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New Perspectives in Biofilm Eradication

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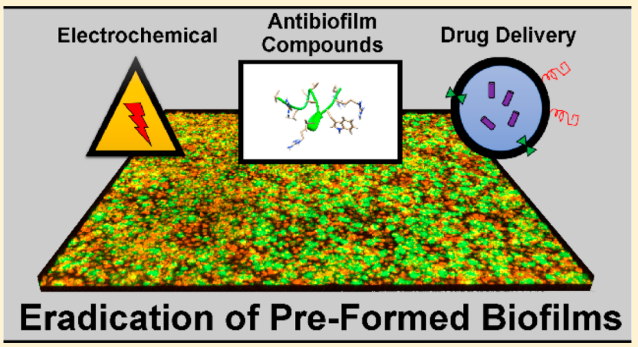
ABSTRACT: Microbial biofilms, which are elaborate and highly resistant microbial aggregates formed on surfaces or medical devices, cause two-thirds of infections and constitute a serious threat to public health. Immunocompromised patients, individuals who require implanted devices, artificial limbs, organ transplants, or external life support and those with major injuries or burns, are particularly prone to become infected. Antibiotics, the mainstay treatments of bacterial infections, have often proven ineffective in the fight against microbes when growing as biofilms, and to date, no antibiotic has been developed for use against biofilm infections. Antibiotic resistance is rising, but biofilm-mediated multidrug resistance transcends this in being adaptive and broad spectrum and dependent on the biofilm growth state of organisms. Therefore, the treatment of biofilms requires drug developers to start thinking outside the constricted "antibiotics" box and to find alternative ways to target biofilm infections. Here, we highlight recent approaches for combating biofilms focusing on the eradication of preformed biofilms, including electrochemical methods, promising antibiofilm compounds and the recent progress in drug delivery strategies to enhance the bioavailability and potency of antibiofilm agents.



KEYWORDS: *biofilms, antibiofilm approaches, biofilm dispersal, electrochemical methods, drug delivery*

BIOFILMS AND THEIR RELEVANCE IN HUMANS

Bacteria exhibit versatile strategies to invade humans. During acute infections, they rapidly proliferate and largely spread as unicellular organisms, whereas in persistent and chronic infections, they predominantly colonize body surfaces and tissues as multicellular aggregates termed biofilms.[1] *Staphylococcus aureus*, *S. epidermidis*, and *Pseudomonas aeruginosa* are prevalent biofilm formers, along with other opportunistic pathogens such as *Klebsiella pneumoniae* and *Escherichia coli*.[2,3] The multicellular and multispecies nature of biofilms renders them particularly difficult to eliminate by the host defenses and to eradicate with antibiotic therapy. According to the US Centers for Disease Control, two-thirds of bacterial infections are due to biofilms, and therefore, they pose a significant problem to human health.[2,3]

THE NATURE OF BIOFILMS

Biofilms are multicellular aggregates of microbes encased in extracellular polymeric substances (EPS) termed the matrix.[4] It is thought that the biofilm lifestyle is a stress adaptation whereby bacteria adapt rapidly to hostile environments. Thus, unfavorable conditions such as stress caused by external attack, physical conditions, or nutrient limitation/starvation can trigger biofilm formation, whereby bacteria colonize body surfaces and then grow into organized communities embedded in a shielding EPS matrix that can be composed of polysaccharides, proteins, and/or extracellular DNA (eDNA).[2−4] Striking changes in bacterial lifestyle and physiology and the complex processes involved in the different steps of biofilm formation are likely mediated by an elaborate, highly regulated biofilm "program".[3] Intercellular communication is largely conducted via signaling molecules that moderate many processes in the biofilm including their physiology, adaptive antibiotic resistance mechanisms, and production of virulence factors.[2,3] Biofilm formation is basically a developmental process whereby bacteria in biofilms exhibit substantially altered gene expression that likely contributes to the above-mentioned biofilm program enabling these biofilm communities to deal with stresses including antibiotics.

After planktonic bacteria attach to surfaces, they adhere, first transiently and then firmly, and grow into aggregates termed microcolonies.[5] Growth, division, and secretion of EPS components ultimately lead to the maturation of heterogeneous three-dimensional matrix cell structures, harboring channels for water and nutrient supply to the inner layers.[2−4] While the outermost region contains largely metabolically active bacteria, the cells in the center are typically in a nongrowing, dormant state and are therefore extremely difficult to eradicate.[6,7] Such dormancy cells fit into the category of persisters that survive antibiotics targeted against growing organisms. The cells in the intermediate layer are heterogeneous displaying different physiologies and/or susceptibilities to antibiotics.[6] Upon maturation or in the face of environmental cues, parts of the

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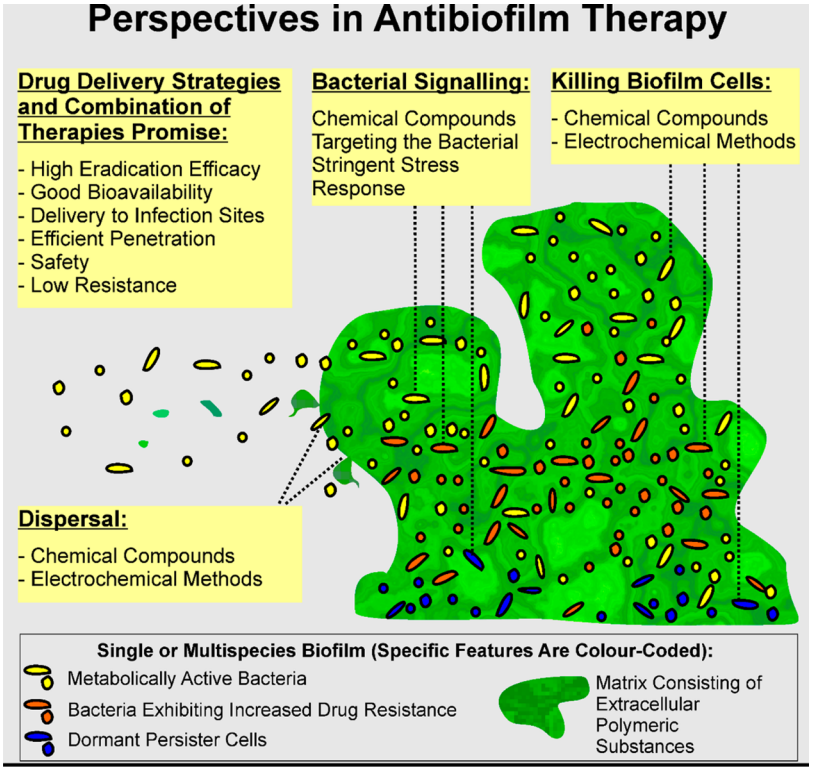

Figure 1. Perspectives in antibiofilm therapy. Chemical compounds, electrochemical methods, drug delivery strategies, and the combination of therapies that target bacterial signaling, biofilm dispersal, and/or killing of biofilm cells promise good results for the eradication of mature biofilms.

biofilm can disperse and bacteria return to a planktonic state to spread and colonize new habitats.[8] Recently, it has been proposed that dispersed cells are highly virulent and, therefore, constitute an intrinsic risk for seeding acute infections.[2,8]

BIOFILMS IN THE HUMAN BODY

Every biological or nonbiological surface in the body is at inherent risk of being colonized by biofilms. For example, urinary and intravascular catheters, prosthetic heart valves, artificial hearts, cardiac pacemakers, cerebrospinal fluid shunts, endotracheal tubes, tissue fillings, and contact lenses are examples of frequently colonized medical devices.[9] Device-related infections are often associated with hospitalization, surgical intervention, and elevated morbidity and mortality, resulting in considerable additional costs for the health care system.[9] Biofilms are often found in acute and chronic wounds and burn injuries thereby impeding the healing process.[10] They also colonize and/or infect biological surfaces in the skin, eyes, ears, nose and throat, the heart and lungs, bones, and the gastrointestinal and urinary tracts.[2] Uropathogenic *E. coli* are able to invade uroepithelial cells of the bladder and form biofilm-like intracellular bacterial communities which can cause recurrent urinary tract infections, despite undetectable bacterial counts in the patient's urine, and these are extremely difficult to diagnose and treat.[11] Furthermore, over the last decades, the incidence of skin abscesses has increased due to the rise in community-acquired infections caused by major etiological agent methicillin-resistant *S. aureus* (MRSA).[12] While abscesses have not been considered biofilm infections, they exhibit similar characteristics since they represent high-density infections embedded in biofilm-like matrices and are similarly recalcitrant to conventional antimicrobials.[13] Biofilms are also habitually found in the oral cavity, known as dental plaque, where they can cause caries or periodontitis, costing $442 billion annually[14] and negatively impacting dental health.[15]

LACK OF EFFICACY OF ANTIBIOTICS IN ERADICATING BIOFILMS IN THE CLINIC

While a sufficient bacterial burden is required to cause overt disease, in the presence of an implantable medical device, even a low bacterial inoculum (e.g., 10^2 CFU/mL of *S. aureus*[16]) can trigger an infection.[17] This is because the indwelling device provides an excellent surface for bacterial colonization. Furthermore, leukocytes isolated from infected implantation sites can become defective in phagocytic and bactericidal responses, which might promote the growth and eventual chronic nature of these infections.[18] Unfortunately, since biofilms are 10- to 1000-fold more adaptively resistant to antimicrobials than planktonic bacteria,[19] indwelling device-associated infections are difficult to eradicate with traditional antibiotic regimens. As such, removal of the foreign body becomes an imperative first step for eradicating the biofilm infection and is often followed by antibiotic treatment to prevent regrowth and to target bacteria released into the bloodstream or surrounding tissues.[17] When indwelling devices cannot be removed, aggressive antimicrobial strategies are implemented. For example, for catheter-related infections, antibiotic lock therapy is used, whereby a high concentration of antibiotics is instilled into the lumen of the catheter.[20] Antibiotics administered in antibiotic lock therapy are typically paired with an anticoagulant such as heparin to interfere with fibrin formation and enhance their penetration into the tissues and biofilm.[20] However, these regimens are implemented sparingly since prolonged exposure to antimicrobials and anticoagulants causes significant toxicity to the host.[21] Other antibiotics such as macrolides, tetracyclines, and quinolones are

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Table 1. List of Selected Electrochemical Methods To Eradicate Biofilms

method	mode of action	biofilm species	biofilm model	safety/drug development	ref.
electrochemical scaffold	H_2O_2	*A. baumannii*, *P. aeruginosa*	porcine explants, glass bottomed Petri dishes	noncytotoxic to mammalian tissue/research stage	24 and 25
low-voltage direct currents (TGON 805 electrode)	active species	*P. aeruginosa*	single chamber electrochemical cell	no information available/ research stage	26
wireless electroceutical dressing (silver/zinc redox fabric)	superoxide radicals	*P. aeruginosa*	human wound exudates	comprehensive wound center/research stage	27
high-voltage pulsed currents (concentric ring electrode)	membrane perturbation	*A. baumannii*, *P. aeruginosa*	synthetic mesh, burn wound murine model	noncytotoxic to mammalian skin/research stage	28
nonthermal plasma (corona discharge, electrospray)	reactive oxygen and nitrogen species, UV light, charged ions	*E. coli*	glass slides	no information available/ research stage	29

also often used due to their ability to penetrate tissues and to reach the site of infection.[17] Furthermore, antibiotic combination therapies[22] have been shown to be more effective than monotherapies when treating biofilm infections largely due to their synergy and their breadth of activity against multispecies biofilms. Nevertheless, treatment is difficult and to date suboptimal.

The lack of antibiotic efficacy against mature biofilms is often attributed to restricted drug accessibility, their predominant mode of action in targeting metabolically active bacteria, cf. the dormant bacteria at the core of the biofilm, and an overall increase in antibiotic resistance in biofilm cells.[19] The exploitation of potential synergy between conventional antibiotics and antibiofilm approaches addressing these shortcomings by facilitating drug penetration, influencing the metabolic state of biofilms, or initiating biofilm dispersal has shown significant promise.

APPROACHES TO REMOVE AND DESTROY RECALCITRANT BIOFILMS

In the following sections, we provide an overview (depicted in Figure 1) of current antibiofilm approaches, their impact on the efficacy of conventional antibiotics (when available), and their stage of development. We specifically focus on medically relevant biofilms as well as recently discovered biofilm eradication approaches. In particular, we discuss electrochemical methods, antimicrobial compounds exhibiting antibiofilm activity, and biomolecules targeting the biofilm architecture, as well as recent progress in the development of biofilm drug delivery methods.

ELECTROCHEMICAL METHODS TO ERADICATE BIOFILMS

The observation that electrical current can decrease biofilms is a longstanding one. Since the first reports more than 40 years ago, contradictory information has been published.[23] However, this approach has recently regained attention due to the urgent need to implement novel antibiofilm strategies. Treatment via electrochemical methods applies a current to an electrically conductive target surface. This surface acts as an electrode and depending on the direction of the current can be either an anode or a cathode. The anode is the point where electrons leave the electrical cell and oxidation occurs while, at the cathode, the electrons re-enter the cell leading to reduction. Electrodes can be used to deliver an electrical current specifically to an infection site or used to disinfect a conductive material, including medical devices and implants.[23]

Electrochemical technologies offer an effective alternative or adjuvant treatment option of contaminated medical devices and biomaterial-associated infections (Table 1).

Although electrochemical administration can kill bacterial pathogens independent of their growth phase, various factors such as current density and electric potential need to be fine-tuned to ensure the success of this approach. Low-intensity of direct or alternating electrochemical currents and pulsed electric fields have been recently investigated for their effects of killing microbes and eliminating associated biofilm-like structures.[30] Electrical stimulation with voltage and electric current can affect the organization of biological membranes, cellular processes,[30] cell behavior,[31] bacterial respiratory rate, and oxidation of proteins, as well as cell electrophysiology.[32] The antibacterial activity of electrical currents has been proposed to be attributed to the bactericidal substances that are produced during electrolysis (e.g., oxidized radicals or H_2O_2). Promisingly, electrochemical treatments have been shown to work synergistically with antibiotics leading to enhanced killing of drug-resistant bacteria.[33]

The choice of the conductive materials, such as stainless steel or carbon fabric, determines the electrochemical properties of the so-called "e-scaffold". Sultana et al.[24] demonstrated that the presence of electrochemically generated H_2O_2, produced by carbon-based Ag/AgCl electrodes, generated approximately 25 μm of H_2O_2 at the e-scaffold surface. By overlaying an e-scaffold onto an existing *Acinetobacter baumannii* biofilm, the authors could achieve a 10 000-fold reduction in viable cells and an 80% decrease in biofilm surface coverage.

In vivo experiments further showed that *A. baumannii* grown as biofilms on porcine explants[24] could be overlaid with the same e-scaffold, and this significantly reduced viable bacteria by about 1000-fold. Subsequently, the same group[25] introduced a constant potential of −600 $mV_{Ag/AgCl}$ to generate a low concentration of H_2O_2 that was continuously delivered into the biofilm. They confirmed that H_2O_2 entered into bacterial cells and induced intracellular production of highly reactive hydroxyl radicals (OH·). Intriguingly, this mirrors a natural mechanism since the production of reactive oxygen species (and H_2O_2) is an intrinsic antimicrobial defense mechanism against invading microbes and is a major mechanism employed by phagocytes such as neutrophils and macrophages,[34] as well as mitochondria[35] and peroxisomes.[36] The contrast between the inability of immune cells to resolve mature biofilms and the efficacy of the e-scaffold might be attributed to the constant exposure of the biofilm cells to low amounts of H_2O_2 and the fact that host cells are exposed to biofilm-associated virulence factors that can compromise their defense mechanisms.[18]

This led to increased cell membrane permeability and degradation of both proteins and DNA in preformed *P. aeruginosa* biofilms within a 24 h treatment window. Consequently, the authors observed a decrease of biofilm cells by approximately 10^5-fold within 24 h and additionally

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demonstrated that persister cells were completely eradicated (10^5-fold reduction in persistence compared to control) within 6 h of e-scaffold treatment.[25] Overall, these data are encouraging and might offer a nonantibiotic treatment strategy for destroying recalcitrant biofilm infections. Moreover, low concentrations of H_2O_2 might be beneficial during the wound healing process, since H_2O_2 produced in wounds as a cellular response encourages healing processes.[37] However, oxidative stress can induce mutations in bacteria rendering them less susceptible to treatment.[38]

Niepa et al.[39] demonstrated that >98% of *P. aeruginosa* persister cells could be eliminated with 1 h treatment of a 70 μA/cm^2 low-level direct current produced by stainless steel 304 electrodes. The authors hypothesized that, due to pitting corrosion of the stainless-steel electrode, ions were released and that persister cells, although resistant to very high concentrations of ions, become susceptible to low concentrations of ions when an electric field was present. However, this is very speculative, and the application in humans might be limited due to the lack of knowledge about the reaction of the human body to the release of ions. Recently, the field has switched to carbon-based biomaterials such as TGON, a high thermal conductive graphite-based sheet that does not release biological active metal cations. Interestingly, just recently, the same group investigated the bactericidal activity of a nonmetallic biomaterial (TGON 805 electrode) on persister and biofilm cells of *P. aeruginosa*.[26] With an application of low-level (70 uA/cm^2) direct electrochemical current, they eradicated *P. aeruginosa* persister cells. Their promising data showed dose and time dependent bactericidal effects, with complete eradication of planktonic persister cells within 40 min of treatment and a 100-fold reduction in viable biofilm cells within 1 h of treatment. Viable cells were eradicated most likely due to reducing agents and/or reactive intermediates of oxygen.

Additional approaches to combine electrochemical methods with drug administration might further enhance the above effects. In this context, Niepa et al.[39] achieved synergistic effects against *P. aeruginosa* through 70 μA/cm^2 direct current (SS304 electrode) combined with 1.5 μg/mL tobramycin. Furthermore, Sultana et al.[25] showed that an e-scaffold combined with 40 μg/mL tobramycin enhanced susceptibility to the antibiotic and completely eradicated *P. aeruginosa* biofilms. Nodzo et al. recently demonstrated that a 1 h application of 1.8 $V_{Ag/AgCl}$ cathodic voltage-controlled electrical treatment with subsequent 1 and 5 weeks-long vancomycin administration (150 mg/kg) reduced viable *S. aureus* by almost 100% for bone and titanium implant-associated infections in a rodent model when compared to the control groups.[40,41] Although the *in vivo* data appears very promising, the authors admitted that they did not test for viable cells to determine complete eradication after the experiment. Their approach has promise as a potential treatment option, especially because they further showed that such a treatment caused no deleterious histological changes in the surrounding tissues.[41]

In the area of low-voltage applications, a recently developed wireless electroceutical dressing (WED) demonstrated promising antibiofilm activity against *P. aeruginosa*. Thus, Banerjee et al.[27] showed that a silver and zinc redox couple WED fabric became electrically active in the presence of wound exudates and generated low voltage (0.3−0.9 V) electrical fields capable of reducing molecular oxygen to produce superoxide radicals. Remarkably, in the presence of WED, the biofilm integrity of a *P. aeruginosa* biofilm was disrupted and its thickness and number of live cells were significantly reduced. In addition, the same group showed that a wireless silver/zinc wound dressing could facilitate wound healing and was safe to use on patients,[40] highlighting the future potential of using electroceuticals.

While antibiotics might be an excellent approach for eliminating dispersed bacteria after electric stimulation, antibiotic-alternative methods might offer additional benefits in the fight against hard-to-treat biofilm infections. In this context, Subramanian et al.[42] demonstrated that the combined treatment of the bacterial quorum sensing inhibitor analog molecule autoinducer-2 with low electric fields could shrink mature *E. coli* biofilms. Preformed (24-h) *E. coli* biofilms were treated for 24 h with this combination leading to a 78% decrease in average biofilm mass and a 76% better treatment efficacy compared to conventional antibiotic therapy.[42] It was hypothesized that electric fields enable more efficient and effective permeation of the inhibitor into the biofilm. Although drug alternatives come with their own limitations, they might provide a way to spare essential antibiotics and delay resistance development.

Other recent advances in the field used high-voltage pulsed currents. This technique applies a high voltage for very short times (less than a millisecond) in a series of very fast pulses. This ensures that that nerve or human cells are not excited and/or damaged.[43] Thus, pulsed electric fields represent a nonchemical approach to potentially eradicating biofilms on implanted medical devices through high voltage that create pores in cell membranes which, if irreversible, can create permanent cell membrane damage.[44] In a study by Khan et al.,[28] concentric ring electrodes were used to treat *P. aeruginosa* biofilms established on a synthetic mesh. Optimized settings led to killing of >80% of the biofilm bacteria.[28] Furthermore, the application of pulsed electric fields in a burn wound murine model resulted in a >10^5-fold reduction of *A. baumannii* in contaminated wounds.[45] One major advantage of pulsed electric fields treatment is depth control that prevents damage of surrounding tissue and organs. Accordingly, skin ablated with pulsed electric fields can heal with no evidence of scarring.[46]

Another interesting decontamination technique uses plasma, a macroscopically neutralized ionized gas[29] that only exists at high-temperature in nature. Nonthermal (i.e., low-temperature) plasma, where the temperature of electrons is high but other particles such as atoms, molecules, and ions remain close to ambient temperature, is a promising agent for decontamination of thermally sensitive surfaces. The biocidal agents produced by plasma sources are reactive oxygen and nitrogen species, UV light, and charged electron ions in electromagnetic fields.[47] Kovalova et al.[29] reported that nonthermal plasma generated by corona discharge in the air could be applied to kill bacteria in the top layer of an *E. coli* biofilm. Plasma treatment could also affect the polymers surrounding bacterial cells, thereby reducing EPS and cell adhesion.[29] However, due to its inability to efficiently eradicate cells in the lower portion of the biofilm, additional hurdles need to be overcome. The production of reactive neutral species can be increased through the addition of water to the discharge causing water vapor or fine liquid droplets to be sprayed from the high-voltage electrode. Liquid from a capillary that is exposed to high electrical potential is called electrospray. It has been shown that it can be used to sterilize polymer surfaces contaminated with biofilm[48] and spores[49] as well as water disinfection. Recently, water electrospray and air corona discharge polarity have been investigated on 2-day old *E. coli* biofilms on glass surfaces.

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Table 2. List of Selected Antimicrobial Compounds Capable of Eradicating Mature Biofilms

compound	mode of action	biofilm species	biofilm model	safety/drug development	ref.
silver oxynitrate (Ag_7NO_{11})	binding to reduced thiols, impairing of membrane function, irreversible denaturing of key enzymes	dual species, *E. coli*, fluoroquinolone-resistant *P. aeruginosa*, MRSA, *P. aeruginosa*, *S. aureus*, uropathogenic *E. coli*	Calgary biofilm device, gauze	nontoxic, nonirritating and nonsensitizing *in vivo*/research stage	50−52
tert-butyl benzoquinone (TBBQ), synergy with antibiotics	membrane perturbation	*Staphylococcus* spp.	living skin equivalent	nontoxic for human skin/preliminary preclinical assessment	53
halogenated phenazines	binding of divalent metal cations, inhibition of protein biosynthesis	methicillin resistant *S. epidermidis*, MRSA, vancomycin-resistant enterococci	Calgary biofilm device	nontoxic to mammalian cells/research stage	54−56
nitroxoline	binding of divalent metal cations, inhibition of protein biosynthesis	*A. baumannii*, *E. coli*, methicillin resistant *S. epidermidis*, MRSA, *P. aeruginosa*, vancomycin-resistant enterococci	Calgary biofilm device, microtiter, porcine skin	safe/approved for urinary tract infections	57−60
IDR-1018, synergy with antibiotics	targeting and blocking the bacterial stringent stress response, modest membrane perturbation	*A. baumannii*, *Burkholderia cenocepacia*, *E. coli*, *Enterococcus faecium*, *Enterobacter* spp., *K. pneumoniae*, MRSA, *P. aeruginosa*, *Salmonella typhimurium*, oral multispecies	flow cell chamber, hydroxyapatite surface, murine cutaneous abscess	minimal cytotoxic to mammalian cells/research stage	61−64
DJK-5, DJK-6, synergy with antibiotics	targeting and blocking the bacterial stringent stress response, membrane perturbation	*A. baumannii*, *P. aeruginosa*, *K. pneumoniae*, *Salmonella enterica*	flow cell chamber, *Caenorhabditis elegans* and *Galleria mellonella* infections, 3D lung epithelium, murine cutaneous abscess	minimal cytotoxic to mammalian cells/research stage	65 and 66
nisin, synergy with antibiotics	membrane perturbation, interrupting of cell-wall biosynthesis	multispecies in human saliva	24-well, microtiter	safe/approved as food additive	67−70
D-Bac8c^{2,5Leu}	membrane perturbation	MRSA, methicillin-sensitive *S. aureus*	microtiter, flow chamber, CLS rat central venous catheter infection	minimal cytotoxic to mammalian cells/research stage	71
medusin-PT1a	membrane perturbation	MRSA	microtiter	modest hemolytic/research stage	72
RNase 3/eosinophil cationic protein	membrane perturbation and cell agglutination activity	*P. aeruginosa*	microtiter	minimal cytotoxic to mammalian cells/research stage	73

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Intriguingly, the biofilm mass was only treated for 15 min but decreased bacterial population by almost 10^5-fold.[29]

Overall, these strategies offer promising treatment alternatives against bacterial biofilms either directly at the site of infection or in biofilm-contaminated devices. The effects of cell migration after electrical stimulation might also improve the wound healing process. Most of these studies are in their infancy, and it remains to be seen whether such methods will prove to be clinically useful, especially since the long-term effects of continuous electrical fields on tissues are unknown. However, considering the positive developments in this area, it seems that they have good potential to progress into the clinic.

ANTIMICROBIAL COMPOUNDS TO ERADICATE BIOFILMS: SUBSTRATES WITH ANTIBIOFILM ACTIVITY

The eradication of pathogens in their protected biofilm growth state is a challenging undertaking. Microorganisms grown in biofilms have been shown to be highly tolerant toward conventional antibiotics,[3] especially nongrowing, dormant persister cells.[7] While many antimicrobial substances have been screened for their ability to annihilate biofilms, with variable success, here we present only a selected list of the most recent and promising biofilm eradication approaches (summarized in Table 2).

One of the longest-known antimicrobial agents is the metal silver (Ag). For millennia, it has been used as a food and water preservative and it was an important antimicrobial agent for a variety of medical purposes prior to the emergence of antibiotics.[74] Several medical devices contain Ag or are coated with Ag-formulations.[74] In the presence of water, Ag ionizes to Ag^+ which harms the bacterial cell by binding to reduced thiols thereby impairing membrane function and irreversibly denaturing key enzymes.[74] The formulation silver oxynitrate (Ag_7NO_{11}), which contains highly oxidized and oxygen-stabilized silver atoms, has been shown to successfully kill mature biofilms after 24 h of treatment.[51] Biofilms that were grown for 24 h in a Calgary biofilm device were eradicated with a minimal biofilm eradication concentration (MBEC) of <50 μM, and even biofilms grown for 4 or 6 days could be eradicated, although at much higher concentrations.[51] In an *in vitro* biofilm model on gauze, wound dressings coated with silver oxynitrate (0.4 mg Ag/cm^2) demonstrated activity against 72 h-old biofilms while being nontoxic, nonirritating, and nonsensitizing *in vivo*.[50] Recently, it has been shown that silver oxynitrate is potent enough to eradicate multispecies biofilm populations composed of *E. coli*, *S. aureus*, and *P. aeruginosa*.[52]

Another promising topical antibiofilm agent is *tert*-butyl benzoquinone (TBBQ), an oxidation product of the antimicrobial food additive *tert*-butyl hydroquinone. Thus, TBBQ eradicated preformed staphylococcal biofilms on the Calgary biofilm device (MBEC $\leq$ 64 mg/L), and topical application was nontoxic.[53] Additionally, TBBQ perturbed the membranes of metabolically active, slow-growing, and persister cells and showed synergy in combination with gentamicin.[53]

Inspired by interspecies competition in the lungs of older cystic fibrosis patients, where *P. aeruginosa* frequently displaces *S. aureus* in part through antimicrobial phenazines, Garrison et al.[54,56] and Yang et al.[55] demonstrated that synthesized halogenated phenazines could efficiently eradicate biofilms grown on a Calgary biofilm device (MBEC $\leq$ 12.5 μM).[55,56] Halogenated phenazines were also able to kill MRSA persister cells in nonbiofilm cultures[54,56] while showing minimal red blood cell hemolysis or cytotoxicity toward epithelial cells.[54,55] The antimicrobial mode of action was proposed to be related to the ability of halogenated phenazines to bind divalent metal cations (cooper and iron), thereby targeting metalloproteins and inhibiting protein biosynthesis.[54,55] Attachment of polyethylene glycol-carbonate conferred desirable drug properties to the halogenated phenazines by improving the water solubility, eliminating residual cytotoxicity, and enhancing its biofilm eradication activity.[55]

Although antibiotics often display poor efficacy against biofilms, nitroxoline (5-nitro-8-hydroxyquinoline) is a promising candidate with broad spectrum antimicrobial and antibiofilm activity.[57,59] Nitroxoline is frequently used to treat urinary tract infections and has been on the market for around 50 years[57] indicating efficacy and a good safety profile[58] and low resistance development.[60] Although *P. aeruginosa* is not considered to be in its spectrum,[60] this compound reduces viable cell numbers in *P. aeruginosa* biofilms[57] and eradicates biofilms formed by various other species *in vitro* as well as *ex vivo* using a porcine skin model.[59] It also shows an ability to kill persister cells of stationary MRSA cultures.[59] Its antimicrobial and antibiofilm efficacy is due to its ability to chelate divalent cations (e.g., iron and zinc), which has also been reported for the metal ion chelating agent EDTA.[75] However, nitroxoline can be applied orally because of its diminished cytotoxicity and is efficacious against biofilms at therapeutic concentrations (plasma 6 μg/mL, urine 300 μg/mL).[57] Apart from its killing capacities, it has also been described as inducing a shift of bacteria from the biofilm to the planktonic lifestyle if applied at subinhibitory concentrations.[57]

Host defense (antimicrobial) peptides are small cationic molecules (12 to 50 amino acids, net charge +2 to +9) with various sequences, structures, and functions. They are produced by many organisms, including humans, mammals, plants, amphibia, and bacteria.[76] These peptides have numerous activities including immunomodulatory, antimicrobial, antibiofilm, and anticancer functions, and despite their overall similarities, each of these properties is differentially determined with distinct structure to function relationships.[77] For example, investigation of small synthetic peptides demonstrated peptides with excellent antibiofilm activity but little or no antimicrobial activity vs planktonic (free-swimming) bacteria[78] while a subsequent, more extensive study showed only modest overlap between good antibiofilm, anti-inflammatory, and chemokine stimulation activity.[79] Furthermore, antibiofilm peptides are highly active against *Burkholderia* spp. biofilms while this species is completely resistant to antimicrobial peptides when grown planktonically.[62] Conversely, human host defense peptide LL-37 is a weak direct antimicrobial but is highly active against *P. aeruginosa* biofilms at one sixteenth the minimal inhibitory concentration and also has excellent anti-inflammatory activity.[62,80]

Recently, we demonstrated that the synthetic 12-amino acid immunomodulatory peptide IDR-1018,[61] derived from the bovine neutrophil peptide bactenecin, eradicated 2-day old biofilms of a wide variety of recalcitrant Gram-negative and Gram-positive bacterial species, at levels that did not inhibit planktonic growth.[62] By targeting and blocking the bacterial stringent stress response, a pathway strongly influencing biofilm initiation and maintenance, IDR-1018 dispersed preformed biofilms at concentrations as low as 0.8 μg/mL,[62] while killing of pathogens occurred at ~10 μg/mL.[62] Subsequently, we synthesized protease-resistant D-enantiomeric peptides (DJK-5

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and DJK-6) that eradicated preformed biofilms, at even lower concentrations (0.5−0.8 μg/mL) than IDR-1018,[65] protected invertebrates from lethal *P. aeruginosa* infections[65] (Figure 2a)

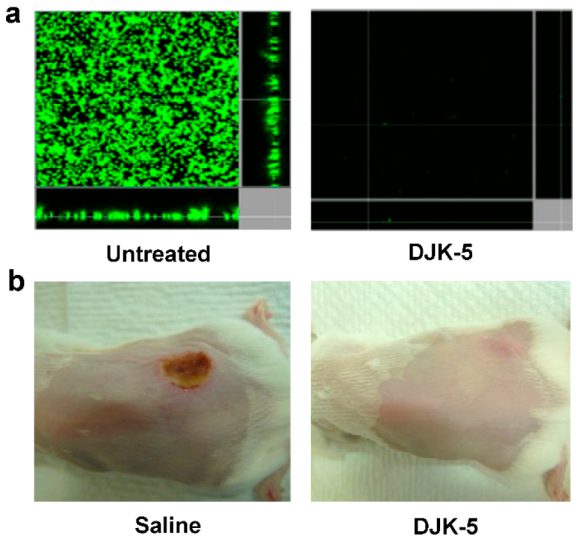

Figure 2. The synthetic host defense peptide DJK-5 efficiently eradicates MRSA infections. (a) Clinical isolate, MRSA SAP0017, biofilms were grown in flow cell chambers for 48 h and then subsequently treated with 2.5 μg/mL of DJK-5 for 24 h. Bacteria were stained with Syto-9 (green, live/dead stain) as well as propidium iodide (red, dead stain) prior to confocal imaging. DJK-5 completely eradicated the preformed biofilm (right panel). (b) Mice were administered 6 mg/kg DJK-5 or saline (as a control) via intraperitoneal injection prior to being infected with MRSA USA300 subcutaneously. DJK-5 treatment was highly efficacious against the high density MRSA infection (right panel). Representative images capture dermonecrotic lesions 72 h postinfection. Images were adapted with permission from Mansour et al.[81] Copyright (2016) Elsevier.

and, like IDR-1018,[63] showed strong synergy with conventional antibiotics in flow cell chambers[65] and in a 3D lung epithelial cell model.[66] DJK-5[64,81] and IDR-1018[64] therapeutically targeted the stringent stress response in a MRSA[81] and a *P. aeruginosa*[64] murine cutaneous abscess model (Figure 2b) leading to the reduction of the bacterial burden and tissue necrosis[64,81] Furthermore, due to their broad-spectrum activity, antibiofilm peptides work potently against multispecies oral biofilms.[82,83] While host defense peptides are known to generally have multiple targets, the conserved bacterial stringent response is a major target of antibiofilm peptides and the basis for their broad spectrum antibiofilm activity.[62,78]

Many other studies examining antibiofilm activity of natural and synthetic peptides have been published recently.[84] Some of these conclude that antimicrobial peptide activity and antibiofilm activities use overlapping mechanisms. For example, the lantibiotic nisin (produced by *Lactococcus lactis* and used as a food preservative)[70] was recently shown to reduce the biomass, thickness, and bacterial survival in preformed multispecies biofilms, which were grown *in vitro* from pooled bacteria present in human saliva. These biofilms were disrupted in a time and dose dependent manner with the best results achieved at 50 μg/mL treatment for 10 min.[70] Nisin also demonstrated synergistic effects as an adjunctive therapy.[67] Zapotoczna et al.[71] investigated the efficacy of several synthetic antimicrobial peptides (AMPs) against mature biofilms of MRSA and methicillin-susceptible *S. aureus* isolates from patients with device-related infections by using a catheter lock solution (CLS) rat central venous catheter infection model.[71] They found that the peptide D-Bac8c^{2,5Leu}, a variant of the bovine bactenecin Bac8c that had both antibiofilm and anti-inflammatory activities, was their most efficacious biofilm eradication compound.[71] Another AMP, medusin-PT1a, a modified analog of the medusin-PT, isolated from the skin secretion of the tarsier leaf frog, *Phyllomedusa tarsius*, was recently shown to eradicate mature MRSA biofilms (MBEC = 64 μg/mL) and to retain its full antimicrobial activity in physiological conditions under which many AMPs considerably lose efficacy.[72] AMPs are often rendered ineffective in the presence of biologically relevant ionic strengths or levels of host proteases or polyvalent anions such as glycosaminoglycans.[85]

AMPs have also been modified to provide additional bioactive properties. Pulido et al.[73] engineered the RN3(5-17P22-36) peptide, which is derived from the eosinophil cationic protein, a RNase with broad antimicrobial activities. The synthetic antimicrobial peptide efficiently eradicated *P. aeruginosa* biofilms through a combination of bacterial agglutination and direct cell killing.[73]

A subset of host defense peptides/AMPs, termed antibiofilm peptides, constitute one of the most promising approaches for the treatment of biofilm infections due to their antibiofilm combined with immunomodulatory properties. Despite promising results *in vitro* and in animal models, peptides have not yet entered clinical trials for future applications as an antibiofilm therapy, although several peptides have been tested in clinical trials as antimicrobial and immunomodulatory agents.[77] To date, limitations to peptide use include lability to host proteases, unknown toxicities, and bioavailability *in vivo*, as well as high cost of production. Several approaches exist to increase safety and bioavailability, including the targeted design of peptides with reduced toxicity and enhanced protease resistance profiles as well as the use of drug delivery strategies, that are addressed in a separate section in this review.

MODULATION OF THE BIOFILM ARCHITECTURE TO ERADICATE BIOFILMS

Biofilms grown under certain *in vitro* conditions have a complex architecture that has been studied microscopically although it should be stated that biofilm appearance varies substantially from experiment to experiment and according to the growth conditions (including carbon source, level of shear stress, starvation, pH, oxygen) and substratum.[86] The complex structuring of biofilms has been suggested to not arise from a stochastic process but rather reflect a careful orchestration of activities employed by bacteria to ensure survival and dissemination. For example, channels are formed within biofilms so that cells deep within the biofilm can receive nutrients and oxygen and expel wastes.[4] It has been further hypothesized that bacteria within a biofilm undergo coordinated events to break down matrix components that allow for biofilm disassembly enabling dispersal to colonize new niches.[4,5]

As mentioned above, the biofilm EPS matrix, which loosely links bacteria within the biofilm, is primarily composed of proteins, polysaccharides, and eDNA. These components mediate what has been termed irreversible cellular attachment; they improve mechanical stability, maintain secreted enzymes, and sequester harmful biocides.[87] In theory, agents that can target the biofilm matrix have the potential to interfere with

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biofilm development, destabilize the biofilm, promote detachment, sensitize biofilm cells, and increase access of antibiotics. EPS-targeting compounds described in this section are summarized in Table 3.

For example, the addition of deoxyribonuclease I (DNase I) at the time of inoculation inhibits biofilm formation by a variety of Gram-positive and Gram-negative organisms, e.g., *S. epidermidis* and *P. aeruginosa*.[103,104] Specifically, DNase I cleaves single-stranded or double-stranded DNA at phosphodiester bonds that make up the phosphate backbone.[87] The role of eDNA in biofilm formation is not always clear, but evidence indicates that it promotes adhesion to abiotic surfaces since removal of eDNA from *S. epidermidis* and *S. mutants* reduces initial colonization and aggregation onto surfaces.[105] It is also crucial for the formation of nonsurface-attached aggregates by cystic fibrosis isolates of *P. aeruginosa*.[106] When administered to preformed mature biofilms, DNase I (100 μg/mL) reduced *Gardnerella vaginalis* biofilm biomass by 50% and furthermore worked in synergy with the antibiotic metronidazole.[90] However, the effects were modest presumably due to the limited penetration of the enzyme. As such, the application of DNase I is often proposed as an adjuvant therapy or a surface coating.[107] DNase I, also called rhDNase or dornase alfa, is available as an inhalational solution with the trade name Pulmozyme. It is used therapeutically in cystic fibrosis patients to improve pulmonary function by reducing sputum viscosity and chest congestion.[91]

Dispersin B, a glycoside hydrolase, produced by *Aggregatibacter actinomycetemcomitans*, degrades poly-*N*-acetylglucosamine, a polysaccharide that is found within the matrix of some bacterial biofilms and mediates attachment to abiotic surfaces.[108] Dispersin B plays an important role in biofilm dispersal as *A. actinomycetemcomitans* mutants unable to produce this enzyme form biofilms that cannot disassemble.[94] Consistent with this, when administered exogenously (at 40–50 μg/mL), it causes the detachment of preformed biofilms (grown for 10 to 24 h in microtiter wells) produced by *A. actinomycetemcomitans* and *S. epidermidis*,[95] *P. fluorescens*, and *E. coli* but not *P. aeruginosa*, *Salmonella enterica*, or *Yersinia pestis*.[93] Dispersin B has been shown to be nontoxic to human cells.[96]

Several exogenously applied proteases have been implicated in biofilm detachment because they degrade accessible cell surface proteins and thus have pleiotropic effects on attachment.[97,99,101,109] For example, proteinase K, a serine protease, triggers the dispersal of (24 and 48 h-old) *S. aureus* biofilms at the highest tested concentration of 250 μg/mL leading to 76% less biofilm mass[97] and has also been shown to prevent attachment of *P. aeruginosa* to wounded corneas.[109] Dispersal often renders cells more susceptible to antimicrobials and, likewise, concurrent use of antibiotics with proteinase K has shown to be very effective at eradicating biofilms.[97] Nonetheless, due to its proteolytic properties and consequent cytotoxicity to host cells, it seems unlikely that the enzyme will make its way to the clinic. However, low concentrations of another serine protease trypsin (0.75 μg/mL) have been shown to be nontoxic for human cells while exhibiting synergy with ceftazidime in destroying biofilms formed by *Pseudomonas* isolates recovered from burn wound infections.[99] Commensal organisms are also known to influence pathogen colonization through related mechanisms. For example, *S. epidermidis* secretes a serine protease, Esp, that destabilizes *S. aureus* biofilms by degrading biofilm-anchoring proteins, fibronectin-

Table 3. List of Selected Compounds Modulating the Biofilm Matrix

compound	mode of action	biofilm species	biofilm model	safety/drug development	ref.
DNase I, rhDNase or dornase alfa, works in synergy with antibiotics	destruction of eDNA	*A. baumannii, E. coli, H. influenzae, K. pneumoniae, P. aeruginosa, S. aureus, S. pyogenes*	flow cell chamber, microtiter, Petri dishes	safe/approved for cystic fibrosis treatment	88–91
dispersin B, works synergy with antibiotics	degradation of poly-*N*-acetylglucosamine	*Aggregatibacter actinomycetemcomitans, E. coli, P. fluorescens, S. epidermidis*	microtiter, polystyrene rods, polyurethane catheters	nontoxic to mammalian cells/approved for use in topical wounds and medical devices	92–96
proteinase K, works in synergy with antibiotics	degradation of cell surface proteins, e.g., biofilm-associated protein (Bap)	*S. aureus, Listeria monocytogenes*	microtiter	cytotoxic to mammalian cells/research stage	89 and 97
trypsin, works in synergy with antibiotics	degradation of cell surface proteins	*P. aeruginosa, G. vaginalis*	microtiter	nontoxic to mammalian cells at low concentrations/research stage	98–100
Esp	degradation of biofilm-anchoring proteins, fibronectin-binding proteins, protein A, and extracellular adherence protein	*S. aureus*	Petri dishes	information not available/research stage	101 and 102

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binding proteins, protein A, and extracellular adherence protein.[101] The effective and safe application of exogenously added Esp *in vivo*, however, remains to be demonstrated.

Likewise, phenol soluble modulins (PSMs) are biosurfactant peptides, secreted by *Staphylococci*, and are required for biofilm structuring and detachment.[110] Like rhamnolipids, PSMs are amphipathic which allow them to oligomerize and interact with cellular membranes.[110,111] The absence of PSMs in *S. aureus* mutants impairs channel formation and prevents biofilms from disseminating.[110] Conversely, other reports have shown that PSM oligomers are critical for biofilm stability.[111] Nevertheless, it is possible that PSMs carry both functions, but their exact biological role may depend on their effective concentrations. Due to these conflicting functions and documented cytolytic activity against neutrophils,[112] the therapeutic application of exogenously added PSMs remains questionable.

The complex and organized structure and altered physiology of biofilms contributes substantially to their resilience against antimicrobials. Therefore, the use of enzymes that degrade components in the EPS matrix or biosurfactants that trigger dispersal is a promising avenue for drug development. Nevertheless, the susceptibility of bacteria to the above agents depends on the chemical composition of the matrix which can vary greatly between species and strains.[113] Since there is great diversity of matrix components, combinations of these agents are likely required to significantly affect biofilms of various species. Furthermore, since certain agents do not possess strong bactericidal activity on their own, but rather disperse bacteria from biofilms, they will need to be paired with antibiotics[89,90] to enhance efficacy and particularly to avoid adverse disseminated infections. For example, dispersin B has been tested in formulations with antibiotics such as gentamicin or the antimicrobial peptide KSL-W for wound care applications.[92] Lastly, while matrix-targeted enzymes have undergone drug development in the cases of dispersin B and Pulmozyme, for example, certain agents (e.g., proteinase K) are likely too cytotoxic to be pursued further.

DRUG DELIVERY METHODS TO ERADICATE BIOFILMS: ENHANCING COMPOUND ACTIVITY

The matrix composition and architecture of biofilms serve to shield the bacteria against therapeutics, although it is important to note that this is not the only issue since biofilms are also adaptively resistant due to stress-coping alterations in gene expression[114] and the dormancy/persister-phenotype of cells in the biofilms.[115] However, targeting of pathogens in biofilms can be impeded by limited drug penetration, slowed diffusion, short exposure times, and chemical or electrostatic interactions with biofilm components (e.g., enzymatic degradation).[116,117] Drug supporting carriers could expand the safety, bioavailability, stability, and compound release over time, thereby ideally increasing its efficacy. A variety of drug delivery approaches, like polymer-, lipid-, and metal-based nanocarriers have been designed in an attempt to improve the penetration and subsequent eradication of mature biofilms.[116,117] Drug formulations discussed here are listed in Table 4.

Vesicular nanosystems composed of naturally occurring lipids are commonly recognized as safe, biocompatible, and biodegradable while exhibiting potent targeting ability.[117] Several studies have addressed the efficacy of liposomal drug formulations in biofilm infections.[116,124−126] Liposomes are spherical vesicles composed of one or more phospholipid bilayers and filled with aqueous solution. Therefore, hydrophilic

Table 4. Examples of Anti-Biofilm Drug Formulations

compound	mode of action	biofilm species	biofilm model	safety/drug development	ref.
amikacin encapsulated in liposomes (Arikace), synergy with antibiotics	binding to ribosomes, impairing protein translation, enhanced delivery	*Mycobaterium* spp., *P. aeruginosa*	clinical application in patients	safe/orphan drug designation for the treatment of cystic fibrosis and nontuberculous mycobacterial lung infections	118
colistin-loaded nanoembedded microparticles	membrane perturbation, sustained delivery	*P. aeruginosa*	microtiter, confocal microscopy	no information available/research stage	119
tobramycin polymeric nanoparticle delivery vehicles, linked with human DNase	blocking protein biosynthesis, enhanced bioavailability, penetration of cystic fibrosis sputum	*P. aeruginosa*	*Galleria mellonella*	low cytotoxity *in vivo*/research stage	120
SLN/NLC-tobramycin	blocking protein biosynthesis, enhanced efficacy to free antibiotic	*P. aeruginosa*	microtiter (pegs)	no information available/research stage	121
SLN-rifampicin	blocking gene transcription, enhanced efficacy to free antibiotic	*S. epidermidis*	microtiter	no information available/research stage	122
dextran-based hydrogels containing cationic biocide	membrane perturbation, prolonged bactericidal effect	*E. coli*, MRSA, *S. aureus*	*in vitro*, superficial MRSA infection mouse model	very good skin compatibility in animal models/research stage	123

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drugs can be encapsulated while lipophilic or amphiphilic compounds can insert inside the bilayer. One of the most promising formulations for the treatment of chronic *P. aeruginosa* lung biofilm infections in cystic fibrosis patients is the liposomal amikacin for inhalation, Arikace.[118] Amikacin is an aminoglycoside that acts by binding to the 30s ribosomal subunit of bacteria thereby shutting off the translation of bacterial proteins.[118] Water-soluble drugs, like amikacin, are located in the liposome's water core. Enclosure of the antibiotic into ~300 nm in size spherical, uncharged liposomes comprised of dipalmitoylphosphatidyl choline and cholesterol improves penetration, retention, and availability of the compound. It has been proposed that the liposomes shield the positively charged amikacin from negatively charged components of cystic fibrosis patient sputum and their cargo is released when lysed by *P. aeruginosa* secreted rhamnolipids at the infection sites.[118] In 2015, Arikace was granted orphan drug designation by the U.S. Food and Drug Administration for the treatment of *Pseudomonas* infections in patients with cystic fibrosis and for the treatment of nontuberculous mycobacterial lung infections.[118]

Besides liposomes, engineered nanoparticles have been explored as drug delivery vehicles. Nanoparticles are solid, colloidal particles made of macromolecular substances and are normally smaller than 200 nm. The compound of interest is adsorbed or attached to a nanomatrix or entrapped and/or encapsulated by it.[127] d'Angelo and co-workers[119] addressed the local delivery of colistin through engineered nanoparticles to improve *P. aeruginosa* clearance in the lung of CF patients. They designed nanoembedded microparticles made of polylactide-*co*-glycolide containing chitosan and polyvinyl alcohol and lactose or mannitol as carriers.[119] The formulation increased the penetration and transport of colistin through artificial CF mucus and exhibited enhanced *P. aeruginosa* biofilm eradication efficacy compared to the free peptide. This effect was ascribed to improved biofilm penetration and sustained drug release of the formulation.[119] Deacon et al.[120] showed that tobramycin polymeric nanoparticle delivery vehicles composed of the natural and biodegradable polysaccharides alginate and chitosan exhibit the same antimicrobial activity against *P. aeruginosa* while being bioavailable for longer periods *in vivo*. Linking of the human recombinant DNase dornase-alfa to the formulation improved DNA degradation and penetration of DNA-rich, thick cystic fibrosis sputum.[120]

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) have been investigated in order to overcome certain limitations of liposomes and polymeric nanoparticles such as shelf life, stability, encapsulation efficacy, drug release, and large-scale production.[128] These spherical particles are composed of solid phase lipid (e.g., fatty acids, steroids, triglycerides, glyceride mixtures or waxes), which varies between SLN and NLC, together with surfactant as an emulsifier. At body or ambient temperatures, SLN matrix lipids are solid while NLC contain solid and liquid lipids in their core.[128] Encapsulation of tobramycin into SLN and NLC led to the retention of its antimicrobial activity against planktonic bacteria, while maintaining or increasing its ability to eradicate preformed biofilm.[121] In this study, NLC-tobramycin was slightly more efficacious at biofilm eradication than SLN-tobramycin.[121] Similarly, various SLN formulations with the antibiotic rifampicin were able to decrease biofilm mass and residual viable bacteria more efficiently than the free antibiotic against preformed *S. epidermidis* biofilms.[122]

Aside from drug delivery via spherical nanovesicles, gel-like delivery systems have been developed, largely for topical treatment of, e.g., biofilm-infected wounds.[129] Recently, dextran-based hydrogels containing a nontoxic cationic biocide were synthesized.[123] These were capable of efficiently eradicating mature *S. aureus*, MRSA, and *E. coli* biofilms *in vitro* and in a MRSA infection model in mice[123] (Figure 3a–c).

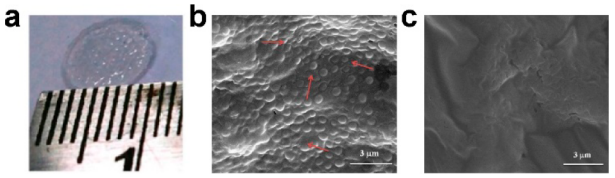

Figure 3. Dextran-based antibacterial hydrogel as a novel drug delivery approach. (a) The hydrogel disk composed of the biocompatible polymer dextran methacrylate and a cationic biocide was synthesized by photopolymerization. The disk was developed for layering onto a biofilm infected wound and to allow the release of the biocide for an extended period of time. (b, c) Scanning electron microscopy images were taken from MRSA-infected mouse skin (b) after 24 h of infection and (c) after 4 days of topical treatment of the infection with the hydrogel which was highly effective in clearing the topical infection. Images were adapted from Hoque et al.[123] Copyright 2017 American Chemical Society.

The optimized gel exhibited very good skin compatibility and was designed to allow layering onto a biofilm-infected wound. Drug release and subsequent bacterial killing was achieved for up to 5 days.[123]

Drug delivery strategies have been shown to be capable of providing antibiofilm compounds with important properties, including safety, bioavailability, and enhanced efficacy, boosting their potential to enter clinical trials. Whereas most compound delivery approaches described in this section are still at the research and development stage, the liposome-encapsulated antibiotic amikacin is a strong example of a formulated drug developed for the treatment of chronic cystic fibrosis lung infections usually caused by biofilms. Cationic biocides embodied in dextran-based hydrogels also show promise for future development toward the treatment of biofilm-infected wounds.

CONCLUSIONS

The eradication of mature bacterial biofilms continues to be an extraordinarily difficult endeavor. Their adaptive multidrug resistance to conventional antibiotics means that these often fail to elicit the desired therapeutic effect, and it is often impossible to apply high enough doses of antibiotics, in part due to adverse side effects. Alternative strategies to eradicate biofilms, as described here, offer promising future perspectives for the fight against these recalcitrant high-density infections. However, drugs that directly kill microorganisms run the risk of initiating the development of resistances, rendering them inefficient in the long term, as has been seen with antibiotics. Compounds that interfere with bacterial signaling and biofilm physiology or dynamics might reduce selective pressures on bacteria and offer a promising new approach to target biofilms. In general, specific treatment options have to be carefully designed, since dispersal of biofilm cells could also lead to severe side effects such as spreading of the infections to other areas of the body, resulting at the worst in systemic disease. Therefore, treatment leading to biofilm dispersal should be accompanied by bactericidal

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therapy. It still remains difficult to predict the outcome of a novel therapy as well as its long-term consequences, including the effects on beneficial organisms of the microbiome. Appropriate prediction models are urgently needed especially for biofilm infections where not a single nonantibiotic compound has been successfully advanced through clinical trials. Studying the dispersal of bacterial biofilm cells *in vivo*, and even assessing activity *in vitro* and *in vivo*, is highly complicated. Therefore, *in vitro* methods, ideally mimicking *in vivo* host−pathogen interactions, are still required to provide starting points for subsequent clinical development. Unfortunately, few novel findings make it to clinical trials, since alternative treatments do not fit the paradigms established for antibiotics, the mainstay of antibacterial therapy. However, the synergistic effects of different antibiofilm approaches with conventional antibiotics, as well as steady progress in delivery strategies that improve safety, bioavailability, and efficacy of the drugs, provide grounds for optimism. We submit it is important to generate an arsenal of different strategies and compounds to more effectively fight against biofilms.

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Notes

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ABBREVIATIONS

AMPs, antimicrobial peptides; DNase I, deoxyribonuclease I; eDNA, extracellular DNA; EPS, extracellular polymeric substances; MRSA, methicillin-resistant *Staphylococcus aureus*; MBEC, minimal biofilm eradication concentration; NLC, nanostructured lipid carriers; PSMs, phenol soluble modulins; SLN, solid lipid nanoparticles; TBBQ, *tert*-butyl benzoquinone; WED, wireless electroceutical dressing

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