controlled trial. J Orthop Traumatol 18(2):159–169
127. Romanò CL, Malizos K, Capuano N, Mezzoprete R, D'Arienzo M, Van Der Straeten C, Scarponi S, Drago L (2016) Does an antibiotic-loaded hydrogel coating reduce early post-surgical infection after joint arthroplasty? J Bone Joint Infect 1:34–41
128. De Giglio E, Cometa S, Ricci MA, Cafagna D, Savino AM, Sabbatini L, Orciani M, Ceci E, Novello L, Tantillo GM et al (2011) Ciprofloxacin-modified electrosynthesized hydrogel coatings to prevent titanium-implant-associated infections. Acta Biomater 7(2):882–891
129. De Giglio E, Cafagna D, Cometa S, Allegretta A, Pedico A, Giannossa LC, Sabbatini L, Mattioli-Belmonte M, Iatta R (2013) An innovative, easily fabricated, silver nanoparticle-based titanium implant coating: development and analytical characterization. Anal Bioanal Chem 405(2–3):805–816
130. Hetrick EM, Schoenfisch MH (2006) Reducing implant-related infections: active release strategies. Chem Soc Rev 35(9):780–789
131. Trautner BW, Darouiche RO (2004) Catheter-associated infections: pathogenesis affects prevention. Arch Intern Med 164(8):842–850
132. Denstedt JD, Wollin TA, Reid G (1998) Biomaterials used in urology: current issues of biocompatibility, infection, and encrustation. J Endourol 12(6):493–500
133. Habash M, Reid G (1999) Microbial biofilms: their development and significance for medical device-related infections. J Clin Pharmacol 39(9):887–898
134. McCoy CP, Irwin NJ, Donnelly L, Jones DS, Hardy JG, Carson L (2018) Anti-adherent biomaterials for prevention of catheter biofouling. Int J Pharm 535(1–2):420–427
135. Lee SY, Lee Y, Le Thi P, Oh DH, Park KD (2018) Sulfobetaine methacrylate hydrogel-coated anti-fouling surfaces for implantable biomedical devices. Biomater Res 22:3–3
136. Fischer M, Vahdatzadeh M, Konradi R, Friedrichs J, Maitz MF, Freudenberg U, Werner C (2015) Multilayer hydrogel coatings to combine hemocompatibility and antimicrobial activity. Biomaterials 56:198–205
137. Lim K, Saravanan R, Chong KKL, Goh SHM, Chua RRY, Tambyah PA, Chang MW, Kline KA, Leong SSJ (2018) Anhydrous polymer-based coating with sustainable controlled release functionality for facile, efficacious impregnation, and delivery of antimicrobial peptides. Biotechnol Bioeng 115(8):2000–2012

138. Vaterrodt A, Thallinger B, Daumann K, Koch D, Guebitz GM, Ulbricht M (2016) Antifouling and antibacterial multifunctional polyzwitterion/enzyme coating on silicone catheter material prepared by electrostatic layer-by-layer assembly. Langmuir 32(5):1347–1359
139. Yang S-H, Lee Y-SJ, Lin F-H, Yang J-M, K-s C (2007) Chitosan/poly(vinyl alcohol) blending hydrogel coating improves the surface characteristics of segmented polyurethane urethral catheters. J Biomed Mater Res B Appl Biomater 83B(2):304–313
140. Singha P, Locklin J, Handa H (2017) A review of the recent advances in antimicrobial coatings for urinary catheters. Acta Biomater 50:20–40
141. Stapleton F, Keay L, Edwards K, Holden B (2013) The epidemiology of microbial keratitis with silicone hydrogel contact lenses. Eye Contact Lens 39(1):79–85
142. Thissen H, Gengenbach T, du Toit R, Sweeney DF, Kingshott P, Griesser HJ, Meagher L (2010) Clinical observations of biofouling on PEO coated silicone hydrogel contact lenses. Biomaterials 31(21):5510–5519
143. Alvarez-Lorenzo C, Yañez F, Concheiro A (2010) Ocular drug delivery from molecularly-imprinted contact lenses. J Drug Delivery Sci Technol 20(4):237–248
144. Tieppo A, White CJ, Paine AC, Voyles ML, McBride MK, Byrne ME (2012) Sustained in vivo release from imprinted therapeutic contact lenses. J Control Release 157(3):391–397
145. Garty S, Shirakawa R, Warsen A, Anderson EM, Noble ML, Bryers JD, Ratner BD, Shen TT (2011) Sustained antibiotic release from an intraocular lens-hydrogel assembly for cataract surgery. Invest Ophthalmol Vis Sci 52(9):6109–6116
146. Shi Y, Lv H, Fu Y, Lu Q, Zhong J, Ma D, Huang Y, Xue W (2013) Preparation and characterization of a hydrogel carrier to deliver gatifloxacin and its application as a therapeutic contact lens for bacterial keratitis therapy. Biomed Mater (Bristol, England) 8(5):055007
147. Huang J-F, Zhong J, Chen G-P, Lin Z-T, Deng Y, Liu Y-L, Cao P-Y, Wang B, Wei Y, Wu T et al (2016) A hydrogel-based hybrid theranostic contact lens for fungal keratitis. ACS Nano 10(7):6464–6473
148. Malakooti N, Alexander C, Alvarez-Lorenzo C (2015) Imprinted contact lenses for sustained release of polymyxin B and related antimicrobial peptides. J Pharm Sci 104(10):3386–3394
149. Hui A, Willcox M, Jones L (2014) In vitro and in vivo evaluation of novel ciprofloxacin-releasing silicone hydrogel contact lenses. Invest Ophthalmol Vis Sci 55(8):4896–4904
150. Sharma A, Taniguchi J (2017) Review: emerging strategies for antimicrobial drug delivery to the ocular surface: implications for infectious keratitis. Ocul Surf 15(4):670–679
151. Lee JH, Kim HW (2018) Emerging properties of hydrogels in tissue engineering. J Tissue Eng 9:2041731418768285
152. Geckil H, Xu F, Zhang X, Moon S, Demirci U (2010) Engineering hydrogels as extracellular matrix mimics. Nanomedicine (Lond) 5(3):469–484
153. Simoes D, Miguel SP, Ribeiro MP, Coutinho P, Mendonca AG, Correia IJ (2018) Recent advances on antimicrobial wound dressing: a review. Eur J Pharm Biopharm 127:130–141
154. Alvarez GS, Helary C, Mebert AM, Wang XL, Coradin T, Desimone MF (2014) Antibiotic-loaded silica nanoparticle-collagen composite hydrogels with prolonged antimicrobial activity for wound infection prevention. J Mater Chem B 2(29):4660–4670
155. Chen HN, Xing XD, Tan HP, Jia Y, Zhou TL, Chen Y, Ling ZH, Hu XH (2017) Covalently antibacterial alginate-chitosan hydrogel dressing integrated gelatin microspheres containing tetracycline hydrochloride for wound healing. Mat Sci Eng C Mater 70:287–295
156. Finnegan S, Percival SL (2015) Clinical and antibiofilm efficacy of antimicrobial hydrogels. Adv Wound Care 4(7):398–406
157. Roy DC, Tomblyn S, Isaac KM, Kowalczewski CJ, Burmeister DM, Burnett LR, Christy RJ (2016) Ciprofloxacin-loaded keratin hydrogels reduce infection and support healing in a porcine partial-thickness thermal burn. Wound Repair Regen 24(4):657–668
158. Mebert AM, Alvarez GS, Peroni R, Illoul C, Helary C, Coradin T, Desimone MF (2018) Collagen-silica nanocomposites as dermal dressings preventing infection in vivo. Mat Sci Eng C Mater 93:170–177
159. Kim JO, Choi JY, Park JK, Kim JH, Jin SG, Chang SW, Li DX, Hwang MR, Woo JS, Kim JA et al (2008) Development of clindamycin-loaded wound dressing with polyvinyl alcohol and sodium alginate. Biol Pharm Bull 31(12):2277–2282

160. Kim JO, Park JK, Kim JH, Jin SG, Yong CS, Li DX, Choi JY, Woo JS, Yoo BK, Lyoo WS et al (2008) Development of polyvinyl alcohol-sodium alginate gel-matrix-based wound dressing system containing nitrofurazone. Int J Pharm 359(1–2):79–86
161. Sung JH, Hwang MR, Kim JO, Lee JH, Kim YI, Kim JH, Chang SW, Jin SG, Kim JA, Lyoo WS et al (2010) Gel characterisation and in vivo evaluation of minocycline-loaded wound dressing with enhanced wound healing using polyvinyl alcohol and chitosan. Int J Pharm 392(1–2):232–240
162. Elviri L, Bianchera A, Bergonzi C, Bettini R (2017) Controlled local drug delivery strategies from chitosan hydrogels for wound healing. Expert Opin Drug Del 14(7):897–908
163. Mahmoud AA, Salama AH (2016) Norfloxacin-loaded collagen/chitosan scaffolds for skin reconstruction: preparation, evaluation and in-vivo wound healing assessment. Eur J Pharm Sci 83:155–165
164. Hu S, Cai X, Qu X, Yu B, Yan C, Yang J, Li F, Zheng Y, Shi X (2019) Preparation of biocompatible wound dressings with long-term antimicrobial activity through covalent bonding of antibiotic agents to natural polymers. Int J Biol Macromol 123:1320–1330
165. Mandy SH (1985) Evaluation of a new povidone-iodine-impregnated polyethylene oxide gel occlusive dressing. J Am Acad Dermatol 13(4):655–659
166. Campoccia D, Montanaro L, Speziale P, Arciola CR (2010) Antibiotic-loaded biomaterials and the risks for the spread of antibiotic resistance following their prophylactic and therapeutic clinical use. Biomaterials 31(25):6363–6377
167. Neibert K, Gopishetty V, Grigoryev A, Tokarev I, Al-Hajaj N, Vorstenbosch J, Philip A, Minko S, Maysinger D (2012) Wound-healing with mechanically robust and biodegradable hydrogel fibers loaded with silver nanoparticles. Adv Healthc Mater 1(5):621–630
168. GhavamiNejad A, Park CH, Kim CS (2016) In situ synthesis of antimicrobial silver nanoparticles within antifouling zwitterionic hydrogels by catecholic redox chemistry for wound healing application. Biomacromolecules 17(3):1213–1223
169. Mekkawy AI, El-Mokhtar MA, Nafady NA, Yousef N, Hamad MA, El-Shanawany SM, Ibrahim EH, Elsabahy M (2017) In vitro and in vivo evaluation of biologically synthesized silver nanoparticles for topical applications: effect of surface coating and loading into hydrogels. Int J Nanomedicine 12:759–777
170. He H, Xia DL, Chen YP, Li XD, Chen C, Wang YF, Shen L, Hu YL, Gu HY (2017) Evaluation of a two-stage antibacterial hydrogel dressing for healing in an infected diabetic wound. J Biomed Mater Res B Appl Biomater 105(7):1808–1817
171. Zhai M, Xu Y, Zhou B, Jing W (2018) Keratin-chitosan/n-ZnO nanocomposite hydrogel for antimicrobial treatment of burn wound healing: characterization and biomedical application. J Photochem Photobiol B 180:253–258
172. Ozaki CK, Hamdan AD, Barshes NR, Wyers M, Hevelone ND, Belkin M, Nguyen LL (2015) Prospective, randomized, multi-institutional clinical trial of a silver alginate dressing to reduce lower extremity vascular surgery wound complications. J Vasc Surg 61(2):419–427. e411
173. Dissemond J, Bottrich JG, Braunwarth H, Hilt J, Wilken P, Munter KC (2017) Evidence for silver in wound care—meta-analysis of clinical studies from 2000-2015. J Dtsch Dermatol Ges 15(5):524–535
174. Choi DS, Kim S, Lim Y-M, Gwon H-J, PArk JS, Nho Y-C, Kwon J (2012) Hydrogel incorporated with chestnut honey accelerates wound healing and promotes early HO-1 protein expression in diabetic (db/db) mice. Tissue Eng Regener Med 9(1):36–42
175. Wang T, Zhu X-K, Xue X-T, Wu D-Y (2012) Hydrogel sheets of chitosan, honey and gelatin as burn wound dressings. Carbohydr Polym 88(1):75–83
176. Tsao C, Chang CH, Lin YY, Wu MF, Wang JL, Young TH, Han JL, Hsieh KH (2011) Evaluation of chitosan/γ-poly(glutamic acid) polyelectrolyte complex for wound dressing materials. Carbohydr Polym 84(2):812–819
177. Sudheesh Kumar PT, Raj NM, Praveen G, Chennazhi KP, Nair SV, Jayakumar R (2013) In vitro and in vivo evaluation of microporous chitosan hydrogel/nanofibrin composite bandage for skin tissue regeneration. Tissue Eng Part A 19(3–4):380–392

178. Ng VW, Chan JM, Sardon H, Ono RJ, Garcia JM, Yang YY, Hedrick JL (2014) Antimicrobial hydrogels: a new weapon in the arsenal against multidrug-resistant infections. Adv Drug Deliv Rev 78:46–62
179. Gan DXT, Xing W, Ge X, Fang L, Wang K, Ren F, Lu X (2018) Mussel-inspired contact-active antibacterial hydrogel with high cell affinity, toughness, and recoverability. Adv Funct Mater 29(1):1805964
180. Zhao X, Wu H, Guo B, Dong R, Qiu Y, Ma PX (2017) Antibacterial anti-oxidant electroactive injectable hydrogel as self-healing wound dressing with hemostasis and adhesiveness for cutaneous wound healing. Biomaterials 122:34–47
181. Dong R, Zhao X, Guo B, Ma PX (2016) Self-healing conductive injectable hydrogels with antibacterial activity as cell delivery carrier for cardiac cell therapy. ACS Appl Mater Interfaces 8(27):17138–17150
182. Koehler J, Brandl FP, Goepferich AM (2018) Hydrogel wound dressings for bioactive treatment of acute and chronic wounds. Eur Polym J 100:1–11
183. Majtan J (2014) Honey: an immunomodulator in wound healing. Wound Repair Regen 22(2):187–192
184. Minden-Birkenmaier BA, Bowlin GL (2018) Honey-based templates in wound healing and tissue engineering. Bioengineering (Basel) 5(2):46
185. Ter Boo GA, Arens D, Metsemakers WJ, Zeiter S, Richards RG, Grijpma DW, Eglin D, Moriarty TF (2016) Injectable gentamicin-loaded thermo-responsive hyaluronic acid derivative prevents infection in a rabbit model. Acta Biomater 43:185–194
186. Ter Boo GJ, Schmid T, Zderic I, Nehrbass D, Camenisch K, Richards RG, Grijpma DW, Moriarty TF, Eglin D (2018) Local application of a gentamicin-loaded thermo-responsive hydrogel allows for fracture healing upon clearance of a high Staphylococcus aureus load in a rabbit model. Eur Cell Mater 35:151–164
187. Dhivya S, Saravanan S, Sastry TP, Selvamurugan N (2015) Nanohydroxyapatite-reinforced chitosan composite hydrogel for bone tissue repair in vitro and in vivo. J Nanobiotechnol 13:40
188. Sani ESP-LR, Spencer A, Yu W, Geilich BM, Noshadi I, Webster T, Annabi N (2018) Engineering adhesive and antimicrobial hyaluronic acid/elastin-like polypeptide hybrid hydrogels for tissue engineering applications. ACS Biomater Sci Eng 4(7):2528–2540

Antibacterial Polymeric and Peptide Gels/Hydrogels to Prevent Biomaterial-Related Infections

Kamal Malhotra and Yashveer Singh

Abstract The emerging threat of antibiotic resistance in pathogenic microbes is a menace to public health. The situation is equally alarming so far as biomaterial-related infections resulting from implantation are concerned. Antibiotics were considered effective in treating bacterial infections and saved millions of lives from infection but the repeated use of antibiotics has led to the development of resistance in microbes. Several strategies have been developed to address the challenge of antibiotic resistance in bacteria. Examples include the use of antiseptics, antiadhesives, metal ions and nanoparticles, carbon nanotubes, graphene and graphene oxide, antimicrobial peptides, and antimicrobial polymers. Even though these approaches offer varying degree of success, they are also associated with serious limitations. Consequently, scientists have focused their efforts toward the development of self-assembled peptide and polymeric gels/hydrogels, as antibacterial biomaterials, to address the challenge of antibiotic resistance in bacteria. This chapter provides a critical review of the developments in the field of antibacterial self-assembled peptides and polymeric gels/hydrogels for treating biomaterial-related infections.

Keywords Antibacterial · Antibiotic resistance · Bacterial infection · Biomaterial-related infection · Polymeric hydrogel · Self-assembled peptide gel

Abbreviations

$SiCl_4$	Silicon tetrachloride
A. baumannii	*Acinetobacter baumannii*
AG	Agarose
AgNPs	Silver nanoparticles
A-lys	Acryloyl-lysine
AMPs	Antimicrobial peptides

K. Malhotra · Y. Singh (✉)
Department of Chemistry, Indian Institute of Technology Ropar, Rupnagar, Punjab, India
e-mail: yash@iitrpr.ac.in

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_23

BMA	n-butyl methacrylate
BP	Bacterial polysaccharide
C. albicans	*Candida albicans*
cfu/dm^2	Colony-forming units/decimeter square
cfu/mL	Colony-forming units per milliliter
CH	Chlorhexidine
CMC/ODex	Carboxymethyl chitosan/oxidized dextran
CNF	Carboxylated cellulose nanofiber
CTX	Ceftriaxone sodium
DMSO	Dimethyl sulfoxide
E. coli	*Escherichia coli*
E. faecalis	*Enterococcus faecalis*
EAK 16-II	AEAEAKAKAEAEAKAK
EPL-MA	Epsilon-poly-L-lysine-graft-methacrylamide
EPS	Extracellular polymeric substances
F. solani	*Fusarium solani*
FDA	Food and Drug Administration USA
G	Gelatin
GO	Graphene oxide
h	Hour
hMSCs	Human mesenchymal stem cells
hRBCs	Human red blood cells
HRTEM	High-resolution transmission electron microscopy
K. pneumonia	*Klebsiella pneumonia*
KLD-12	Ac-KLDLKLDLKLDL-NH_2
L. ivanovii	*Listeria ivanovii*
M. smegmatis	*Mycobacterium smegmatis*
M. tuberculosis	*Mycobacterium tuberculosis*
MAX-1	VKVKVKVK^{D}PPTKVKVKVKV-NH_2
MDR	Multidrug resistance
MRSA	Methicillin-resistant *S. aureus*
MWNTs	Multiwalled carbon nanotubes
NCG	Natural cashew gum
NH007	Boc-D-Phe-γ^4-L-Phe-PEA
NH009	Boc-L-Phe-γ^4-L-Phe-PEA
NVP	*N*-vinylpyrrolidone
P. aeruginosa	*Pseudomonas aeruginosa*
P. gingivalis	*Porphyromonas gingivalis*
P1	Boc-AUDA-Phe-COOH
P2	Boc-AUDA-Phg-COOH
pCBOH1	Poly(2-((2-hydroxyethyl) (2-(methacryloyloxy) ethyl) (methyl) ammonio) acetate
$pCBOH_2$	Poly(2-(bis(2-hydroxyethyl) (2-(methacryloyloxy)ethyl) ammonio) acetate)
PDMAEMA	Poly(2-dimethylamino) ethylmethacrylate

PDR	Pandrug resistance
PEG	Polyethylene glycol
PEGDA	Poly(ethylene glycol) diacrylate
PES	Poly(ether sulfone)
PET	Polyethylene terephthalate
PF 127	Pluronic F-127
PHMB	Polyhexamethylene biguanide
PLLA-PEG-PLLA	Poly(L-lactide)-*b*-poly(ethylene glycol)-*b*-poly(lactide)
PNIPAAm	Poly(*N*-isopropylacrylamide)
QAC	Quaternary ammonium compounds
QCS	Quaternized chitosan
RBCs	Red blood cells
rBMSC	Rat bone mesenchymal stem cell
rGO	Reduced graphene oxide
ROS	Reactive oxygen species
S. aureus	*Staphylococcus aureus*
S. epidermidis	*Staphylococcus epidermidis*
S. mutans	*Streptococcus mutans*
S. pyogenes	*Streptococcus pyogenes*
SEM	Scanning electron microscopy
SPAAC	Strain-promoted alkyne–azide cycloaddition
SWCNTs	Single-wall carbon nanotubes
UV	Ultraviolet
WHO	World Health Organization
XRD	Extensively drug resistant

Introduction

The burden of infectious diseases has significantly reduced due to the promising developments in the healthcare sector, but infectious diseases remain a serious threat to mankind, and it poses many socioeconomic problems. As per the World Health Organization (WHO) report, infections caused by pathogenic bacteria are the leading cause of mortality worldwide [1]. India was the highest consumer of antibiotics in 2010, significantly raising the threat of antibiotic resistance in the country [2]. Among 195 countries, India ranked 154, thus reflecting the poor state of the healthcare index [3]. According to the report, the mortality rate in India was 416.75 per 100,000 people in 2010, which was twice the mortality rate in the USA (200 per 100,000 people) [3]. To combat increasing infections, antibiotics are being used repeatedly, thus raising the threat of antibiotic resistance worldwide. The Centre for Disease Control and Prevention (CDC) has classified a series of bacteria as a serious burden on the US healthcare system [4]. Infections due to antibiotic-resistant bacteria are responsible for about 23,000 deaths annually in the USA [5] and may lead to ten million annual deaths by 2050, which will be higher than the

deaths caused by cancer [5]. Thus, there is an urgent need to develop innovative solutions to prevent this looming disaster.

This chapter provides a brief overview on the current status of bacterial infections and biomaterial-related infections along with the challenges in effectively treating bacterial infections. It is followed by a critical summary of methods, such as antibiotics, antiseptics, antiadhesives, metal ions and nanoparticles, carbon nanotubes, graphene and graphene oxide, antimicrobial peptides (AMPs), and antimicrobial polymers, in treating bacterial infections. A major focus of this chapter is the use of polymeric and peptide hydrogels/gels for treating bacterial infections. These biomaterials have been critically reviewed for their potential in treating biomaterial-related infections and combating antibiotic-resistance in bacteria.

Biomaterial-Related Infections

The demand for biomaterial-based medical devices, like artificial stents, catheters, prosthetic joints, artificial hearts, vascular prosthesis, orthopedic and dental implants, has increased tremendously [6]. The biomaterial composition of these devices varies significantly depending upon their use. However, a common feature of these devices is that they attract microbes and act as a niche for infection during surgical intervention (biomaterial-related infections) [7]. If bacteria grow and proliferate as unicellular organisms, it leads to acute infections, whereas the colonization of bacteria on the surface of devices and tissues as an agglomerate, leads to biofilm formation, which is persistent and chronic in nature [8].

The dry mass of bacteria in these biofilms is about 10% only and the remaining 90% comprises the matrix made up of extracellular polymeric substances (EPS) produced by the bacteria to sustain themselves in the 3D structure of biofilm [9]. The EPS promotes the immobilization of the biofilm structure to help the bacterial cells to communicate and adhere on the surface, providing nutrients to bacteria via sequestering particulate and dissolved nutrients from the water phase, and acts as a recycling center [9]. EPS is mainly composed of lipids, proteins, nucleic acid, and polysaccharides, which protect the bacterial cells from desiccation, charged biocides, host immune defense, and ultraviolet radiation, and does not allow for the penetration of antibiotics to the target site [8]. The bacteria residing in the outer layer of the biofilm are metabolically active but as it goes deeper, cells become more dormant and nongrowing and burdensome to eliminate [10]. The biofilms even evade the host immune system [11].

Challenges in Treating Infection

Bacterial strains, such as *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumonia* (*K. pneumonia*), and *Escherichia coli* (*E. coli*) are the pathogens responsible for forming a biofilm on the biomaterial/medical device surfaces. ESKAPE

pathogens, such as *Enterococcus faecium* (*E. faecium*), *S. aureus*, *K. pneumoniae*, *Acinetobacter baumannii* (*A. baumannii*), *P. aeruginosa*, and *Enterobacter* species are the leading cause of nosocomial-related infections and contribute to the high mortality rate resulting from bacterial infections [12, 13]. The treatment of a biofilm involves the scrapping of bacteria during the surgery and delivery of a high dose of antibiotics at the target site [14]. Earlier, vancomycin along with other antibiotics, like rifampin or gentamicin, was used to treat infection, as they possessed the ability to penetrate the bacterial biofilms. However, this combination gradually became ineffective against the biofilm due to a lack of active metabolism in bacteria residing there [15].

Antibiotic was first invented by Alexander Fleming with the discovery of penicillin in 1929, which led to the era of modern antibiotics [16]. Different antibiotics exhibit different mode of actions—beta-lactam and glycopeptide-based antibiotics target cell wall synthesis, tetracyclines (tetracycline, doxycycline, chlortetracycline) target protein synthesis, quinolones inhibit DNA replication, and sulfonamides interfere with folic acid metabolism [17]. Prior to the discovery of antibiotics, infections often led to the death of individuals. However, the repeated use and misuse of antibiotics has led to the development of antibiotic resistance, which is currently a serious concern worldwide [2]. This resistance occurs because bacteria have the tendency to alter the natural pathways to overcome the effect of antibiotics, and it usually includes alteration in the ribosome unit (30S or 50S) and cell wall precursors, mutation in DNA gyrase and topoisomerase IV involved in DNA replication, mutation in ribosome polymerase, and overexpression of the efflux pump to pump out the antibiotics entering into the bacterial cell [17]. India has been the biggest consumer of antibiotics and, therefore, the problem of antibacterial resistance is more severe [2]. Antibiotic resistance is not only restricted to developing countries. In fact, more than 23,000 deaths are reported in the USA and 28,000 deaths in Europe each year, resulting from the antibiotic resistance [18]. Bacterial strains, like *Mycobacterium tuberculosis* (*M. tuberculosis*), *Streptococcus pyogenes* (*S. pyogenes*), *K. pneumonia*, *P. aeruginosa*, *S. aureus*, *A. baumannii*, and *E. coli*, are known resistant pathogens, whereas *A. baumannii*, *P. aeruginosa*, *S. aureus*, and *E. coli* are becoming multidrug resistant [19].

To deal with the emerging problem of antibiotic resistance, the WHO has made an action plan, which includes the development of novel antibacterial drugs [20]. For the ease of assessment, bacterial resistance is categorized into multidrug resistance (MDR), extensive drug resistance (XDR), and pan drug resistance (PDR). The MDR bacteria are resistant to three or more antibacterial agents, XDR, which is resistance to the first line agents and at least one of the three second class of antimicrobial agents, and PDR, which is resistance to all antibacterial agents [21].

Current Antibacterial Approaches

In this section, an overview of approaches currently in use or under development to treat bacterial infections, particularly antibiotic-resistance infections and biomaterial-related infections, is provided.

Antibiotics

As mentioned earlier, Penicillin was the first antibiotic discovered by Alexander Fleming in 1928 when he noticed a zone of inhibition around an invading fungus on the agar plate where the bacteria could not grow. On isolation and identification, he found that it belongs to genus *Penicillium*. He determined that the extract from the mold named "Penicillin" had an antibacterial effect against *Staphylococci* and other gram-positive pathogens [22]. Thereafter, a German pathologist discovered prontosil (sulfamidochrysoïdine), a chemical derivative from the oil dye, which achieved great success in treating bacterial infections, and he received the Nobel Prize in 1939. Within the next 2 years, sulfanilamide and several derivatives of sulfa drugs became available in the market [16], thus opening the era of antibiotics.

The use of antibiotics gradually increased and it was able to bring down the death rate due to infectious diseases by 20-fold, from 1900 to 1980 [23]. High doses of antibiotics were used as a standard practice to treat the high rate of infections (biofilm) and this repeated use and/or misuse of antibiotics led to the problem of antibiotic resistance [23]. Combinations of antibiotics were used to treat biofilm but later studies found them to be ineffective due to the lack of metabolism in bacteria [15].

Later, efforts were made to develop formulations, which could provide sustained and controlled release of antibiotics. Lujan and coworkers designed a new device for the local delivery of antibiotics to the target site. The implant device was made with a stainless-steel reservoir filled with an antibiotic, and the desired number of orifices was tailored depending on the requirement of the release profile (rapid initial release/slow sustained release). It showed excellent bactericidal action with complete eradication of the bacteria within 8 h (cefazolin with 6 orifices) [24]. Self-defensive antibiotic-loaded coatings were explored to inhibit the growth of pathogenic bacteria on the surface of implants and devices. The pH-triggered coatings, made from the chemical cross-linking of poly(methacrylic acid) with pH-sensitive SNARF-1 (fluorescent label) and unlabeled antibiotic (gentamicin or polymyxin B), exhibited the release of antibiotics on localized acidification by *S. aureus* and *E. coli* [25]. Table 1 lists selected antibiotics and their antibacterial applications. Several antibiotic release coatings were developed but the threat of infection with resistant strains or the development of antibiotic resistant strains due to the continuous release of antibiotics was persistent. Consequently, the use of such techniques was discouraged by the FDA [26].

Antiseptics

Antiseptics and disinfectants have been widely used in healthcare settings for topical disinfection and prevention of nosocomial diseases [28]. The risk of microbial contaminants and infection in food and consumables has increased the use of antiseptics even for the general public. There are a wide variety of products containing

Table 1 Antibiotics used to treat bacterial infections

Antibiotics	Targets	Remarks	References
Penicillin	*Staphylococci* and other gram-positive pathogens	First true antibiotic discovered and was used to kill pathogenic bacterial infections	[16]
Prontosil (sulfamidochrysoïdine) and sulfa drugs	*Streptococcal* and *Staphylococcal* origins	First antibacterial chemical derivative (azobenzenes and sulfonamides) showing bactericidal activities in animals but not in vitro	[16, 27]
Vancomycin	Methicillin-resistant *S. aureus* (MRSA)	Used in combination with rifampin or gentamicin to treat serious bacterial infections	[15]
Formulations to deliver antibiotics locally	*S. aureus* ATCC 6538	Linezolid and cefazolin showed controlled release from orifice to reduce infections	[24]
Antibiotic-loaded coatings	*S. aureus* ATCC 12600 and *E. coli* O2K2	Layer-by-layer hydrogel coatings of poly(methacrylic acid) attached with gentamicin or polymyxin to reduce bacterial infections	[25]

biocides that have been used for centuries to prevent infection and are associated with broad-spectrum activity, less cytotoxicity, and lower chances of developing resistance. The commonly used antiseptics are alcohol (ethyl or isopropyl), dilute iodine solutions, iodophors, and chlorhexidine [28]. Chlorhexidine is the most commonly used antiseptic for topical medical applications, like dental irrigant fluid [29]. However, these antiseptics must not be used in excess.

Galvez and coworkers showed that the repeated use of chlorhexidine (CH) and quaternary ammonium compounds (QAC) may promote bacterial drug resistance against these biocides and clinically relevant antibiotics. The study also suggested that the change in the membrane fluidity may be the major reason for the development of tolerance against biocides [30].

Antiadhesives

Implant surfaces provide an ideal substrate for the attachment of bacteria. It involves the initial attraction of cells toward the surface, followed by adsorption and attachment. The adherence of bacteria to surfaces is controlled by van der Waals, electrostatic, hydration, and hydrophobic interactions. After attachment, bacterial cells start colonizing and form a biofilm on the implant, which is difficult to treat [31]. Variation in the property of implant surfaces, like hydrophobicity and charge, may lead to decreased bacterial adhesion to surfaces. With this thought, polyethylene glycol (PEG) has been extensively explored as a coating for implants because of its

hydrophilicity and steric hindrance, which prevent the adherence of bacteria to the surface [32]. PEGs of different molecular weights (Mw: 200, 400, 600, 2000, and 4600) were grafted on the polyethylene terephthalate (PET) surfaces using silicon tetrachloride ($SiCl_4$) plasma and all the PEG-coated surfaces showed reduction in the attachment of *Salmonella enterica* sv Typhimurium, with maximum antifouling activity observed for PEG2000 [33].

Several bacterial polysaccharides (A101 isolated from marine bacterium *Vibrio* sp. QY101, Ec111p from *E. coli* Ec111, Ec300p from *E. coli* Ec300, Pel from *P. aeruginosa* PAO1), owing to their hydrophilicity, have shown the potential to inhibit biofilm formation by a wide range of bacteria and fungi, and could potentially be used in antibacterial applications [34]. The conjugate of the Pluronic F-127 (PF127) polymer functionalized with antibacterial peptide and RGD was explored for antiadhesive and antibacterial properties along with the induction of host tissue integration. This polymer-peptide conjugate not only showed good antibacterial and antiadhesive properties against *S. aureus*, *S. epidermis*, and *P. aeruginosa* but also promoted the growth of host cells [35]. Table 2 lists selected antiadhesives to prevent bacterial attachment and infection.

Metal Ions and Nanoparticles

Silver is the most widely used metal in the form of ions, salts, and nanoparticles for antibacterial applications [39]. Silver, a noble metal, releases silver ions upon oxidation, which makes the metal surfaces antibacterial. The antibacterial activity can be increased by enhancing the oxidation of silver by complexation with inorganic ions or organic molecules [39]. Silver-coated catheters have been used as a cost-effective

Table 2 Antiadhesives used to prevent bacterial attachment and infection

Antiadhesives	Targets	Remarks	References
Poly(ethylene glycol) or PEG	*Salmonella enterica* sv. Typhimurium and *Listeria monocytogenes*	PEG2000-grafted polyethylene terephthalate (PET) showed effective biofilm inhibition	[33]
Bacterial polysaccharide (BP) A101	*P. aeruginosa*	Inhibited biofilm formation by increasing aminoglycosides antibiotics' capability and inhibiting cell aggregation	[34, 36]
BP Ec111p	*S. aureus*	Inhibited biofilm formation and showed antiadhesive property	[34, 37]
BP Ec300p	*S. aureus*	Inhibited biofilm formation and showed antiadhesive property	[34, 37]
BP Pel	*S. epidermidis*	Disrupted the biofilm formation	[34, 38]
Pluronic F-127 (PF127)	*S. aureus*, *S. epidermis*, and *P. aeruginosa*	PF127 conjugated with RGD showed antiadhesive, antibacterial, and host tissue integration properties	[35]

strategy to prevent nosocomial associated urinary tract infections [40]. Polymer scaffolds containing silver or coated with metallic silver/silver salt have been shown to exhibit fast and broad-spectrum antibacterial activities against gram-positive and negative bacterial strains [41].

The coating of a hydrophilic copolymer, *N*-vinylpyrrolidone (NVP) and n-butyl methacrylate (BMA), with silver nanoparticles and sodium heparin, has been used to treat bacterial bloodstream infections in central venous catheters. The incorporation of silver nanoparticles in the coating prevented the adhesion of clinically isolated *S. aureus* to the surface of the catheter [42]. Silver ions (released from metallic silver, silver nanoparticles, and a sparingly soluble silver salt) have been extensively explored in medicine and healthcare settings because the development of silver resistance in bacteria is quite difficult, as it engages multiple targets (blockage of respiratory enzymes and alteration in DNA or cell walls) [39]. The mechanism of action of silver varies, depending on the formulation [43]; silver nanoparticles may penetrate the bacterial cell membrane, causing cell death. The formation of free radicals by silver nanoparticles is also considered to be responsible for cell death by penetrating the bacterial cell membrane and making it porous [43]. Some studies also suggested that silver nanoparticles release silver ions, which interact with thiol groups of vital enzymes to inactivate them [44]. However, there are instances where the development of resistance against silver has been shown. Kvítek and coworkers reported the development of bacterial resistance against silver nanoparticles in *P. aeruginosa* and *E. coli* after exposure [45]. Epple and coworkers found that silver ions and silver nanoparticles exhibited toxicity at 0.5–5 ppm and 12.5–50 ppm in bacterial strains, human mesenchymal stem cells (hMSCs), and mononuclear cells, which limits their applicability [46].

Copper nanoparticles also exhibit interesting biological, chemical, and physical properties along with antibacterial characteristics. Ibrahim and coworkers investigated the potent antibacterial activities of Cu-chitosan nanoparticles against methicillin-resistant strains along with the antifungal activity but the rapid oxidation of copper in air limits its utility in antibacterial applications [47]. The mechanism behind the antibacterial activities of different metal nanoparticles include the generation of ROS (reactive oxygen species), induction of pits and gaps in the bacterial membrane, disruption of cell walls, DNA damage, and interruption of electron transport, as shown in Fig. 1. The silver/copper nanoparticle coating on catheters was found to be effective in preventing methicillin-resistant *S. aureus* (MRSA) infection both in vitro and in vivo [48]. The biocompatible copper bearing titanium implant was used to prevent peri-implantitis and provide antibacterial and antibiofilm activities against oral bacterial strains, *Streptococcus mutans* and *Porphyromonas gingivalis* [49]. The Ti and copper-bearing Ti-implants supported the adhesion of mesenchymal stem cells with more filopodia detected on the Ti-Cu implant, thus showing the biocompatibility of the surfaces [49].

As discussed earlier, the antibacterial activities of copper nanoparticles involve multiple targets, like DNA damage, ROS generation, and lipid and protein oxidation. Therefore, the chances of developing bacterial resistance against copper are minimal [50]. Interestingly, the use of copper as an antibacterial agent has reached

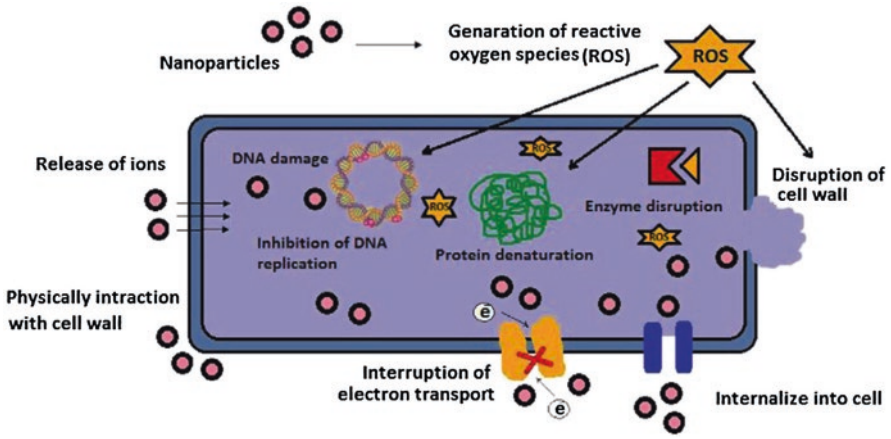


Fig. 1 The mechanism of antibacterial activities of metal nanoparticles [47]. (Reprinted with permission from Dizaj SM, Lotfipour F, Barzegar-Jalali M, Zarrintan MH, Adibkia K. Antimicrobial activity of the metals and metal oxide nanoparticles. Materials Science and Engineering: C. 2014; 44: 278–284 (© 2014, Elsevier))

clinical trials where it was used as a coating in hospital settings to prevent nosocomial-related infections [51]. Although, as discussed earlier, the bacterial resistance against copper is scarce, one study depicted the potential threat of developing bacterial resistance against copper nanoparticles after repeated exposure [52]. Zinc oxide nanoparticles have also shown antibacterial potential against skin infection. Intradermal administration of zinc oxide nanoparticles reduced bacterial load and inflammation in mice along with improvements in skin architecture [53]. The mechanisms behind the antibacterial activities of ZnO nanoparticles involve the disruption of cell membrane and induction of oxidative stress [54]. Table 3 lists selected metal ions and nanoparticles with potential for antibacterial applications. Several other metal oxides like TiO_2, Fe_2O_3, and MgO have also been explored against bacterial infections [47], but they all are associated with the problem of cytotoxicity toward mammalian cells and development of bacterial resistance [55].

Carbon Nanotubes

Carbon nanotubes (CNTs) are classified as single-wall carbon nanotubes (SWCNTs) and multiwalled carbon nanotubes (MWCNTs). SWCNTs have single graphene layer whereas MWCNT possess concentric layers of graphene as shown in Fig. 2 [57]. The CNTs possesses functional groups at end tips and highly aromatic side wall, and it is the aromatic side wall which imparts the antibacterial activity. Elimelech and coworkers reported for the first time strong antibacterial activities of SWCNTs [58]. Other studies also reported similar antibacterial activities of SWCNTs and MWCNTs [59, 60]. Individually, dispersed SWCNTs were found to

Table 3 Metal ions and nanoparticles for antibacterial applications

Metal ions/ nanoparticles	Targets	Remarks	References
Silver ions and nanoparticles	*Candida albicans* (*C. albicans*) I, II, *C. tropicalis*, *E. coli* DH5a, *P. aeruginosa* ATCC 10145, and *Legionella pneumophila*	Engage multiple pathways: blockage of respiratory enzymes, alteration in DNA, penetration of bacterial cell wall, generation of oxidative stress via release of reactive oxygen species (ROS)	[39]
Copper nanoparticles	MRSA, *B. subtilis*, *P. aeruginosa*, *Salmonella choleraesuis*, *C. albicans*, *Streptococcus mutans*, and *Porphyromonas gingivalis*	Penetrate bacterial membrane, induce pores in the membrane, damage vital enzymes of bacteria, release intracellular materials, and shrink bacterial cells causing lysis	[47–49]
Zinc oxide nanoparticles	*Aspergillus niger, Bacillus megaterium*, *B. subtilis*, *C. albicans*, *Campylobacter jejuni*, *E. coli*, *K. pneumonia*, *P. aeruginosa*, *Pseudomonas vulgaris, S. aureus,* and *Sarcina lutea*	Disrupt the bacterial cell membrane and generate oxidative stress via ROS	[53–56]

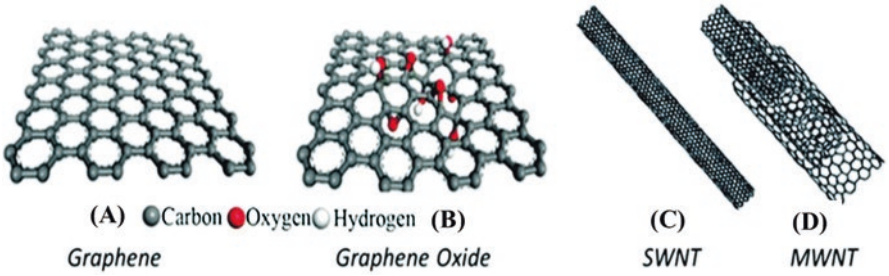


Fig. 2 Structure of (**A**) graphene, (**B**) graphene oxide, (**C**) single-walled carbon nanotube (SWCNT), and (**D**) multiwalled carbon nanotube (MWCNT) [57]. (Reprinted with permission from Li N, Su X, Lu Y. Nanomaterial-based biosensors using dual transducing elements for solution phase detection. Analyst. 2015; 140(9):2916–2943 (© 2015, Royal Society of Chemistry, Great Britain))

be more toxic to both gram-positive and negative bacteria than SWCNT aggregates [61]. These dispersed SWCNTs acted as nanodarts, which penetrated the bacterial membrane to cause cell death [61]. MWCNTs with a smaller diameter ($MWNTs_{20–40}$) showed excellent toxicity toward bacterial viability and the electrostatic repulsion occurred at the interface of MWCNTs and the bacterial surface, resulting in the repulsion of bacteria [62]. The mechanisms behind the antibacterial activities of CNTs lied in the disruption of bacterial membranes via electrostatic interactions and generation of oxidative stress by reactive oxygen species [63]. Although these materials exhibited promising antibacterial activities, they were found to be toxic toward mammalian cells even at low concentrations [64].

Graphene and Graphene Oxide

Graphene (2D monolayer of carbon) and graphene oxide (epoxy, hydroxyl, and carboxyl functionalized graphite) (Fig. 2) possess interesting properties due to which they can be used for drug delivery and antibacterial applications [65]. The water-dispersible formulations of graphene oxide (GO) and reduced graphene oxide (rGO) showed excellent antibacterial activities against *E. coli* with minimum cytotoxicity toward A549 cell lines. Fan and coworkers [65] formulated GO into low-cost antibacterial paper, with an extensive potential for environmental and clinical applications. The graphene coating on titanium implant was used to enhance in vivo osteogenesis and osteointegration in a white rabbit femoral condyle defect model. The coating of GO was achieved by chemical and physical etching, which improved the bone defect and biocompatibility of the scaffold in vivo. The antibacterial mechanism of the coating involved the penetration of sharp edges of graphene into the bacterial membrane, causing cell death [66].

Lochab and coworkers [67] showed that GO-coated surface exhibited promising antibacterial activity against *E. coli* and *S. aureus* to prevent biofilm formation. The studies also reported that the mechanism behind the antibacterial activity of the coated GO surface is different from graphene in suspension. It was revealed that besides reactive-oxygen species generated oxidative stress, physical properties of the GO-coated surface helped to mitigate bacterial load [67]. In terms of cytotoxicity of graphene-based materials, different reports exist. Akhavan and coworkers [68] suggested a side-dependent genotoxicity of rGO nanoplatelets in human stem cells through DNA fragmentation and chromosomal abbreviation even at a very low concentration of 0.1 μg/mL [68], whereas a study by Pelin et al. [69] suggested that only high concentrations and longer exposure (72 h) of graphene and GO materials induced plasma membrane damage, thus implying lower cytotoxicity toward keratinocytes [69].

Antimicrobial Peptides (AMPs)

Direct acting and cationic AMPs are the two classes of host-defense peptides [70]. Among them, direct acting host defense peptides showed great potential as a broad-spectrum antibacterial agent and, hence, could be used as a new antibiotic. But clinical studies showed their potential for topical applications only and there is a need to reduce their cytotoxicity, and improve their proteolytic stability and serum half-life. Cationic peptides are ideal candidates to treat bacterial infections because these peptides may boost the immune response against infection, consequently decreasing the proinflammatory responses [70]. AMPs interact with the bacterial membrane to cause cell death via depolarization, disruption of membrane or intracellular targets [70]. The proposed mechanism of antibacterial activities of AMP was explained using different confirmations, like barrel-stave, carpet, and toroidal-pore

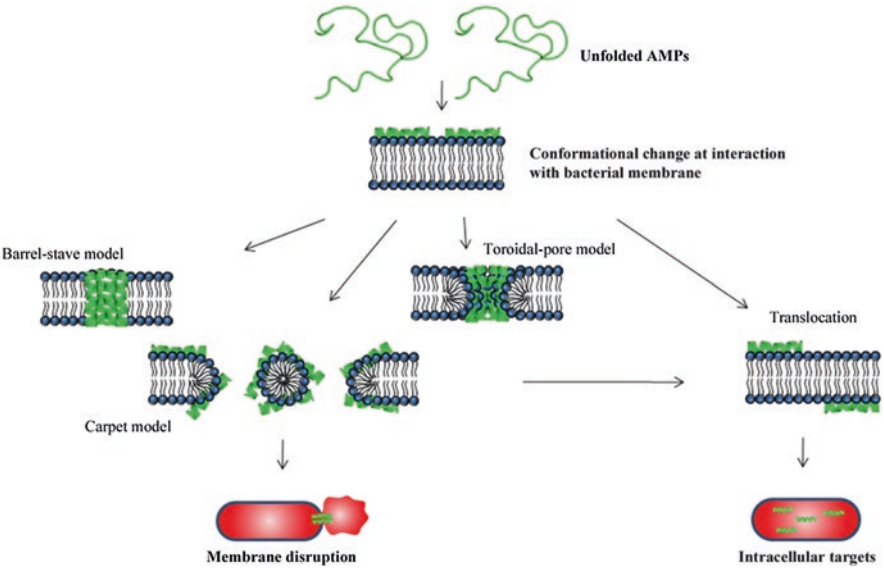


Fig. 3 Killing of bacteria by antimicrobial peptides (AMPs) [71]. (Reprinted with permission from Mahlapuu M, Håkansson J, Ringstad L, Björn C. Antimicrobial Peptides: An Emerging Category of Therapeutic Agents. Front Cell Infect Microbiol. 2016; 6: 194 (© 2016, Authors))

model, adopted by the peptides [71]. According to barrel-stave model, peptide is inserted perpendicularly into the bilayer leading to the formation of transmembrane pores. Toroid model suggest that peptide insertion forces the phospholipids to bend from one leaflet to other, creating transmembrane pores. Further, carpet model suggests accumulation of peptide on the membrane surface, causing tension in the bilayer and leading to the micelle formation (Fig. 3) [71]. A synthetic peptide, cathelicidin, has been shown to have potential to inhibit biofilm formation by *S. aureus*, with a potential to treat chronic wound infections [72].

Surface modification of titanium (Ti), with Ti-binding peptides and AMPs, was found to be effective in reducing biofilm formation by *Porphyromonas gingivalis* and preventing peri-implantitis [73]. Studies showed that the specific binding of AMPs to the surface not only inhibited bacterial growth but also reduced biofilm formation [73]. The chimeric AMPs were tested against nosocomial, drug-resistant pathogenic strains of *A. baumannii*, and it showed efficient antibacterial activities and this activity was synergistically increased when administered with ciprofloxacin [74]. Moreover, the AMPs tested revealed no cytotoxicity toward HaCaT human skin keratinocytes, and the reduction of biofilm formation by *A. baumannii* was quite promising [74].

For preventing biomaterial-related infections, AMPs can be tethered to the surface or applied as a controlled or sustained release formulation. The tethering of AMPs to the surface can be achieved using the physical or chemical immobilization methods discussed below.

Physical Immobilization of AMPs

The physical immobilization of AMPs includes adsorption or the formation of self-assembled monolayers (SAMs) through noncovalent interactions, like hydrogen bonding, van der Waals, and hydrophobic or ionic interactions. Gold [75], titanium/titanium dioxide [76], silicon or polymeric brushes [77] are the various substrates that could be used for immobilization of AMPs without any shape constrains. Egles and coworkers [78] developed a new approach, where they inserted AMP, defensin, into a polycationic and polyanionic polyelectrolyte multilayer film, which showed 98% inhibition against *E. coli* D22. Thus, functionalized coatings can be built on implantable surfaces to prevent infections [78]. However, the incorporation of water-soluble peptides along with electrostatic interactions of peptides and the matrix made the system degradable and reduced its motility.

To overcome this limitation, a water-insoluble peptide, gramicidin A, was introduced into the negatively charged layer-by-layer assembly with poly-(L-lysine) on the outermost layer, which acted as a bactericidal agent against gram-positive bacteria, *Enterococcus faecalis* [79]. This biofunctionalized film killed bacteria by direct contact as well as by the release of AMPs into the surrounding medium [79]. Although this technique was efficient in inhibiting bacterial growth, it showed poor release profiles. Therefore, Hammond and coworkers [80] incorporated ponericin G1, an AMP, into the hydrolytically degradable polyelectrolytic film, which showed a sustained release for up to 10 days against *S. aureus* and was found to be biocompatible toward NIH 3T3 and human umbilical vein endothelial cells [80]. This physical entrapment of the peptide into a multilayer complex is associated with the limitation of gradual loss of peptide [79], which raises the threat of antibiotic resistance and hemolytic activity of such films. The long-term stability and activity of such biofunctionalized films have also not been investigated.

Chemical Immobilization of AMPs

Chemical immobilization of AMPs on biomaterial surface improves peptide stability and long-term efficiency of the antimicrobial surface [81]. Chemical immobilization involves the introduction of chemical bonds between the AMPs and the surface to impart additional stability and prevent the leaching of AMPs, thus preventing cytotoxicity [80]. Kizhakkedathu and coworkers [82] reported a polymeric brush-based antibacterial coating, consisting of covalently conjugated hydrophilic polymeric chains with a series of AMPs, to provide infection resistant coatings. Such coating did not impart cytotoxicity or elicit immune responses [82] and showed significant potential in preventing biomaterial-related infections.

The antibacterial peptide, magainin 1, was covalently conjugated to the SAMs of 11-mercaptoundecanoic acid and 6-mercaptohexanol (1:3) and showed a 50% reduction of bacterial adhesion to the surface along with killing of bacterial strains, *Listeria ivanovii*, *Enterococcus faecalis*, and *S. aureus* [83]. The leaching of AMP

was not observed and activity was retained for up to 6 months [83]. A similar study by Berjeaud and coworkers [84] reported the covalent conjugation of AMP gramicidin A, to cysteine-based SAMs, which exhibited 60% inhibition against *E. coli* and 90% against *C. albicans* along with a reduction in bacterial adhesion. The activity was persistent for over 6 months [84]. For the AMPs to be effective, the peptide should be tethered to the surface and other factors, which affect the efficacy include the length and orientation of the peptide, and spacer connecting the peptide to the surface [85]. However, tethering of the AMP to the surface leads to a decrease in activity, a challenge that needs to be addressed [86].

Antimicrobial Polymers

Polymers, like proteins and cellulose, are the building blocks of life and there is an increasing interest in using them for antibacterial applications [87]. Polymers have been explored for antibacterial applications to prevent the increasing bacterial resistance [88]. Antibacterial polymer was first described in 1965, when Cornell and Dunraruma [89] reported a polymer, 2-methacryloxytroponones, that killed bacteria. Ekzemplyaro and coworkers in 1971 reported a polymer with quaternary ammonium groups, which exhibited antibacterial activities against *S. aureus* and *E. coli* [90]. Several synthetic conventional amphiphilic polymers, like poly(vinyl pyridine), poly(vinyl alcohol), polyacrylates, and polystyrenes showed promising antibacterial activities by disrupting the bacterial membrane and transmembrane potential, causing the leakage of cytoplasmic contents, and thus leading to the cell death [91].

An amphiphilic polymethacrylate derivative has been explored for antibacterial activity against *E. coli* D31 [91]. Antibacterial polymers are promising alternatives to antibiotics as their mode of actions lies in membrane disruption [92]. Studies by Perrier and coworkers [92] explained the effect of sedimentation and cationic comonomer on antibacterial activity. They found that the triblock polymer with low cationic comonomer content showed antibacterial activity against *P. aeruginosa* and *S. epidermidis* [92]. Liu and coworkers [93] designed an antibacterial and antiadhesive cotton fabric coated by a cationic fluorinated polymer. Such polymer-coated fabrics exhibited a synergistic antibacterial effect due to the presence of an organic quaternary ammonium salt, and antiadhesive and antibacterial effect of fluorine, resulting from hydrophobicity and low surface energy [93].

Despite the substantial progress in the development of antibacterial polymers, the exact mechanisms behind the activity of these polymers have not been fully understood [94]. Moreover, there is a need to develop reusable or long-lasting antimicrobial polymers to treat biomaterial-related infections [94]. Therefore, lots of strategies are being developed to reduce infection but they are associated with invariable cytotoxicity and other side effects.

Gels and Hydrogels for Biomaterial-Related Infections

Hydrogels/gels are cross-linked hydrophilic polymers, with the ability to absorb water from ten to thousand of times of their dry weight in water, and swell [95]. Hydrogels/gels could be stable or may eventually disintegrate or degrade, depending upon the nature of material used in their fabrication. Hydrogels/gels have been prepared by employing covalent cross-linking or noncovalent interactions, like H-bonding and hydrophobic and/or ionic interactions [95]. Hydrogels/gels could be stimuli-responsive and respond to a change in pH, temperature, and concentration. The term hydrogel was first coined in 1894 and its use in biologics was explored in 1960 by Wichterle and Lim [96]. Hydrogels/gels have generated considerable attention because of their potential to mimic physiological and biological properties of the naturally occurring ECM [97]. Their low interfacial tension, biomimetic nature, and high permeability to small molecules make them attractive for biomedical applications, like drug delivery, tissue engineering, and wound healing [97]. The drugs or antibacterial agents can be entrapped within the hydrogel matrix employing covalent bonds or noncovalent interactions (Fig. 4). The biomaterials also show great potential in antibacterial applications, such as for preventing biomaterial-related infections.

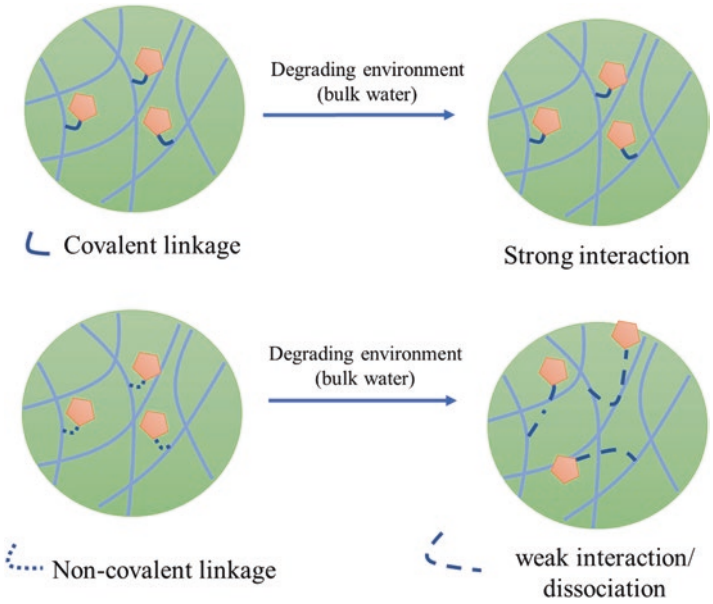


Fig. 4 Hydrogel/gel exhibiting covalent and noncovalent interactions with active moieties

Polymeric Hydrogels

Polymeric hydrogels can be categorized into the following groups.

Polymeric Hydrogels Containing Antibiotics

Hydrogels provide a hydrophilic environment for the entrapment of hydrophilic antibiotics/drugs, which are then released at the site to reduce bacterial infections and minimize side effects. Two electrosynthesized polyacrylic hydrogels, poly(2-hydroxyetheyl methacrylate) and poly(ethylene glycol) diacrylate and acrylic acid-based copolymer, were coated on a titanium implant and loaded with ciprofloxacin to test the inhibition of MRSA growth in vitro [98]. The studies indicated that the antibiotic-loaded hydrogel coatings could be effectively used to prevent bacterial infections associated with orthopedic implants, without affecting osteoblast function related to the new bone formation [98]. The antibiotic entrapped into the hydrogel through physical encapsulation led to an initial burst release that emptied the depot, which then acted as a reservoir for the bacterial growth [99]. To prevent this, PEG-based hydrogels covalently conjugated with vancomycin through poly(β-amino ester) chemistry were developed and found to maintain the release of antibiotic along with the degradation of the hydrogel matrix. These antibiotic-loaded hydrogels showed release of antibiotics for long durations (7–21 days) and exhibited inhibition of planktonic *S. aureus* [99].

Wu and coworkers [100] developed a carboxymethyl-chitosan (CM-chitosan) hydrogel loaded with gentamicin sulphate, which not only inhibited the growth of *S. aureus* but also aided in the adhesion, proliferation, and differentiation of MC3T3-E1 cells. The hydrogel showed dual function: antibacterial activity with osteoblastic cell response and hence could potentially be used for various orthopedic applications [100]. A new polysaccharide salecan/poly(*N*-3-dimethylamino propyl acrylamide-*co*-acrylamide)-based hydrogel was fabricated by free radical polymerization for the controlled release of amoxicillin, in a pH-sensitive manner [101]. Zhang and coworkers [102] developed a marine mussel inspired bio-adhesive hybrid hydrogels containing a nanoparticle–hydrogel combination, where antibiotics were loaded into the polymeric nanoparticles and then embedded into the 3D hydrogel matrix. The hydrogels were tailored for imparting adhesion and nanoparticles provided the controlled and prolonged release of antibiotics to inhibit the proliferation of *E. coli* film. The application of hybrid hydrogels on the mouse tissue for 7 consecutive days did not elicit any toxicity, indicating a safe and effective drug delivery platform against infection [102].

Co-delivery of membrane disrupting polymer along with commercial antibiotics were used to kill opportunistic bacteria [103]. Yang and coworkers [103] developed vitamin E conjugated cationic polycarbonates, which exhibited synergistic antimicrobial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans*. The studies suggested that the optimal balance between the hydrophobicity and cationic

charge is essential for improved antimicrobial activity and reduced cytotoxicity toward mammalian cells. The cationic polymer increased the bacterial membrane permeability, facilitating the penetration of the antibiotic into the bacterial membrane to cause lysis at concentrations significantly below the minimum bactericidal concentration of polymer and antibiotic alone [103]. Forsythe and coworkers [104] developed photocleavable caged antibiotic-conjugated, in situ forming PEG hydrogels through strain-promoted alkyne–azide cycloaddition (SPAAC) chemistry. The studies reported temporal and sequential controlled release of native ciprofloxacin on exposure to UV light of low intensity at 365 nm and was found to be effective against infected wounds [104]. There has been an increasing interest in using antibiotic-loaded biomaterials for implant-related infections [105]. Studies by Moriarty and coworkers reported the use of thermoresponsive hyaluronic acid derivative-based hydrogels loaded with gentamicin, which showed the inhibition of *S. aureus* colonization on the implant in a rabbit model of osteosynthesis [105]. The carboxymethyl chitosan oxidized dextran (CMC/ODex) polysaccharide-based hydrogels were prepared by a Schiff based cross-linking reaction (Fig. 5) [106]. The polysaccharide hydrogel was loaded with the drug ceftriaxone sodium (CTX), which showed gradual degradation over 60 days with a better anti-infective effect when used in murine models (infection and a cecal ligation and puncture model) [106].

Cheng and coworkers [107] developed aminoglycoside-based hydrogels formed by cross-linking oxidized polysaccharides (such as alginate, carboxymethyl cellulose, dextran and chondroitin) with aminoglycoside as cross-linkers. The hydrogels showed fast gelation and self-healing ability, with good injectability [107]. These aminoglycoside-based hydrogels released the antibiotic based on the acidity generated by the growing bacteria, which cleaved the Schiff base linkage between the

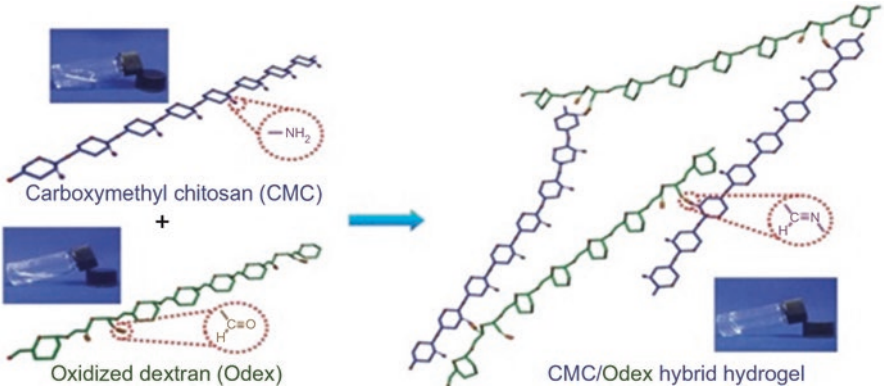


Fig. 5 Formation of a cross-linked network of CMC/ODex through Schiff base-based reactions [106]. (Reprinted with permission from Li Z, He C, Yuan B, Dong X, Chen X. Injectable Polysaccharide Hydrogels as Biocompatible Platforms for Localized and Sustained Delivery of Antibiotics for Preventing Local Infections. Macromolecular Bioscience. 2017; 17(4): 1600347 (© 2017, John Wiley & Sons, Inc.))

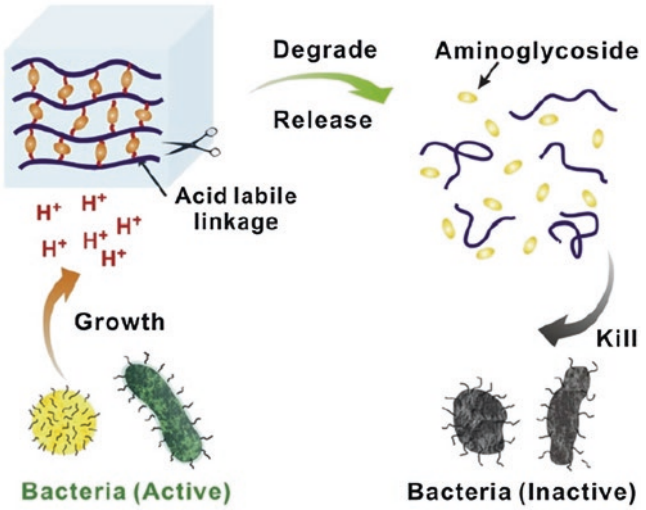


Fig. 6 The mechanism of release of aminoglycoside from the hydrogel to treat bacterial infections [107]. (Reprinted with permission from Hu J, Quan Y, Lai Y, et al. A smart aminoglycoside hydrogel with tunable gel degradation, on-demand drug release, and high antibacterial activity. Journal of Controlled Release. 2017; 247: 145–152 (© 2017, Elsevier))

polysaccharide and the aminoglycoside, thus providing an on-demand antibiotic release with tunable release kinetics (Fig. 6). The increasing growth of bacteria accelerated the release of aminoglycosides along with the degradation of the hydrogel backbone. Once sufficient release of aminoglycoside was achieved to kill bacteria, the recovered pH slowed down the drug release, thus avoiding the immune reaction. The smart, tunable hydrogels showed promising antibacterial activities against *Streptococcus pyogenes* [107]. The injectable hydrogels, fabricated using polydextran aldehyde and *N*-(2-hydroxypropyl)-3-trimethylammonium chitosan chloride (antibacterial polymer), were encapsulated with vancomycin using reversible imine bonds (Fig. 7) and exhibited sustained release of the antibiotic for an extended time period in a pH-dependent manner [108]. The studies revealed a dual killing mechanism via the contact mode and the release of antibiotic into the surroundings. These materials showed killing of >99.99% *S. aureus* when implanted into a mice model [108].

Polymeric Hydrogels Containing Metal Nanoparticles

Several metal and metal nanoparticles, such as Ag, Au, CuO, Ag_2O, MgO, TiO_2, and ZnO, have been studied for antibacterial activities [47]. Silver nanoparticles (AgNPs) have been most extensively used for potential healthcare applications but they are associated with the induction of apoptosis and necrosis in mammalian cells. Silver is also associated with irreversible discoloration of skin (argyria) [47]. There

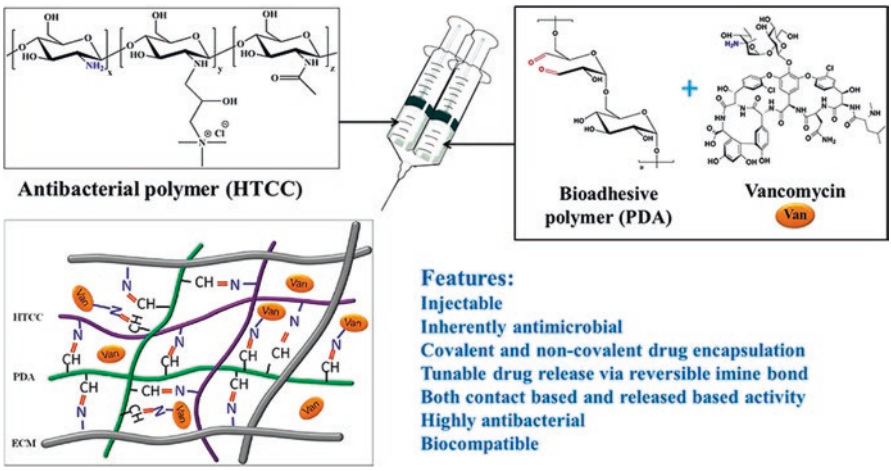


Fig. 7 The structure of hydrogel precursors and vancomycin-loaded hydrogels formed using covalent as well as noncovalent encapsulation [108]. (Reprinted with permission from Hoque J, Bhattacharjee B, Prakash RG, Paramanandham K, Haldar J. Dual Function Injectable Hydrogel for Controlled Release of Antibiotic and Local Antibacterial Therapy. Biomacromolecules. 2018; 19(2): 267–278 (© 2018, American Chemical Society))

are ongoing efforts to reduce the cytotoxicity and side effects of silver and other nanoparticles. Mohan and coworkers studied the combined effect of hydrogels, nanoparticles, and natural compounds to overcome such limitations [109]. AgNP–curcumin composite hydrogels, comprising of poly(acrylamide)-poly(vinyl sulfonic acid sodium salt), were prepared, which showed antibacterial activity against *E. coli* and exhibited sustained release of curcumin [109]. Prokopovich and coworkers [110] studied the encapsulation of silver nanoparticles into the methacrylate hydrogels containing calcium phosphate to develop mineralized biomaterials, which imparted antibacterial activity against *S. epidermidis* and MRSA along with osteoconductive properties for bone graft applications [110].

Parkin and coworkers [111] developed medical grade antibacterial silicone surfaces by incorporating crystal violet and di(octyl)-phosphinic-acid-capped zinc nanoparticles to inhibit bacterial infection against *S. aureus* and *E. coli*, the causative agents in hospital-acquired infections. The antibacterial activity of the silicone increased on illuminating the surface with a standard light source in the hospital [111]. The zwitterionic antifouling hydrogels were prepared by mixing the AgNPs and dopamine methacrylamide (DMA) monomer in an aqueous solution of sodium tetraborate decahydrate [112]. It was protected and solubilized by increasing the pH, and used to induce cross-linking among zwitterionic monomers to form the hydrogel [112]. The hydrogels produced silver nanoparticles upon coming in contact with silver nitrate ($AgNO_3$), without any reducing agent. The hydrogels provided excellent antifouling and antibacterial activities with strong potential in wound healing applications [112]. Silver nanoparticles have been widely used for broad-spectrum antibacterial activities as they liberate silver ions by a slow release mechanism [113].

Leite and coworkers [113] developed carboxymethyl cellulose-based hydrogels containing silver nanoparticles, which showed the formation of a zone of inhibition against *S. aureus* and *P. aeruginosa*. The nanoparticles were encapsulated into hydrogels in situ using two formulations, cashew gum (CG) as a capping agent and sodium borohydride as a reducing agent. The CG with phthalic anhydride (PhCG) hydrogels showed promising wound healing ability in vivo as compared to a natural cashew gum (NCG) formulation, and hydrogel based formulations were tested in the wound healing model of rats with surgical wounds [113]. To prevent the formation of a biofilm on a poly(ether sulfone) (PES) membrane, the antibacterial layer of poly(sulfobetaine methacrylate)/poly(sodium acrylate) were covalently attached to the membrane and loaded with AgNPs (Fig. 8) [114]. Zhao and coworkers [114] created double bonds on the PES membrane to provide an anchoring site for the incorporation of Ag ions, which were reduced to AgNPs after treatment with sodium borohydride. The hydrogels not only resisted bacterial attachment to the surface but also killed *E. coli* and *S. aureus* without eliciting cytotoxicity toward L929 cells. The coated surface showed hemocompatibility as there was no sign of plasma protein adsorption, suppressed platelet adhesion, suppressed blood related complement system, low hemolysis ratio, and prolonged clotting time [114].

Another study reported the use of polyvinyl alcohol/chitosan (PVA/chitosan) hydrogels containing 1 or 3% of lignin nanoparticles, where a 1% lignin-containing hydrogel exhibited enhanced thermal and mechanical properties due to better interactions between the hydrogel and nanoparticles [115]. The presence of lignin

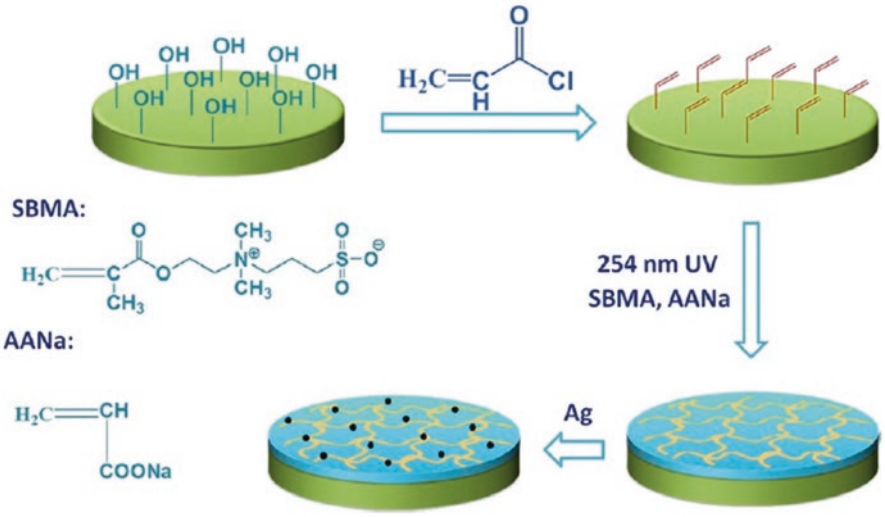


Fig. 8 Attachment of antibacterial hydrogels on a PES membrane using covalent linkages [114]. (Reprinted with permission from He M, Wang Q, Wang R, Xie Y, Zhao W, Zhao C. Design of Antibacterial Poly(ether sulfone) Membranes via Covalently Attaching Hydrogel Thin Layers Loaded with Ag Nanoparticles. ACS Applied Materials & Interfaces. 2017; 9(19): 15962–15974 (© 2017, American Chemical Society))

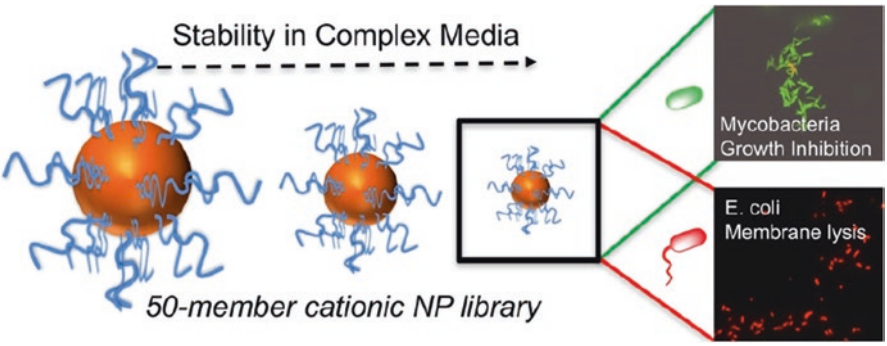


Fig. 9 The mechanism of antibacterial activity exhibited by a multivalent cationic polymer against *E. coli* and bacteriostatic activity against *Mycobacterium smegmatis* [117]. (Reprinted with permission from Richards S-J, Isufi K, Wilkins LE, Lipecki J, Fullam E, Gibson MI. Multivalent Antimicrobial Polymer Nanoparticles Target Mycobacteria and Gram-Negative Bacteria by Distinct Mechanisms. Biomacromolecules. 2018; 19(1): 256–264 (© 2018, American Chemical Society))

nanoparticles along with the chitosan into the hydrogel induced an antioxidative response and showed efficient antibacterial activities against *E. coli* and *S. aureus* [115]. The green nanocomposite hydrogels were made from aminated silver nanoparticles (Ag-NH_2-NPs), gelatin (G), and carboxylated cellulose nanofibers (CNF) [116]. This nanocomposite hydrogel contained 0.5 mg/mL Ag-NH_2 NPs and exhibited good mechanical strength, self-healing ability, and efficient antibacterial and hemostatic characteristics both in vitro and in vivo. The hydrogels showed efficient biocompatibility (100% cell viability) and wound healing efficacy (~90%) even after 14 days. It showed great promise as a dressing for wound healing [116]. Fullam, Gibson, and coworkers [117] studied the effect of multivalent cationic antibacterial polymer at nanoparticle surface on antibacterial activity and their mode of action. Poly(2-dimethylamino)ethyl methacrylate (PDMAEMA) was used as the cationic polymer to form a library of nanoparticles (>50 nm), with diameters varying from 2 to 32 nm [117]. The results depicted that a stable formulation was achieved with 2 nm nanoparticles and promising antibacterial activity against *E. coli* was demonstrated, as compared to the polymer alone. Mechanistic studies revealed that the antibacterial nanoparticles induced the killing of *E. coli* by disrupting the bacterial membrane and caused a bacteriostatic effect against *Mycobacterium smegmatis* (a model of *M. tuberculosis*) (Fig. 9) [117].

Polymeric Hydrogels Containing Antimicrobials

Several polymeric hydrogels have been investigated to deliver antimicrobials so as to eliminate the problem of antibacterial resistance by avoiding the use of antibiotics. Poly(ethylene glycol) cross-linked poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogels containing acryoyl-lysine (A-lys) were developed to improve its swelling and thermoresponsive properties [118]. Polyhexamethylene biguanide (PHMB)

was encapsulated into the polymeric hydrogel as an antimicrobial agent, which resulted in a decreased bacterial count within 2 h [118]. In vivo studies using a rodent cutaneous wound healing model revealed an enhanced healing rate as compared to the control. The polymeric hydrogel containing antibacterial agent could be used as a platform to promote wound healing and deliver the antimicrobial agent to prevent infections [118]. Gilmore and coworkers [119] developed poly(2-hydroxyethyl methacrylate) hydrogels containing AMP, maximin-4 (H-Orn-Orn-TrpTrp-NH_2) and lipopeptide (C_{12}-Orn-Orn-Trp-Trp-NH_2), to prevent the adherence of *S. epidermidis* on the biomaterial. The addition of lipopeptide resulted in decreased adhesion of bacteria to the surface, when tested at 1, 4, and 24 h [119]. Both peptides showed a decrease in bacterial growth by rupturing the bacterial membrane [119].

Gemeinhart and coworkers [120] developed poly(ethylene glycol) diacrylate (PEGDA) hydrogel microspheres, which were attached to the antibacterial peptide, melittin, by glutathione S-transferase. The linkage was cleaved by activating the enzyme thrombin, which released the recombinant protein, melittin, to inhibit the growth of *Streptococcus pyogenes* [120]. A cationic antibacterial hydrogel consisting of poly(ethylene glycol) as a backbone containing poly(hexamethylene guanidine) linked through thiol–ene click chemistry imparted the broad-spectrum antibacterial activities against both gram-positive and negative bacteria [121]. The hydrogel showed outstanding biocompatibility as compared to cationic polymeric hydrogels reported earlier [121].

Natural Antibacterial Polymeric Hydrogels

Studies have been carried out to explore natural polymers for antibacterial applications. Chitosan–dextran hydrogels have been explored for antibacterial activities against *S. aureus*, *Streptococcus pyogenes*, *E. coli*, and *Clostridium perfringens* at a concentration of 50,000 mg/L [122]. Interestingly, the studies confirmed that the antibacterial activities of chitosan-dextran hydrogels were due to the presence of dextran, with minimum bactericidal concentration ranging from 2000 to 32,000 mg/L. It was found that the mechanism of antibacterial activity involved membrane rupture, loss of cytoplasmic content, binding to cell wall proteins, and cleavage of peptide bonds [122]. The hydrogel killed *S. aureus* at a much faster rate than *E. coli*, suggesting that it could potentially be used for preventing gram-positive infections [122]. Chan-Park and coworkers [123] reported dimethyldecylammonium chitosan (with high quaternization)-graft-poly(ethylene glycol) methacrylate and poly(ethylene glycol) diacrylate as excellent antibacterial and antifungal polymeric hydrogels to prevent infection against *P. aeruginosa*, *E. coli*, *S. aureus*, and *Fusarium Solani* [123]. The polycationic antimicrobial hydrogels acted as an anion sponge and exhibited the inhibition by the disruption of the microbial membrane, leading to cell death. Moreover, in vivo studies on rabbit conjunctiva exhibited biocompatibility and no toxicity toward epithelial cells [123]. Chitosan/γ-poly(glutamic acid) complex has been explored for wound healing, where chitosan was used as a

cationic polyelectrolyte and γ-poly(glutamic acid) as an anionic polyelectrolyte [124]. The complex provided optimum moisture and mechanical strength that allowed for the easy removal of dressing from the wound surface, without damaging the new regenerative tissues [124].

Neoh and coworkers [125] used natural polymers as antibacterial agents using thiol–ol chemistry. Agarose (AG) and quaternized chitosan (QCS)-based antibacterial coatings were developed on polymer and metal surfaces using a thiol–ol reaction involving oxidative conjugation between thiol and hydroxy groups [125]. FDA-approved drug, dimercaprol, was used as a surface anchor for cross-linking of polymeric chains. The agarose-coated surface inhibited biofilm formation by *S. aureus* and *P. aeruginosa*, whereas QCS-coated surfaces decreased contamination by bacteria by >95% [125]. Amino acid-based cationic polymers were found to be effective against inhibiting both gram-positive (*B. subtilis*) and negative bacteria (*E. coli*) [126]. The antibacterial polymer induced a change in the morphology of *E. coli* from a rod to spherical shape (Fig. 10), whereas *B. subtilis* remained intact with stacks of cells formed after treatment [126].

Leucine-based antibacterial cationic polymers with different shapes (linear, hyperbranched, and star) containing both hydrophilic and hydrophobic components have been used against *E. coli* [127]. The highest activity was observed in hyperbranched and star architectures because of the prevalence of higher cationic and hydrophobic segments, which enhanced the penetration of cationic polymers into the bacterial membrane (Fig. 11). The switching of morphology from a rod shape to sphere and lengthening due to stacking was also observed in case of *E. coli*, after the treatment with the cationic polymer [127].

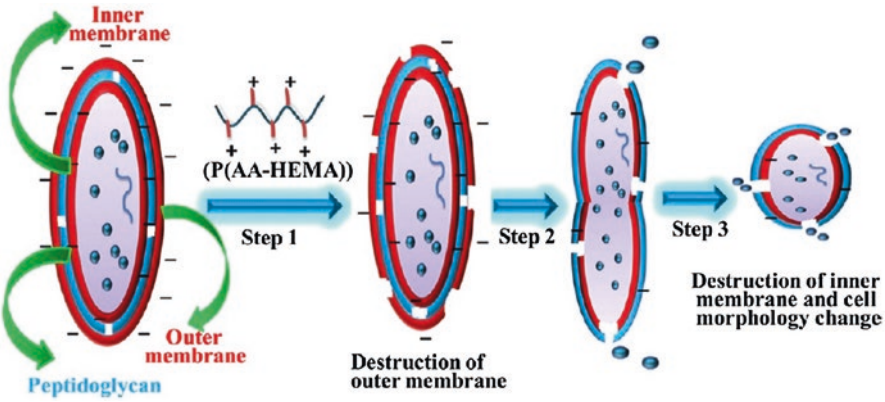


Fig. 10 Mechanism of disruption of the outer membrane of gram-negative bacteria after treatment with an antibacterial cationic polymer [126]. (Reprinted with permission from Mukherjee I, Ghosh A, Bhadury P, De P. Side-Chain Amino Acid-Based Cationic Antibacterial Polymers: Investigating the Morphological Switching of a Polymer-Treated Bacterial Cell. ACS Omega. 2017; 2(4): 1633–1644 (© 2017, American Chemical Society))

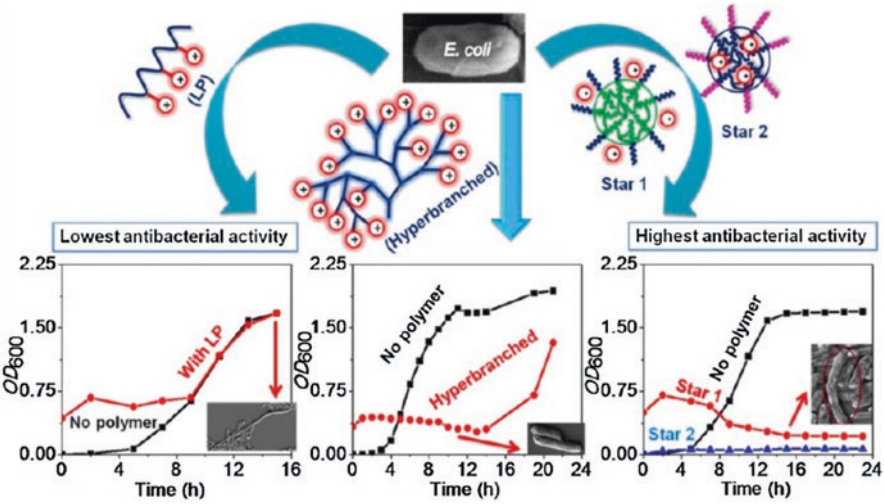


Fig. 11 The antibacterial activities of linear, hyperbranched, and star-shaped cationic polymers against *E. coli* [127]. (Reprinted with permission from Mukherjee I, Ghosh A, Bhadury P, De P. Leucine-Based Polymer Architecture-Induced Antimicrobial Properties and Bacterial Cell Morphology Switching. ACS Omega. 2018; 3(1): 769–780 (© 2018, American Chemical Society))

Synthetic Antibacterial Polymeric Hydrogels

Synthetic hydrogels fabricated from antibacterial polymers have been developed to prevent bacterial resistance. These hydrogels possess inherent antibacterial activities, which could be used to prevent biomaterial-related infections in various biomedical applications. Yang and coworkers [128] developed non-fouling synthetic antimicrobial polymeric hydrogels, which could be used as a coating for catheters and wound dressings, to prevent infections. The hydrogels were fabricated using PEG employing Michael addition chemistry and contained an antimicrobial cationic block polymer of PEG and a polycarbonate with quaternary ammonium groups (APC) [128]. They exhibited 99.9% killing efficiency against clinical isolates of multidrug resistant gram-positive and -negative bacteria (*S. aureus* and *E. coli*) along with fungus (*C. albicans*). The hydrogels did not show any toxic hemolytic activity and demonstrated biocompatibility in an animal model [128].

Hedrick and coworkers [129] developed stimuli-responsive antibacterial hydrogels from biodegradable poly(L-lactide)-*b*-poly(ethylene glycol)-*b*-poly(lactide)/(PLLA-PEG-PLLA) and charged polycarbonate triblock polymers (PDLA-CPC-PDLA) [129]. These stereo complexes existed as a solution at room temperature but upon heating to 37 °C underwent a sol-to-gel transformation. The hydrogels exhibited antibacterial activity against both gram-positive (*S. aureus*) and negative bacteria (*E. coli*) and could potentially be used to prevent biofilm formation on implants [129]. Another study by Cheng and coworkers [130] developed switchable antibacterial and antifouling hydrogels from zwitterionic materials poly(2-((2-hydroxyethyl)

(2-(methacryloyloxy) ethyl) (methyl) ammonio) acetate (pCBOH1) and poly(2-(bis(2-hydroxyethyl) (2-(methacryloyloxy)ethyl) ammonio) acetate (pCBOH2) [130]. This material remained in zwitterionic form under basic/neutral condition but transformed into cationic hydrogel at acidic conditions, which exhibited antibacterial activity. They killed the bacteria (*E. coli* K12) on contact with the charged surface and killed bacterial cells could be released on changing the pH to neutral/basic [130]. Injectable and biodegradable hydrogels were developed from poly(hexamethylene guanidine) hydrochloride and poly(ethylene glycol) as a backbone through a thiol–ene click reaction and they exhibited excellent antibacterial activities against gram-positive (*S. aureus*) and gram-negative (*E. coli*) strains under physiological conditions [121]. Hemolytic experiments revealed outstanding biocompatibility properties as compared to other antibacterial cationic hydrogels [121].

Thus, polymeric hydrogels exhibit potential against biomaterial-related infections and are not cytotoxic but they do not support the adhesion of mammalian cells, which is very much needed for tissue engineering applications. The first defensive antibacterial coating, DAC® HA-g-PLA, composed of covalently linked hyaluronan and poly-D,L-lactide (DAC®, Novagenit SRL, Mezzolombardo, Italy), cleared clinical trials in Europe. It provides protection to implanted biomaterials used in orthopedics, dentistry, traumatology, and maxillofacial surgery from bacterial colonization [131].

Self-Assembled Peptide Gels/Hydrogels

Molecular self-assembly of peptide is a spontaneous process, which leads to the formation of ordered nanostructures and these nanostructures show extensive potential in biomedical applications [132]. Self-assembled peptide gels/hydrogels offer biocompatibility, high drug loading capability, chemical diversity, and an ability to target a molecular recognition site. Hence, such self-assembled nanostructures can be exploited for drug delivery and tissue engineering applications [132]. The first self-assembled peptide hydrogel was serendipitously discovered by Zhang et al. at MIT in 1989, while working on a yeast protein, Zuotin [133], which has the ability to bind to the left-handed Z-DNA in the presence of salmon DNA. They found that this protein contains a 16-residue repetitive sequence motif, AEAEAKAKAEAEAKAK (EAK 16-II), which self-assembled into ordered nanostructures [134]. The 16-residue peptide sequence was synthesized to analyze its structural and biological functions. Out of curiosity, the team developed four ionic peptide sequences, RADA 16-I, RADA 16-II, EAK I, and EAK 16-II, possessing a positive and negative charge on one side and hydrophobic side chain on the other side, thus containing both hydrophilic and hydrophobic regions [135, 136]. These peptide sequences formed a β-sheet and self-assembled in water to form nanoscaffolds. It was also found that the nanofiber held a large amount of water and could be used to entrap cells in the scaffold to support mammalian cell attachment [135].

Grodzinsky and coworkers [137] developed self-assembled peptide hydrogels, Ac-KLDLKLDLKLDL-NH_2 (KLD-12), for cartilage repair by encapsulating chondrocytes into the hydrogel. The hydrogels seeded with the chondrocytes retained their morphology and the development of cartilage-like ECM enriched in type-II collagen and proteoglycans, representing stable chondrocytes, was observed [137]. The time-dependent studies revealed that the accumulation of ECM led to an increase in material stiffness, which then led to the deposition of neo-tissues. Overall, the studies indicated that the self-assembled peptide gel could be used as a 3D scaffold for cartilage tissue repair [137]. Schneider and coworkers [138] developed self-assembled β-hairpin peptide hydrogels (Fig. 12), VKVKVKVKV^{D}PPTKVKVKVKV-NH_2 (MAX-1), containing 20 residues of alternating lysine and valine, and connected by a tetrapeptide sequence (-V^{D}PPT-). The hydrogels were investigated for broad-spectrum antibacterial activities against strains implicated in hospital acquired infections, like *S. epidermidis*, *S. aureus*, *S. pyogenes*, *K. Pneumonia*, and *E. coli*, with concentrations ranging from 2×10^3 to 2×10^9 cfu/dm^2 [138]. The mechanistic studies were done using β-galactosidase and revealed that the hydrogel disrupted the *E. coli* outer and inner membrane, causing cell death [138]. The cytotoxicity studies revealed that the gel surface was not toxic toward NIH3T3 cells even though it caused the lysis of bacterial cells, thus indicating selectivity for bacterial cells. Overall, this study raised the hope of using self-assembled peptide hydrogels against bacterial infections, without eliciting significant mammalian cytotoxicity [138].

Schneider et al. developed another self-assembled β-hairpin peptide hydrogel, VKVKVRVKV^{D}PPTKVKVRVKV-NH_2 (MARG1), by changing the two arginine residues at positions 6 and 17 [139]. They introduced more positively charged amino acids (lysine and arginine) to make it more efficient against bacterial infections. The MARG1 showed an excellent killing mechanism against the MRSA without eliciting significant toxicity to the mammalian cells. The MARG1 peptide hydrogels showed a shear-thinning behavior and, hence, could be easily injected at the target site using a syringe [139]. High arginine containing self-assembled β-hairpin peptide hydrogels were explored for broad-spectrum antibacterial activities against both gram-positive and negative strains, including the multidrug resistance *P. aeruginosa* [140]. The PEP8R peptide hydrogel exhibited excellent

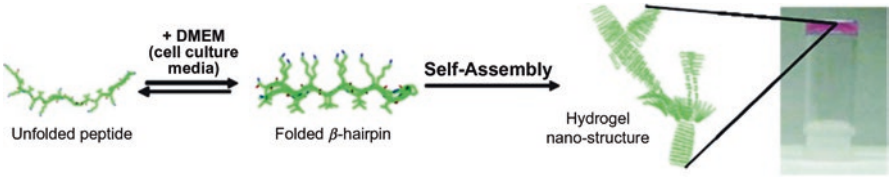


Fig. 12 Formation of self-supporting hydrogels through folding and self-assembly of MAX-1 [138]. (Reprinted with permission from Salick DA, Kretsinger JK, Pochan DJ, Schneider JP. Inherent Antibacterial Activity of a Peptide-Based β-Hairpin Hydrogel. Journal of the American Chemical Society. 2007; 129(47): 14793–14799 (© 2007, American Chemical Society))

antibacterial activity but showed lytic activity toward red blood cells (RBCs) [140]. Other peptides containing less arginine content or having lysine in place of arginine (PEP6R, PEP4R, and PEP2R) were also investigated and demonstrated slightly lower antibacterial activities but significantly lower hemolytic activity [140]. The VKVRVRVRV^{D}PPTRVRVRVKV (PEP6R, 1.5% w/v) hydrogels showed the highest potency against bacteria with minimum cytotoxicity toward human erythrocytes and mammalian cells. Mechanistic studies indicated the disruption of cell membrane via dissociation of metal ions in the bacterial cell wall in contact with the gel surface [140].

Another study reported the use of Epsilon-poly-L-lysine-graft-methacrylamide (EPL-MA) hydrogels as a broad-spectrum antibacterial and antifungal agent against *E. coli*, *P. aeruginosa*, *S. marcescens*, *S. aureus*, *C. albicans*, and *F. solani* [141]. EPL-MA exhibited in vitro biocompatibility and non-hemolytic activity towards human red blood cells (hRBCs) [141]. This study also revealed that the selectivity for the pathogenic microorganism over hRBCs was 230–1560 times, which implied that the hemolysis occurred when the concentration of peptide exceeded 12,500 μg/mL [141]. Zhao and coworkers in another study observed that the balance between hydrophobic and electrostatic interactions was the main factor determining the formation of secondary structures and individual nanofibers [142]. The study utilized the antibacterial peptide sequence, $KIGAKI_3$-NH_2, with a central tetrapeptide linker. The self-assembly of the peptide gel occurred by balancing the electrostatic repulsion, hydrophobic interactions, and hydrogen bonding, which led to a phase transition and the formation of individually dispersed nanofibers [142]. This hydrogel showed antibacterial activity against *E. coli* and was further explored for drug delivery and tissue engineering applications [142]. The long chain peptide hydrogels discussed above showed excellent antibacterial activities but they were associated with tedious and costly synthetic procedures.

Therefore, the short lysine containing peptide amphiphile, PA-Kn, was fabricated and it demonstrated a pH-sensitive self-assembly, gelation, and antibacterial activity against gram-negative, *E. coli* [143]. The peptide amphiphile exhibited a decrease in self-assembly on increasing lysine content. Hence, sodium alginate was added to improve the rheological properties through electrostatic interactions between the -COOH group of sodium alginate and -NH_2 group of lysine-containing peptide amphiphiles. This antibacterial hybrid peptide hydrogels showed potential in biomedical applications [143]. Das and coworkers [144] developed a Fmoc amino acid/peptide-based amphiphile having a pyridinium moiety at the C-terminal and it showed gelation due to the presence of π–π interactions and intermolecular hydrogen bonding [144]. The pyridinium derived peptide amphiphile showed broad spectrum antibacterial activities against *E. coli*, *P. aeruginosa*, *B. subtilis*, and *S. aureus*; as pyridinium possess bacterial cell penetration capabilities [144]. Another study reported the use of naphthalene protected self-assembled peptide gels for antibacterial applications [145]. The gels did not show cytotoxicity toward murine fibroblasts and exhibited limited hemolysis of hRBCs. The lysine-conjugated variant, NapFFKK, displayed gelation at 2% (w/v) and showed reduction in biofilm formation by *S. epidermidis* (up to 94%) [145]. This study also found that the reduction of chain length by removing the methylene groups in R, decreased the antibacterial activity [145].

Laverty and coworkers [146] developed ultra-short Fmoc-derived peptide gels, Fmoc-FFKK, Fmoc-FFKKK, and Fmoc-FFOO, to eliminate biofilm formation on medical devices/implants [146]. These peptide gels were tested against the strains most prevalent in biomaterial-related infections, like *S. aureus*, *S. epidermidis*, *E. coli*, and *P. aeruginosa*. Most of the Fmoc (0.5–2%) gels showed inhibition of biofilm formation by gram-positive and negative pathogens along with the toxicity towards red blood cells at the majority of concentrations tested [146]. Although the short self-assembled peptide gels showed excellent antibacterial activities, they were prone to degradation by proteolytic enzymes under in vivo conditions, which limited their utility in biomedical applications [147]. To impart the proteolytic stability to ultrashort self-assembled peptide gels discussed above, a long chain fatty acid, such as undecanoic acid, was incorporated into two ultra-sort peptide sequences, Boc-AUDA-Phe-COOH (P1) and Boc-AUDA-Phg-COOH (P2) [147]. Both peptide gels showed improved proteolytic stability against proteinase K and chymotrypsin, biocompatibility towards mammalian cells, and antibacterial activities against gram-negative (*E. coli* and *P. aeruginosa*) strains [147]. Another study employed unnatural β- and γ-amino acid to the short peptides, which exhibited antibacterial activity and proteolytic stability up till 24 h of incubation [148].

Singh and coworkers [149] developed nonnatural α/γ hybrid dipeptides, Boc-D-Phe-γ^4-L-Phe-PEA (NH007) and Boc-L-Phe-γ^4-L-Phe-PEA (NH009), to obtain self-assembled gels with improved proteolytic stability and antibacterial activities [149]. Both peptide gels showed gelation in aqueous dimethyl sulfoxide (DMSO) (3–5% w/v), and exhibited good viscoelastic and self-healing characteristics. The peptide gels also showed excellent proteolytic stability in the mocktail of proteolytic enzymes, proteinase K, pepsin, and chymotrypsin [149]. Moreover, the gels showed broad-spectrum antibacterial activities against the strains implicated in biomaterial-related infections (gram-negative: *P. aeruginosa* and *E. coli*; and gram-positive: *S. aureus* and *B. subtilis*) even at a high inoculum of 10^7–10^8 colony forming units per milliliter (cfu/mL) [149]. The cytotoxicity studies revealed that the gels were not toxic towards NIH 3T3 mouse embryonic fibroblast cells. Mechanistic studies suggested the entrapment of bacterial cells into the gel networks, followed by the interaction with bacterial cell membrane components, causing the cell lysis. These gels could potentially be used for preventing biomaterial-related infections [149].

Another study by Singh and coworkers reported the potential of α/γ hybrid self-assembled peptide/chitosan gels for biomaterial-related infections [150]. The hybrid peptide/polymer gels showed broad-spectrum antibacterial activities against the strains associated with biomaterial infections, like *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. subtilis*. It was also observed that the complexation of chitosan not only enhanced the antibacterial activities but also helped in sustaining it for longer durations. Ghosh and coworkers [151] developed biocompatible lipopeptide hydrogels, which showed inhibition of *E. coli* and *S. aureus*, by inducing cell lysis and necrotic cell death. The construct was composed of a short peptide, NH_2-NAVSIQKKK-$CONH_2$, with a hydrophobic long chain at the *N*-terminal and hydrophilic triple lysine unit at the C-terminal. This amphiphilic hydrogel exhibited proteolytic stability against proteinase K and was not cytotoxic towards WI-38 (normal lung cell line) and HeLa (cancer cell line) cells [151]. Table 4 provides the representative examples of self-assembled peptide gels for antibacterial applications.

Table 4 Self-assembled peptide gels for antibacterial applications

Self-assembled peptide gels	Target	Remarks	References
VKVKVKVK^{D}PPTKVKVKVKV-NH_2 (MAX-1)	*S. epidermidis*, *S. aureus*, *S. pyogenes*, *K. Pneumonia*, and *E. coli*	Disrupts the outer and inner membrane, causing cell death	[138]
VKVKVRVKV^{D}PPTKVKVRVKV-NH_2 (MARG1)	MRSA	Kills on contact by a mechanism not known	[139]
VKVRVRVRV^{D}PPTRVRVRVKV (PEP6R)	Both gram-positive and -negative strains, MDR *P. aeruginosa*	Disrupts the membrane on contact	[140]
$KIGAKI_3$-NH_2	*E. coli*	Mechanism not known	[142]
NapFFKK	*S. epidermidis*	Inhibits biofilm by a mechanism not known	[145]
Fmoc-FFKK, Fmoc-FFKKK, and Fmoc-FFOO	*S. aureus*, *S. epidermidis*, *E. coli*, and *P. aeruginosa*	Inhibits biofilm by a mechanism not known	[146]
Boc-AUDA-Phe-COOH (P1) and Boc-AUDA-Phg-COOH (P2)	*E. coli*, *P. aeruginosa*	Mechanism not known	[147]
Boc-D-Phe-γ^4-L-Phe-PEA (NH007) and Boc-L-Phe-γ^4-L-Phe-PEA (NH009)	*P. aeruginosa*, *E. coli*; *S. aureus* and *B. subtilis*	Disrupts membrane components, causing cell lysis	[149]
NH_2-NAVSIQKKK-$CONH_2$	*E. coli* and *S. aureus*	Induces cell lysis and necrotic cell death	[151]

Overall, the ultra-short self-assembled peptide gels possess enormous potential against bacterial infections but they are still associated with problems, like proteolytic instability [147], poor mechanical strength, and cytotoxicity towards mammalian cells [146]. The exact mechanism of antibacterial activity exhibited by the peptide gels is not fully understood yet [146]. Thakur and coworkers [152] investigated the antibacterial activity of Fmoc-F entrapped in hydrogels and in solution phase against gram-positive bacteria, including the clinical isolates of MRSA and *Mycobacteria* [152]. The small molecular hydrogelator not only inhibited the bacteria in vitro but also reduced skin wound infections in mice [152]. The antibacterial activity was due to the release of Fmoc-F from hydrogels, which induced oxidative and osmotic stress. The stress led to a change in the permeability and integrity of bacterial membrane that induced cell death (Fig. 13) [152].

A comparison of different antibacterial approaches discussed in this chapter along with their advantages and limitations is provides in Table 5.

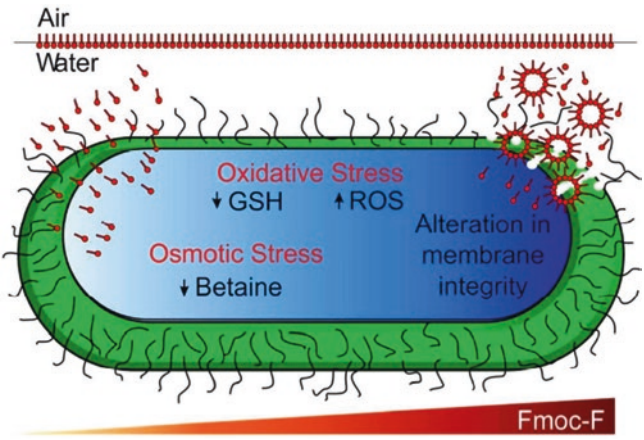


Fig. 13 Plausible mechanism of antibacterial activity exhibited by self-assembled Fmoc-F gels against gram-positive bacteria [152]. (Reprinted with permission from Gahane AY, Ranjan P, Singh V, et al. Fmoc-phenylalanine Displays Antibacterial Activity Against Gram-positive Bacteria in Gel and Solution Phases. Soft Matter. 2018; 14(12): 2234–2244 (© 2018, Royal Society of Chemistry, Great Britain))

Table 5 Overview of different antibacterial approaches

Approaches	Advantages	Limitations	References
Antibiotics	Reduces infectious diseases by 20-fold	Repeated use leads to the development of antibiotic resistance in bacteria, cytotoxic to mammalian cells at higher concentrations	[23]
Antiseptics	Broad spectrum activity, lower cytotoxicity towards mammalian cells	Bacteria develops tolerance on repeated exposure	[28, 30]
Antiadhesives	Prevents bacterial adhesion	Lack of data on long term safety to mammalian cells and comparative studies	[32, 153]
Metal ions and nanoparticles	Fast broad-spectrum activities at lower concentration, active against drug-resistant strains	Cytotoxic to mammalian cells and development of resistant strains reported in some cases	[39, 55]
Carbon nanotubes	Excellent broad-spectrum activities against drug-resistant strains	Cytotoxic to mammalian cells at lower concentrations	[59, 60, 64]
Antimicrobial peptides	Broad-spectrum antibacterial, active against MDR bacterial strains	Cytotoxic to mammalian cells, proteolytically unstable, expensive	[70, 154]
Antimicrobial polymers	Broad-spectrum antibacterial, active against resistant bacterial strains	Exact mechanism of action not known, variable cytotoxicity to mammalian cells	[88, 94, 154]
Antibacterial polymeric hydrogels	Inherent antibacterial activity, active against MDR bacterial strains, biocompatible	Lack of control over backbone structure, does not support the growth of mammalian cells	[121, 128, 154]
Antibacterial peptide hydrogels	Inherent broad-spectrum activities, biocompatible	Expensive, tedious synthetic procedures, proteolytic instability, mechanism not fully understood	[138, 146, 149, 155]

Conclusions

In summary, the major aim of this chapter was to critically evaluate the potential of self-assembled peptides and polymeric hydrogels/gels in treating biomaterial-related infections. Infections caused by pathogenic bacteria are the leading cause of deaths worldwide. Similarly, biomaterial-related infections resulting from the implantation of medical devices, like artificial stents, catheters, prosthetic joints, artificial hearts, vascular prosthetics, and orthopedic and dental implants, are equally challenging. Antibiotics have been effectively used to treat such infections. However, the repeated use and/or misuse of antibiotics has led to the problem of antibiotic resistance, which is a major challenge in the field. Scientists around the world have employed different strategies, like antiseptics, antiadhesives, metal ions and nanoparticles, carbon nanotubes, graphene and graphene oxide, AMPs, and antimicrobial polymers, to counter the problem of antibiotic-resistance in bacteria, with varying degrees of success. Unfortunately, each of these strategies suffer from one or another problem that limits their use in biomedical applications. In this context, polymeric and self-assembled peptide hydrogels/gels have generated tremendous interest for treating bacterial infections, in particular, biomaterial-related infections. The cationic/hydrophobic polymeric hydrogels/gels demonstrate excellent antibacterial activities, without eliciting significant cytotoxicity and hemolytic responses but these biomaterials do not support the adhesion of mammalian cells to the surface. Self-assembled peptide hydrogels/gels exhibit excellent antibacterial activities and also provide extracellular mimicking scaffolds for the adhesion and growth of mammalian cells but suffer from poor proteolytic stability and mechanical strength along with the hemolytic activity at higher concentrations. There is also a need to fully understand the mechanism of antibacterial activity of these hydrogels/gels. Ideally, the biomaterials for treating biomaterial-related infections must prevent bacterial infections on the implant surface and provide ECM-mimicking 3D environment to support the growth and proliferation of mammalian cells. The hybrid peptide/polymeric hydrogels/gels could be the way forward to tackle this problem.

Acknowledgements We gratefully acknowledge our students (PhD, MSc, and MTech) and colleagues who contributed to this work and the financial support to YS from the **CSIR, New Delhi** (grant # 02(0245)/15/EMR-II) and **SERB, New Delhi** (grant # EMR/2017/000045).

References

1. (2008) The global burden of disease: 2004 update. World Health Organization, Geneva, Switzerland
2. Laxminarayan R, Chaudhury RR (2016) Antibiotic resistance in India: drivers and opportunities for action. PLoS Med 13(3):e1001974
3. Kumar SG, Adithan C, Harish B, Roy G, Malini A, Sujatha S (2013) Antimicrobial resistance in India: a review. J Nat Sci Biol Med 4(2):286
4. Ventola CL (2015) The antibiotic resistance crisis. P&T 40(4):277–283

5. Gupta A, Mumtaz S, Li C-H, Hussain I, Rotello VM (2019) Combatting antibiotic-resistant bacteria using nanomaterials. Chem Soc Rev 48(2):415–427
6. Cooper IR (2015) Introduction to biomaterials and medical device-associated infections. In: Barnes L, Cooper IR (Eds) Biomaterials and Medical Device – Associated Infections. pp 3–17
7. Busscher HJ, van der Mei HC, Subbiahdoss G, Jutte PC, van den Dungen JJ, Zaat SA et al (2012) Biomaterial-associated infection: locating the finish line in the race for the surface. Sci Transl Med 4:153rv10
8. Wolfmeier H, Pletzer D, Mansour SC, Hancock REW (2018) New perspectives in biofilm eradication. ACS Infect Dis 4:93–106
9. Flemming HC, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8:623–633
10. Stewart PS, Franklin MJ (2008) Physiological heterogeneity in biofilms. Nat Rev Microbiol 6:199
11. Roilides E, Walsh TJ, Simitsopoulou M, Katragkou A (2015) How biofilms evade host defenses. In: Mukherjee PK, Ghannoum M, Whiteley M, Parsek M (eds) Microbial biofilms, 2nd edn. American Society of Microbiology, pp 287–300
12. Boucher HW, Talbot GH, Bradley JS et al (2009) Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 48(1):1–12
13. Paramythiotou E, Routsi C (2016) Association between infections caused by multidrug-resistant gram-negative bacteria and mortality in critically ill patients. World J Crit Care Med 5(2):111
14. Ciofu O, Rojo-Molinero E, Macià MD, Oliver A (2017) Antibiotic treatment of biofilm infections. APMIS 125(4):304–319
15. Deresinski S (2009) Vancomycin in combination with other antibiotics for the treatment of serious methicillin-resistant Staphylococcus aureus infections. Clin Infect Dis 49(7):1072–1079
16. Gaynes R (2017) The discovery of Penicillin—new insights after more than 75 years of clinical use. Emerg Infect Dis 23(5):849–853
17. Kapoor G, Saigal S, Elongavan A (2017) Action and resistance mechanisms of antibiotics: a guide for clinicians. J Anaesthesiol Clin Pharmacol 33(3):300
18. Petchiappan A, Chatterji D (2017) Antibiotic resistance: current perspectives. ACS Omega 2(10):7400–7409
19. Elbossaty WF (2017) Antibiotic drugs and multidrug resistance bacteria. Int J Pub Health Safe 2(3):3
20. Tangcharoensathien V, Sattayawutthipong W, Kanjanapimai S et al. (2017) Antimicrobial resistance: from global agenda to national strategic plan. Thailand Bull World Health Organ 95(8):599–603
21. Magiorakos A-P, Srinivasan A, Carey RB et al (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18(3):268–281
22. Fleming A (1929) On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzae. Br J Exp Pathol 10(3):226–236
23. Walsh W (2005) Introduction: antibiotic resistance. Chem Rev 105(2):391–394
24. Gimeno M, Pinczowski P, Pérez M et al (2015) A controlled antibiotic release system to prevent orthopedic-implant associated infections: an *in vitro* study. Eur J Pharm Biopharm 96:264–271
25. Albright V, Zhuk I, Wang Y et al (2017) Self-defensive antibiotic-loaded layer-by-layer coatings: imaging of localized bacterial acidification and pH-triggering of antibiotic release. Acta Biomater 61:66–74
26. (2007) Premarket notification [510(k)] submissions for medical devices that include antimicrobial agents—draft guidance for industry and FDA staff. 510:1–18
27. Science History Institute. (2017). Gerhard Domagk. https://www.sciencehistory.org/historical-profile/gerhard-domagk

28. Mcdonnell G, Russell AD (1999) Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 12:33
29. Iqbal A (2012) Antimicrobial irrigants in the endodontic therapy. Int J Health Sci 6(2):1–7
30. Gadea R, Glibota N, Pérez Pulido R, Gálvez A, Ortega E (2017) Adaptation to biocides cetrimide and chlorhexidine in Bacteria from organic foods: association with tolerance to other antimicrobials and physical stresses. J Agric Food Chem 65(8):1758–1770
31. Ong YL, Razatos A, Georgiou G, Sharma MM (1999) Adhesion forces between *E. coli* Bacteria and biomaterial surfaces. Langmuir 15(8):2719–2725
32. Thebault P, Jouenne T (2015) The battle against microbial pathogens: basic science, technological advances and educational programs. ISBN-13 Vol. 1: 978-84-942134-6-5
33. Dong B, Manolache S, Wong ACL, Denes FS (2011) Antifouling ability of polyethylene glycol of different molecular weights grafted onto polyester surfaces by cold plasma. Polym Bull 66(4):517–528
34. Rendueles O, Kaplan JB, Ghigo J-M (2013) Antibiofilm polysaccharides. Environ Microbiol 15(2):334–346
35. Muszanska AK, Rochford ETJ, Gruszka A et al (2014) Antiadhesive polymer brush coating functionalized with antimicrobial and RGD peptides to reduce biofilm formation and enhance tissue integration. Biomacromolecules 15(6):2019–2026
36. Jiang P, Li J, Han F, Duan G, Lu X, Gu Y et al (2011) Antibiofilm activity of an exopolysaccharide from marine bacterium Vibrio sp. QY101. PLoS One 6:e18514
37. Rendueles O, Travier L, Latour-Lambert P, Fontaine T, Magnus J, Denamur E et al (2011) Screening of Escherichia coli species biodiversity reveals new biofilm-associated antiadhesion polysaccharides. mBio 2:e00043–e00011
38. Qin Z, Yang L, Qu D, Molin S, Tolker-Nielsen T (2009) Pseudomonas aeruginosa extracellular products inhibit staphylococcal growth, and disrupt established biofilms produced by Staphylococcus epidermidis. Microbiology 155:2148–2156
39. Chernousova S, Epple M (2013) Silver as antibacterial agent: ion, nanoparticle, and metal. Angew Chem Int Ed 52(6):1636–1653
40. Rupp ME, Fitzgerald T, Marion N et al (2004) Effect of silver-coated urinary catheters: efficacy, cost-effectiveness, and antimicrobial resistance. Am J Infect Control 32(8):445–450
41. Rigo C, Ferroni L, Tocco I et al (2013) Active silver nanoparticles for wound healing. Int J Mol Sci 14(3):4817–4840
42. Stevens KNJ, Croes S, Boersma RS et al (2011) Hydrophilic surface coatings with embedded biocidal silver nanoparticles and sodium heparin for central venous catheters. Biomaterials 32(5):1264–1269
43. Prabhu S, Poulose EK (2012) Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int Nano Lett 2(1):32
44. Matsumura Y, Yoshikata K, Kunisaki S-I, Tsuchido T (2003) Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. Appl Environ Microbiol 69(7):4278–4281
45. Panáček A, Kvítek L, Smékalová M et al (2018) Bacterial resistance to silver nanoparticles and how to overcome it. Nat Nanotechnol 13(1):65–71
46. Greulich C, Braun D, Peetsch A et al (2012) The toxic effect of silver ions and silver nanoparticles towards bacteria and human cells occurs in the same concentration range. RSC Adv 2(17):6981
47. Dizaj SM, Lotfipour F, Barzegar-Jalali M, Zarrintan MH, Adibkia K (2014) Antimicrobial activity of the metals and metal oxide nanoparticles. Mater Sci Eng C 44:278–284
48. Ballo MKS, Rtimi S, Pulgarin C et al (2016) In vitro and in vivo effectiveness of an innovative silver-copper nanoparticle coating of catheters to prevent methicillin-resistant Staphylococcus aureus infection. Antimicrob Agents Chemother 60(9):5349–5356
49. Liu R, Memarzadeh K, Chang B et al (2016) Antibacterial effect of copper-bearing titanium alloy (Ti-Cu) against *Streptococcus mutans* and *Porphyromonas gingivalis*. Sci Rep 6(1):29985

50. Chatterjee AK, Chakraborty R, Basu T (2014) Mechanism of antibacterial activity of copper nanoparticles. Nanotechnology 25(13):135101
51. Casey AL, Adams D, Karpanen TJ et al (2010) Role of copper in reducing hospital environment contamination. J Hosp Infect 74(1):72–77
52. Guo J, Gao S-H, Lu J, Bond PL, Verstraete W, Yuan Z (2017) Copper oxide nanoparticles induce lysogenic bacteriophage and metal-resistance genes in Pseudomonas aeruginosa PAO1. ACS Appl Mater Interfaces 9(27):22298–22307
53. Pati R, Mehta RK, Mohanty S et al (2014) Topical application of zinc oxide nanoparticles reduces bacterial skin infection in mice and exhibits antibacterial activity by inducing oxidative stress response and cell membrane disintegration in macrophages. Nanomedicine 10(6):1195–1208
54. Xie Y, He Y, Irwin PL, Jin T, Shi X (2011) Antibacterial activity and mechanism of action of zinc oxide nanoparticles against campylobacter jejuni. Appl Environ Microbiol 77(7):2325–2331
55. Jeng HA, Swanson J (2006) Toxicity of metal oxide nanoparticles in mammalian cells. J Environ Sci Health A 41(12):2699–2711
56. Siddiqi KS, Ur Rahman A, Tajuddin, Husen A (2018) Properties of zinc oxide nanoparticles and their activity against microbes. Nanoscale Res Lett 13:141
57. Li N, Su X, Lu Y (2015) Nanomaterial-based biosensors using dual transducing elements for solution phase detection. Analyst 140(9):2916–2943
58. Kang S, Pinault M, Pfefferle LD, Elimelech M (2007) Single-walled carbon nanotubes exhibit strong antimicrobial activity. Langmuir 23(17):8670–8673
59. Hirschfeld J, Akinoglu EM, Wirtz DC et al (2017) Long-term release of antibiotics by carbon nanotube-coated titanium alloy surfaces diminish biofilm formation by *Staphylococcus epidermidis*. Nanomedicine 13(4):1587–1593
60. Zardini HZ, Amiri A, Shanbedi M, Maghrebi M, Baniadam M (2012) Enhanced antibacterial activity of amino acids-functionalized multi walled carbon nanotubes by a simple method. Colloids Surf B: Biointerfaces 92:196–202
61. Liu S, Wei L, Hao L et al (2009) Sharper and faster "nano darts" kill more bacteria: a study of antibacterial activity of individually dispersed pristine single-walled carbon nanotube. ACS Nano 3(12):3891–3902
62. Yang F, Jiang Q, Xie W, Zhang Y (2017) Effects of multi-walled carbon nanotubes with various diameters on bacterial cellular membranes: cytotoxicity and adaptive mechanisms. Chemosphere 185:162–170
63. Kang S, Herzberg M, Rodrigues DF, Elimelech M (2008) Antibacterial effects of carbon nanotubes: size does matter! Langmuir 24(13):6409–6413
64. Jia G, Wang H, Yan L et al (2005) Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene. Environ Sci Technol 39(5):1378–1383
65. Hu W, Peng C, Luo W et al (2010) Graphene-based antibacterial paper. ACS Nano 4(7):4317–4323
66. Li K, Wang C, Yan J et al (2018) Evaluation of the osteogenesis and osseointegration of titanium alloys coated with graphene: an *in vivo* study. Sci Rep 8(1):1843
67. Yadav N, Dubey A, Shukla S et al (2017) Graphene oxide-coated surface: inhibition of bacterial biofilm formation due to specific surface–interface interactions. ACS Omega 2(7):3070–3082
68. Akhavan O, Ghaderi E, Akhavan A (2012) Size-dependent genotoxicity of graphene nanoplatelets in human stem cells. Biomaterials 33(32):8017–8025
69. Pelin M, Fusco L, León V et al (2017) Differential cytotoxic effects of graphene and graphene oxide on skin keratinocytes. Sci Rep 7:40572
70. Hancock REW, Sahl H-G (2006) Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol 24(12):1551–1557
71. Mahlapuu M, Håkansson J, Ringstad L, Björn C (2016) Antimicrobial peptides: an emerging category of therapeutic agents. Front Cell Infect Microbiol 6:194

72. Dean SN, Bishop BM, van Hoek ML (2011) Natural and synthetic cathelicidin peptides with anti-microbial and anti-biofilm activity against Staphylococcus aureus. BMC Microbiol 11(1):114
73. Yoshinari M, Kato T, Matsuzaka K, Hayakawa T, Shiba K (2010) Prevention of biofilm formation on titanium surfaces modified with conjugated molecules comprised of antimicrobial and titanium-binding peptides. Biofouling 26(1):103–110
74. Gopal R, Kim YG, Lee JH et al (2014) Synergistic effects and antibiofilm properties of chimeric peptides against multidrug-resistant *Acinetobacter baumannii* strains. Antimicrob Agents Chemother 58(3):1622–1629
75. Lombana A, Raja Z, Casale S et al (2014) Temporin-SHa peptides grafted on gold surfaces display antibacterial activity. J Pept Sci 20(7):563–569
76. Godoy-Gallardo M, Mas-Moruno C, Fernández-Calderón MC et al (2014) Covalent immobilization of hLf1-11 peptide on a titanium surface reduces bacterial adhesion and biofilm formation. Acta Biomater 10(8):3522–3534
77. Glinel K, Jonas AM, Jouenne T, Leprince J, Galas L, Huck WTS (2009) Antibacterial and antifouling polymer brushes incorporating antimicrobial peptide. Bioconjug Chem 20(1):71–77
78. Etienne O, Picart C, Taddei C et al (2004) Multilayer polyelectrolyte films functionalized by insertion of defensin: a new approach to protection of implants from bacterial colonization. Antimicrob Agents Chemother 48(10):3662–3669
79. Guyomard A, Dé E, Jouenne T, Malandain J-J, Muller G, Glinel K (2008) Incorporation of a hydrophobic antibacterial peptide into amphiphilic polyelectrolyte multilayers: a bioinspired approach to prepare biocidal thin coatings. Adv Funct Mater 18(5):758–765
80. Shukla A, Fleming KE, Chuang HF et al (2010) Controlling the release of peptide antimicrobial agents from surfaces. Biomaterials 31(8):2348–2357
81. Andrade CA (2016) Chemical immobilization of antimicrobial peptides on biomaterial surfaces. Front Biosci 8(1):129–142
82. Gao G, Lange D, Hilpert K et al (2011) The biocompatibility and biofilm resistance of implant coatings based on hydrophilic polymer brushes conjugated with antimicrobial peptides. Biomaterials 32(16):3899–3909
83. Humblot V, Yala J-F, Thebault P et al (2009) The antibacterial activity of Magainin I immobilized onto mixed thiols self-assembled monolayers. Biomaterials 30(21):3503–3512
84. Yala J-F, Thebault P, Héquet A, Humblot V, Pradier C-M, Berjeaud J-M (2011) Elaboration of antibiofilm materials by chemical grafting of an antimicrobial peptide. Appl Microbiol Biotechnol 89(3):623–634
85. Hilpert K, Elliott M, Jenssen H et al (2009) Screening and characterization of surface-tethered cationic peptides for antimicrobial activity. Chem Biol 16(1):58–69
86. Onaizi SA, Leong SSJ (2011) Tethering antimicrobial peptides: current status and potential challenges. Biotechnol Adv 29(1):67–74
87. Pal K, Banthia AK, Majumdar DK (2009) Polymeric hydrogels: characterization and biomedical applications. Des Monomers Polym 12(3):197–220
88. Siedenbiedel F, Tiller JC (2012) Antimicrobial polymers in solution and on surfaces: overview and functional principles. Polymers 4(1):46–71
89. Cornell RJ, Donaruma LG (1965) 2-Methacryloxytropones. Intermediates for the synthesis of biologically active polymers. J Med Chem 8(3):388–390
90. Panarin EF, Solovskii MV, Ekzemplyarov ON (1971) Synthesis and antimicrobial properties of polymers containing quaternary ammonium groups. Pharm Chem J 5(7):406–408
91. Kuroda K (2005) Amphiphilic polymethacrylate derivatives as antimicrobial agents. J Am Chem Soc 125(12):4128–4129
92. Kuroki A, Sangwan P, Qu Y et al (2017) Sequence control as a powerful tool for improving the selectivity of antimicrobial polymers. ACS Appl Mater Interfaces 9(46):40117–40126
93. Lin J, Chen X, Chen C et al (2018) Durably antibacterial and bacterially antiadhesive cotton fabrics coated by cationic fluorinated polymers. ACS Appl Mater Interfaces 10(7):6124–6136
94. Huang K-S, Yang C-H, Huang S-L, Chen C-Y, Lu Y-Y, Lin Y-S (2016) Recent advances in antimicrobial polymers: a mini-review. Int J Mol Sci 17(9):1578

95. Hoffman AS (2012) Hydrogels for biomedical applications. Adv Drug Deliv Rev 64:18–23
96. Ng VWL, Chan JMW, Sardon H et al (2014) Antimicrobial hydrogels: a new weapon in the arsenal against multidrug-resistant infections. Adv Drug Deliv Rev 78:46–62
97. Eslahi N, Abdorahim M, Simchi A (2016) Smart polymeric hydrogels for cartilage tissue engineering: a review on the chemistry and biological functions. Biomacromolecules 17(11):3441–3463
98. De Giglio E, Cometa S, Ricci MA et al (2011) Ciprofloxacin-modified electrosynthesized hydrogel coatings to prevent titanium-implant-associated infections. Acta Biomater 7(2):882–891
99. Lakes AL, Peyyala R, Ebersole JL, Puleo DA, Hilt JZ, Dziubla TD (2014) Synthesis and characterization of an antibacterial hydrogel containing covalently bound vancomycin. Biomacromolecules 15(8):3009–3018
100. Wu F, Meng G, He J, Wu Y, Wu F, Gu Z (2014) Antibiotic-loaded chitosan hydrogel with superior dual functions: antibacterial efficacy and osteoblastic cell responses. ACS Appl Mater Interfaces 6(13):10005–10013
101. Qi X, Wei W, Li J et al (2017) Design of Salecan-containing semi-IPN hydrogel for amoxicillin delivery. Mater Sci Eng C 75:487–494
102. Zhang Y, Zhang J, Chen M, Gong H, Thamphiwatana S, Eckmann L, Gao W, Zhnag L (2016) A bioadhesive nanoparticle−hydrogel hybrid system for localized antimicrobial drug delivery. ACS Appl Mater Interfaces 8:18367–18374
103. Ng VWL, Ke X, Lee ALZ, Hedrick JL, Yang YY (2013) Synergistic co-delivery of membrane-disrupting polymers with commercial antibiotics against highly opportunistic bacteria. Adv Mater 25(46):6730–6736
104. Shi Y, Truong VX, Kulkarni K et al (2015) Light-triggered release of ciprofloxacin from an in situ forming click hydrogel for antibacterial wound dressings. J Mater Chem B 3(45):8771–8774
105. ter Boo G-JA, Arens D, Metsemakers W-J et al (2016) Injectable gentamicin-loaded thermoresponsive hyaluronic acid derivative prevents infection in a rabbit model. Acta Biomater 43:185–194
106. Li Z, He C, Yuan B, Dong X, Chen X (2017) Injectable polysaccharide hydrogels as biocompatible platforms for localized and sustained delivery of antibiotics for preventing local infections. Macromol Biosci 17(4):1600347
107. Hu J, Quan Y, Lai Y et al (2017) A smart aminoglycoside hydrogel with tunable gel degradation, on-demand drug release, and high antibacterial activity. J Control Release 247:145–152
108. Hoque J, Bhattacharjee B, Prakash RG, Paramanandham K, Haldar J (2018) Dual function injectable hydrogel for controlled release of antibiotic and local antibacterial therapy. Biomacromolecules 19(2):267–278
109. Varaprasad K, Mohan YM, Vimala K, Mohana Raju K (2011) Synthesis and characterization of hydrogel-silver nanoparticle-curcumin composites for wound dressing and antibacterial application. J Appl Polym Sci 121(2):784–796
110. González-Sánchez MI, Perni S, Tommasi G et al (2015) Silver nanoparticle based antibacterial methacrylate hydrogels potential for bone graft applications. Mater Sci Eng C 50:332–340
111. Noimark S, Weiner J, Noor N et al (2015) Dual-mechanism antimicrobial polymer-ZnO nanoparticle and crystal violet-encapsulated silicone. Adv Funct Mater 25(9):1367–1373
112. GhavamiNejad A, Park CH, Kim CS (2016) In situ synthesis of antimicrobial silver nanoparticles within antifouling Zwitterionic hydrogels by Catecholic Redox chemistry for wound healing application. Biomacromolecules 17(3):1213–1223
113. Lustosa A, de Jesus Oliveira A, Quelemes P et al (2017) In situ synthesis of silver nanoparticles in a hydrogel of Carboxymethyl cellulose with Phthalated-cashew gum as a promising antibacterial and healing agent. Int J Mol Sci 18(11):2399
114. He M, Wang Q, Wang R, Xie Y, Zhao W, Zhao C (2017) Design of antibacterial poly(ether sulfone) membranes via covalently attaching hydrogel thin layers loaded with Ag nanoparticles. ACS Appl Mater Interfaces 9(19):15962–15974

115. Yang W, Fortunati E, Bertoglio F et al (2018) Polyvinyl alcohol/chitosan hydrogels with enhanced antioxidant and antibacterial properties induced by lignin nanoparticles. Carbohydr Polym 181:275–284
116. Liu R, Dai L, Si C, Zeng Z (2018) Antibacterial and hemostatic hydrogel via nanocomposite from cellulose nanofibers. Carbohydr Polym 195:63–70
117. Richards S-J, Isufi K, Wilkins LE, Lipecki J, Fullam E, Gibson MI (2018) Multivalent antimicrobial polymer nanoparticles target mycobacteria and Gram-negative Bacteria by distinct mechanisms. Biomacromolecules 19(1):256–264
118. Jiang B, Larson JC, Drapala PW, Pérez-Luna VH, Kang-Mieler JJ, Brey EM (2012) Investigation of lysine acrylate containing poly(N-isopropylacrylamide) hydrogels as wound dressings in normal and infected wounds. J Biomed Mater Res B Appl Biomater 100B(3):668–676
119. Laverty G, Gorman SP, Gilmore BF (2012) Antimicrobial peptide incorporated poly(2-hydroxyethyl methacrylate) hydrogels for the prevention of Staphylococcus epidermidis-associated biomaterial infections. J Biomed Mater Res A 100A(7):1803–1814
120. Buhrman JS, Cook LC, Rayahin JE, Federle MJ, Gemeinhart RA (2013) Proteolytically activated antibacterial hydrogel microspheres. J Control Release 171(3):288–295
121. Du H, Wang Y, Yao X et al (2016) Injectable cationic hydrogels with high antibacterial activity and low toxicity. Polym Chem 7(36):5620–5624
122. Aziz MA, Cabral JD, Brooks HJL, Moratti SC, Hanton LR (2012) Antimicrobial properties of a chitosan dextran-based hydrogel for surgical use. Antimicrob Agents Chemother 56(1):280–287
123. Li P, Poon YF, Li W et al (2011) A polycationic antimicrobial and biocompatible hydrogel with microbe membrane suctioning ability. Nat Mater 10(2):149–156
124. Tsao CT, Chang CH, Lin YY et al (2011) Evaluation of chitosan/γ-poly (glutamic acid) polyelectrolyte complex for wound dressing materials. Carbohydr Polym 84(2):812–819
125. Li M, Mitra D, Kang E-T, Lau T, Chiong E, Neoh KG (2017) Thiol-ol chemistry for grafting of natural polymers to form highly stable and efficacious antibacterial coatings. ACS Appl Mater Interfaces 9(2):1847–1857
126. Mukherjee I, Ghosh A, Bhadury P, De P (2017) Side-chain amino acid-based cationic antibacterial polymers: investigating the morphological switching of a polymer-treated bacterial cell. ACS Omega 2(4):1633–1644
127. Mukherjee I, Ghosh A, Bhadury P, De P (2018) Leucine-based polymer architecture-induced antimicrobial properties and bacterial cell morphology switching. ACS Omega 3(1):769–780
128. Liu SQ, Yang C, Huang Y et al (2012) Antimicrobial and antifouling hydrogels formed in situ from polycarbonate and poly (ethylene glycol) via Michael addition. Adv Mater 24(48):6484–6489
129. Li Y, Fukushima K, Coady DJ et al (2013) Broad-spectrum antimicrobial and biofilm-disrupting hydrogels: stereocomplex-driven supramolecular assemblies. Angew Chem Int Ed 52(2):674–678
130. Cao B, Tang Q, Li L et al (2013) Switchable antimicrobial and antifouling hydrogels with enhanced mechanical properties. Adv Healthc Mater 2(8):1096–1102
131. Gaetano G, Giuseppe P, Salvatore PF, Susanna M, Sara S, Luca RC (2018) Hyaluronic-based antibacterial hydrogel coating for implantable biomaterials in orthopedics and trauma: from basic research to clinical applications. In: Haider S, Haider A (eds) Hydrogels. InTech
132. Habibi N, Kamaly N, Memic A, Shafiee H (2016) Self-assembled peptide-based nanostructures: smart nanomaterials toward targeted drug delivery. Nano Today 11(1):41–60
133. Zhang S, Lockshin C, Herbert A, Winter E, Rich A (1992) Zuotin, a putative Z-DNA binding protein in Saccharomyces cerevisiae. EMBO J 11(10):3787–3796
134. Holmest T, Lockshin C, Rich A (1993) Spontaneous assembly of a self-complementary oligopeptide to form a stable macroscopic membrane. Proc Natl Acad Sci U S A 90(8):3334–3338
135. Zhang S, Holmes TC, DiPersio CM, Hynes RO, Su X, Rich A (1995) Self-complementary oligopeptide matrices support mammalian cell attachment. Biomaterials 16(18):1385–1393

136. Zhang S, Lockshin C, Cook R, Rich A (1994) Unusually stable β-sheet formation in an ionic self-complementary oligopeptide. Biopolymers 34(5):663–672
137. Kisiday J, Jin M, Kurz B et al (2002) Self-assembling peptide hydrogel fosters chondrocyte extracellular matrix production and cell division: implications for cartilage tissue repair. Proc Natl Acad Sci 99(15):9996–10001
138. Salick DA, Kretsinger JK, Pochan DJ, Schneider JP (2007) Inherent antibacterial activity of a peptide-based β-hairpin hydrogel. J Am Chem Soc 129(47):14793–14799
139. Salick DA, Pochan DJ, Schneider JP (2009) Design of an Injectable β-hairpin peptide hydrogel that kills methicillin-resistant Staphylococcus aureus. Adv Mater 21(41):4120–4123
140. Veiga AS, Sinthuvanich C, Gaspar D, Franquelim HG, Castanho MARB, Schneider JP (2012) Arginine-rich self-assembling peptides as potent antibacterial gels. Biomaterials 33(35):8907–8916
141. Zhou C, Li P, Qi X et al (2011) A photopolymerized antimicrobial hydrogel coating derived from epsilon-poly-l-lysine. Biomaterials 32(11):2704–2712
142. Liu Y, Yang Y, Wang C, Zhao X (2013) Stimuli-responsive self-assembling peptides made from antibacterial peptides. Nanoscale 5(14):6413
143. Wan Y, Liu L, Yuan S, Sun J, Li Z (2017) pH-responsive peptide supramolecular hydrogels with antibacterial activity. Langmuir 33(13):3234–3240
144. Debnath S, Shome A, Das D, Das PK (2010) Hydrogelation through self-assembly of Fmoc-peptide functionalized cationic amphiphiles: potent antibacterial agent. J Phys Chem B 114(13):4407–4415
145. Laverty G, McCloskey AP, Gilmore BF, Jones DS, Zhou J, Xu B (2014) Ultrashort cationic naphthalene-derived self-assembled peptides as antimicrobial nanomaterials. Biomacromolecules 15(9):3429–3439
146. McCloskey AP, Draper ER, Gilmore BF, Laverty G (2017) Ultrashort self-assembling Fmoc-peptide gelators for anti-infective biomaterial applications: Fmoc-peptides demonstrate selective activity against biofilms. J Pept Sci 23(2):131–140
147. Baral A, Roy S, Ghosh S, Hermida-Merino D, Hamley IW, Banerjee A (2016) A peptide-based mechano-sensitive, proteolytically stable hydrogel with remarkable antibacterial properties. Langmuir 32(7):1836–1845
148. Wani NA, Singh G, Shankar S, Sharma A, Katoch M, Rai R (2017) Short hybrid peptides incorporating β- and γ-amino acids as antimicrobial agents. Peptides 97:46–53
149. Malhotra K, Shankar S, Rai R, Singh Y (2018) Broad-spectrum antibacterial activity of proteolytically stable self-assembled αγ-hybrid peptide gels. Biomacromolecules 19:782–792
150. Malhotra K, Shankar S, Rai R, Singh Y. Design, characterization, and evaluation of antibacterial gels, Boc-D-Phe-γ^4-L-Phe-PEA/chitosan and Boc-L-Phe-γ^4-L-Phe-PEA/chitosan, for biomaterial-related infections. Unpublished work
151. Adak A, Ghosh S, Gupta V, Ghosh S (2019) Biocompatible lipopeptide-based antibacterial hydrogel. Biomacromolecules 20(5):1889–1898
152. Gahane AY, Ranjan P, Singh V et al (2018) Fmoc-phenylalanine displays antibacterial activity against Gram-positive bacteria in gel and solution phases. Soft Matter 14(12):2234–2244
153. Romanò CL, Tsuchiya H, Morelli I, Battaglia AG, Drago L (2019) Antibacterial coating of implants: are we missing something? Bone Joint Res 8:199–206
154. Santos M, Fonseca A, Mendonça P, Branco R, Serra A, Morais P et al (2016) Recent developments in antimicrobial polymers: a review. Materials 9:599
155. Huang R, Qi W, Feng L, Su R, He Z (2011) Self-assembling peptide–polysaccharide hybrid hydrogel as a potential carrier for drug delivery. Soft Matter 7:6222

Antibacterial Hydroxyapatite: An Effective Approach to Cure Infections in Orthopedics

Prabaha Sikder and Sarit B. Bhaduri

Abstract Several statistical reports confirm that surgical site infections (SSI) remain one of the predominant reasons for the failure of orthopedic implant working. Indeed, foreign implant materials are preferential sites for bacterial infestation and formation of biofilms causing deadly infections. It results in delayed recovery with significant prolongation in hospital stays, expensive bills, patient stress, and revision surgeries, which may not solve the problem but drastically increase the chances of infection reoccurrence. Perioperative antibiotic prophylaxes have been used for decades to prevent and treat SSI, simultaneously also contributing to the mutation of antibiotic-resistant bacterial strains. Thus, the need for developing alternative therapeutics to treat wide-spectrum SSI is essential. In this attempt, transition metals, especially silver (Ag), copper (Cu), zinc (Zn), and their complexes have garnered significant attention as effective antibacterial agents in the orthopedic industry. Given the fact that hydroxyapatite (HA) is a traditional and benchmark material for developing orthopedic scaffolds and coatings, a diverse range of studies has been focused on developing antibacterial HA. This chapter presents an overview of those advancements that have been made in developing antibacterial HA with the help of transition metals over the years. The first part focuses on the fabrication of material-specific antibacterial HA, and in the second part, various kinds of conventional and state-of-the-art antibacterial HA coatings are discussed.

Keywords Surgical site infections (SSI) · Orthopedics · Hydroxyapatite (HA) · Antibacterial · Transition metal-doped HA

P. Sikder (✉) · S. B. Bhaduri
Department of Mechanical, Industrial and Manufacturing Engineering,
The University of Toledo, Toledo, OH, USA
e-mail: Prabaha.sikder@rockets.utoledo.edu

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_24

Introduction

Bacterial contamination or infection is a serious threat to the healthcare industry. It not only disrupts the actual healing action of therapeutics but results in significant distress in the patients. In general, orthopedic surgeries involve strict aseptic techniques and antimicrobial prophylaxis. However, the incidence of surgical site infections (SSI) seems to be somewhat inescapable. In orthopedics, prosthetic infections can result in serious complications and can sometimes be life-threatening. The following are the most prominent effects of orthopedic SSI: (1) they prolong the recovery of patients thus increasing hospital stays ranging from 12 to over 20 days, (2) approximately double the chances of rehospitalization and revision surgeries, and (3) exorbitantly increase healthcare costs [1, 2]. In a report by Kurtz et al. in 2012, the authors focused on the economic burden of periprosthetic joint infection (PJI) in the USA [3]. The Nationwide Inpatient Sample (NIS), a nationally representative inpatient database maintained by the Agency for Healthcare Quality and Research, was used for the study. The relative incidence of PJI was reported to be in the range of 2.0–2.4% in case of total hip arthroplasty (THA) and total knee arthroplasty (TKA), and it was predicted to increase over time substantially. The study reported that the annual cost of infected revisions to US hospitals increased from $320 million to $566 million during the study period (2001–2009) and was projected to exceed $1.62 billion by 2020. To certify this prediction, the American College of Surgeons and Surgical Infection Society published a recent article in 2017. The study highlighted the following points: (1) SSIs result in a 2- to 11-fold increase in mortality risks, (2) their incidence has been calculated to be 2–5% in patients undergoing surgery and finally, (3) the annual cost for treating SSIs is estimated at $3.5 to $10 billion in the USA alone [4]. Thus, it is important to develop preventive and effective treatment strategies for SSIs in orthopedics.

It is well known that hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$), due to its chemical similarity to the mineral component of bone, is one of the most widely explored synthetic materials in the orthopedics industry. More than any other calcium phosphate (CaP) phase, it has been extensively used to develop various kinds of orthopedic prostheses like bone cement and bone fillers, and as coatings on orthopedic implants like femoral stems and acetabular component. Given the widespread incidence of SSI and the grievous concerns related to it, significant efforts have been focused on developing antibacterial HA materials. The bactericidal agents used in developing antibacterial HA include a diverse range of materials, and various kinds of innovative materials are still being explored to achieve maximum antibacterial efficacy and biological properties.

Several antibiotics have been developed over decades to treat SSI in orthopedics. It remains one of the primary real-time prophylaxis routes in any surgery. Many reports exist which suggest critical steps to maximize the beneficial effect of prophylactic antibiotics [5]. Cefazolin and cefuroxime are generally the preferred antibiotics for prophylaxis in patients undergoing THA or TKA. Various studies report the inclusion of a wide range of antibiotics like cephalothin, carbenicillin,

amoxicillin, cefamandole, tobramycin, gentamicin, gentamicin sulfate, vancomycin, metronidazole, and simvastatin into HA to make it antibacterial [6–8]. However, the remarkable increase in multidrug-resistant bacteria is not new and is growing worse over time [5, 9]. A recent review shows the global jeopardy due to the evolution of antibiotic-resistant bacterial strains and the deadly infection risks in orthopedic surgeries [10]. The repeated usage of antibiotics over time is the primary reason. One classic example is *methicillin-resistant Staphylococcus aureus* (MRSA). Also, most of the pharmaceutical drugs are developed in a way such that they are useful either against gram-positive strains or gram-negative strains. In most cases, the existing drugs are tailored to be effective against *Staphylococcus aureus* (*S. aureus*), as it is the most common infection causing strain. However, those same drugs render ineffective in curing infections which are caused by gram-negative strains, for example, *Pseudomonas agenesis* (*P. agenesis*) or *Escherichia coli* (*E. coli*).

Thus, the healthcare industry demands the need for antibacterial agents which is effective against a wide spectrum of infection-causing pathogens. Transition metals, especially silver (Ag), copper (Cu), and zinc (Zn) have been recognized to be some of the most effective bactericidal agents in a wide range of industries. Identifying their outstanding antibacterial capabilities, the orthopedic industry has also taken advantage. Over the last two decades, significant efforts have been focused on developing transition metal-doped antibacterial HA materials. This chapter presents an overview of those accomplishments. The first part deals with the development of bulk antibacterial HA. The various doping processes and criticalities in developing those compositions have been described. In the second part, we discuss metal-doped antibacterial HA coatings. The research in developing such coatings is remarkably vast. We discuss some of the most common coating techniques and their related advancements in the field.

Transition Metals as Antibacterial Agents

Various forms of the well-known antibacterial transition metals like (1) elemental, (2) salts (3) ionic, (4) oxides, (5) nanoparticles (NPs), and (6) complex forms (ligands of diverse structures with transition metal centers) have been evidenced to provide excellent antitumor, antibacterial, and antifouling properties. For instance, metallic Ag and Ag salts have been used historically as a disinfectant to either conserve food and water [11] or prevent infection of burnt skin [12]. Not to mention, the ionic forms of Ag and Cu are considered to be one of the most prevalent and active antibacterial agents in a physiological (aqueous) medium. Certain metal oxides and multi-metal oxides have also shown promising antibacterial properties against both gram-positive and gram-negative bacterial strains. Fe_3O_4, TiO_2, CuO, and ZnO are the most common single metal oxides which have been extensively explored, while $Zn_xMg_{1-x}O$, Ta-doped ZnO, and Ag/Fe_3O_4 are the multi-metal nanocomposites that have shown excellent antibacterial functionality. Noteworthy, with the advancement

of technology, several efforts have been made to synthesize various kinds of stabilized transition metal NPs like Ag/Cu/Zn-NPs.

However, the fear of toxicity in living cells always remains when dealing with the metals or their NPs in vivo. Keeping that in mind, various counteractive and adaptive mechanisms for cytocompatibility have been discussed by Stankic et al. [13] and Díaz-Visurraga et al. [14]. In another perusal, metal complexes with antitumor properties have also shown promising antibacterial effects. The well-known metal complex cis-diamminedichloridoplatinum (II), also known as cisplatin, and its derivatives paved the way for the subsequent development of metal-based chemotherapeutic agents [15]. Ruthenium complexes are evidenced to exhibit both anticancer and antibacterial properties [16]. Nevertheless, it is worth mentioning that in the effort of developing antibacterial materials for orthopedics, most of the attention has been focused on certain metals (Ag, Cu, and Zn), their oxides (ZnO), and their NPs (Ag/Cu/Zn-NPs) [17–19]. Ag has been explored the most as a bactericidal agent.

The literature on the biological effect of Ag is extensive and diverse. Lemire et al. reviewed the antibacterial effect of this metal, and it is believed that Cu and Zn also exhibit the same antibacterial mechanism [20]. Numerous studies have confirmed the release of Ag^+ ions from metallic Ag, Ag-NPs, and Ag salts when they are placed in an aqueous solution. In the case of Ag-NPs, the release kinetics of Ag^+ ions is critically dependent on the size of the NPs, their surface functionalization, the temperature, and the composition of the surrounding medium [21]. Ag^+ ions are the actual bactericidal agent, and they can effectively make their way into the bacteria by damaging their cell walls. The ions can bind to the thiol groups, nucleic acids, and mitochondria and influence their function and structure. This mechanism eventually disrupts the overall healthy functioning of the bacterial cells. Moreover, the ions can also bind and alter specific proteins or enzymes, which are crucial for cellular respiration and metabolism. Ag^+ ions are also believed to interfere with DNA, thus hampering cell division and replication [22]. In some instances, the formation of reactive oxygen species (ROS) was also observed, though dependent on the cell type [23]. However, in some cases, Ag-NPs can directly enter through the nano-pores of the bacterial cell wall and disrupt the internal working of the cell. Indeed, there have been instances where Ag-NPs were located inside cells [24]. A recent study has shown evidence to suspend the "nano effect" of Ag [25]. Figure 1 shows the possible antibacterial mechanisms of Ag-NPs. The antibacterial activity of Ag^+ ions is also hypothesized to work in the same way [26].

Metal Doping in HA

It is well known that the doping of different ions into the CaP lattice can influence important material properties like bioactivity, biocompatibility, and solubility in a biological medium. However, not all CaP based compounds provide an easy route for doping. Apart from its chemical similarity to the bone which makes HA the

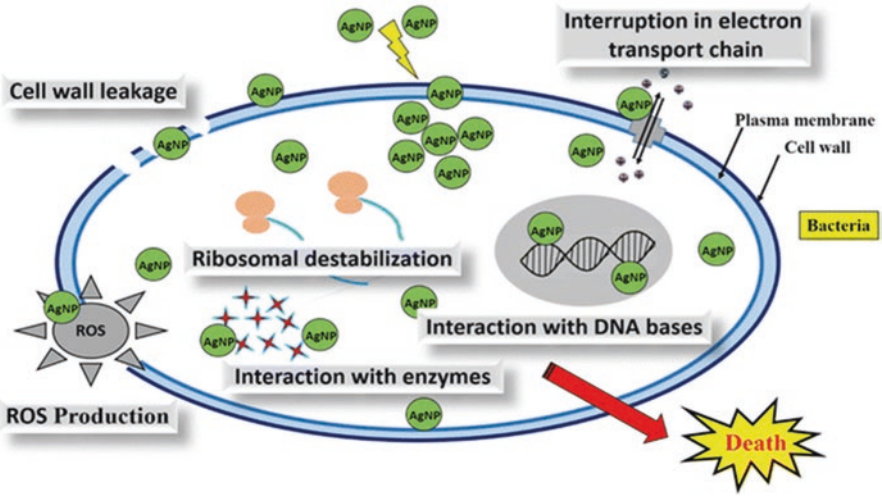


Fig. 1 Possible antibacterial mechanisms by Ag-NPs [26]. (Reproduced with suitable rights and permission)

biomaterial of choice for most of the orthopedic application, it is also one of the most preferred and explored compounds in the arena of metal doping. The choice is motivated by specific material properties of HA like (1) high cation exchangeability enabling easy substitution of dopant, (2) favorable adsorption capacity resulting in excellent adhesion of the dopant to the chemical structure, (3) hydrophilic nature which allows smooth reactions under aqueous conditions, and (4) robust structure which does not easily enable the dopant to leach [27]. These advantages have been identified by chemist long back, and the application was mainly in the sector of catalysis. The concept of doping HA dates back to the 1970s when Suzuki and coworkers found that the surface structure and bulk properties of HA can be altered by ion-exchange of Ca^{2+} with metal ions like Cu^{2+}, Ni^{2+}, and Pb^{2+} [28]. However, the focus was never mainly on improving the biological aspects of HA by metal ion doping. It was much after in 2000 when Ito et al. and Kawamura et al. doped Zn^{2+} in a tricalcium phosphate–HA composite ceramic and showed enhanced bone formation both in vitro and in vivo, respectively [29, 30]. Subsequent to this study, significant efforts were made to dope various metal cations (Mg^{2+}, Zn^{2+}, La^{3+}, Y^{3+}, In^{3+}, and Bi^{3+}) to enhance HA's capability in bone regeneration properties pertinent to orthopedic and dental applications [31].

Simultaneously, the infection incidences in orthopedics were growing at an alarming rate and antibiotics were not doing the job. Thus, supplemental to the enhancement of biocompatibility properties, significant attention was also focused on developing HA with active antibacterial properties. In this context, transition metals became the most potential bactericidal agent due to its strong antibacterial properties against wide-spectrum pathogens. From the late 1990s to date, significant studies have been focused on developing transition metal-doped antibacterial

HA. There were primarily three motivations for this start. First, the long-term antibiotic usage led to the mutation of antibiotic-resistant bacteria which were very difficult to treat. Sometimes, patients were under antibiotics medication for years which not only degraded their innate immunity but also resulted in side effects. Second, the prophylaxis was systemic in nature which never ensured a site-specific treatment. Third, transition metals, especially Ag, have been explored for a long time in the biological sector as an outstanding antibacterial agent against a broad spectrum of microbial species. It was readily available and did not demand substantial financial investments. Thus, there could not be a better choice for an active infection treatment. Third, previous research showed the ease of doping metal cations into HA.

Several studies took advantage of the bactericidal and bacteriostatic effect of the transition metals, metal complexes, and metal ions of Ag, Cu, and Zn incorporated with HA. Initially, it started with a focus in developing the bulk material. Simultaneously or subsequently, significant amount of efforts has been involved in developing HA-based antibacterial products like scaffolds and coatings. In majority of the cases, the metals in its ionic form were doped into the HA lattice to form single-phasic or multiphasic composites (Ag/Cu/Zn-HA). In other instances, NPs or micro elemental particles were also incorporated with HA. The following sections describe some of the critical progress in this field.

Transition Metal-Doped Antibacterial HA

The late 1980s or early 1990s marked the beginning of research in transition metal-doped HA. A group of Japanese researchers patented some of the very first studies in the development of antibacterial CaPs or HA [32–34]. The inventions primarily focused on the synthesis of Ag/Cu/Zn-HA powders using either ion exchange or co-precipitation methods. In some cases, the initial powders were heat-fired to reduce the elution extent of the dopants. The very minimal dopant elution helps in sustaining the antibacterial property until a year and made it safe and favorable for applications in the fields of cosmetics, pharmaceuticals, and food packaging. However, such low elution properties might not be beneficial to inhibit SSIs in orthopedics. In a study conducted by Radovanović et al., the authors observed a much-decreased release of dopant from the sintered compacts of doped HA/α--tricalcium phosphate (TCP) composites as compared to the as-synthesized single-phasic powders [35]. Further, the authors also showed that the Ag^{+} release was much higher than the Cu^{2+} version from the powders prepared by autoclaving. This occurrence could be due to the differences in the dopant radii. The hydrated radii of Ca^{2+}, Cu^{2+}, and Ag^{+} are 0.412 nm, 0.419 nm, and 0.341 nm, respectively [36], indicating that in an aqueous solution during the HA synthesis, it is much easier for Ag^{+} to replace Ca^{2+}. However, in the powder form, the crystal radii of Ca^{2+}, Cu^{2+}, and Ag^{+} are 0.099 nm, 0.072 nm, and 0.126 nm, respectively, and hence, Ag^{+} is more difficult to be retained in the HA structure than Cu^{2+} [35]. The scenario is different when

the powders were sintered, and TCP phases were formed. The Ca(II) position in α-TCP (Ca(II)–O distance 0.268 nm with six oxygen atoms) [37] is much more tolerant for substitution than the Ca(I) and Ca(II) sites in the HA and thus fewer ions are released from the sintered compacts [35]. However, on the other hand, Zn-doped sintered HA/TCP compacts exhibited higher dopant release resulting in enhanced antibacterial efficacy as compared to as-synthesized powders [38]. There have been studies which state that single-phasic materials are more beneficial than composites in term of sustained dopant release; however, it is not yet clear due to the lack of comparison between the same groups of materials. Indeed, the aim is to have a low or sustainable release which might eventually help in a long-term antibacterial effect, but care should be taken to design materials with sufficient dopant release during the first few days or weeks after the surgery when the chances of biofilm formation are at its peak.

The kind of transition metal ions chosen, and its doping concentration are critical parameters for effective infection prevention or treatment. Kim et al. demonstrated a noticeable antibacterial effect with Ag^{+} ion-doped HA against *E. coli* [39]. However, the powders with the same doping concentration of Cu^{2+} and Zn^{2+} were not useful. This result makes it evident that the minimum inhibitory concentration (MIC) of metal ions varies from one another. On the other hand, Stanic et al. synthesized Cu/Zn-nano-HA by neutralization method and showed noteworthy antibacterial effect against *E. coli* and *Candida albicans* (*C. albicans*) but not *S. aureus* [40]. In another study, the same group synthesized Ag-HA and showed outstanding antibacterial activity against all three strains [41]. In general, most of the studies have achieved satisfactory results with Ag as the antibacterial agent against a broad spectrum of bacteria. The number of studies involving Ag is much higher compared to Cu and Zn. This is because of the multiple antibacterial routes that can be adapted by Ag to either inhibit or kill pathogens. However, the type of the microorganism to be treated is another essential and critical factor when antibacterial materials are designed. Especially, when infections can be caused by a wide variety of microorganisms with different tolerances towards antibacterial agents. *S. aureus*, a gram-positive bacterium, has been the primary infection causing pathogen in orthopedic surgeries as opposed to gram-negative bacteria like *E. coli*. Many studies have reported the presence of thicker cell walls in gram-positive strains to be the reason for decreased antibacterial effectiveness, as compared to gram-negative bacteria. However, in a study by Tank et al., the authors showed that Zn-HA samples were significantly antibacterial against the gram-positive strains of *Micrococcus luteus* (*M. luteus*) and *S. aureus*, as opposed to moderate effectiveness against gram-negative strains [42]. Shanmugam and Gopal synthesized Cu-HA, which exhibited outstanding antibacterial activity against *C. albicans* and *S. aureus*, but not against *E. coli* [43]. Such results have not been shown by Ag-doped specimens as Ag has exhibited prominent antibacterial mechanism against both gram-positive and gram-negative strains. At this point, it should also be noted that most of the studies have always focused on the in vitro antibacterial mechanisms and effects. Very few studies exist in the literature which has explored the in vivo antibacterial activity of these materials. The efficacy of the agents and their biological effects in vivo might

be very different from the in vitro scenario. Honda et al. varied the Ag doping content and employed ultrasonic spray pyrolysis route to develop Ag-HA [44]. It is one of those studies which explored both the in vitro and in vivo studies to analyze the antibacterial effect as shown in Fig. 2a–d. Nevertheless, the right concentration is always critical to initiate the antibacterial effect. Numerous studies have reported the MIC of metal ions against *S. aureus* and *E. coli* in vitro. In a recent review by Ferraris and Spriano, the authors summarized those values as shown in Table 1 [45].

In addition to the antibacterial effect, care should be taken to secure the biocompatibility properties of HA. It is well known that transition metals above a specific

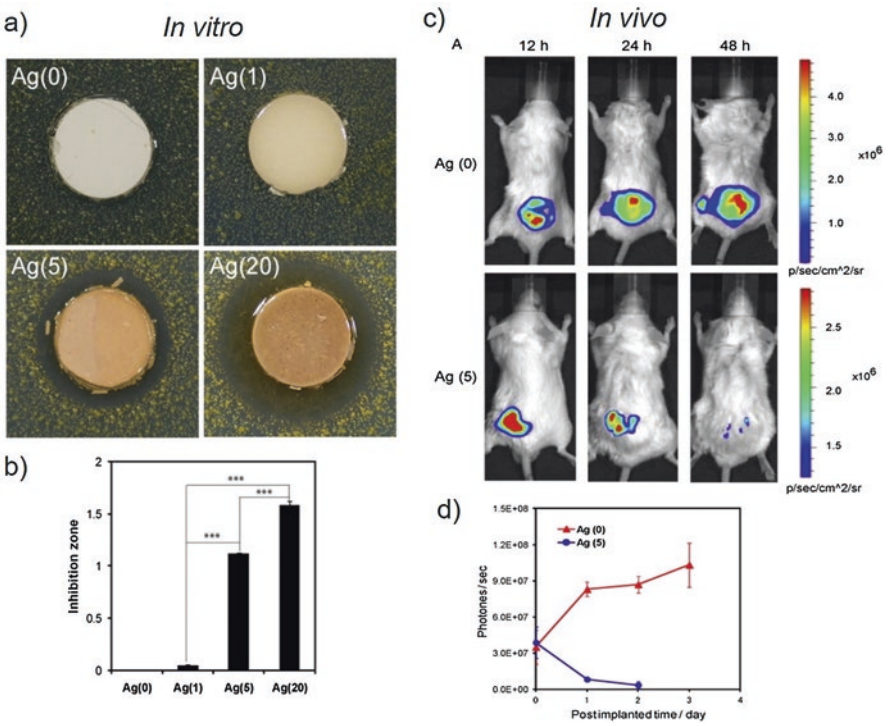


Fig. 2 In vitro and in vivo results of Ag-HA developed by ultrasonic spray pyrolysis synthesis route. **(a)** In vitro antibacterial evaluations show the zone of inhibition (ZOI) formed by various Ag-HA discs (1, 5, and 20 are the mol.% of Ag) when incubated with *S. aureus*. **(b)** ZOI area was calculated by the equation $(D_1\text{-}D_2)/D_2$ where D_1 represented the diameter of the total ZOI and D_2 represented the diameter of the disc. Results are presented as mean of three samples and error bars indicate standard deviation. **(c)** In vivo evaluation of antibacterial activity by IVIS imaging analysis. Real-time monitoring of the antibacterial functionality of Ag-HA(5) on *S. aureus*, Xen 29 infection in mice. The mice were imaged periodically over a 2-day period by using an IVIS camera. A representative animal from the group receiving Ag-HA(5) or the control group [i.e., Ag(0)] is shown. The color bar indicates the signal intensity. **(d)** The bacterial photon intensity from ROI (region of interest) was sequentially measured on 1–4 days after surgery ($n = 5$ for each point). Error bars indicate standard error of the mean ($n = 5$). The asterisks show $***P < 0.001$ by Student's t-test [44]. (Reproduced with suitable rights and permission)

Table 1 Minimum inhibitory concentrations (MIC), against various bacterial strains (including *S. aureus* and *E. coli*) and lethal dose 50 (LD50) for L929 mouse fibroblast cells [45]

	MIC (μg/mL)	LD50 (mmol/L)
Ag^{+}	0.03–8	3.5×10^{-3}
Cu^{2+}	256–448	2.3×10^{-1}
Zn^{2+}	768	3.6×10^{-3}

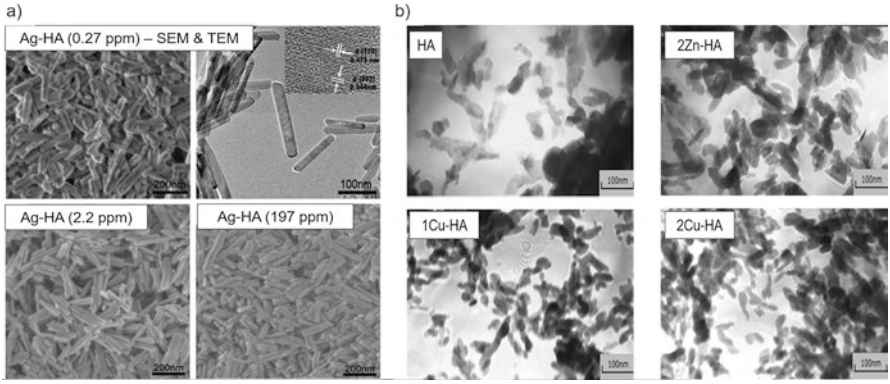


Fig. 3 (**a**) Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) micrographs of various Ag-HA concentration in the shape of nano-rods developed by a hydrothermal technique [46]. (**b**) Irregular shaped HA, Zn-HA and Cu-HA NPs developed by neutralization method (Atomic ratios, Zn/(Zn + Ca) = 0.004 (2Zn-HA), Cu/(Ca + Cu) = 0.0004 (1Cu-HA), 0.004 (2Cu-HA) [40]. (Reproduced with suitable rights and permission)

concentration can be cytotoxic. It is usually referred to as Lethal Doses 50 or LD50. A material exhibiting outstanding antibacterial efficacy supplemented with cytotoxic nature is no good. Shi et al. synthesized Ag-HA nanocrystals, specifically in the shape of nanorods (Fig. 3a) and increased the antibacterial efficacy against *E. coli* and *S. aureus* from 63 to 99% but decreased the viability of L929 cells from 97% to 62% [46]. Singh et al. observed cytotoxicity in case of sintered compacts when he increased Ag doping level ($x \geq 0.3$ in $Ca_{10-x}Ag_x(PO_4)_6(OH)_2$). On the other hand, sintered HA composites with a higher level of Zn doping showed much better cytocompatibility results as compared to lower doping levels [38]. Table 1 contains some of the LD50 values as measured against L929 mouse fibroblast cells. It should be noted that many studies in the literature which focused on the development of antibacterial HA, did not always check the cytocompatibility behavior of the antibacterial specimens. Thus, there are several important factors which should be taken into consideration while designing the "optimized" metal-doped antibacterial HA products. In orthopedics, an optimized antibacterial product would exhibit the required antibacterial properties needed to inhibit biofilm formation for a required amount of time and also help in osseointegration (bind to neighboring bone), thus resulting in a speedy recovery.

Processing Techniques for Developing Metal-Doped HA

Material synthesis plays one of the most important factors to influence the biological properties of doped HA. In the first place, the synthesis process plays an important influence on the extent of doping within the lattice, which further affects its release kinetics. If the transition metal dopants are not well substituted at the Ca^{2+} sites of the HA lattice in a similar manner throughout, the chances of an outburst or un-regulated release are high. Thus, it is important to develop doped HA which would promote a sustained release of the transition metals, thus resulting in effective antibacterial efficacy and no cytotoxicity. Some of the common processing techniques for developing transition metal-doped HA are discussed in the following.

The ion-exchange method was quite prevalent during the initial years of research for doping Ag, Cu, and Zn into HA. This occurs because of the prior research work which employed ion-exchange as one of the established processes for doping metal cations into HA. In 2004, a group of researchers from the Nano Ceramic Center and Kyungpook National University Hospital, South Korea reported a series of studies which compared the physical and biological properties of Ag-HA developed by two different synthesis routes [47] [48, 49]. Their studies prove that in ion-exchange, the dopant only gets substituted at the surface Ca^{2+} sites, rather than the core. On the contrary, in co-precipitation, Ag replaces the Ca sites while the HA crystals are getting formed in the solution, thus confirming a much more homogenous substitution [49]. In another study conducted by Dubnika et al., the authors suggested that even choosing the raw materials like Ca and PO_4 salts and the kind of co-precipitation method influence the thermal stability, thermo-dynamical properties, dopant release and thus the antibacterial properties of the materials [50]. Generally, an optimized substitution of Ag^{+} at the Ca^{2+} is desired. The substitution effect in most cases is confirmed by the change in lattice parameters as calculated from the X-ray diffraction (XRD) of specimens. Ag^{+} (1.28 Å) ions replace the Ca^{2+} (0.99 Å) sites preferentially in the Ca(1) site of HA, and this leads to an increase in the lattice parameters (*a* and *c* axes) of doped HA. On the contrary, a slight decrease in the lattice parameters was observed when Cu was doped into HA. This occurs because of the lower ionic radius of copper Cu^{2+} (0.72 Å) compared to Ca^{2+} [43]. Similar results were observed when Zn with a smaller ionic radius of (0.74 Å) was doped into HA [51]. It should be noted that in most cases, these measurements were not done with the single-phasic as-synthesized powders but with heat-treated composites containing multiple phases. The presence of different phases or impurities might not yield a promising result. However, this being said, the low dopant concentration might be too negligible to induce any significant changes in the lattice parameters.

Over the last decade, co-precipitation has evolved to be one of the preferable methods to synthesize homogeneously doped HA powders. Ciobanu et al. developed various single-phase Ag-HA compositions ($Ca_{10-x}Ag_x(PO_4)_6(OH)_2$, $0.05 \leq x \leq 0.4$) using a co-precipitation route at 100 °C in 2 h [52–54]. Singh et al. synthesized the same kind of compositions using a similar technique at room temperature [55]. In another instance, Cu was also doped into HA ($Ca_{10-x}Cu_x(PO_4)_6$

x = 0.05–2) using co-precipitation at room temperature [43]. Ag/Zn-HA powders were synthesized using co-precipitation and calcined at 650 °C before loading them with Ag-NPs [56]. However, apart from co-precipitation, various other techniques have also been used to develop doped HA. These were not widely used but promised the synthesis of homogeneously doped HA. Irregularly shaped Cu, Zn, and Ag-HA nanocrystals with no impurities were prepared by a 24 h long neutralization method as shown in Fig. 3b [40, 41]. Single-phase Ag-HA powders, with an average particle size below 100 nm, were produced by electrostatic spray-pyrolysis process [57]. Similarly, an ultrasonic spray pyrolysis route was used to synthesize Ag-HA powders with micron-sized spherical particles [44]. The single-phasic nature of HA was retained till 20 mol.% Ag doping. However, in spray pyrolysis synthesis, Ag does not enter the HA lattice. Instead, it gets deposited on the surface of HA particles. A 12 h long modified sol–gel technique was employed to synthesize Ag-HA nano-rods with favorable hemocompatible and antibacterial properties [58]. Similar kind of nano-rods was also synthesized by a hydrothermal method using a drying oven at 150 °C [46]. In another instance, a kind of hydrothermal method which included autoclaving at 160 °C was used to synthesize Zn-HA spherical particles from a modified precursor solution involving urea. The doped spherical HA particles converted into crystalline particles of various shapes after annealing [38]. The various morphologies of Ag/Cu/Zn-HA as prepared by different synthesis techniques are presented in Fig. 3a, b.

However, most of the synthesis procedures involved multiple steps with long processing times of at least 2 h. Recently, we employed the rapid microwave irradiation technique to synthesize antibacterial single-phasic compositions [59, 60]. Microwaves increase the temperature of the precursor solution rapidly and play a significant role in enhancing the precipitation kinetics, thus resulting in the formation of precipitates in minutes as opposed to hours in conventional co-precipitation and hydrothermal methods. Further, the benign processing temperature of 100–160 °C does allow the creation of other phases or impurities. We could synthesize single-phasic nano-sized and nano-crystalline Ag-HA particles with flake-like morphology [60]. The energy barrier in microwaves does not allow the crystals to mature during the synthesis. But, we were also successful in developing highly crystalline and micron-sized particles with the microwaves [61]. However, it should be noted that while synthesizing the powders in the first place, the processing temperature might not play an important role. Especially, given the fact that most of the synthesis processes involve temperatures not more than 200 °C. It is the concentration of the dopant which matters the most during the synthesis. An excess in the dopant amount will lead to the formation of secondary phases even if the processing temperature is benign. For example, silver phosphate (Ag_3PO_4) was formed in the as-synthesized powders developed by microwave–irradiation when the precursor solutions were doped with Ag concentrations equal to and above 0.4 at% [62]. The formation of a secondary phase makes the system unstable which might lead to outbursts and inhomogeneous dopant release, thus causing cytotoxicity.

Developing as-synthesized compositions with single-phase nature and no impurities is one of essential aims of various studies, including the ones conducted

by our group. Single-phasic compositions are a confirmation for a homogenous dopant distribution in the HA lattice. This homogeneity is believed to promote a sustained dopant release, thus promising long-term antibacterial effect and reduced chances of cytotoxicity. Also, they are an indication that the dopant amount was sufficient for the substitution and there was no excess. However, in most cases, the as-synthesized single-phasic powders were sintered for compaction. Exposure to high temperatures (≥700 °C) degraded the actual phase of the powders and resulted in the formation of multiphasic composites. These composites mainly consisted of HA, β/α-TCP and Ag. An important consideration in this context is the role of Ag substitution in lowering the thermal stability of HA. In a study conducted by Rameshbabu et al., the authors heat-treated varying concentrations of Ag-HA samples at 700, 750, and 800 °C. A secondary phase of elemental Ag was formed only when the Ag-HA with 0.5 at% doping was heated at 800 °C. Whereas, Ag-HA with 1.5 at% doping degraded to form β-TCP right at 750 °C. In another perusal, the evolution of Ag peaks has been debated to be dependent on the kind of fabrication method followed to develop the as-synthesized powders. Singh et al. observed α-TCP phases, but no Ag phases, when the Ag-HA compositions (3 at.%—5 at.% doping) were prepared through co-precipitation and sintered at 1200 °C [55]. The intensity of the TCP peaks also increased with an increase in Ag doping. Whereas, Ag phase was found at 5 at % doped samples prepared by microwaves and heat-treated at 900 °C [63]. In some studies, silver oxide (AgO) was also formed after sintering, with a change in the selection of raw materials [50]. β-TCP phases were formed when co-precipitated Cu-HA powders were calcined at 700 °C [43]. In the case of Zn-HA samples, however mainly α-TCP phases were observed after sintering as the secondary phase [38]. Sintering is essential for compaction, especially when the applications are in the field of hard-tissue replacements. However, that being said, various scaffolds like bone-cements can also be prepared such that their strength matches to that of cortical bone [64]. Indeed, care should be taken to choose the raw materials, powder synthesis procedure, transition metal doping content and the sintering temperature to rightfully achieve the desired properties in the finished antibacterial products.

Transition Metal-Doped Antibacterial HA Coatings

The development of antibacterial coatings on various kinds of orthopedic implants remains one of the most common techniques to develop biofilm resistant surfaces [123]. The concept was to load the primary coating materials with antibacterial agents and deliver them locally at the implant site to render the bactericidal or bacteriostatic effect in that region. Indeed, HA being the most preferred material-of-choice for coatings on orthopedic implants, a significant amount of research was focused on developing antibacterial HA coatings. Researchers first focused on antibiotics loaded HA, given the fact that antibiotics are one of the most well-known and certified ways of infection treatment in the healthcare industry. Hence, the past decades witnessed

various efforts in developing antibiotics loaded HA. Dip-coating and biomimetic co-precipitation from a drug containing supersaturated CaP solution were the most common techniques employed to develop the antibiotics loaded HA coatings. In many cases, the antibiotics were soaked (or loaded) onto the coatings subsequent to the development of the HA coatings [8]. Such coatings resulted in an outburst release resulting in losing most of the drugs within the first few days. On the contrary, coatings developed by co-precipitation of HA and drugs simultaneously promoted a much more sustained release of the antibiotics over a long time. In certain instances, a polymeric overlayer/overcoat was developed on the drug-loaded HA coatings to control the outburst, subsidize the release and exhibit a more prolonged release of the drugs from the coatings. For instance, Neut et al. developed a composite coating which consisted of three layers. The layer containing the gentamicin drug was sandwiched between the innermost HA layer and outermost poly(lactic-co-glycolic acid) (PLGA) overcoat [65]. Of late, multi or dual drug loading is being explored to enhance the antibacterial efficacy against both gram-positive and gram-negative bacterial strains. Recently, HA-coated titanium was functionalized with a polymer of cyclodextrin and was loaded with two antibiotics—tobramycin and rifampicin [66]. Even though the drugs were loaded by soaking method, the coatings promoted a very controlled release and exhibited outstanding antibacterial efficacy against *S. aureus* and *Enterobacter cloacae* (*E. cloacae*). However, the grievous concern of infections caused by antibiotic-resistant bacteria stands out. Therefore, transition metal-doped HA coatings are the best alternatives for effective antibacterial efficacy against a diverse range of disease-producing strains.

On a classical approach, Ag has been introduced onto Ti surfaces with the help of conventional ion-exchange process, wherein the Na^+ ions from the sodium titanate layer formed in a NaOH solution, exchanges with the Ag^+ ions [67]. In contrast to conventional co-precipitation or ion-exchange methods, the recent decade has witnessed several interesting routes for developing transition metal-doped HA coatings on orthopedic implant surfaces. Some of the most common surface modification techniques and their related work are discussed in the following.

High Temperature Coating Processes (Plasma and Thermal Spraying)

Plasma spraying is a widely established method to coat orthopedic implants with HA. The HA powder is prepared and compounded/substituted with transition metals or other additional elements before the actual coating process by either physical or chemical doping methods like milling [68, 69] or ion-exchange [70]. After the doping process, the powders are fed into the hopper which delivers them to the spraying nozzle. The nozzle ejects the HA-based compositions at a high speed via a plasma channel and deposits the coating on the substrate. The mechanism of forming plasma sprayed coatings is shown in Fig. 4a and an image (Fig. 4b) which shows commercially used plasma prayed HA coatings on orthopedic implants. In a tradi-

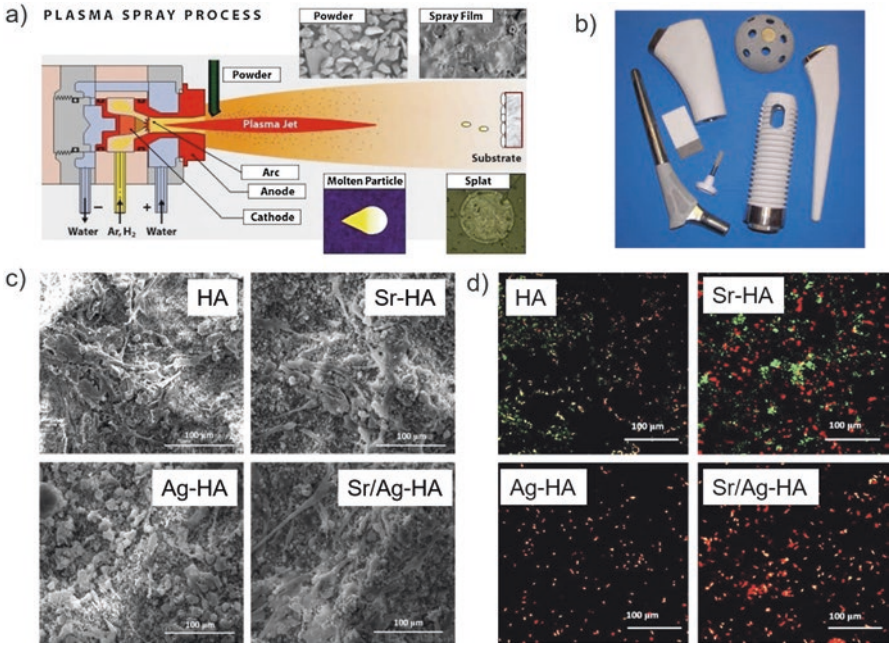


Fig. 4 **(a)** Mechanism of plasma spray process for developing coatings (Courtesy: Science Learning Hub—Pokapū Akoranga Pūtaiao, University of Waikato, www.sciencelearn.org.nz). (**b**) Plasma-sprayed undoped HA coatings on real-time orthopedic implants (Courtesy: APS Materials, Inc). (**c**) FESEM images depicting hFOB cell morphology after 3 days in culture on various undoped and doped HA coatings prepared by plasma-spraying technique. (**d**) Live/dead confocal images of adhered bacteria on the doped and undoped HA coatings prepared by the same process, after 24 h. Dead bacteria appear red, while live bacteria appear green [68]. (Reproduced with suitable rights and permission)

tional plasma spraying process, the coating compositions reside in the plasma for 5 ms, whereas, in a supersonic nozzle, the compositions reside for 290 µs. The supersonic plasma nozzle helps in reduced heating of the HA particles as compared with a conventional plasma nozzle. Fielding et al. developed Ag and strontium-doped plasma sprayed hydroxyapatite coatings and achieved favorable in vitro cytocompatibility and antibacterial properties as shown in Fig. 4c, d. In another instance, the same group mixed HA and Ag_2O via ball-milling and employed plasma spraying to develop the coatings [69]. The plasma sprayed coatings adhered firmly to the roughened Ti substrates via mechanical bonding, but the scenario is disturbed when polymeric substrates are used. The high processing temperatures of plasma can significantly degrade the substrate surface, resulting in poor adhesion of the coatings to the substrates [71]. Thermal spraying, another high temperature coating process, works similar to the plasma spraying and uses a flame temperature of around 2700 °C. This technique has also been used to develop Ag-HA coatings which were proven to exhibit sustainable Ag^+ ion release in vitro [72] and outstanding antibacterial efficacy against MRSA in vivo [73].

The involvement of high processing temperatures does not help in retaining the single-phasic nature of HA. Apart from HA, the developed coatings comprise of various other phases like α, β-TCP, Ag_2O, and elemental Ag. Such phase impurities might result in a non-homogeneous dopant release which might result in cytotoxicity. For example, the 6 wt.% silver-doped plasma sprayed HA coatings exhibited outstanding antibacterial efficacy against *P. aeruginosa* but were cytotoxic against human derived osteoblast cells. In order to regulate or minimize the cytotoxic effects of Ag, the authors doped strontium (Sr) in addition to the Ag (Sr/Ag-HA) [68]. In another study, Geng et al. similarly developed Sr/Ag-HA coatings using a hydrothermal method [74]. In both cases, the cytocompatibility results were much favorable in case of Sr/Ag-HA as opposed to Ag-HA coatings when the coatings were cultured in osteoblast cells. These studies highlight the fact that additional cytocompatible materials can be added to subdue the cytotoxic effects of the bactericidal metals.

Cold Spraying Process

As opposed to processes which involve high processing temperatures, cold-spraying offers many advantages and holds a strong potential to become one of the most efficient low-temperature coating techniques for orthopedic implants. It is mainly cost-effective, appropriate for oxygen-sensitive materials, and environmentally green. The feedstock material remains in its powder form which is supersonically sprayed onto the substrate but without any melting as it occurs in conventional thermal spray processes [75]. It is mainly beneficial for polymeric materials which degrade at high temperatures. It can be a useful technique to develop Ag, Cu, Zn-doped HA coatings on polymers like polyether ether ketone (PEEK). In another instance, gentamicin sulfate loaded HA coatings were developed by the same method [76].

Sol–Gel Coating Process

The sol–gel coating technique first involves the development of a precursor solution responsible for the development of either HA or doped HA. The precursor solution usually contains calcium, phosphorous, and Cu/Zn/Ag containing salts mixed in an aqueous medium and is aged for 24–48 h. After aging, the coatings are developed on the substrates either by spin-coating, where the solution is applied onto the surface of a high-speed substrate rotator/spinner, or dip-coating, where the substrates are dipped into the solution and retrieved at a determined rate. Spin-coating is usually used for metallic or silicon substrates while dip-coating is preferable for glass. The solution is converted to gel either through hydrolysis or condensation reactions. Subsequently, the gels are dried at around 60 °C and heat treated (calcination) at

around 700 °C for the conversion of the gel into dense coatings. Ag/Zn-HA gels were spin-coated on Ti6Al4V alloys followed by drying and a rapid heat treatment procedure [77]. Similarly, Samani et al. developed Zn/Ag/(Zn + Ag)-HA gels but used dip-coating to produce the coatings on glass substrates [78]. In both cases, the coatings prove their outstanding antibacterial efficacy against antibiotic-resistant strains or a common infection causing strain. Further, the presence of two metal ions promoted an excellent synergistic effect for increasing antibacterial efficiency and enhancing cytocompatibility [78]. On the contrary, only Zn-HA coatings showed significant antifungal behavior against *C. albicans* only under daylight and UV light illumination and not under dark conditions [79]. This coating technique is quite efficient in uniformly coating complex or porous structures. In a study conducted by Qu et al., the authors developed uniform Ag-HA composite coatings on the surface of Ti scaffolds with macro-pores and interconnected structures with the help of dip-coating [80]. Coatings doped with 0.8 wt.% Ag were found to be the optimum composition for sufficient antibacterial efficacy while preserving cytocompatibility. The antibacterial efficacy against *E. coli* and *S. albus* were outstanding. As opposed to transition metals, a recent study shows the antibacterial efficacy of HA-titanium dioxide (TiO_2) composite coatings developed by dip coating method [81]. However, the efficiency of coating the inside of the pores has not yet been critically studied.

Sputtering Coating Process

Sputtering is a kind of coating technique where energized gas ions strike a target material and cause the atoms from the target to be ejected. These ejected atoms then travel in plasma with enough energy towards the substrate and bonds with its surface, forming a coating. Chen et al. reported one of the early studies where Ag-HA coatings were developed on Ti by co-sputtering, that is, simultaneously sputtering from two targets—Ag and HA [82]. The Ag-HA coatings were not cytotoxic and showed satisfactory antibacterial efficacy against *S. aureus*. In some cases, co-sputtering was not used to develop the doped HA coatings. Instead, Ag wires with different diameters were affixed to the top of the HA target to form Ag-HA coatings [83]. The various wire diameters contribute to the different concentrations of Ag doping.

Ion beam assisted deposition (IBAD) is a kind of coating technique which combines ion implantation with simultaneous sputtering. Bai et al. reported the fabrication of robust Ag-HA coatings on roughened Ti with the help of IBAD; however, the study focused on the mechanical properties and not on the antibacterial capabilities [83]. The sputtering system also had the ability of in-situ substrate heating (550 °C for the first 4 h and 450 °C for the last 2 h during the process) which helped in developing the functionally graded microstructure. To enhance the adhesion strength of the magnetron sputtered coatings onto the substrates, plasma-based etching has been proven to be an effective method to etch the substrate surface with topography in the micro and nanoscale. Trujillo et al. employed a similar technique to etch the

Ti substrates at −700 eV and sputter deposited with 0.5 wt.% and 1.5 wt.% Ag-doped HA [84]. The results indicated the uniform and even distribution of Ag all over the doped HA coatings which helped in developing bacterial anti-adhesive and biofilm-resistant surfaces. A three-layer system of HA (innermost)/Ag NPs(middle)/amorphous CaP (outermost) was developed by a combination of electrophoretic deposition and radio frequency (RF) magnetron sputtering [85]. The schematics of the hybrid coating process and the composite coating are shown in Fig. 5a. The SEM micrographs show the cross-sectional distribution of the multiple coatings in Fig. 5b. The antimicrobial effect against *E. coli* was found in the 150 nm thickness profile of the CaP and also showed excellent resistance to coating delamination and cracking as shown in Fig. 5c, d. Recently, Ivanova et al. explored the physicomechanical properties of magnetron sputtered Ag-HA coatings [86]. Interestingly, the authors reported that the addition of silver to HA coatings resulted in an increase of the coating nano-hardness and elastic modulus when compared with those of pure HA coatings. The same group previously developed a hybrid composite containing a bottom Ag-NPs layer followed by a HA layer using the same process [87]. The Ag^{+} ion release from the coatings was sustained and after 7 days, the concentration was $0.27 \pm 0.02\ \mu g\ mL^{-1} - 0.54 \pm 0.02\ \mu g\ mL^{-1}$ in phosphate and acetate buffers, respectively. These values corresponded well with the MIC range in the literature. It should be noted that the number of studies focused on developing sputtered antibacterial Ag/Zn/Cu-HA coatings are very few when compared to the studies related to sputtered Ag-TiO_2 coatings, Ag/Cu-Titanium Nitride (TiN) or just Ag/Cu-NPs coatings on various substrates. Mostly, sputtering is used in the healthcare industry to develop coatings on instruments or implants which require enhanced mechanical and tribological properties rather than improving osseointegrability.

Electrochemical Coating Processes

Electrochemical deposition is a room-temperature coating process in which the coatings are developed on a conductive substrate by simple electrolysis method. Electrophoretic deposition (EPD) is a kind of electrochemical method which is widely used to fabricate ceramic based coatings on metallic substrates. HA powders and transition metal powders (chemical complex) /ions/NPs are mixed in an aqueous solution to form a suspension, which forms the electrolyte. These colloidal particles are mainly positively charged in the aqueous medium, and they migrate under the influence of an electric field (electrophoresis) towards the cathode. The cathode is usually the substrate on which the coating develops within 20–30 min, and the process is known as cathodic EPD. Metal–ceramic nanocomposite coatings comprising of Cu-HA was developed using this method within 10 min [88]. The presence of Cu above a specific concentration degraded the bioactivity of the coatings but increased the adhesive strength. Also, the highest Cu-doped (5 wt.%) compositions were cytotoxic against MG63 cells. The study concluded that 3 wt.% Cu-HA were the optimum coatings with active antibacterial properties against

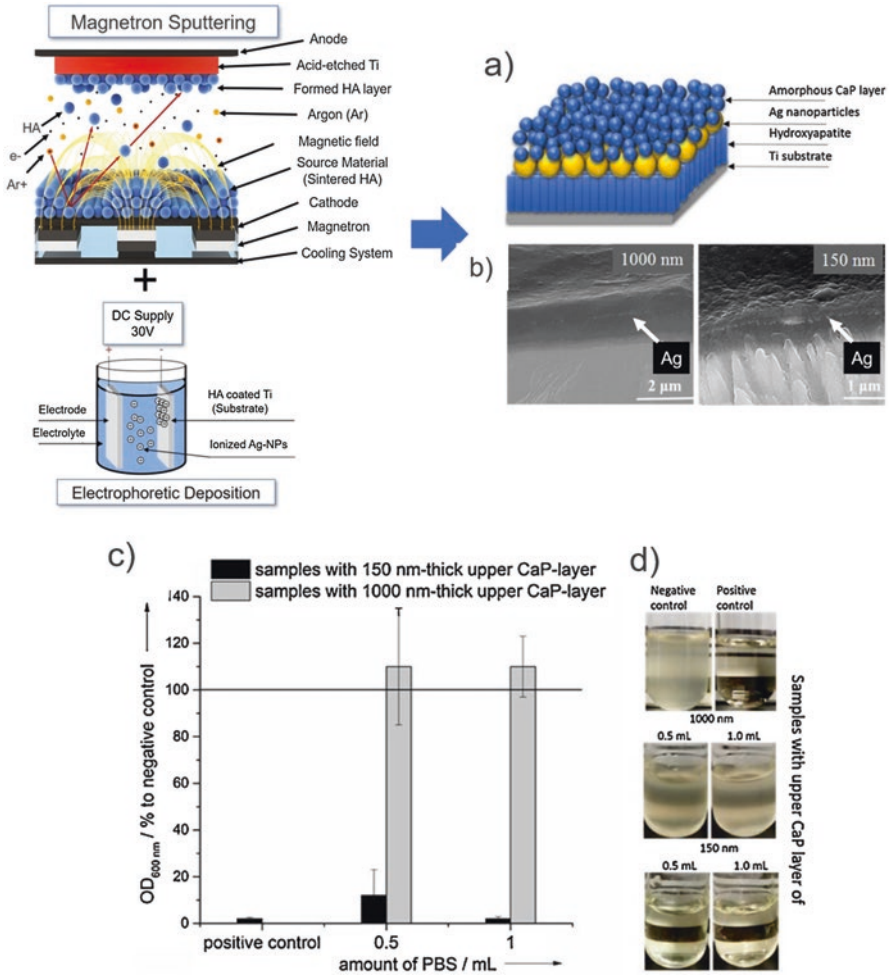


Fig. 5 (**a**) Schematic representation of the working of magnetron sputtering (Courtesy: Visual Science, Client: Rusano) and EPD [88] and their synergy to form the three-layer antibacterial coatings on Ti. Three sequential steps produced multilayer coatings. The first step was the preparation of a nanocrystalline HA by RF magnetron sputtering. The second step was EPD of Ag-NPs, and the third and final step was the deposition of a CaP layer (either 150 nm or 1000 nm thick) by sputtering. (**b**) Backscattered electron images of cross sections of the three-layer coatings. (**c**) Planktonic growth of *E. coli* on the multilayer coatings (OD600 nm measurements normalized to the negative control, 100%) shows that the coatings with a thinner (150 nm) outer layer have an antibacterial effect. (**d**) Representative examples of the bacterial suspensions (*E. coli*) in contact with the incubation media from different Ag-containing coatings. The turbidity indicates bacterial growth. As a positive control, the antibiotic ampicillin was added. Each value represents the mean ± standard deviation of the three determinations [85]. (Reproduced with suitable rights and permission)

E. coli and *S. aureus* and desired cytocompatibility. Similar results of good cytocompatibility towards MC3T3 cells were obtained when the Cu^{2+} ion release was low from electroplated Cu-HA coatings [89]. In another study, electrochemical deposition was used to develop composite coatings of chitosan (CS)/HA/Ag [90]. In this case, CS was used to reduce the cytotoxic effects of Ag, and it also acted as the stabilizing agent to chelate Ag^{+} ions and generate Ag-NPs. Further, the room-temperature processing conditions were taken advantage of, and bone morphogenetic protein-2 (BMP-2) was loaded onto the coatings via electrostatic immobilization. The BMP/CS/HA/Ag coatings favored bone formation in vivo and showed high antibacterial properties against common infection causing strains like *S. epidermidis* and *E coli* in vitro. Interestingly, lignin (Lig), a well-known biopolymer, was also conjugated with Ag-HA to form Ag/HA/Lig composite coatings via EPD to resist corrosion and increase bioactivity and surface porosity [91]. On the other hand, Mirzaee et al. anodized Ti substrates at 40 V for 2 h before employing EPD to develop antibacterial and corrosion resistant Ag-HA coatings [92]. Anodization forms TiO_2 nanotubes on the substrates and significantly improves the bond strength (>16 MPa) of the coatings to the substrates. Of late, graphene oxide (GO) based coatings are being developed as potential antibacterial surfaces. Shi et al. developed GO/CS/HA composite coatings by EPD [93]. Not only were the coatings corrosion resistant, but they also exhibited excellent biocompatibility with osteoblasts and antibacterial properties against *S. aureus*.

Plasma electrolytic oxidation (PEO) or better known as micro-arc oxidation (MAO) is another kind of electrochemical technique which works as the same principle of EPD; however, it involves higher potentials, thus resulting in higher discharges, which creates a plasma. The plasma helps in influencing the coating microstructure and morphology. However, it is difficult to get single phasic HA as the oxidation results in the formation of calcium orthophosphate phases ($Ca_2P_2O_7$) along with TCP phases [94]. This process is mainly used to develop antibacterial oxide-based [95] or metal NP-based [96] coatings, and very few works are present for developing antibacterial CaP phases. Necula et al. developed multifunctional CaP coatings with micro/nano-interconnected porosity, and antibacterial properties on plasma sprayed TiO_2 surfaces using PEO [97]. The coatings were developed in electrolytes based on calcium acetate and calcium glycerophosphate salts containing Ag-NPs. Results showed that the CaP (which had a Ca/P ratio close to 1.67) coatings and Ag-NPs were uniformly deposited on the top of the roughened surface and inside the macro-pores of the TiO_2 layer, highlighting the efficiency of PEO to coat porous and intricate structures. Efforts have been made to develop coatings via plasma electrolytic process (PEP) which is a combination of PEO and EPD. This method has evolved primarily to create thick coating layers. About 83 μm thick Ag-HA coatings were developed by the PEP technique which involved an electrolytic solution containing 2.5 wt.% Ag-HA NPs, prepared by microwaves [98].

The coatings were corrosion resistant both at physiological and acidic medium. Further, they were bioactive in nature and showed prominent zone of inhibition (ZOI) against *E. coli*.

Microwave Irradiation Coating Process

In most of the coating processes described above, the techniques involved: (1) high processing temperature which resulted in degradation of polymeric substrates (plasma spraying), (2) an aging process and additional heat-treatment step to densify the coatings which prolonged the processing time (sol–gel), (3) the development of compositions which sometimes did not release sufficient dopant (sputtering), and (4) complex setups and stringent processing conditions (electrochemical techniques) to develop antibacterial surfaces. Most importantly, in all the cases, the as-developed coatings are primarily multiphasic in nature, which plays a major role in sudden release of dopants leading to cytotoxicity. Additionally, given the fact that 3D-printed scaffolds are widely used in the implant industry now, developing uniform coatings all over the complex structures is very crucial. Presently, the literature does not have enough published studies to discuss that. Over the last few years, our research group has developed a novel "microwave assisted biomimetic coating" technique to coat implant surfaces [99]. The conventional "biomimetic coating" process deposits coatings on implants by a precipitation method from a supersaturated solution. However, it takes about 1–2 weeks. In comparison, we combine the biomimetic technique with microwave-irradiation exposure. This combined process accelerates the coating kinetics, thus making the hybrid process much faster, efficient, simple, and reliable. Importantly, the processing temperature is equal to that of boiling water and this temperature makes it suitable for coating polymers like PEEK [59, 100] and even metallic implants like Magnesium alloy (Mg AZ31) [125]. Also, this helps in retaining the stable single-phase nature of the coating compositions as opposed to unstable composite coatings developed in high-temperature processes. In particular, we synthesized a customizable supersaturated solution (which gives the ease to dope with different ions) and exposed it to microwaves [101]. The solution also contained the pretreated substrates and the microwaves helped in nucleating the crystals rapidly onto the substrate surfaces, thus developing the coating within minutes. The technique also possesses the potential to coat complex 3D structures. Recently, we prepared a range, 2, 4, 6 wt.% Ag-Calcium Deficient HA (Ag-CDHA) coatings on etched Ti6Al4V by using doped CaP precursor solutions and exposed it to 1200 W microwave-irradiation for 8 min [60]. The as-synthesized coatings were single-phasic in nature and promoted a sustainable release of Ag^{+} ions, which helped in showing outstanding antibacterial efficacy against *E. coli* and *S. aureus*. It should also be noted that the 6 wt.% Ag-HA single-phasic coatings were favorably cytocompatible against MC3T3 cells, while the plasma sprayed HA coatings with the same Ag concentrations were cytotoxic [69]. The results of the

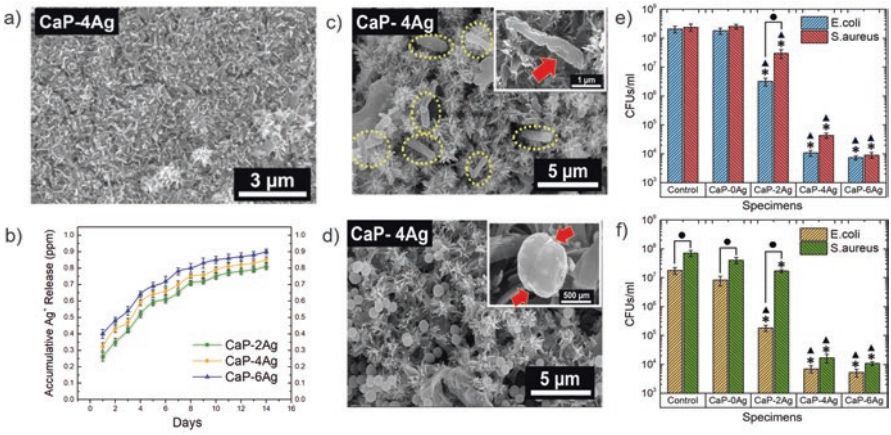


Fig. 6 Antibacterial coatings as developed by microwave-irradiation technique. (**a**) SEM image of 4wt.%Ag-HA (CaP-4Ag) showing the nano-sized flake-like morphology. (**b**) ICP results indicating the sustainable Ag^+ ion release from the coatings. Adhered (**c**) *E. coli* and (**d**) *S. aureus* on the coatings with the insets showing the breakage of cell walls due to the Ag^+ ion effect. (**e**) Results of the plate count method showing the number of viable bacteria formed after 24 h incubation. (**f**) The number of viable surface adherent bacteria formed after 24 h incubation. (*Asterisk* means statistically significant with respect to the control, *filled triangle* means statistically significant with respect to CaP-0Ag, and *filled circle* means statistically significant within the same group, $p < 0.05$.) [60] (Reproduced with suitable rights and permission)

microwave irradiated Ag-HA coatings are shown in Fig. 6a–f. The ICP results show the sustainable nature of the Ag^+ release (Fig. 6b) which result in the antibacterial effect as shown in Fig. 6c–f.

Recent Progresses in Metal-Doped Antibacterial HA

In recent years, the research in metal-doped antibacterial HA has evolved to be a synergistic approach between conventional antibacterial agents like Ag, Cu, Zn and newer multifunctional materials like polymers, drugs, or NPs. This result might occur due to some of the adverse effects (like outbursts and cytotoxicity) involved in using the transition metals. Thus, to counterbalance the potential cytotoxic effects, other elements/materials are used along with doped HA. For example, as mentioned before, Sr, well-known for enhancing cellular activities, has been employed in various studies along with Ag/Cu/Zn-HA to offset the cytotoxic effects of the bactericidal agents [74, 102, 103]. Ag-HA layer combined with calcium silicate (Ca_2O_4Si) were developed on TiO_2 nanotubes using electrodeposition [104]. The idea was to take advantage of the bioactive agent Si^{4+} which helps in enhancing cytocompatibility of the coatings. Also, the novel coatings had excellent corrosion resistance and antibacterial properties. Nonetheless, the availability of innovative

manufacturing techniques, contemporary functional materials, and the growing trend of collaborative research are sufficient motivating factors to develop novel materials with multifunctional properties. For instance, 3D printing was recently used to prepare β-TCP scaffolds modified with graphene oxide containing Ag-NPs [105]. The research in developing hybrid materials like polymer/HA/antibacterial agents has gained significant attention. CS, a well-known polymer, has evolved to be a promising antibacterial agent by itself, along with its excellent cytocompatible properties. Shen et al. developed well-defined microspheres of carboxylated CS/Ag-HA via a simple gas diffusion method [106]. The microspheres exhibited excellent bacteriostatic activity against *S. aureus* due to the combined effects of CS and Ag. The compositions which showed the highest antibacterial activity also were the most cytocompatible with MG63 cells. It should be noted that apart from contributing in the antibacterial effect, the presence of CS also plays an essential role in suppressing the transition metal release which can sometimes lead to cytotoxicity. Bhowmick et al. reported the synthesis of organically modified montmorillonite clay containing chitosan, HA and ZnO materials. The nanocomposites showed enhanced mechanical, antibacterial and cytocompatibility properties which could be beneficial for bone tissue engineering [107]. Dubnika et al. developed a multifunctional porous Ag-HA scaffold which was impregnated with lidocaine hydrochloride, an anesthetic drug, and finally coated them with sodium alginate and CS [108]. The scaffolds had the potential to inhibit bacterial infections up to 1 year as well as sustained release of the anesthetic drug for up to 2 weeks. In another study, Ag-loaded Sr-HA/porous CS scaffolds with 3D interconnected macro-pores provided a robust platform for human bone marrow mesenchymal stem cells to adhere, proliferate, and differentiate [109]. Specifically, in the effort of developing antibacterial metal-doped HA coatings, the employment of collaborative coating technologies is evident. As mentioned before, multilayer hybrid coatings were developed by a combination of magnetron sputtering and electrophoretic deposition [85]. In another instance, a hybrid coating containing HA exhibited good synergistic bactericidal effect with lysozyme, CS, and Ag-NPs as the coating materials [110]. Based on pulsed electrochemical technology, a composite coating consisting of HA, Ag-NPs, and co-hybridized CS was developed, which not only had a long-lasting antibacterial efficacy but also exhibited favorable wear-resistant properties [111]. Innovative techniques like laser processing have also been used to develop bioactive and antibacterial HA coatings containing Ag-NPs on Ti [112]. In another study, pulsed laser deposition was used to fabricate Cu/Zn-HA [113].

A significantly growing interest in transition metal NPs is quite evident. Several efforts have been made to synthesize Ag/Cu/Zn-NPs, with special focus on Ag-NPs based antibacterial materials. This rapid growth is primarily because of the technological advancement which helps in developing stable Ag-NPs of uniform size. The large surface area of Ag-NPs results in a great Ag^+ ion release which is beneficial for the rapid antibacterial effect. Moreover, because of their nano dimension, they can directly attach on to the bacterial cell membrane and penetrate through their cell wall. Most importantly, their antibacterial activity mainly depends on their size and shape. Andrade et al. synthesized Ag-NPs in a colloidal suspension in the presence of CS and further immersed the HA into the as-prepared Ag-NP

colloids [114]. Tian et al. developed Ag-NP loaded HA coatings for implants with different morphologies [115]. In another study, the same group designed the same kind of NP based coatings which exhibited oriented block array morphology [116]. Ag-NPs were also loaded with polydopamine (PDA) coated HA nanowires with the help of a reduction technique [117]. These innovative nanowires were prepared for dental applications and had the potential to be adequate reinforcements in a resin matrix with good interfacial adhesion strength. Other transition metal NPs have also been explored. An optimum composition of Cu-NP incorporated HA coatings developed by EPD, showed good antibacterial activity against *E. coli* and *S. aureus* along with favorable cytocompatibility in MG63 cells [88]. However, not much progress has been made in synthesizing ZnO-NP based materials. Recently, Karbowniczek [118] et al. developed bioglass-based coatings with ZnO-NPs as the antibacterial agent. The coatings were antibacterial against *S. aureus* and *Salmonella enteric* and were also corrosion resistant. On a final note, research in HA, or in general CaP has reached a saturation point. Hence, there is a need to explore other materials which are equally favorable. Magnesium phosphates (MgPs) are another class of bioceramics which have shown outstanding biocompatibility properties. In several instances, MgPs performed better than CaPs on grounds of biocompatibility [61, 119, 120]. Considering this beneficial aspect, recently we doped Ag into MgPs and wanted to explore the antibacterial and cytocompatibility aspects of such materials [59, 121]. We developed antibacterial Ag-doped MgP coatings on both implant types—metallic [121] and polymeric [59]. Further, we also developed Ag-doped MgP particulates [124]. The results were outstanding, which highlights that it is time that we explore doping of other transition metals and other phases of MgPs as opposed to conventional CaPs.

The availability of various material-synthesizing technologies, effortless chances of collaboration, and the charm of innovation thrive the quest of developing newer antibacterial materials each and every day. Nonetheless, the recent statistical reports and hospital surveys confirm that SSIs are still prevalent in the orthopedic surgeries, and their incidences are wreaking havoc. Apart from routine antibiotic prophylaxes (which in many cases fail), clinicians use irrigation and debridement to remove bacteria or dead tissues in case of PJI, without satisfactory results. Scientists in the academic sector are well aware of these failures and promise persistent research and development in antibacterial materials. However, the big question is, "Are those innovations getting commercialized to make a real-time impact on the society?" Indeed, the literature holds a diverse repository of various antibacterial HA materials, but the real success would be to see those reliable antibacterial orthopedic prostheses employed in patients so that the latter directly benefit from them. Indeed, there are strict regulatory pathways which need to be abided by to successfully launch a new biomaterial product in the market. We have made detailed discussions about those in a recent article [122]. The hope lies in the future where scientists, academicians, industrialists, clinicians, and, most importantly, FDA would collaboratively work towards commercializing HA scaffolds or implant coatings with "optimum" antibacterial and osseointegrable properties, in the market.

Acknowledgements This work is funded by NSF Grant No. 1706513.

References

1. Whitehouse JD, Friedman ND, Kirkland KB, Richardson WJ, Sexton DJ (2002) The impact of surgical-site infections following orthopedic surgery at a community hospital and a university hospital adverse quality of life, excess length of stay, and extra cost. Infect Contr Hosp Epidemiol 23:183–189
2. Bachoura A, Guitton TG, Smith RM, Vrahas MS, Zurakowski D, Ring D (2011) Infirmity and injury complexity are risk factors for surgical-site infection after operative fracture care. Clin Orthop Relat Res 469:2621–2630
3. Kurtz SM, Lau E, Watson H, Schmier JK, Parvizi J (2012) Economic burden of periprosthetic joint infection in the United States. J Arthropl 27:61–65. e61
4. Ban KA, Minei JP, Laronga C, Harbrecht BG, Jensen EH, Fry DE et al (2017) American College of Surgeons and Surgical Infection Society: surgical site infection guidelines, 2016 update. J Am Coll Surg 224:59–74
5. Prokuski L (2008) Prophylactic antibiotics in orthopaedic surgery. JAAOS J Am Acad Orthop Surg 16:283–293
6. Stigter M, Bezemer J, De Groot K, Layrolle P (2004) Incorporation of different antibiotics into carbonated hydroxyapatite coatings on titanium implants, release and antibiotic efficacy. J Control Release 99:127–137
7. Stigter M, De Groot K, Layrolle P (2002) Incorporation of tobramycin into biomimetic hydroxyapatite coating on titanium. Biomaterials 23:4143–4153
8. Brohede U, Forsgren J, Roos S, Mihranyan A, Engqvist H, Strømme M (2009) Multifunctional implant coatings providing possibilities for fast antibiotics loading with subsequent slow release. J Mater Sci Mater Med 20:1859–1867
9. Greene LR (2012) Guide to the elimination of orthopedic surgery surgical site infections: an executive summary of the Association for Professionals in Infection Control and Epidemiology elimination guide. Am J Infect Control 40:384–386
10. Li B, Webster TJ (2018) Bacteria antibiotic resistance: new challenges and opportunities for implant-associated orthopedic infections. J Orthop Res 36:22–32
11. Alexander JW (2009) History of the medical use of silver. Surg Infect 10:289–292
12. Klasen H (2000) Historical review of the use of silver in the treatment of burns. I. Early uses. Burns 2:117–130
13. Stankic S, Suman S, Haque F, Vidic J (2016) Pure and multi metal oxide nanoparticles: synthesis, antibacterial and cytotoxic properties. J Nanobiotechnol 14:73
14. Díaz-Visurraga J, Gutiérrez C, Von Plessing C, García A (2011) Metal nanostructures as antibacterial agents. In: Science and technology against microbial pathogens: research, development and evaluation. Formatex, Badajoz, pp 210–218
15. Wong E, Giandomenico CM (1999) Current status of platinum-based antitumor drugs. Chem Rev 99:2451–2466
16. Li F, Collins JG, Keene FR (2015) Ruthenium complexes as antimicrobial agents. Chem Soc Rev 44:2529–2542
17. Hajipour MJ, Fromm KM, Ashkarran AA, de Aberasturi DJ, de Larramendi IR, Rojo T et al (2012) Antibacterial properties of nanoparticles. Trends Biotechnol 30:499–511
18. Chapman J, Weir E, Regan F (2010) Period four metal nanoparticles on the inhibition of biofouling. Colloids Surf B: Biointerfaces 78:208–216
19. Paladini F, Pollini M, Sannino A, Ambrosio L (2015) Metal-based antibacterial substrates for biomedical applications. Biomacromolecules 16:1873–1885
20. Lemire JA, Harrison JJ, Turner RJ (2013) Antimicrobial activity of metals: mechanisms, molecular targets and applications. Nat Rev Microbiol 11:371
21. Chernousova S, Epple M (2013) Silver as antibacterial agent: ion, nanoparticle, and metal. Angew Chem Int Ed 52:1636–1653
22. Feng QL, Wu J, Chen G, Cui F, Kim T, Kim J (2000) A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. J Biomed Mater Res 52:662–668

23. Greulich C, Diendorf J, Gessmann J, Simon T, Habijan T, Eggeler G et al (2011) Cell type-specific responses of peripheral blood mononuclear cells to silver nanoparticles. Acta Biomater 7:3505–3514
24. Lorenz MR, Holzapfel V, Musyanovych A, Nothelfer K, Walther P, Frank H et al (2006) Uptake of functionalized, fluorescent-labeled polymeric particles in different cell lines and stem cells. Biomaterials 27:2820–2828
25. Xiu Z-M, Zhang Q-B, Puppala HL, Colvin VL, Alvarez PJ (2012) Negligible particle-specific antibacterial activity of silver nanoparticles. Nano Lett 12:4271–4275
26. Patil MP, Kim G-D (2017) Eco-friendly approach for nanoparticles synthesis and mechanism behind antibacterial activity of silver and anticancer activity of gold nanoparticles. Appl Microbiol Biotechnol 101:79–92
27. Kaneda K, Ebitani K, Mizugaki T, Mori K (2006) Design of high-performance heterogeneous metal catalysts for green and sustainable chemistry. Bull Chem Soc Jpn 79:981–1016
28. Suzuki T, Hatsushika T, Hayakawa Y (1981) Synthetic hydroxyapatites employed as inorganic cation-exchangers. J Chem Soc Faraday Trans 1 F 77:1059–1062
29. Ito A, Ojima K, Naito H, Ichinose N, Tateishi T (2000) Preparation, solubility, and cytocompatibility of zinc-releasing calcium phosphate ceramics. J Biomed Mater Res 50:178–183
30. Kawamura H, Ito A, Miyakawa S, Layrolle P, Ojima K, Ichinose N et al (2000) Stimulatory effect of zinc-releasing calcium phosphate implant on bone formation in rabbit femora. J Biomed Mater Res 50:184–190
31. Webster TJ, Massa-Schlueter EA, Smith JL, Slamovich EB (2004) Osteoblast response to hydroxyapatite doped with divalent and trivalent cations. Biomaterials 25:2111–2121
32. Atsumi K, Saito T, Komori M (1992) Process for producing an antibacterial ceramic material. Google Patents
33. Sakuma S, Atsumi K, Fujita K (1991). Antimicrobial hydroxyapatite powders and methods for preparing them. Google Patents
34. Atsumi K, Saito T, Komori M (1993) Antibacterial calcium phosphate ceramic. Google Patents
35. Radovanović Ž, Jokić B, Veljović D, Dimitrijević S, Kojić V, Petrović R et al (2014) Antimicrobial activity and biocompatibility of Ag+-and Cu2+-doped biphasic hydroxyapatite/α-tricalcium phosphate obtained from hydrothermally synthesized Ag+-and Cu2+-doped hydroxyapatite. Appl Surf Sci 307:513–519
36. Nightingale E Jr (1959) Phenomenological theory of ion solvation. Effective radii of hydrated ions. J Phys Chem 63:1381–1387
37. Knowles J, Gibson I, Abrahams I (1999) High temperature phase transitions in CA 3 (PO 4) 2 measured by neutron diffraction. In: Bioceramics, vol 12. World Scientific, pp 341–344
38. Radovanović Ž, Veljović D, Jokić B, Dimitrijević S, Bogdanović G, Kojić V et al (2012) Biocompatibility and antimicrobial activity of zinc (II)-doped hydroxyapatite, synthesized by a hydrothermal method. J Serb Chem Soc 77:1787–1798
39. Kim T, Feng QL, Kim J, Wu J, Wang H, Chen G et al (1998) Antimicrobial effects of metal ions (Ag+, Cu2+, Zn2+) in hydroxyapatite. J Mater Sci Mater Med 9:129–134
40. Stanić V, Dimitrijević S, Antić-Stanković J, Mitrić M, Jokić B, Plećaš IB et al (2010) Synthesis, characterization and antimicrobial activity of copper and zinc-doped hydroxyapatite nanopowders. Appl Surf Sci 256:6083–6089
41. Stanić V, Janaćković D, Dimitrijević S, Tanasković SB, Mitrić M, Pavlović MS et al (2011) Synthesis of antimicrobial monophase silver-doped hydroxyapatite nanopowders for bone tissue engineering. Appl Surf Sci 257:4510–4518
42. Tank KP, Chudasama KS, Thaker VS, Joshi MJ (2014) Pure and zinc doped nanohydroxyapatite: synthesis, characterization, antimicrobial and hemolytic studies. J Cryst Growth 401:474–479
43. Shanmugam S, Gopal B (2014) Copper substituted hydroxyapatite and fluorapatite: synthesis, characterization and antimicrobial properties. Ceram Int 40:15655–15662
44. Honda M, Kawanobe Y, Ishii K, Konishi T, Mizumoto M, Kanzawa N et al (2013) In vitro and in vivo antimicrobial properties of silver-containing hydroxyapatite prepared via ultrasonic spray pyrolysis route. Mater Sci Eng C 33:5008–5018

45. Ferraris S, Spriano S (2016) Antibacterial titanium surfaces for medical implants. Mater Sci Eng C 61:965–978
46. Shi C, Gao J, Wang M, Fu J, Wang D, Zhu Y (2015) Ultra-trace silver-doped hydroxyapatite with non-cytotoxicity and effective antibacterial activity. Mater Sci Eng C 55:497–505
47. Oh KS, Kim KJ, Jeong YK, Park EK, Kim SY, Kwon J, et al (eds) (2004) Cytotoxicity and antimicrobial effect of Ag doped hydroxyapatite. Key Engineering Materials. Trans Tech Publ
48. Oh KS, Park SH, Jeong YK (eds) (2004) Antimicrobial effects of Ag doped hydroxyapatite synthesized from co-precipitation route. Key Engineering Materials. Trans Tech Publ
49. Oh KS, Park SH, Jeong YK (eds) (2004) Durability in antimicrobial effects of silver doped hydroxyapatite depending on the synthesis route. Materials Science Forum. Trans Tech Publ
50. Dubnika A, Loca D, Salma I, Reinis A, Poca L, Berzina-Cimdina L (2014) Evaluation of the physical and antimicrobial properties of silver doped hydroxyapatite depending on the preparation method. J Mater Sci Mater Med 25:435–444
51. Popa CL, Deniaud A, Michaud-Soret I, Guégan R, Motelica-Heino M, Predoi D (2016) Structural and biological assessment of zinc doped hydroxyapatite nanoparticles. J Nanomater 2016:1062878
52. Ciobanu CS, Massuyeau F, Constantin LV, Predoi D (2011) Structural and physical properties of antibacterial Ag-doped nano-hydroxyapatite synthesized at 100 C. Nanoscale Res Lett 6:613
53. Ciobanu CS, Iconaru SL, Le Coustumer P, Constantin LV, Predoi D (2012) Antibacterial activity of silver-doped hydroxyapatite nanoparticles against gram-positive and gram-negative bacteria. Nanoscale Res Lett 7:324
54. Ciobanu CS, Iconaru SL, Chifiriuc MC, Costescu A, Le Coustumer P, Predoi D (2013) Synthesis and antimicrobial activity of silver-doped hydroxyapatite nanoparticles. Biomed Res Int 2013:916218
55. Singh B, Dubey AK, Kumar S, Saha N, Basu B, Gupta R (2011) In vitro biocompatibility and antimicrobial activity of wet chemically prepared Ca10− xAgx (PO4) 6 (OH) 2 (0.0≤ x≤ 0.5) hydroxyapatites. Mater Sci Eng C 31:1320–1329
56. Mocanu A, Furtos G, Rapuntean S, Horovitz O, Flore C, Garbo C et al (2014) Synthesis; characterization and antimicrobial effects of composites based on multi-substituted hydroxyapatite and silver nanoparticles. Appl Surf Sci 298:225–235
57. Hwang K-S, Hwangbo S, Kim J-T (2008) Silver-doped calcium phosphate nanopowders prepared by electrostatic spraying. J Nanopart Res 10:1337–1341
58. Jadalannagari S, Deshmukh K, Ramanan SR, Kowshik M (2014) Antimicrobial activity of hemocompatible silver doped hydroxyapatite nanoparticles synthesized by modified sol–gel technique. Appl Nanosci 4:133–141
59. Sikder P, Grice CR, Lin B, Goel VK, Bhaduri SB (2018) Single-phase, antibacterial trimagnesium phosphate hydrate coatings on polyetheretherketone (PEEK) implants by rapid microwave irradiation technique. ACS Biomater Sci Eng 4:2767–2783
60. Sikder P, Koju N, Ren Y, Goel VK, Phares T, Lin B et al (2018) Development of single-phase silver-doped antibacterial CDHA coatings on Ti6Al4V with sustained release. Surf Coat Technol 342:105–116
61. Sikder P, Bhaduri SB (2018) Microwave assisted synthesis and characterization of single-phase tabular hexagonal newberyite, an important bioceramic. J Am Ceram Soc 101:2537–2544
62. Rameshbabu N, Sampath Kumar T, Prabhakar T, Sastry V, Murty K, Prasad Rao K (2007) Antibacterial nanosized silver substituted hydroxyapatite: synthesis and characterization. J Biomed Mater Res A 80:581–591
63. Iqbal N, Kadir MRA, Malek NANN, Mahmood NH, Murali MR, Kamarul T (2012) Rapid microwave assisted synthesis and characterization of nanosized silver-doped hydroxyapatite with antibacterial properties. Mater Lett 89:118–122
64. Koju N, Sikder P, Gaihre B, B Bhaduri S (2018) Smart injectable self-setting monetite based bioceramics for orthopedic applications. Materials 11:1258

65. Neut D, Dijkstra R, Thompson J, Kavanagh C, van der Mei H, Busscher H (2015) A biodegradable gentamicin-hydroxyapatite-coating for infection prophylaxis in cementless hip prostheses. Eur Cell Mater 29:42–55
66. Taha M, Chai F, Blanchemain N, Neut C, Goube M, Maton M et al (2014) Evaluation of sorption capacity of antibiotics and antibacterial properties of a cyclodextrin-polymer functionalized hydroxyapatite-coated titanium hip prosthesis. Int J Pharm 477:380–389
67. Lee S-B, Otgonbayar U, Lee J-H, Kim K-M, Kim K-N (2010) Silver ion-exchanged sodium titanate and resulting effect on antibacterial efficacy. Surf Coat Technol 205:S172–S176
68. Fielding GA, Roy M, Bandyopadhyay A, Bose S (2012) Antibacterial and biological characteristics of silver containing and strontium doped plasma sprayed hydroxyapatite coatings. Acta Biomater 8:3144–3152
69. Roy M, Fielding GA, Beyenal H, Bandyopadhyay A, Bose S (2012) Mechanical, in vitro antimicrobial, and biological properties of plasma-sprayed silver-doped hydroxyapatite coating. ACS Appl Mater Interfaces 4:1341–1349
70. Guimond-Lischer S, Ren Q, Braissant O, Gruner P, Wampfler B, Maniura-Weber K (2016) Vacuum plasma sprayed coatings using ionic silver doped hydroxyapatite powder to prevent bacterial infection of bone implants. Biointerphases 11:011012
71. Ha S-W, Mayer J, Koch B, Wintermantel E (1994) Plasma-sprayed hydroxylapatite coating on carbon fibre reinforced thermoplastic composite materials. J Mater Sci Mater Med 5:481–484
72. Noda I, Miyaji F, Ando Y, Miyamoto H, Shimazaki T, Yonekura Y et al (2009) Development of novel thermal sprayed antibacterial coating and evaluation of release properties of silver ions. J Biomed Mater Res B Appl Biomater 89:456–465
73. Shimazaki T, Miyamoto H, Ando Y, Noda I, Yonekura Y, Kawano S et al (2010) In vivo antibacterial and silver-releasing properties of novel thermal sprayed silver-containing hydroxyapatite coating. J Biomed Mater Res B Appl Biomater 92:386–389
74. Geng Z, Cui Z, Li Z, Zhu S, Liang Y, Liu Y et al (2016) Strontium incorporation to optimize the antibacterial and biological characteristics of silver-substituted hydroxyapatite coating. Mater Sci Eng C 58:467–477
75. Vilardell AM, Cinca N, Concustell A, Dosta S, Cano I, Guilemany J (2015) Cold spray as an emerging technology for biocompatible and antibacterial coatings: state of art. J Mater Sci 50:4441–4462
76. Li D, Gong Y, Chen X, Zhang B, Zhang H, Jin P et al (2017) Room-temperature deposition of hydroxyapatite/antibiotic composite coatings by vacuum cold spraying for antibacterial applications. Surf Coat Technol 330:87–91
77. Chung RJ, Hsieh MF, Huang CW, Perng LH, Wen HW, Chin TS (2006) Antimicrobial effects and human gingival biocompatibility of hydroxyapatite sol–gel coatings. J Biomed Mater Res B Appl Biomater 76:169–178
78. Samani S, Hossainalipour S, Tamizifar M, Rezaie H (2013) In vitro antibacterial evaluation of sol–gel-derived Zn-, Ag-, and (Zn+ Ag)-doped hydroxyapatite coatings against methicillin-resistant Staphylococcus aureus. J Biomed Mater Res A 101:222–230
79. Iconaru S, Prodan A, Buton N, Predoi D (2017) Structural characterization and antifungal studies of zinc-doped hydroxyapatite coatings. Molecules 22:604
80. Qu J, Lu X, Li D, Ding Y, Leng Y, Weng J et al (2011) Silver/hydroxyapatite composite coatings on porous titanium surfaces by sol-gel method. J Biomed Mater Res B Appl Biomater 97:40–48
81. Kaviyarasu K, Mariappan A, Neyvasagam K, Ayeshamariam A, Pandi P, Palanichamy RR et al (2017) Photocatalytic performance and antimicrobial activities of HAp-TiO2 nanocomposite thin films by sol-gel method. Surf Interfaces 6:247–255
82. Chen W, Liu Y, Courtney H, Bettenga M, Agrawal C, Bumgardner J et al (2006) In vitro anti-bacterial and biological properties of magnetron co-sputtered silver-containing hydroxyapatite coating. Biomaterials 27:5512–5517
83. Bai X, More K, Rouleau CM, Rabiei A (2010) Functionally graded hydroxyapatite coatings doped with antibacterial components. Acta Biomater 6:2264–2273

84. Trujillo NA, Oldinski RA, Ma H, Bryers JD, Williams JD, Popat KC (2012) Antibacterial effects of silver-doped hydroxyapatite thin films sputter deposited on titanium. Mater Sci Eng C 32:2135–2144
85. Surmeneva MA, Sharonova AA, Chernousova S, Prymak O, Loza K, Tkachev MS et al (2017) Incorporation of silver nanoparticles into magnetron-sputtered calcium phosphate layers on titanium as an antibacterial coating. Colloids Surf B: Biointerfaces 156:104–113
86. Ivanova AA, Surmeneva MA, Tyurin A, Pirozhkova T, Shuvarin I, Prymak O et al (2016) Fabrication and physico-mechanical properties of thin magnetron sputter deposited silver-containing hydroxyapatite films. Appl Surf Sci 360:929–935
87. Ivanova AA, Surmenev RA, Surmeneva MA, Mukhametkaliyev T, Loza K, Prymak O et al (2015) Hybrid biocomposite with a tunable antibacterial activity and bioactivity based on RF magnetron sputter deposited coating and silver nanoparticles. Appl Surf Sci 329:212–218
88. Hadidi M, Bigham A, Saebnoori E, Hassanzadeh-Tabrizi S, Rahmati S, Alizadeh ZM et al (2017) Electrophoretic-deposited hydroxyapatite-copper nanocomposite as an antibacterial coating for biomedical applications. Surf Coat Technol 321:171–179
89. Huang Y, Zhang X, Zhao R, Mao H, Yan Y, Pang X (2015) Antibacterial efficacy, corrosion resistance, and cytotoxicity studies of copper-substituted carbonated hydroxyapatite coating on titanium substrate. J Mater Sci 50:1688–1700
90. Xie C-M, Lu X, Wang K-F, Meng F-Z, Jiang O, Zhang H-P et al (2014) Silver nanoparticles and growth factors incorporated hydroxyapatite coatings on metallic implant surfaces for enhancement of osteoinductivity and antibacterial properties. ACS Appl Mater Interfaces 6:8580–8589
91. Eraković S, Janković A, Veljović D, Palcevskis E, Mitrić M, Stevanović T et al (2012) Corrosion stability and bioactivity in simulated body fluid of silver/hydroxyapatite and silver/hydroxyapatite/lignin coatings on titanium obtained by electrophoretic deposition. J Phys Chem B 117:1633–1643
92. Mirzaee M, Vaezi M, Palizdar Y (2016) Synthesis and characterization of silver doped hydroxyapatite nanocomposite coatings and evaluation of their antibacterial and corrosion resistance properties in simulated body fluid. Mater Sci Eng C 69:675–684
93. Shi Y, Li M, Liu Q, Jia Z, Xu X, Cheng Y et al (2016) Electrophoretic deposition of graphene oxide reinforced chitosan–hydroxyapatite nanocomposite coatings on Ti substrate. J Mater Sci Mater Med 27:48
94. Song WH, Ryu HS, Hong SH (2009) Antibacterial properties of Ag (or Pt)-containing calcium phosphate coatings formed by micro-arc oxidation. J Biomed Mater Res Part A 88:246–254
95. Hu H, Zhang W, Qiao Y, Jiang X, Liu X, Ding C (2012) Antibacterial activity and increased bone marrow stem cell functions of Zn-incorporated TiO2 coatings on titanium. Acta Biomater 8:904–915
96. Necula B, Apachitei I, Tichelaar F, Fratila-Apachitei L, Duszczyk J (2011) An electron microscopical study on the growth of TiO2–Ag antibacterial coatings on Ti6Al7Nb biomedical alloy. Acta Biomater 7:2751–2757
97. Necula B, Apachitei I, Fratila-Apachitei L, Van Langelaan E, Duszczyk J (2013) Titanium bone implants with superimposed micro/nano-scale porosity and antibacterial capability. Appl Surf Sci 273:310–314
98. Venkateswarlu K, Rameshbabu N, Bose AC, Muthupandi V, Subramanian S, MubarakAli D et al (2012) Fabrication of corrosion resistant, bioactive and antibacterial silver substituted hydroxyapatite/titania composite coating on Cp Ti. Ceram Int 38:731–740
99. Koju N, Sikder P, Ren Y, Zhou H, Bhaduri SB (2017) Biomimetic coating technology for orthopedic implants. Curr Opin Chem Eng 15:49–55
100. Ren Y, Sikder P, Lin B, Bhaduri SB (2018) Microwave assisted coating of bioactive amorphous magnesium phosphate (AMP) on polyetheretherketone (PEEK). Mater Sci Eng C 85:107–113
101. Bhaduri SB, Goel VK, Ren Y, Sikder P (2018) Bifunctional bioactive antibacterial coatings, and process for coating implant surfaces therewith. Google Patents

102. Huang Y, Hao M, Nian X, Qiao H, Zhang X, Zhang X et al (2016) Strontium and copper co-substituted hydroxyapatite-based coatings with improved antibacterial activity and cytocompatibility fabricated by electrodeposition. Ceram Int 42:11876–11888
103. Geng Z, Wang R, Zhuo X, Li Z, Huang Y, Ma L et al (2017) Incorporation of silver and strontium in hydroxyapatite coating on titanium surface for enhanced antibacterial and biological properties. Mater Sci Eng C 71:852–861
104. Huang Y, Xu Z, Zhang X, Chang X, Zhang X, Li Y et al (2017) Nanotube-formed Ti substrates coated with silicate/silver co-doped hydroxyapatite as prospective materials for bone implants. J Alloys Compd 697:182–199
105. Zhang Y, Zhai D, Xu M, Yao Q, Zhu H, Chang J et al (2017) 3D-printed bioceramic scaffolds with antibacterial and osteogenic activity. Biofabrication 9:025037
106. Shen J, Jin B, Qi Y-c, Q-y J, X-f G (2017) Carboxylated chitosan/silver-hydroxyapatite hybrid microspheres with improved antibacterial activity and cytocompatibility. Mater Sci Eng C 78:589–597
107. Bhowmick A, Banerjee SL, Pramanik N, Jana P, Mitra T, Gnanamani A et al (2018) Organically modified clay supported chitosan/hydroxyapatite-zinc oxide nanocomposites with enhanced mechanical and biological properties for the application in bone tissue engineering. Int J Biol Macromol 106:11–19
108. Dubnika A, Loca D, Rudovica V, Parekh MB, Berzina-Cimdina L (2017) Functionalized silver doped hydroxyapatite scaffolds for controlled simultaneous silver ion and drug delivery. Ceram Int 43:3698–3705
109. Xu Z-L, Lei Y, Yin W-J, Chen Y-X, Ke Q-F, Guo Y-P et al (2016) Enhanced antibacterial activity and osteoinductivity of Ag-loaded strontium hydroxyapatite/chitosan porous scaffolds for bone tissue engineering. J Mater Chem B 4:7919–7928
110. Yu W-Z, Zhang Y, Liu X, Xiang Y, Li Z, Wu S (2018) Synergistic antibacterial activity of multi components in lysozyme/chitosan/silver/hydroxyapatite hybrid coating. Mater Des 139:351–362
111. Yan L, Xiang Y, Yu J, Wang Y, Cui W (2017) Fabrication of antibacterial and antiwear hydroxyapatite coatings via in situ chitosan-mediated pulse electrochemical deposition. ACS Appl Mater Interfaces 9:5023–5030
112. Liu X, Man H (2017) Laser fabrication of Ag-HA nanocomposites on Ti6Al4V implant for enhancing bioactivity and antibacterial capability. Mater Sci Eng C 70:1–8
113. Hidalgo-Robatto B, López-Álvarez M, Azevedo A, Dorado J, Serra J, Azevedo N et al (2018) Pulsed laser deposition of copper and zinc doped hydroxyapatite coatings for biomedical applications. Surf Coat Technol 333:168–177
114. Andrade FAC, de Oliveira Vercik LC, Monteiro FJ, da Silva Rigo EC (2016) Preparation, characterization and antibacterial properties of silver nanoparticles–hydroxyapatite composites by a simple and eco-friendly method. Ceram Int 42:2271–2280
115. Tian B, Chen W, Dong Y, Marymont JV, Lei Y, Ke Q et al (2016) Silver nanoparticle-loaded hydroxyapatite coating: structure, antibacterial properties, and capacity for osteogenic induction in vitro. RSC Adv 6:8549–8562
116. Tian B, Chen W, Yu D, Lei Y, Ke Q, Guo Y et al (2016) Fabrication of silver nanoparticle-doped hydroxyapatite coatings with oriented block arrays for enhancing bactericidal effect and osteoinductivity. J Mech Behav Biomed Mater 61:345–359
117. Ai M, Du Z, Zhu S, Geng H, Zhang X, Cai Q et al (2017) Composite resin reinforced with silver nanoparticles–laden hydroxyapatite nanowires for dental application. Dent Mater 33:12–22
118. Karbowniczek J, Cordero-Arias L, Virtanen S, Misra SK, Valsami-Jones E, Tuchscherr L et al (2017) Electrophoretic deposition of organic/inorganic composite coatings containing ZnO nanoparticles exhibiting antibacterial properties. Mater Sci Eng C 77:780–789
119. Nabiyouni M, Brückner T, Zhou H, Gbureck U, Bhaduri SB (2018) Magnesium-based bioceramics in orthopedic applications. Acta Biomater 66:23–43
120. Touny AH, Sikder P, Saleh MM, Bhaduri SB (2019) Facile synthesis and characterization of biphasic magnesium phosphate bioceramic nanosheets by a reflux approach. Mater Res Express 6(9)

121. Sikder P, Grice C, Bhaduri SB (2019) Processing-structure-property correlations of crystalline antibacterial magnesium phosphate (newberyite) coatings and their in vitro effect. Surf Coat Technol 374:276–290
122. Sikder P, Bhaduri SB (2019) Regulatory aspects of medical devices and biomaterials. In: Biomaterials in translational medicine. Elsevier, pp 23–36
123. Sikder P, Liu L, Jayatissa AH, Bhaduri SB (2019) Antibacterial silver (Ag) containing titanium oxynitride (TiOxNy) coatings for inhibiting surgical site infections (SSI). Medi Devices & Sensors
124. Prabaha Sikder, Sarit B. Bhaduri, Joo L. Ong, Teja Guda (2019) Silver (Ag) doped magnesium phosphate microplatelets as next-generation antibacterial orthopedic biomaterials. Journal of Biomedical Materials Research Part B: Applied Biomaterials
125. Sikder P, Ren Y, Bhaduri SB. Synthesis and evaluation of protective poly (lactic acid) and fluorine-doped hydroxyapatite–based composite coatings on AZ31 magnesium alloy. Journal of Materials Research:1-1

3D Printed Ceramic-Polymer Composites for Treating Bone Infection

Anusha Elumalai, Yangyang Lou, Ahmed Humayun, A. J. McFarland, and David K. Mills

Abstract It is well known that bacteria and host cells are in a competitive race for the surfaces of dental and orthopedic implants. If bacteria win the race and a biofilm forms, this can lead to infection, and postsurgical complications that may include revision procedures and increased hospital stays can cost thousands of dollars for a single patient, significant lost time from work, altered and restricted lifestyles, and, death. Bone infections are treated with antibiotics given intravenously or orally, via antibiotic-releasing bone cement or collagen sponges placed directly within the infected area. Collectively, these approaches have limited effectiveness due to the lack of site specificity, uncontrolled release, and additional surgeries. Antibiotics currently in use suffer from systemic toxicity, short half-life, and increases in bacterial resistance. This chapter will cover topics related to antimicrobial biomaterials (e.g., antibiotics, antimicrobial peptides, etc.), antimicrobial coatings, antimicrobial drug delivery vehicles, as well as research integrating both antimicrobial and osteoinductive/osteoconductive properties. Antibiotic resistance and implants ineffective in inhibiting antimicrobial growth offer to shift the race in favor of bacteria. Strategies designed to increase bacterial resistance and offer a supportive environment for resistant pre-osteoblasts and osteoblasts will also be discussed.

Keywords 3D printing · Bone infection · Bone regeneration · Ceramics · Composites · Polymers

A. Elumalai · Y. Lou · A. Humayun · A. J. McFarland
Molecular Science and Nanotechnology, Louisiana Tech University, Ruston, LA, USA
e-mail: ael028@latech.edu; ylu003@latech.edu; ahu005@latech.edu

D. K. Mills (✉)
Biomedical Engineering, Louisiana Tech University, Ruston, LA, USA

School of Biological Sciences, Louisiana Tech University, Ruston, LA, USA
e-mail: dkmills@latech.edu

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7_25

The Need for Customized and Personalized Treatments

Bone defects due to trauma, age, or bacterial infection must be surgically treated due to their low potential of repair [1, 2]. It is well known that bacteria and host cells are in a competitive race for the surfaces of dental and orthopedic implants. This chapter will cover topics related to antimicrobial biomaterials (e.g., antibiotics, antimicrobial peptides, vaccines, immunotherapeutic approaches, etc.), antimicrobial coatings, antimicrobial drug delivery vehicles, as well as research integrating both antimicrobial and osteoinductive/osteoconductive properties. Antibiotic resistance, which shifts the race toward bacteria, and strategies to reduce antibiotic resistance will also be included.

Several strategies have used implant surface modification or coating with antimicrobial agents to prevent bacterial colonization and biofilm formation [3, 4]. The antibiotics used to treat bone infections are given intravenously or orally, through antibiotic-releasing bone cement, or collagen sponges [5, 6]. Antibiotics currently used suffer from systemic toxicity, short half-life, and may lead to an increase in antibiotic resistance [5, 7]. Collectively, these approaches have shown limited effectiveness due to the lack of site specificity, uncontrolled release, and ineffective control over microbial growth.

Impaired fracture healing or nonunion bone defects often result in functional disability and represents a major clinical challenge [8, 9]. Currently, autografts, allografts, and bone implant substitutes represent 80% of all transplantations worldwide [10, 11]. While autografts represent the gold standard; there are the attendant risks of donor site morbidity and limited availability [6]. Allografting also has limitations such as potential infection, a high nonunion rate, and high costs [7, 8]. Orthopedic and reconstructive surgeries are using tissue engineering as a more effective alternative to current grafting materials [12, 13]. The development of a biodegradable, multi-functional package that combats infection, while simultaneously initiating bone tissue formation, is a reasonable solution to the current situation. Such a package requires four components: a morphogenetic signal, responsive host cells, a suitable carrier, and a “tunable” character [14, 15]. Further, this system must be capable of providing controlled and sustained release of growth factors, bioactive molecules, and antimicrobial agents, and in a combinatorial fashion. When delivered to affected sites it must act as a scaffold for cell growth leading to accelerated healing and enhanced tissue formation.

An Overview of Three-Dimensional (3D) Printing

3D printing is being rapidly adopted in the biomedical field, with extensive research focused on the development of novel materials and combinatorial techniques that will enhance product functionality. 3D printing offer design freedom, faster product development, local production, and carries the promise of revolutionizing the quality and efficiency of healthcare [16, 17]. It enables the printing of an object from a single polymer or polymer composites with controlled spatial heterogeneity

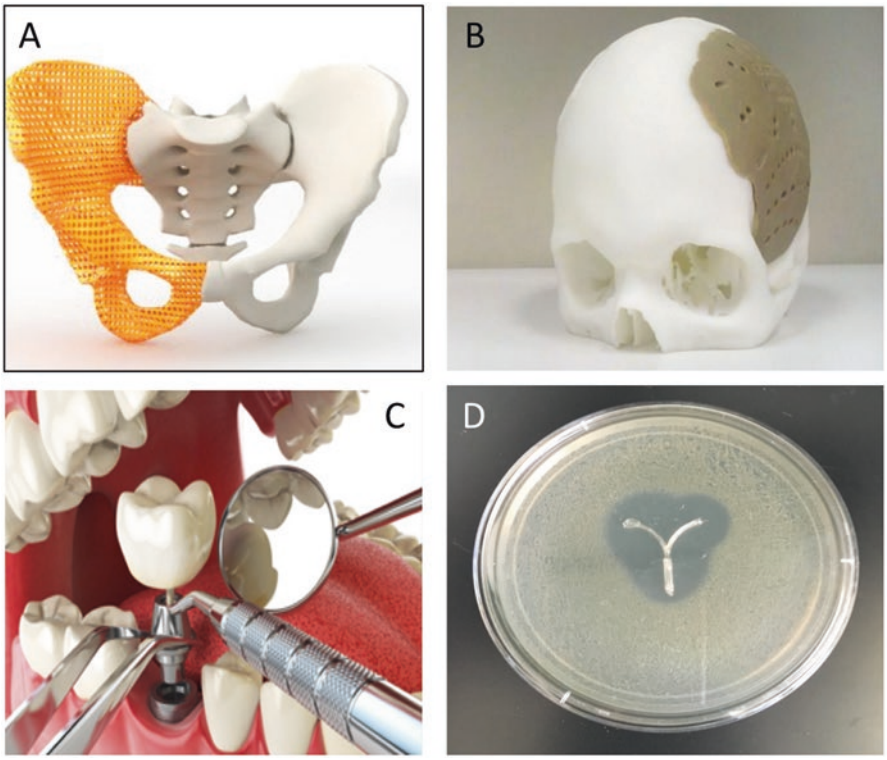


Fig. 1 Images of 3D printed medical devices. (**A**). Right innominate bone; (**B**) Parietal and temporal bones of the skull; (**C**) Mandibular crown; (**D**) Antibiotic-doped IUD

for superior structure–function relationships, specific internal and external geometries, and customized designs (Fig. 1).

3D printing has the potential to offer patient-specific solutions and personalized medical devices designed using images acquired from a patient and fabricated to fit their treatment needs. Complex shapes, articulations, and miniaturized geometries of implantable medical devices and instruments have the potential to radically enhance treatment effectiveness and posttreatment recovery.

Antimicrobial Materials

Clay Nanoparticles

Nanoclays are inexpensive materials widely used in biomedical and industrial applications and can be classified into two categories: natural and synthetic clays [18]. Nanoclays are clay minerals with at least one dimension in the order of 1–100 nm

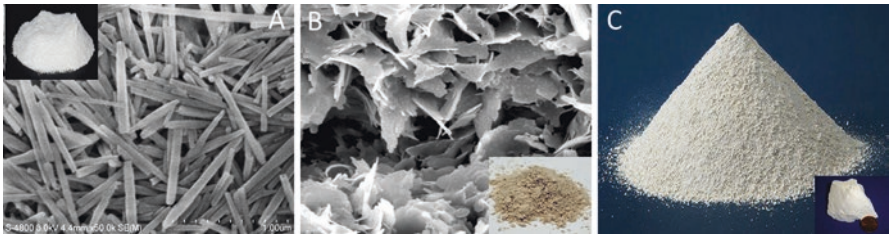


Fig. 2 Examples of widely used clay nanoparticles. (**A**). Scanning electron microscopy (SEM) image of halloysite; insert halloysite in native powder form. (**B**) SEM image of montmorillonite, inert—native powder ; (**C**) Kaolin powder and native form

and usually can be obtained by the ion exchange reaction of hydrophilic clay [19]. The beneficial properties of these kinds of nanoparticles are their high aspect ratio, no mutagenic effect on the body, cyto- and biocompatibility, their "green" environmentally friendly nature, thickness of less than one nanometer, and a large surface area in the range of 700 square meters per gram [20, 21] (Fig. 2).

In order to increase polymeric matrix properties, additionally, nanoclays have been used for synthesizing polymer matrix–nanoclay composites and used for varied applications including as part of an antimicrobial drug delivery system.

Nanoclays

Halloysite

Halloysite is an aluminosilicate clay chemically similar to kaolin, but different in having a hollow microtubular structure [22, 23]. It is found abundantly in naturally occurring deposits in the United States, China, Brazil, Japan, and South Korea. Within individual countries, there may be significant differences in halloysite morphology in terms of size, specific surface area, and porosity because of the exact forces of nature involved in their formation [23]. Compared to other nanomaterials, halloysite is easily obtained and is an inexpensive nanoscale container [5]. Since 2008, numerous studies have established HNTs as a viable nanocontainer capable of entrapping a range of active agents within the inner lumen, followed by their retention and slow release [23]. HNTs have been used in various applications including antimicrobial formulations, dental and orthopedic implants, in drug delivery systems and in anticorrosion, flame-retardant, and toxic absorption application [24].

The surface chemistry of HNTs is versatile for the targeted chemical modification of the inner lumen and outer surface. Functionalized halloysite constitutes a valuable support for metal nanoparticles, promoting catalytic applications with tunable properties. The peculiar tubular shape of HNTs favors the dispersion and surface availability of the supported metal nanoparticles that are active in the catalytic

path. HNTs have been shown to be non-cytotoxic on several cell types (up to concentrations of 0.1 mg/mL) [25]. Mesenchymal stem cells, fibrochondrocytes, osteoblasts, and human dermal fibroblasts cultured on halloysite nanofilms showed no cytotoxic effects; all cells proliferated and expressed proteins showing that they maintained cellular phenotype on the HNT thin films [25, 26].

HNTs have shown use in a number of 3D printed applications including regenerative medicine [27] and tissue engineering [28]. Weisman et al. (2017) used gentamicin-doped HNTs and poly(lactic acid) (PLA) to fabricate GS releasing disks, beads, and pellets [29]. Gentamicin was released from 3D printed constructs in a sustained manner and had a superior antibacterial growth inhibition effect that was dependent on GS doping concentration. 3D printed antimicrobial devices have been produced but few have been directed at treating bone infections [30, 31]. While most 3D printing applications have focused on tissue regeneration, doped HNTs incorporated into 3D printed thermoplastic polymers holds great potential for the development of devices with multiple functionalities.

Laponite

Laponite (LAP) is a form of isomorphous substituted smectite clay and is a layered aluminosilicate disk-like clay material [32]. Because the interlayer space can be used for effective drug encapsulation with high retention capacity, LAP and other smectite clays have been used as drug carriers [33]. It has not seen much use, as yet, in 3D printed medical devices.

Montmorillonite

Montmorillonite (MMT) appears in nature in varied colors (yellow-green, yellow-white, gray and white) due to the presence of trace metals [34]. MTT is structured as multiple layered sheets with each layer comprised on an octahedral alumina sheet sandwiched between two tetrahedral silica sheets [34]. MTT's large specific surface area, exhibits good absorbance ability, high cation exchange capacity, standout adhesiveness, and drug-carrying capability. Thus, MMT has been used in antimicrobial applications targeting infections of bone usually complexed with another polymer such as chitosan [34] or metals such as copper and zinc [35]. Its application in 3D printing of medical devices has been limited to date.

Metal Nanoparticles

Reports of evolving multidrug resistant bacterial strains and increasing case of antibiotic poisoning due to use of high dosage are increasing at an alarming rate which has made development of newer alternative methods to address this situation

which is the need of the hour [35]. Antibiotics exert antimicrobial effect by affecting bacterial genome and protein translation machinery, however, over time mutant strains have developed antibiotic resistant genes expressing antibiotic degrading enzymes [36] (Fig. 3).

The antimicrobial effects of heavy metals have been known and used over many centuries. Silver was used in wound care, and as self-sanitizing cutlery similarly, copper has been used in brass for storing water, gold has been used in dental fillings, and arsenic has been used in the treatment of syphilis [37]. This can be attributed damage to bacterial cell by production of reactive oxygen species, protein denaturing, and cell membrane damage [38]. Additionally, metals, such as copper, silver, and zinc, exert toxicity on mammalian cells even at low concentrations. Reduction in the size of a metal particle amplifies its toxicity even in small amounts. In this regard, nanotechnology holds great promise; silver, copper, zinc, and titanium oxide nanoparticles have shown increased efficacy against prokaryotes [39].

Metal nanoparticles find utility in various medical applications including creating anti-biofouling surfaces, implant coatings, and use as topical agents for cutaneous skin disorders. They have the potential to act as a structural reinforcer, stimulate angiogenesis, promote extracellular matrix synthesis, inhibit bone resorption, and many have inherent antimicrobial activity [40]. Multiple factors affect the success of antimicrobial devices including osseointegration, degradation, anti-biofouling, and angiogenesis capability [41].

Incorporation of several metallic nanoparticles, having an inherent antimicrobial activity, such as silver [41], strontium [42], zinc oxide [43], and copper [44], have been further shown to improve implant success by exhibiting anti-biofouling effects. Metal nanoparticles at higher concentrations exhibit a bactericidal effect but also toxicity to eukaryotic cell lines as well. Several studies have reported that combinations of the metal nanoparticles can be lethal even at low concentrations [45]. Combinations of silver, copper, and zinc nanoparticles have been shown to exhibit synergistic antimicrobial activity, which can be attributed to increased prokaryotic cell permeability [46, 47]. Furthermore, metal nanoparticles when combined with

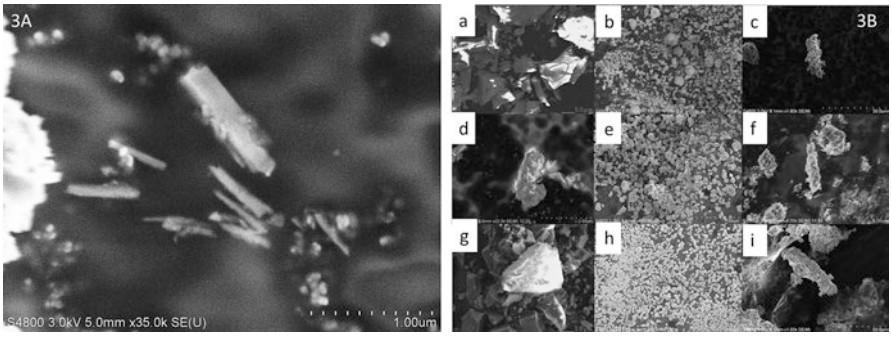


Fig. 3 (**a**) Scanning electron micrographs (SEM). Left SEM is of an uncoated halloysite nanotubes (HNTs). Right SEM is a composite figure of (**a**, **d**, **g**) silver nitrate, copper sulfate, and zinc oxide nanoparticles, respectively; **b**, **e**, **h**). silver, copper and zinc nanoparticles, respectively; (**c**, **f**, **i**) silver, copper and zinc nanoparticle composites, respectively

antibiotics and a biopolymer have shown similar augmented antimicrobial effects [48–50]. Metal nanoparticles have also been combined with ceramics and clay nanoparticles. Metal nanoparticles, for example, deposited on the outer surface of halloysite nanotubes have shown an enhanced antimicrobial activity [51].

Many biomaterials for bone tissue engineering suffer drawbacks, such as low antimicrobial properties and poor mechanical qualities leading to fracture fatigue, are not biodegradable or lack osteogenic potential, making them impractical candidates for many applications and often require structural modification. These shortcomings can be improved by blending/modification with metal nanoparticles. Hydroxyapatite (HA), incorporated with strontium, zinc, silver, iron, and titanium nanoparticles, respectively, have shown not only an increased anti-biofouling effect but also improved tensile properties after their addition [52].

Metal nanoparticles including copper [53], strontium [54], and cobalt [55] have also shown strong angiogenic effects. Similarly, the addition of silicon [56], zinc [57], cobalt [58], and copper [59] to another promising bone regeneration material, bioactive glass ceramic has been shown to promote angiogenesis. Metal nanoparticles have also been shown to alter the surface topological properties and assist in cellular attachment and thus help in cellular proliferation. Addition of titanium [60] and iron [61] has been shown to promote collagen synthesis and calcium deposition resulting in improved mechanical properties. Several nanoparticles including bisphosphate-conjugated gold nanoparticles have been shown to inhibit bone resorption by reducing osteoclasts [62]; copper [62] and gold [63], has likewise shown to increase the expression of mesenchymal stem cells and; HA-coated magnetite nanoparticles having magnetic properties have been shown to enhance calcium, collagen, and protein synthesis [47]. Incorporation of several metallic nanoparticles having an inherent antimicrobial activity such as silver [51], strontium [64], zinc oxide, and titanium [65, 66], have been shown further help improve implants by exhibiting anti-biofouling effects.

Applications in Dental and Orthopedic Surgery

Antimicrobial Coatings

At the present time with advancements in surgical environment standards, sterilization technology, and antibiotic therapies, postsurgical infections have dramatically decreased. Nevertheless, the bacterial dormancy is very long, and an attendant infection may appear weeks after the initial surgery [67]. *Staphylococcus* species are responsible for nearly 30% of all orthopedic implant-related infections [67]. Once the infection breaks out, the formation of bacterial colonies and biofilm initiate host immune responses. Conventional debridement treatments are not always effective on infections that are already established [68]. Improper dealing with infection results in tissue morbidity, implant failure, and making patients surfer in pain. The severe infection even risk patients' life. Currently, antibiotic administra-

tion and surgical intervention are only provided after an infection is present. Current research is directed toward the design of an implant surface that improves osseointegration and simultaneously inhibits infection. This has come in implant research as the "Holy Grail."

Antimicrobial Coatings that Prevent Microbial Adhesion

Utilizing the specific physical properties of biomaterials has attracted more attention to prevent bacterial attachment. For instance, coating with poly(ethylene glycol) (PEG) or PEGylated material, allows for maintenance of interfacial water, which inhibits microbes from strongly attaching via chemical bonds. Simultaneously, it can reduce the adhesion of proteins and mammalian cells [69–71]. Hydrophilic polymer brush coating is another way to repel bacteria. One or more polymers are grafted or tethered to a solid substrate at high density; due to volume-excluded effects polymers stretch away from the surface and exhibit different properties from the original chain conformation. Incorporating with antimicrobial peptide Tet213, a functional brush consisting of poly (DMA-coAPMA) for Ti surface coating against *Pseudomonas aeruginosa* was designed by Gao et al. [72] Surface coating with multiple biodegradable layers has also shown great promise on bacterial resistance. A variety of strategies are involved in limiting bacterial adhesion and promoting bone healing potential of cellulose derivatives in the presence of cellulose, which is chiefly produced by numerous microbial strains [73, 74]. The degradation of polymer composites can remove any bacteria that initially adhered to the original surface and pregrafted antimicrobial groups (metals, peptides) can provide further antimicrobial activity [75] (Fig. 4).

Antimicrobial Coatings That Kill Bacteria

Repelling bacteria from implants surface based on alteration of physicochemical properties has been a long-standing approach. A more preemptive approach is also to kill bacteria in the surrounding implant microenvironment with antimicrobial agents embedded in coating materials or directly deposited on the implant surface. Tet213 and HHC36, for example, are cationic peptides, that have broadly antimicrobial activity spectra against both Gram-negative (*Pseudomonas aeruginosa)* and Gram-positive (*Staphylococcus aureus*) bacteria. They are loaded into calcium phosphate (CaP) for Ti coating and provide local drug delivery [72, 73]. Quaternary ammonium chitosan, which presents positive charge, also exhibits a strong antibacterial activity (Fig. 5).

In a recent study of Xu et al. the synthesized *N,O*-quaternary ammonium chitosan performed an excellent and the best antibacterial activity compared with all other non-quaternized chitosan groups [75]. A detailed introduction of quaternized chitosan properties and applications were reviewed by Tan et al. [76] Despite of the

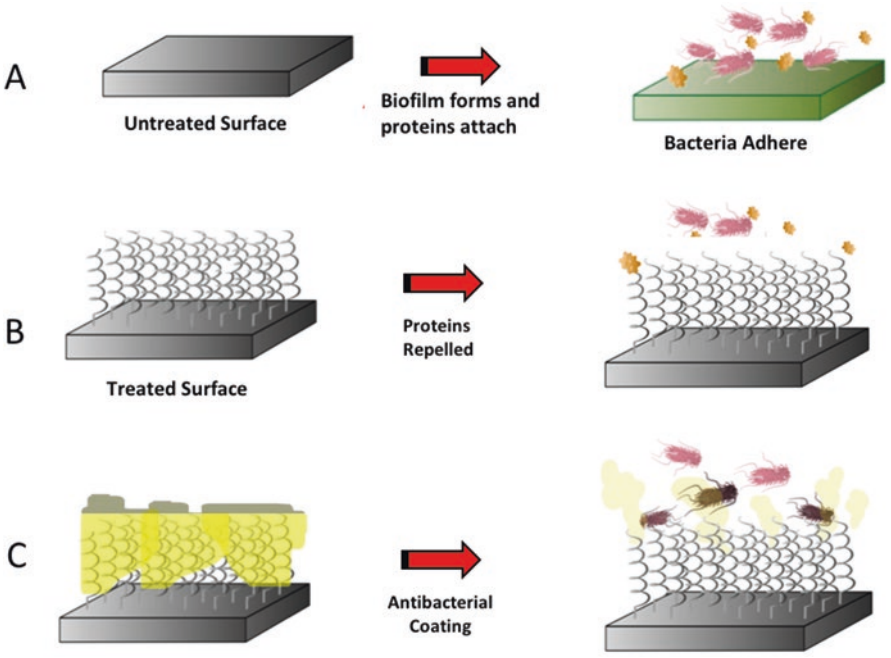


Fig. 4 This graphic depicts several strategies aimed at preventing bacterial colonization of an implant surface. (**A**) An untreated implant surface leads to protein absorption followed by bacterial adhesion and then colonization with subsequent biofilm formation. (**B**) Preventing protein attachment can aid in the prevention of bacterial adhesion and colonization. (**C**) The incorporation of antimicrobial agents and drugs on the implant surface aides in killing bacteria within the surrounding microenvironment. Many surface modification strategies combine elements of prevention and killing, which promotes colonization by osteoblasts leading to bone tissue formation and osseointegration

covalent attachment of polycationic groups, the direct implantation of numerous cations (Ca+, N+, F+) on implants surface [77, 78] or various metal-impregnated coatings (Ag, CuO, Cu_2O, ZnO, TiO_2) are also commonly used for killing bacteria [79–81] (Fig. 6).

Antimicrobial Coatings That Promote Bone Healing

Bacterial infection is initiated by the adhesion of microorganisms on implant surfaces, and prosthesis-associated infections established by further interactions between bacteria and biomaterial surfaces [82]. Thus, another efficient way to prevent implant-related infections is to accelerate the adhesion of desired osteocytes, enabling these cells to dominate over bacteria in the race for the implants surface. Biomaterials, such as HA and CaP ceramics, have been demonstrated to have favorable osteoconductive properties [83, 84]. Their incorporation, with transforming

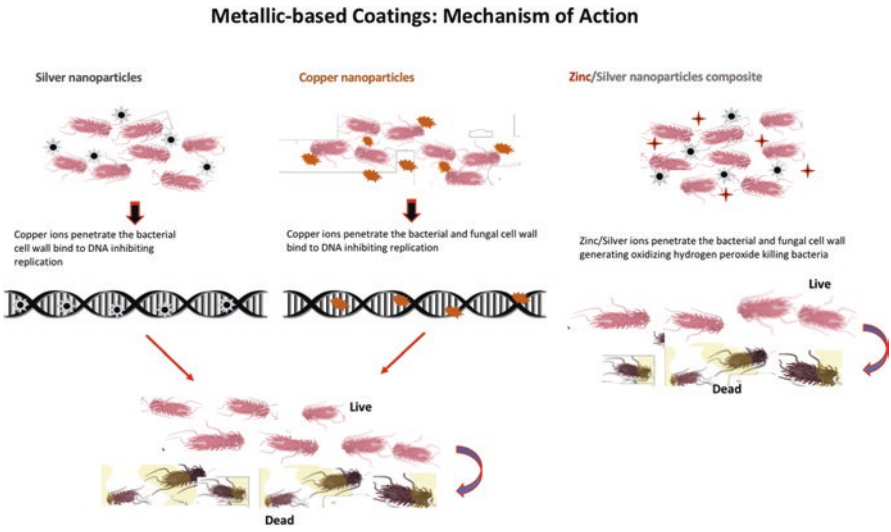


Fig. 5 Applications of metal nanoparticle in implant coatings have varying antimicrobial effects from DNA disruption to prevention of cell replication

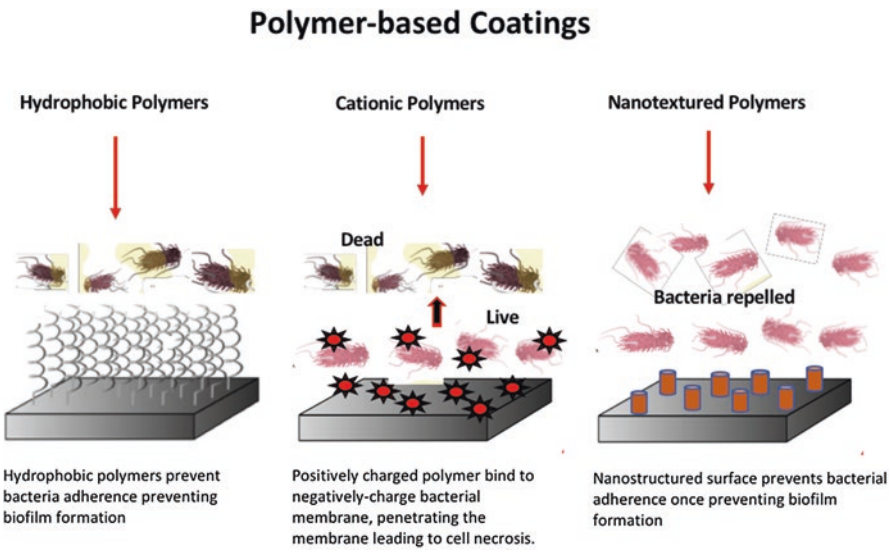


Fig. 6 Polymer-based coatings are designed to either kill or repel bacteria and thus prevent formation of a biofilm

growth factor-β-1 [85] or one of the bone morphogenic proteins (BMPs) [86], has shown improved osteoinductivity leading to enhanced bone reformation.

Coating biomaterials that biomimick native ECM is a huge temptation to attract cells setting. Implants coated by collagen, RGD proteins, and chondroitin sulfate have been observed successfully to enhance cell adhesion and bone growth [87, 88].

As integrins take an important role in regulating cellular interactions with ECM [89], biomaterials anchored with peptides targeting integrin receptor α5β1, ũFC;2β1, and αvβ3 could stimulate cell adhesion and osteogenesis [90, 91] (Fig. 7).

Antimicrobial Coatings with Multiple Roles

In clinical conditions, there is usually more than one medical problem that needs to be addressed, and coatings with multiple properties are a promising technique for resolving these problems. Many studies combined the beneficial properties of the above methods used more than one coating materials to improve osseointegration and antimicrobial efficacy. In the report of Uskoković [92], clindamycin was loaded in poly- (D, L-lactide-co-glycolide)-coated calcium phosphate nanoparticles. The antibiotic-containing powders exhibited sustained drug release against *Staphylococcus aureus*. Simultaneously, the increased expression of osteogenic markers including osteocalcin, osteopontin, Runx2, and procollagen type I, suggested their ability to promote osteogenesis and enhance remineralization of the infected site. A similar research of multiple functionalized coating is mentioned in this chapter; the antimicrobial peptides-loaded CP (AMP-CaP) [73, 74] and AMP took the role of protecting the surface from bacterial adhesion, and calcium phosphate offers osteoconductivity. Due to the complication issues along with the infected sites, the biocoatings that prevent infection as well as enable bone growth show great potential for application in orthopedic implants and wound care.

Osteoconductive and Osteoinductive Biomaterials

Identification of the mechanical, technical, and medical restrictions is essential in order to design an optimal bone regenerative system. It should be bioactive and able to recruit neighboring osteoblasts and stem cells and stimulate in their histogenic

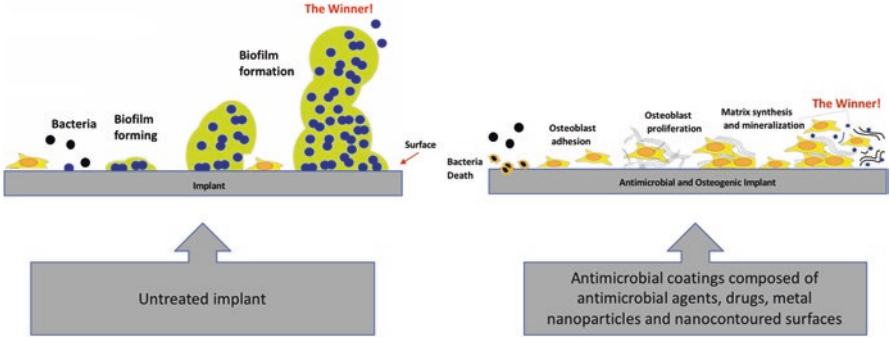


Fig. 7 After implant insertion, there is a "race for the surface" between bacteria and osteoblastic cells. The surface composition and structure can often predict who the winner will be

properties [1]. The mechanical properties should match the application and remain sufficiently strong until the surrounding tissue has healed. The biomaterial used should provoke no inflammatory, toxic, or carcinogenic response [2]. It should be easily sterilized and completely metabolized by the body after fulfilling its purpose. Commercially, the properties of the materials must be consistently reproducible, and it must be scalable for industrial production.

Therapeutic Strategies and Delivery Vehicles for Osteoconductive and Osteoinductive Agents

The successful regeneration of bone requires a "complete package" consisting of three integral components: osteoprogenitor cells, osteogenic factors, and osteoinductive/osteoconductive scaffolds [93]. Inherent in any bioengineered design are these requirements in the scaffold structure: interconnected porosity, adequate mechanical properties, and stimulating healing by inducing chemotaxis, proliferation, and osteoblast differentiation [94, 95]. A significant issue with the use of growth factors is specific delivery to the target site and in the required amounts. Many antibiotics or chemotherapeutics are delivered systemically, but their efficacy is limited due the insufficient quantities of a drug reaching the affected site [15, 96, 97]. Previous studies have shown that HNTs can stimulate chemotaxis, proliferation, and osteoblast differentiation [97–100], and can be doped with antibiotics, chemotherapeutics, and steroids, and 3D printed into various devices [101, 102]. These studies have demonstrated that fabricating a complete delivery package that includes antibiotics, growth factors, cells, and other materials to support bone tissue regeneration, is possible [102, 103].

Bioactive Glass

Researchers have integrated 3D printing techniques and various materials including microspheres, metal nanoparticles, bioactive glass, and other additives to develop customized implants and scaffolds and add additional functionalities with the goal of tissue repair and regeneration. Two such examples are bioactive glasses and metal nanoparticles. Bioactive glasses contains calcium and sodium and bioactive glass (BG) strongly adheres to the targeted tissues, and has a large surface as it promotes cell and tissue growth before dissolving leading to the formation of hydroxylapatite [103]. In bone tissue regeneration, it enables customization leading to a composition that is antimicrobial, therapeutic, or aides in cell recruiting effects [104–106]. Bioactive glasses can be integrated with other polymers (bioplastics, calcium phosphate, etc.) or via various bioprinting methods to develop composites with many different properties, leading to a host of medical applications [107–109]. Through 3D printing, bioactive glass structures can assist with increasing polymer mechanical strength and fine-tuned pore structures. With specifically designed, 3D-printed architectures made from bioactive glass can be employed for innovative solutions in tissue scaffolding, medical and dental implants, and tumor therapy [110].

Metal Nanoparticles

Metal nanoparticles with low toxicity, contrasting agent properties, tailorable characteristics, drug delivery potential, and other functionalities such as conductivity, magnetism, or histogenic capabilities can be used for bioengineering bone tissues and also imparting antimicrobial capabilities [111]. A common approach is to combine metal nanoparticles such as zinc, copper, or silver parts with a polymer(s) and use the composites to 3D print antimicrobial constructs [112]. In an interesting study, Lee et al. (2018) grew gold nanoparticle on a polydopamine-coated 3D printed PCL scaffold using only mild aqueous conditions [57]. Their in vitro study used adipose stem cells in a rabbit defect model and showed osteoblast differentiation and bone tissue formation.

3D Printed Antimicrobial Medical Devices

3D printing's ability to make optimized and customized parts that are very precise and complex, incorporating designed features will usher in an era of new and novel biomedical and clinical applications [113]. In the field of 3D printing, several major groups of biomaterials are currently being investigated for their use in 3D printed antimicrobial medical devices and others are being actively developed [102]. Many natural and synthetic polymers, which are used in 3D printing for bone repair and regeneration, are mainly used as a scaffold for cells, providing mechanical support and also possessing osteogenic properties [104]. These include hydroxyapatite (HA) [114, 115], calcium phosphate [116, 117], poly-(e-caprolactone) (PCL) [118], etc. However, the same polymers are being used to print a diverse array of antimicrobial medical devices. Some examples of the range of methods and polymers used are described below.

3D Printed Antimicrobial Medical Devices Using Bioplastics

Fused depositional modeling, in particular, that uses readily extrudable thermoplastic polymers. The ability to extrude thermoplastic filaments for FDM fabrication methods has led to the creation of customized filaments. Current advances in 3D printing of anti-infective medical devices have emphasized device functionalization directed toward specific diseases and disorders [117]. In many clinical applications, implantable devices can be made using biodegradable materials. This allows a patient to avoid a second surgery for implant removal.

Using FDM, Weisman et al., developed a novel method for 3D printing bioactive implants that release chemotherapeutics or antibiotics [30]. They demonstrated the ability to fabricate antibiotic beads, catheters, or custom inserts to desired specifications for the treatment of osteomyelitis and catheter-related infections. Drug patches

have been developed to deliver antimicrobial drugs or apply antimicrobial agents such as antimicrobial metal ions. In one such study, a nose patch was fabricated with either drug loaded polycaprolactone (PCL) using FDM or silver using PEGDA and a stereolithography (SLA) method for drug delivery. Mills et al. (2018) demonstrated a method for 3D printing antibiotic-doped PMMA for the prevention and remediation of bone infection and biofilm formation [31]. Gentamicin, tobramycin, and vancomycin were successfully doped into PMMA and antibiotic-doped 3D printed beads, disks, and filaments were easily printed. This methodology was also used to 3D print antimicrobial nasal stents for use in treating cleft lip/palate patients [118]. The growth inhibition capacity of the antibiotic-loaded PMMA 3D printed constructs was also demonstrated. Gentamicin was also incorporated into PLA and customized filaments were used to 3D print antibacterial hernia meshes, bioactive mesh, and other constructs [119].

In another approach, Horst et al. (2017) fabricated a bioactive disc of mixed oleo-gum-resins of metal oxides that showed effectiveness in inhibiting the bacterial growth with B + MoO3, P + MoO3, and M + TiO_2 showing the higher inhibition rates [120].

There are several critical design considerations in the 3D printing of antimicrobial devices. These include the need to provide sustained drug release, preventing denaturation of drugs during the FDM printing process, optimal device material properties, and proper size and shape. Accordingly, custom filament extrusion requires matching the material properties of the additive with the desired plastic or polymer that is meant to serve as the core binder of the filament. PLA pellets are typically extruded at around 170–180 °C, although these temperatures may vary depending on ambient conditions. Though these extrusion temperatures are standard for raw PLA filament extrusion, the plastic may still flow below or above these thresholds, and the addition of external cooling or controlled environment may alter these temperatures further [121].

When considering additives, the temperature must again be reconsidered to ensure no degradation of the additive as in the case of bioactive compounds. All compounds undergo thermal decomposition at specific temperatures. Beyond this decomposition temperature, the molecules undergo structural changes. In other words, thermal energy causes cleavage of bonds resulting in fragmentation [30, 122, 123]. Bioactive compounds need to retain their specific molecular structure to be bioactive, and few bioactive compounds are insensitive to temperature as many degrade even at room temperature, and only a select few are stable at higher temperatures [123].

3D Printed Antimicrobial Calcium Phosphate Scaffolds

The field of 3D printed bone tissue engineering is advancing rapidly and the inclusion of antibiotics or metals with antimicrobial properties into calcium phosphate is an often investigated approach [124]. Inzana et al. (2016) fabricated rifampin- and

vancomycin-laden calcium phosphate scaffolds (CPS) by three-dimensional (3D) printing to treat an implant-associated *Staphylococcus aureus* bone infection in a murine model [111]. Some reduction in inhibition of bacterial growth but biofilm persistence on the fixation hardware reaffirmed the need to complement local antibiotic therapy with other biofilm eradication strategies to complement. Zhang et al. (2017) prepared β-tricalcium phosphate (β-TCP) 3D printed scaffolds containing silver nanoparticles dispersed on graphene oxide. In vitro studies showed that the scaffolds had excellent antibacterial activity and accelerated osteogenic differentiation of rabbit bone marrow stromal cells [125]. Correira et al. (2016) used robocasting to fabricate tricalcium phosphate and sodium alginate scaffolds and functionalized with silver nanoparticles (AgNPs) that possessed the needed mechanical properties, biocompatibility, and bactericidal activity [126]. In another approach, 3D printed PLA scaffolds multi-functionalized with collagen, minocycline, and bioinspired citrate-hydroxyapatite nanoparticles. The resulting scaffolds had excellent antimicrobial properties and enhanced osteogenic activity [127]. Finally, a novel 3D printed composite scaffold with antibacterial efficacy for treating bone infections has been recently published. In this study, a polycaprolactone/hydroxyapatite 3D construct with antibiotics added was used as a scaffold base onto which a hydroxyapatite-based hydrogel seeded with macrophages was then printed onto its surfaces. The composite was then inserted into a bone defect *S. aureus* craniotomy-associated biofilm model [128]. While not as effective as treatment with only antibiotics, the investigators did demonstrate a potential new therapeutic benefit for the treatment of established biofilms.

Adding Antimicrobial Functionalities to 3D Printed Medical Devices

Recent efforts have also seen some unique approaches toward creating antimicrobial devices by fabricating 3D printed parts and surface modification techniques to apply antimicrobial agents. Vargas-Alfredo et al. (2017) used high-impact polystyrene (HIPS) to fabricate antimicrobial 3D objects after surface functionalization methodologies were applied [129]. 3D parts, produced via FDM, were dipped in a polymeric solution, composed of antimicrobial cationic polymers bearing two quaternary ammonium groups per monomeric unit, for less than 30 s. Due to the porous nature of the constructs, the antimicrobial materials localized toward the surface of the construct.

Polymer solutions, consisting of PS and PS-b-PAA blends and a quaternized PS-b-poly(dimethylaminoethyl methacrylate) and a quaternized PS-b-poly(dimethylaminoethyl methacrylate were used to chemically modify the scaffold and incorporate antimicrobial functional groups into the scaffold surface [130]. Such a design could easily target a variety of devices including biocompatible/antifouling tubes, osteofixators, surgical screws, or scaffolds. Finally, polymer

composites have been studied for the fabrication of antimicrobial 3D printed devices and have used graphene [131] and metal nanoparticles (copper, silver, titanium dioxide zinc) [112, 132, 133]. Chen et al. (2017) created a composite composed of thermoplastic polyurethane (TPU), poly(lactic acid) (PLA), and graphene oxide (GO), an excellent antimicrobial agent that also enhanced the mechanical properties of printed part [112]. Incorporating antimicrobial metals such as zinc, copper, and silver incorporated polymer such as polycaprolactone or polylactic acid to produce filaments 3D printing with antibacterial efficacy tested against *S. aureus* and *E. coli.* To perform this type of study, a circular dressing was created using Tinkercad, which is a browser-based 3D design and modeling tool. According to these results, the silver and copper wound dressing had the most potent bactericidal properties. Please see González-Henríquez et al. [134] for a thorough review of 3D printing polymers for antimicrobial applications.

What Does the Future Hold?

Lim et al. (2019), in a recent publication, asked the question "Are 3D printed drug delivery and testing systems—a passing fad or the future? [135]. Several reports have demonstrated that medical-grade, biodegradable, and biocompatible PLA and PCL beads, discs, and filaments can be loaded with antibiotics or chemotherapy drugs for a more focused drug-delivery system. This breakthrough could generate improved drug-delivery devices, implants, and catheters. We are beginning to see the gradual introduction of customized 3D printed implants for orofacial, cranial, dental, and orthopedic repair applications. In the next 5 years, more patient-specific 3D printed implants will likely follow using new methods and biomaterials [136].

Furthermore, as researchers seek to customize 3D printing processes, a more comprehensive array of materials and the ability to individually tailor material properties to create customized, controlled drug-delivery devices designed to transport therapeutic drugs directly to targeted areas and then degrade safely in the body and be expelled. As the regulation of 3D printed devices becomes more defined, medical applications of 3D printing biomaterials and technologies and 3D printed biomaterials in clinical applications will rapidly advance. The future will bring state-of-the-art developments in dental and orthopedic surgery and the commercial development of new and novel 3D printing materials, tools, and applications and future developments, and how 3D printing will design our future.

Acknowledgments The authors wish to acknowledge the funding assistance provided by the Center for Dental, Oral and Craniofacial Tissue and Organ Regeneration (CDOCTOR) with the support of NIH NIDCR (U24DE026914).

Disclosures: Dr. David K Mills is a co-inventor on Methods and Devices for Three-Dimensional Printing or Additive Manufacturing of Bioactive Medical Devices. United States Patent Application No. 14/822,275 filed on August 10, 2015.

References

1. Christenson E, Anseth K, van den Beucken J, Chan CB, Orcan B (2007) Nanobiomaterial applications in orthopedics. J Orthop Res 25:1122
2. Cram P, Lu X, Kates S, Singh J, Li Y, Wolf B (2012) Total knee arthroplasty volume, utilization, and outcomes among Medicare beneficiaries, 1991–2010. JAMA 308(12):1227–1236
3. Ruiz D, Koeinig L, Dall T, Gallo P, Narzikul A, Parvizi P, Tongue J (2013) The direct and indirect costs to society of treatment for end-stage knee osteoarthritis. J Bone Joint Surg Am 95:1473–1480. https://doi.org/10.2106/JBJS.L.01488
4. Romanò C, Romanò D, Logoluso N, Drago L (2011) Bone and joint infections in adults: a comprehensive classification proposal. Eur Orthop Traumatol 1(6):207–217. https://doi.org/10.1007/s12570-011-0056-8
5. Bejon P, Robinson E (2013) Bone and joint infection. Medicine 41(12):719–722
6. Sanchez CJ, Ward CL, Romano DR, Hurtgen BJ, Hardy SK, Woodbury RL, Trevino AV, Rathbone CR, Wenke JC (2013) Staphylococcus aureus biofilms decrease osteoblast viability, inhibits osteogenic differentiation, and increases bone resorption in vitro. BMC Musculoskelet Disord 14:187
7. Hatzenbueler J, Pulling TJ (2011) Diagnosis and management of osteomyelitis. Am Fam Physician 84(9):1027–1033
8. Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A (2007) Incidence and economic burden of osteoporosis-related fractures in the United States, 2005–2025. J Bone Miner Res 22:465–475
9. Ensrud KE (2013) Epidemiology of fracture risk with advancing age. J Gerontol A Biol Sci Med Sci 68:1236–1242
10. Marino JT, Ziran BH (2010) Use of solid and cancellous autologous bone graft for fractures and nonunions. Orthop Clin North Am 41:15–26
11. Wang W, Yeung KWK (2017) Bone grafts and biomaterials substitutes for bone defect repair: a review. Bioactive Mater 2:224–247
12. O'Keefe RJ, Mao J (2011) Bone tissue engineering and regeneration: from discovery to the clinic—an overview. Tissue Eng Part B Rev 17:389–392
13. Amini AR, Laurencin CT, Nukavarapu SP (2012) Bone tissue engineering: recent advances and challenges. Crit Rev Biomed Eng 40:363–408
14. Szpalski C, Wetterau M, Barr J, Warren SM (2012) Bone tissue engineering: current strategies and techniques−part I: scaffolds. Tissue Eng Pt B 18(4):246–257
15. Tiffany N, Kasper FK, Mikos AG (2012) Strategies for controlled delivery of growth factors and cells for bone regeneration. Adv Drug Deliv Rev 64(12):1292–1309. https://doi.org/10.1016/j.addr.2012.01.016
16. Ventola CL (2014) Medical applications for 3D printing: current and projected uses. P T 39(10):704–711
17. Mills DK (2015) Future medicine: the impact of 3D printing. J Nanomater Mol Nanotechnol 4(3):1–3. https://doi.org/10.4172/2324-8777.1000163
18. Suresh R, Borkar SN, Sawant VA, Shende VS, Dimble SK (2010) Nanoclay drug delivery system. Int J Pharma Sci Nanotech 3(2):901–905
19. Kotal M, Bhowmick AK (2015) Polymer nanocomposites from modified clays: recent advances and challenges. Prog Polym Sci 51:127–187
20. Li P-R, Wei J-C, Chiu Y-F, Su H-L, Peng F-C, Lin J-J (2010) Evaluation on cytotoxicity and genotoxicity of the exfoliated silicate nanoclay. ACS Appl Mater Interfaces 2(6):1608–1613
21. Peña-Parás L, Sánchez-Fernández JA, Vidaltamayo R (2017) Nanoclays for biomedical applications. Handb Ecomater:1–19
22. Zhanga Y, Tang A, Yang H, Jing Ouyanga J (2016) Applications and interfaces of halloysite nanocomposites. Appl Clay Sci 119(1):8–17
23. Lvov Y, Wang W, Zhang L, Fakhrullin R (2016) Halloysite clay nanotubes for loading and sustained release of functional compounds. Adv Mater 28:1227–1250

24. Du M, Guo B, Jia D (2010) Newly emerging applications of halloysite nanotubes: a review. Polym Int 59:574–582
25. Rawtani D, Agrawal YK (2012) Multifarious applications of halloysite nanotubes: a review. Rev Adv Mater Sci 30:282–295
26. Yuan P, Tan D, Annabi-Bergaya F (2015) Properties and applications of halloysite nanotubes: recent research advances and future prospects. Appl Clay Sci 112–113:75–93
27. Liu M, Wu C, Jiao Y, Xiong S, Zhou C (2013) Chitosan-halloysite nanotubes nanocomposite scaffolds for tissue engineering. J Mater Chem B 1(15):2078–2089
28. Santos AC et al (2018) Halloysite clay nanotubes for life sciences applications: from drug encapsulation to bioscaffold. Adv Colloid Interface Sci 257:58–70
29. Weisman J, Jammalamadaka U, Tappa K, Mills D (2017) Doped halloysite nanotubes for use in the 3D printing of medical devices. Bioengineering 4(4):96
30. Weisman JA, Nicholson JC, Tappa K, Jammalamadaka U, Wilson CG, Mills D (2015) Antibiotic and chemotherapeutic enhanced three-dimensional printer filaments and constructs for biomedical applications. Int J Nanomedicine 10:357–370
31. Mills DK, Jammalamadaka U, Tappa K, Weisman J (2018) Studies on the cytocompatibility, mechanical and antimicrobial properties of 3D printed poly(methyl methacrylate) beads. Bioact Mater 3(2):157–166
32. Li Y, MacIel D, Tomás H, Rodrigues J, Ma H, Shi X (2011) PH sensitive Laponite/alginate hybrid hydrogels: swelling behaviour and release mechanism. Soft Matter 7(13):6231–6238
33. Viseras C, Cerezo P, Sanchez R, Salcedo I, Aguzzi C (2010) Current challenges in clay minerals for drug delivery. Appl Clay Sci 48(3):291–295
34. Uddin F (2018) Montmorillonite: an introduction to properties and utilization. IntechOpen, London
35. Katti KS, Katti DR, Dash R (2008) Synthesis and characterization of a novel chitosan/montmorillonite/hydroxyapatite nanocomposite for bone tissue engineering. Biomed Mater 3(3):034122
36. Jiao L et al (2017) Preparation, characterization, antimicrobial and cytotoxicity studies of copper/zinc- loaded montmorillonite. J Anim Sci Biotechnol 8(1):27
37. Dijkshoorn L, Nemec A, Seifert H (2007) An increasing threat in hospitals: Multidrug-resistant Acinetobacter baumannii. Nat Rev Microbiol 5(12):939
38. Turner RJ (2017) Metal-based antimicrobial strategies. J Microbial Biotechnol 10(5):1062–1065
39. Slavin YN, Asnis J, Häfeli UO, Bach H (2017) Metal nanoparticles: understanding the mechanisms behind antibacterial activity. J Nanobiotechnol 15(1):65
40. Paladini F, Pollini M, Sannino A, Ambrosio L (2015) Metal-based antibacterial substrates for biomedical applications. Biomacromolecules 16(7):1873–1885
41. Ewald A, Glückermann SK, Thull R, Gbureck U (2006) Antimicrobial titanium/silver PVD coatings on titanium. Biomed Eng Online 5(1):22
42. Cheng H et al (2016) Strontium (Sr) and silver (Ag) loaded nanotubular structures with combined osteoinductive and antimicrobial activities. Acta Biomater 31:388–400
43. Memarzadeh K, Sharili AS, Huang J, Rawlinson SCF, Allaker RP (2015) Nanoparticulate zinc oxide as a coating material for orthopedic and dental implants. J Biomed Mater Res Pt A 103(3):981–989
44. Wan YZ, Raman S, He F, Huang Y (2007) Surface modification of medical metals by ion implantation of silver and copper. Vacuum 81(9):1114–1118
45. Garza-Cervantes JA, Chávez-Reyes A, Castillo EC et al (2017) Synergistic antimicrobial effects of silver/transition-metal combinatorial treatments. Sci Rep 7(1):903
46. Vaidya MY, McBain AJ, Butler JA, Banks CE, Whitehead KA (2017) Antimicrobial efficacy and synergy of metal ions against enterococcus faecium, klebsiella pneumoniae and acinetobacter baumannii in planktonic and biofilm phenotypes. Sci Rep 7(1):5911
47. Patil PA, Bhutkar BR, Dange YD, Kharat SV (2016) Screening of most effective nano metal between AgNP, CuNP and Ag-Cu NP's synergistic by In vitro antibacterial comparison. J Nanomed Nanotechnol 7(353):2

48. Katva S, Das S, Moti HS, Jyoti A, Kaushik S (2017) Antibacterial synergy of silver nanoparticles with gentamicin and chloramphenicol against Enterococcus faecalis. Pharmacogn Mag 13(Suppl 4):S828
49. Hwang I, Hwang JH, Choi H, Kim K-J, Lee DG (2012) Synergistic effects between silver nanoparticles and antibiotics and the mechanisms involved. J Med Microbiol 61(12):1719–1726
50. Mohiti-Asli M, Pourdeyhimi B, Loboa EG (2014) Novel, silver-ion-releasing nanofibrous scaffolds exhibit excellent antibacterial efficacy without the use of silver nanoparticles. Acta Biomater 10(5):2096–2104
51. Yu L, Zhang Y, Zhang B, Liu J (2014) Enhanced antibacterial activity of silver nanoparticles/halloysite nanotubes/graphene nanocomposites with sandwich-like structure. Sci Rep 4:4551
52. Ciobanu CS, Iconaru SL, Chifiriuc MC, Costescu A, Le Coustumer P, Predoi D (2013) Synthesis and antimicrobial activity of silver-doped hydroxyapatite nanoparticles. Biomed Res Int 2013:916218
53. Barralet J, Gbureck U, Habibovic P, Vorndran E, Gerard C, Doillon CJ (2009) Angiogenesis in calcium phosphate scaffolds by inorganic copper ion release. Tissue Eng Part A 15(7):1601–1609
54. Wu C, Zhou Y, Xu M et al (2013) Copper-containing mesoporous bioactive glass scaffolds with multifunctional properties of angiogenesis capacity, osteostimulation and antibacterial activity. Biomaterials 34(2):422–433
55. Zhou J, Zhao L (2016) Multifunction Sr, Co and F co-doped microporous coating on titanium of antibacterial, angiogenic and osteogenic activities. Sci Rep 6:29069
56. Shie MY, Chang HC, Ding SJ (2012) Effects of altering the Si/Ca molar ratio of a calcium silicate cement on in vitro cell attachment. Int Endod J 45(4):337–345
57. Zhang J, Park Y-D, Bae W-J et al (2015) Effects of bioactive cements incorporating zinc-bioglass nanoparticles on odontogenic and angiogenic potential of human dental pulp cells. J Biomater Appl 29(7):954–964
58. Kargozar S, Baino F, Lotfibakhshaiesh N et al (2018) When size matters: biological response to strontium-and cobalt-substituted bioactive glass particles. Mater Today Proc 5(7):15768–15775
59. Li J, Zhai D, Lv F et al (2016) Preparation of copper-containing bioactive glass/eggshell membrane nanocomposites for improving angiogenesis, antibacterial activity and wound healing. Acta Biomater 36:254–266
60. Kim M, Kim C, Lim Y, Heo S (2006) Microrough titanium surface affects biologic response in MG63 osteoblast-like cells. J Biomed Mater Res Pt A 79(4):1023–1032
61. Tran N, Webster TJ (2011) Increased osteoblast functions in the presence of hydroxyapatite-coated iron oxide nanoparticles. Acta Biomater 7(3):1298–1306
62. Lee D, Heo DN, Kim H-J et al (2016) Inhibition of osteoclast differentiation and bone resorption by bisphosphonate-conjugated gold nanoparticles. Sci Rep 6:27336
63. Yi C, Liu D, Fong C-C, Zhang J, Yang M (2010) Gold nanoparticles promote osteogenic differentiation of mesenchymal stem cells through p38 MAPK pathway. ACS Nano 4(11):6439–6448
64. Lin K, Liu P, Wei L et al (2013) Strontium substituted hydroxyapatite porous microspheres: surfactant-free hydrothermal synthesis, enhanced biological response and sustained drug release. Chem Eng J 222:49–59
65. Yang F, Dong W, He F, Wang X, Zhao S, Yang G (2012) Osteoblast response to porous titanium surfaces coated with zinc-substituted hydroxyapatite. Oral Surg Oral Med Oral Pathol Oral Radiol 113(3):313–318
66. Mo A, Liao J, Xu W, Xian S, Li Y, Bai S (2008) Preparation and antibacterial effect of silver–hydroxyapatite/titania nanocomposite thin film on titanium. Appl Surf Sci 255(2):435–438
67. Kargupta R, Bok S, Darr CM, Crist BD, Gangopadhyay K, Gangopadhyay S, Sengupta S (2014) Coatings and surface modifications imparting antimicrobial activity to orthopedic implants. Wiley Interdiscip Rev Nanomed Nanobiotechnol 6(5):475–495

68. Kilgus DJ, Howe DJ, Strang A (2002) Results of periprosthetic hip and knee infections caused by resistant bacteria. Clin Orthop Relat Res 404:116–124
69. Page K, Wilson M, Parkin IP (2009) Antimicrobial surfaces and their potential in reducing the role of the inanimate environment in the incidence of hospitalacquired infections. J Mater Chem 19:3819–3831
70. Park KD, Kim YS, Han DK, Kim YH, Lee EHB, Suh H, Choi KS (1998) Bacterial adhesion on PEG mod- ified polyurethane surfaces. Biomaterials 19:851–859
71. Bridges AW, García AJ (2008) Anti-inflammatory polymeric coatings for implantable biomaterials and devices. J Diabetes Sci Technol 2:984–994
72. Gao G, Yu K, Kindrachuk J, Brooks DE, Hancock RE, Kizhakkedathu JN (2011) Antibacterial surfaces based on polymer brushes: investigation on the influence of brush properties on antimicrobial peptide immobilization and antimicrobial activity. Biomacromolecules 12(10):3715–3727
73. Kazemzadeh-Narbat M, Noordin S, Masri BA, Garbuz DS, Duncan CP, Hancock Robert EW, Wang R (2012) Drug release and bone growth studies of antimicrobial peptide-loaded calcium phosphate coating on titanium. J Biomed Mater Res Pt B 2012(00B):000–000
74. Kazemzadeh-Narbat M, Kindrachuk J, Duan K, Jenssen H, Hancock RE, Wang R (2010) Antimicrobial peptides on calcium phosphate-coated titanium for the prevention of implant-associated infections. Biomaterials 31(36):9519–9526
75. Bayramov DF, Neff JA (2017) Beyond conventional antibiotics—new directions for combination products to combat biofilm. Adv Drug Deliv Rev 112:48–60. https://doi.org/10.1016/j.addr.2016.07.010
76. Tan H, Ma R, Lin C, Liu Z, Tang T (2013) Quaternized chitosan as an antimicrobial agent: antimicrobial activity, mechanism of action and biomedical applications in orthopedics. Int J Mol Sci 14(1):1854–1869
77. Yoshinari M, Oda Y, Kato T, Okuda K (2001) Influence of surface modifications to titanium on antibacterial activity in vitro. Biomaterials 22:2043–2048
78. Tsuchiya H, Shirai T, Nishida H, Murakami H, Kabata T, Yamamoto N, Watanabe K, Nakase J (2012 Sep 1) Innovative antimicrobial coating of titanium implants with iodine. J Orthop Sci 17(5):595–604
79. Montanaro L, Speziale P, Campoccia D, Ravaioli S, Cangini I, Pietrocola G, Giannini S, Arciola CR (2011) Scenery of Staphylococcus implant infections in ortho- pedics. Future Microbiol 6:1329–1349
80. Colon G, Ward BC, Webster TJ (2006) Increased osteoblast and decreased Staphylococcus epidermidis functions on nanophase ZnO and TiO2. J Biomed Mater Res A 78:595–604
81. Ren G, Hu D, Cheng EW, Vargas-Reus MA, Reip P, Allaker RP (2009) Characterisation of copper oxide nanoparticles for antimicrobial applications. Int J Antimicrob Agents 33(6):587–590
82. Campoccia D, Montanaro L, Arciola CR (2006) The significance of infection related to orthopedic devices and issues of antibiotic resistance. Biomaterials 27(11):2331–2339
83. Barrère F, Layrolle P, van Blitterswijk CA, de Groot K (2001) Biomimetic coatings on titanium: a crystal growth study of octacalcium phosphate. J Mater Sci Mater Med 12:529–534
84. Shadanbaz S, Dias GJ (2012) Calcium phosphate coatings on magnesium alloys for biomedical applications: a review. Acta Biomater 8:20–30
85. Blom E, Klein-Nulend J, Wolke J, Van Waas M, Driessens F, Burger E (2002) Transforming growth factor-β1 incorporation in a calcium phosphate bone cement: material properties and release characteristics. J Biomed Mater Res 59:265–272
86. Sumner DR, Turner TM, Urban RM, Turek T, Sherman H, Wozney JM (2004) Locally delivered rhBMP-2 enhances bone ingrowth and gap healing in a canine model. J Orthop Res 22:58–65
87. Bernhardt R, van den Dolder J, Bierbaum S, Beutner R, Scharnweber D, Jansen J, Beckmann F, Worch H (2005) Osteoconductive modifications of Ti-implants in a goat defect model: characterization of bone growth with SR muCT and histology. Biomaterials 26:3009–3019

88. Rammelt S, Illert T, Bierbaum S, Scharnweber D, Zwipp H, Schneiders W (2006) Coating of titanium implants with collagen, RGD peptide and chondroitin sulfate. Biomaterials 27:5561–5571
89. Van der Flier A, Sonnenberg A (2001) Function and interactions of integrins. Cell Tissue Res 305(3):285–298
90. Petrie TA, Raynor JE, Reyes CD, Burns KL, Collard DM, García AJ (2008) The effect of integrin- specific bioactive coatings on tissue healing and implant osseointegration. Biomaterials 29(19):2849–2857
91. Huebsch N, Arany PR, Mao AS, Shvartsman D, Ali OA, Bencherif SA et al (2010) Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. Nat Mater 9(6):518
92. Uskoković V, Hoover C, Vukomanović M, Uskoković DP, Desai TA (2013) Osteogenic and antimicrobial nanoparticulate calcium phosphate and poly-(D, L-lactide-co-glycolide) powders for the treatment of osteomyelitis. Mater Sci Eng C 33(6):3362–3373
93. Ducheyne P, Mauck RL, Smith DH (2012) Biomaterials in the repair of sports injuries. Nat Mater 11:652–654
94. Issa JP, Bentley M, Iyomasa VMM, Sebald W, De Albuquerque RF (2008) Sustained release carriers used to delivery bone morphogenetic proteins in the bone healing process. Anat Histol Embryol 2008(37):181–187
95. Hensleea AM, Specera PP, Yoon DM, Naira MB, Meretoja VV (2011) Biodegradable composite scaffolds incorporating an intramedullary rod and delivering bone morphogenetic protein-2 for stabilization in segmental long bone defects. Acta Biomater 7:3627–3637
96. Petrovic V, Zivkovic P, Petrovic D, Stefanovic V (2006) Craniofacial bone tissue engineering. Dent Clin N Am 50:175–190
97. Karnik S, Mills DK (2015) Nanoenhanced hydrogel system with sustained release capabilities. J Biomed Mat Pt A 103(7):2416–2426
98. Karnik S, Jammalamadaka UM, Tappa K, Mills DK (2016) Performance evaluation of nanoclay enriched antimicrobial hydrogels for biomedical applications. Heliyon 2:e00072. https://doi.org/10.1016/j.heliyon.2016.e00072
99. Jammalamadaka U, Tappa K, Mills DK (2018) Calcium phosphate/clay nanotube bone cement with enhanced mechanical properties and sustained drug release. In: Zoveidavianpoor M (ed) Clay science and engineering. InTech, Zagreb, Croatia
100. Patel S, Jammalamadaka U, Sun L, Tappa K, Mills DK (2015) Sustained Release of Antibacterial Agents from Doped Halloysite Nanotubes. Bioengineering (Basel) 3(1):1. https://doi.org/10.3390/bioengineering3010001
101. Wei W, Minullina R, Abdullayev E, Fakhrullin R, Mills D, Lvov Y (2014) Enhanced efficiency of antiseptics with sustained release from clay nanotubes. RSC Adv 4:488–494
102. Mills DK, Tappa K, Jammalamadaka UM, Mills PAS, Alexander JS, Weisman JA (2018) Chapter 8. Medical applications for 3D printing. In: Bar-Cohen Y (ed) Advances in manufacturing and processing of materials and structures. CRC, Taylor & Francis Group, Boca Rotan, FL
103. El-Rashidy A, Roether J, Hrhaus L, Kneser U, Boccinni A (2017) Regenerating bone with bioactive glass scaffolds: a review of in vivo studies in bone defect models. Acta Biomater 62:1–28. https://doi.org/10.1016/j.actbio.2017.08.030. Epub 2017 Aug 24
104. Sawkins M, Mistry Brown P, Shakesheff K, Bonassar J, Yang J (2015) Cell and protein compatible 3D bioprinting of mechanically strong constructs for bone repair. Biofabrication 7:035004
105. Zhao F, Xie W, Zhang W, Fu X, Gao W, Lei B, Chen X (2018) 3D Printing nanoscale bioactive glass scaffolds enhance osteoblast migration and extramembranous oosteogenesis through stimulating immunomodulation. Adv Healthc Mater 7(16):e1800361. https://doi.org/10.1002/adhm.201800361. Epub 2018 Jun 27
106. Murphy C, Kolan KCR, Long M, Li W, Leu MC, Semon JA, Day DE (2016) 3D printing of a polymer bioactive glass composite for bone repair. In: Solid Freedom Fabrication 2016: Proceedings of the 27th Annual International Solid Freedom Fabrication Symposium

107. Bergmann C, Lindner M, Zhang W, Koczur K, Kirsten A, Telle R, Fischer H (2010) 3D printing of bone substitute implants using calcium phosphate and bioactive glasses. J Eur Ceram Soc 30(12):2563–2567
108. Kolana K, Liuae Y, JakebBaldridge J, Murphy C, Semon J, Day D, Leu D (2017) Solvent based 3D printing of biopolymer/bioactive glass composite and hydrogel for tissue engineering applications. Proc CIRP 65:38–43
109. Zhang J, Chen Y, Jing Y, Wang J, Li C, Wang L (2018) Tissue engineering using 3D printed nano-bioactive glass loaded with NELL1 gene for repairing alveolar bone defects. Regener Biomater 5:213–220. https://doi.org/10.1093/rb/rby015
110. Ma H, Feng C, Chang J, Wu C (2018) 3D-printed bioceramic scaffolds: from bone tissue engineering to tumor therapy. Acta Biomater 79:3759. https://doi.org/10.1016/j.actbio.2018.08.026. Epub 2018 Aug 28
111. Inzana JA, Trombetta RP, Schwarz EM, Kates SL, Awad HA (2015) 3D printed bioceramics for dual antibiotic delivery to treat implant-associated bone infection. Eur Cell Mater 30:232–247. (Published 2015 Nov 4. 121)
112. Zuniga J (2018) 3D printed antibacterial prostheses. Appl Sci 8:1651
113. Liaw CY, Guvendiren M (2017) Current and emerging applications of 3D printing in medicine. Biofabrication 9:024102
114. Cox SC, Thornby JA, Gibbons GJ, Williams MA, Mallick KK (2015) 3D printing of porous hydroxyapatite scaffolds intended for use in bone tissue engineering applications. Mater Sci Eng C 47:237–247
115. Cheng H, Chabok R, Guan X, Chawla Y, Li A, Khademhosseini, A, Jang H (2018) Synergistic interplay between the two major bone minerals, hydroxyapatite and whitlockite nanoparticles, for osteogenic differentiation of mesenchymal stem cells, Acta Biomater. 69: 342–351.
116. Mok S-W, Nizak R, Fu S-C, Ho K-WK, Qin L, Saris DBF, Chan KM, Malda J (2016) From the printer: potential of three-dimensional printing for orthopaedic applications. J Orthop Transl 6:42–49
117. Wang X, Ao Q, Tian X, Fan J, Wei Y, Hou W, Tong H, Bai S (2016) 3D bioprinting technologies for hard tissue and organ engineering. Materials 9(10):802
118. Kim BS, Jang J, Chae S, Gao G, Kong J-S, Ahn M, Cho D-W (2016) Three dimensional bioprinting of cell-laden constructs with polycaprolactone protective layers for using various thermoplastic polymers. Biofabrication 8(3):035013
119. Mills DK, Tappa K, Jammalamadaka U, Weisman JA, Woerner RJ (2018) The use of 3D printing in the fabrication of nasal stents. Inventions 3:1. https://doi.org/10.3390/inventions3010001
120. Horst DJ, Tebcherani SM, Kubaski ET, de Almeida Vieira R (2017) Bioactive potential of 3Dprinted oleo-gum-resin disks: B. papyrifera, C. myrrha, and S. benzoin loading nanooxides-TiO_2, P25, Cu_2O, and MoO_3. Bioinorg Chem Appl 2017:6398167
121. Boyer CJ, Ballard DH, Weisman JA, Hurst S, McGee DJ, Mills DK, Woerner JE, Jammalamadaka U, Tappa K, Alexander JS (2018) Three dimensional printing antimicrobial and radiopaque constructs. 3D Print Add Manuf 5(1):29–35
122. Weisman J, Ballard D, Jammalamadaka U, Tappa K, Dagostino H, Mills DK, Woodard P (2018) 3D printed antibiotic and chemotherapeutic eluting catheters for potential use in interventional radiology: in vitro proof of concept study. Acad Radiol 26(2):270–274
123. Tappa K, Jammalamadaka U, Weisman JA, Ballard DH, Wolford DD, Pascual-Garrido C, Wolford LM, Woodard PK, Mills DK (2019) 3D printing custom bioactive and absorbable surgical screws, pins, and bone plates for localized drug delivery. J Funct Biomater 10:17. https://doi.org/10.3390//jfb10020017
124. Bose S, Vahabzadeh S, Bandyopadhyay A (2013) Bone tissue engineering using 3D printing. Mater Today 16(12):396–406
125. Zhang Y, Zhai D, Xu M, Yao Q, Chang J, Wu C (2017) 3D-printed bioceramic scaffolds with antibacterial and osteogenic activity. Biofabrication 9(2):025037. https://doi.org/10.1088/1758-5090/aa6ed6

126. Correia TR, Figueira DR, de Sá KD, Miguel SP, Fradique RG, Mendonça AG, Correia IJ (2016) 3D Printed scaffolds with bactericidal activity aimed for bone tissue regeneration. Int J Biol Macromol 93(Pt B):1432–1445. https://doi.org/10.1016/j.ijbiomac.2016.06.004. Epub 2016 Jun 3
127. Martin V, Ribeiroa I, Alves M, Concalves L, Claudio R et al (2019) Engineering a multifunctional 3D-printed PLA-collagen-minocyclinenanohydroxyapatite scaffold with combined antimicrobial and osteogenic effects for bone regeneration. Mater Sci Eng C 101:15–26
128. Aldrich A, Kuss M, Duan B, Kielian T (2019) 3D bioprinted scaffolds containing viable macrophages and antibiotics promote clearance of Staphylococcus aureus craniotomy-associated biofilm infection. ACS Appl Mater Interfaces 11(13):12298–12307. Epub 2019 Mar 21
129. Alfredo N, Dorronsoro A, Cortajarena AL, Rodríguez-Hernández J (2017) Antimicrobial 3D porous scaffolds prepared by additive manufacturing and breath figures. ACS Appl Mater Interfaces 9:37454–37462
130. Vargas-Alfredo N, Reinecke H, Gallardo A, del Campo A, RodríguezHernández J (2018) Fabrication of 3D printed objects with controlled surface chemistry and topography. Eur Polym J 98:21–27
131. Chen Q, Mangadlao JD, Wallat J, De Leon A, Pokorski JK, Advincula RC (2017) 3D printing biocompatible polyurethane/poly(lactic acid)/graphene oxide nanocomposites: anisotropic properties. ACS Appl Mater Interfaces 9:4015–4023
132. Tiimob BJ, Mwinyelle G, Abdela W, Samuel T, Jeelani S, Rangari VK (2017) Nanoengineered eggshell-silver tailored copolyester polymer blend film with antimicrobial properties. J Agric Food Chem 65:1967–1976
133. Totu EE, Nechifor AC, Nechifor G, Aboul-Enein HY, Cristache CM (2017) Poly(methyl methacrylate) with TiO2 nanoparticles inclusion for stereolitographic complete denture manufacturing—the future in dental care for elderly edentulous patients? J Dent 59:68–77
134. González-Henríquez CM, Sarabia-Vallejos MA, Rodríguez HJ (2019) Antimicrobial polymers for additive manufacturing. Int J Mol Sci 20(5):1210. Published 2019 Mar 10. https://doi.org/10.3390/ijms20051210
135. Lim S, Kathuria H, Tan J, Kang L (2018) 3D printed drug delivery and testing systems—a passing fad or the future? Adv Drug Deliv Rev 132:139–168
136. Park S-H, Jung CS, Min B-H (2016) Advances in three-dimensional bioprinting for hard tissue engineering. Tissue Eng Regen Med 13(6):622–635

Index

B. Li et al. (eds.), *Racing for the Surface*,
https://doi.org/10.1007/978-3-030-34475-7