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REVIEW

# Prevention and treatment of biofilms by hybrid- and nanotechnologies



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**Abstract:** Bacteria growing as adherent biofilms are difficult to treat and frequently develop resistance to antimicrobial agents. To counter biofilms, various approaches, including prevention of bacterial surface adherence, application of device applicators, and assimilation of antimicrobials in targeted drug delivery machinery, have been utilized. These methods are also combined to achieve synergistic bacterial killing. This review discusses various multimodal technologies, presents general concepts, and describes therapies relying on the principles of electrical energy, ultrasound, photodynamics, and targeted drug delivery for prevention and treatment of biofilms.

**Keywords:** biofilm, antimicrobial, drug carrier, hybrid technology, nanotechnology

## Introduction

Biofilms are an aggregate of microorganisms (eg, *Pseudomonas* spp., *Escherichia* spp., *Staphylococcus* spp., etc) attached to a substratum or an interface in moist environments. The substratum is composed of extracellular polymeric substances produced by microorganisms; the latter have a distorted phenotype with respect to growth rate and gene transcription. The presence of this distorted phenotype can cause a high forbearance to exogenous stress and resistance (up to 1000-fold increase) to antibiotic therapy.[1,2] Many planned events can predispose bacteria to adhere and form a biofilm (Table 1).[3] In general, biofilm formation is initiated by surface attachment of planktonic free-swimming bacteria on a surface that subsequently differentiate into mushroom- or pillar-like structures interspersed with fluid-filled channels.[4] Although these differentiated structures are genetically homogenous, a small fraction of bacteria can randomly survive challenge to lethal concentrations of an antibiotic (Table 2). These bacteria, referred to as "persisters", exist in a transient dormant state that protects them from antibiotics, and allows random switching back to a growth phase under favorable conditions.[5,6] These switch events have important roles in tolerance to antimicrobial therapy and drug resistance. For example, *Escherichia coli* persisters are tolerant to several antibiotics (eg, Ofloxacin ciporfloxacin and Mitomycin C).[7] Thus, it is clear that an understanding of antimicrobial tolerance mechanisms is important to institute novel therapeutic approaches. Most importantly, failure of antimicrobial therapy should not be perceived as a lack of clinical management tools. The genesis of phenotypic distortion and resistance to antimicrobials is partly associated with our inability to achieve sufficient antibiotic concentrations and induce changes in the microenvironment at the site of infection. In this review, we address clinically relevant methods for biofilm

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**Table 1** Essential factors in cell attachment and biofilm formation[3]

| Properties of the substratum | Properties of the bulk fluid | Properties of the cell |
|---|---|---|
| 1. Texture or roughness | 1. Flow velocity immediately below substratum | 1. Microbial cell and substractum surface hydrophobicity |
| 2. Hydrophobicity | 2. pH | 2. Fimbriae (cell surface hydrophobicity and attachment) |
| 3. Conditioning film | 3. Temperature (seasonal effect) | 3. Flagella (motile versus non-motile) |
| | 4. Cations (ionic strength; reducing the repulsive forces between the negatively charged bacterial cells and the glass surfaces) | 4. Extracellular polymeric substances |
| | 5. Presence of antimicrobial agents | |
| | 6. Nutrients | |

**Table 2** Factors contributing to resistance to antibiotics.

Biofilm-grown cells express increased resistance to antimicrobials in such a fashion that this property is distinct from planktonic cells

- Production of an exopolysaccharide matrix
  - Limit the transport of antimicrobial agents to the cells within the biofilm
  - Production of inactivating enzymes such as catalases and beta-lactamases
  - Oxygen deprivation and anaerobic growth
- Slow growth and the stress response
  - Related to nutrients
  - Unrelated to nutrients – physiological changes that provide microenvironment to protect the cell from various environmental stresses, eg, heat shock, cold shock, changes in pH, and many chemical agents
- Heterogeneity within the biofilm
  - Relative RNA content and growth rate
  - Pattern of respiratory activity
  - Protein synthesis
- Induction of general stress response
  - Activating quorum sensing systems – an RNA polymerase subunit (rpoS)-dependent process
  - Induction of a biofilm phenotype – a biofilm-specific phenotype is induced in a subpopulation of the community that results in expression of active mechanisms to combat the detrimental effects of antimicrobial agents
    - Increasing expression of multidrug resistance pumps
    - Changing profiles of outer membrane proteins

control that rely on complementary killing approaches as a means of prevention.

## Approaches in biofilm control

Conventional antimicrobial agents are based on standardized antimicrobial susceptibility test results, and are usually performed with planktonic cells. Translation of these methods to biofilm is difficult due to poor penetration and decreased susceptibility of bacteria to antimicrobial agents. Thus, complementary approaches that are based on surface modifications, use of device applicators (electrical, ultrasound, photodynamic, etc), and nanomaterials (liposomes, polymers, nanoparticles, and phage therapies) are being investigated as a means of prevention. These methods can achieve synergistic (hybrid) killing of resident pathogens, as described below.

### Biofilm surface modification

Attachment of a microbe to a favorable surface is a key step in biofilm formation. Thus, many interventional strategies focus on surface modification methods. Surface modification is defined as altering the functionality to produce specific biological and chemical interactions that prevent initiation of biofilm formation. Introduction of desired chemical functionality requires a thorough understanding of the structure and chemistry of the solid/environment interface. In general, biofilms typically form on a resident conditioning layer present before the influx of microorganisms. When microbes in an aqueous medium (eg, blood or water) make contact with the conditioning film, a weak and often reversible binding occurs due to Brownian motion (random movement of particles suspended in a fluid resulting from their bombardment by fast-moving atoms or molecules in the liquid or gas), gravity, microbial movement, and diffusion (Table 3).[8–33] The longevity of weak binding depends on the sum total of several variables, including electrostatic and hydrophobic interaction, steric hindrance, van der Waals forces, temperature, hydrodynamic forces, microbial cell surface, and the nature of the adherent surface. As organic substances in conditioning film concentrate near a surface, the adhesion strengthens due to congregation of microorganisms in nutrient-rich environments. As this happens, loosely bound organisms consolidate adhesion by producing exopolysaccharides that form complexes with surface materials and/or receptor-specific ligands located on pili and on fimbriae and fibrillae, or both. This phenomenon, termed the "bio-recognition processes",[6] is mediated by the specific binding of the receptors on cell conditioning surface with corresponding ligands in the microbe. This is achieved by a variety of extracellular matrix recognition molecules (eg, fibronectin, vitronectin, laminin, and collagen) that in

**Table 3** Properties favoring pathogen adhesion and its effect on adherence

| Property favoring adhesion | Bacteria | Effect | Reference |
|---|---|---|---|
| Cell surface hydrophobicity | *E. coli* | Hydrophobicity of cell surface reduced attachment | Zita and Hermansson[8] |
| | *Cryptosporidium parvum* and *Giardia lamblia* | Hydrophobicity of cell surface reduced attachment | Dai et al[9] |
| Negative charge | *S. epidermidis* | Intercellular adhesion | Mack et al[10] |
| pH (3) | *Bacillus sp.* | Hydrophilic surface enhanced adhesion | Husmark and Ronner[11] |
| **Surface conditioning** | | | |
| Presence of skim milk | *S. aureus, L. monocytogenes* | Inhibited attachment | Parker et al[12] |
| Presence of albumin, gelatin, and fibrinogen | *Pseudomonas sp.* | Inhibited attachment | Fletcher[13] |
| Presence of β-lactoglobulin | *L. monocytogenes, S. typhimurium* | Increased adherence | Helke et al[14] |
| Presence of *Pseudomonas fragi* | *L. monocytogenes, Caulobacter spp.* | Increased adherence | Sasahara and Zottola[15] |
| Presence of *Enterococcus* | *Campylobacter* | Increased adherence | Trachoo and Brooks[16] |
| Mass transport | *E. coli* | Mutagenesis to disrupt flagella and enhance attachment | Davies[17] |
| Surface charge | *L. monocytogenes* | High ionic strengths suppressed surface charge and enhance attachments | Mafu et al[18] |
| | *S. enteric* | High Na concentration inhibited adherence | Giaouris et al[19] |
| | *Bacillus cereus* | pH 3 enhanced attachment | Husmark and Ronner[11] |
| | *Streptococci* and *E. Coli* | Negative surface charge inhibit attachment | Flint et al[20,21] and Gilbert et al[22] |
| Hydrophobicity | *Cryptosporidium parvum* and *Giardia lamblia* | Hydrophobicity of cell surface reduced attachment | Dai et al[9] |
| | *L. monocytogenes* | Hydrophobicity of cell surface correlated to attachment with polystyrene | Chae et al[23] Chavant et al[24] Briandet et al[25] |
| | | *L. monocytogenes* has dynamic and highly changing cell surface; proteolytic enzyme decreased attachment | Giovannacci et al[26] |
| | *Vibrio proteolytica* | Proteolytic enzyme decreased attachment | Paul and Jeffrey[27] |
| | *Streptococcus sanguis* | Trypsin treatment reduced attachment | Oakley et al[28] |
| Surface roughness and surface micro-topography | *Staphylococcus aureus* | Harboring the BAP (Biofilm Associated Protein) gene were highly adherent | Cucarella et al[29] Arrizubieta et al[30] Tormo et al[31] |
| | *S. epidermidis* | Mutants with Tn917 transposon inserted decreases attachment; phenotype change between high adherent and low adherent by the proteolytic cleavage of SSP1 to SSP2 | Heilmann et al[32] |
| | *S. aureus, S. epidermidis, S. chromogenes, S. xylosus, S. simulans,* and *S. hyicus* | Ultrastructural organization and regulation of biomaterial adhesion of *Staphylococcus epidermidis* | Veenstra et al[33] |

addition to physical support, also adjust cell behaviors by presenting various growth factors in vivo.[34,35] This results in irreversible adhesion in the absence of physical or chemical intervention.[36] Critical target points include modulation of material surface properties, including chemical composition, hydrophilicity/hydrophobicity, surface charge, and roughness to a state that the adsorbed proteins can maintain their normal bioactivities (Table 4).

Due to complexities associated with biofilm formation, one approach is incorporation of broad-spectrum antimicrobials on attachment surfaces to attack early instituting bacteria. For example, rifampin and amoxicillin have been incorporated on a polyurethane surface through introduction of polymer side-chain functional groups; this results in bacterial inhibition that can persist for several months, especially from rifampin-coated polymer.[37] It may be noted that antimicrobial efficacy is

**Table 4** Properties and functionality of cell-extracellular matrix interface

| Properties | Factors to improve or control |
|---|---|
| Hydrophilicity, hydrophobicity | Adhesion |
| Ability to form covalent bonds | Bonding of reactive components |
| Formation of protective barriers | Cell response |

dependent on surface type. Incorporation of antimicrobials on an unstable surface may cause rapid release of drug. To address this, triggered release in the presence of infection has been developed. For example, neutrophil-derived factors can achieve triggered ciprofloxacin release. Similarly, macrophage-derived enzyme cholesterol esterase recognizes hydrophobic moieties and achieves drug release in polyurethanes surfaces synthesized with 1,1 diisocyanatododecane with long hydrophobic monomers.[38] Despite enhanced bacterial killing, incorporation of antimicrobials on surfaces is limited by encapsulation efficiencies.[39] Ideally, high levels of antimicrobial incorporation on modified surfaces should not affect material properties of the surface. However, a failure can cause contrasting outcomes. For example, sub-inhibitory concentrations of tetracycline and quinupristin-dalfopristin may favor *Staphylococcus* biofilm formation, as well as development of antibiotic resistant organisms.[40] One approach of addressing this is the sequestration of biological agents on the surfaces. For example, usnic acid, a secondary lichen metabolite, has been sequestered into modified polyurethane to achieve comparatively superior antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[41] Although this technology is in preclinical stages, and requires more investigation, preliminary data are very promising.

## Role of device applicators in biofilm prevention

### Electrical energy

The lethal effects of electric current (EC) and electrochemical potentials to microorganisms have been known for decades.[42–45] Electrical energy can increase antimicrobial activity against established biofilms, and may synergistically enhance antibiotic killing efficiencies. For example, simultaneous application of antibiotics and a low level EC between 1.5 and 20 V/cm can enhance the efficacy of aminoglycosides, quinolones, and oxytetracycline against *P. aeruginosa, Klebsiella pneumoniae, Staphylococcus epidermidis, E. coli,* and *Streptococcus gordonii* and may reduce minimum inhibitory concentrations of antibiotics by approximately 1000-fold.[46] These bioelectric effects can occur due to pH modifications, production, and transportation of antimicrobial agents into the biofilm by electrophoresis, generation of biocide ions, and hyperoxygenation.[47–49] Hyperoxygenation is mainly through hydrolysis of water that initiates oxygen production, improves oxygen tension, and increases the minimum inhibitory concentrations required to kill some bacteria.[50–52] Additionally, ECs can interact with charged particles and molecular chains within polar subsystems[53] to enhance bacterial killing.[54] There are more direct effects in polyionic antimicrobials. For example, gentamicin, a cationic antimicrobial, has improved activity against *S. epidermidis* biofilms in the presence of pulsed electromagnetic fields.[55] Thus, electrical energy can improve susceptibility and synergism in killing various bacterial pathogens.

### Enhancement of antimicrobial transport using ultrasound

Ultrasound (US) consists of non-invasive acoustic energy (pressure waves) with frequencies exceeding 20 kHz. Ultrasound waves can be focused through the skin and tissue and directed to the desired target in the body. Whereas low frequency US waves (<500 kHz) are not attenuated and produce heating, higher frequency (>1 MHz) ultrasound can achieve medical imaging and physical therapy. Similar to EC, low frequency US can significantly enhance the bactericidal activity of antibiotics in both planktonic and biofilm forms.[56] Ultrasonic energy can also release drugs from delivery devices (drug release by passive diffusion resulting in rapid dissipation to sublethal concentrations) in a triggered manner, increase

**Table 5** Antimicrobial nanomaterials

| Nanomaterial | Antibacterial mechanism | Application |
|---|---|---|
| Ag | Disruption of cell membranes and electron transport | Surgical dressing; surface coating of medical device |
| ZnO | Cell membrane damage | Surface coating of medical device |
| $TiO_2$ | Cell membrane damage | Antibacterial |
| Au | Cell membrane damage and electrostatic attraction | Photothermal therapy; antibacterial and antifungal agent |
| Chitosan | Increased permeability and rupture of membrane | Bacteria immobilizer; microbicide |
| Fullerenes | Dell membrane damage; increase infiltration of neutrophil | Disinfectant |
| Carbon nanotubes (CNTs) | Cell membrane damage; oxidation of cell membrane proteins and lipids | Antibacterial; surface coating |
| Nitric oxide releasing nanoparticles | Reactive oxygen species production | Surgical and wound treatment |
| Nanoemulsions | Membrane disruption | Antibiofilm agent |

cell membrane permeability, enhance microconvection by heating, and stimulate active or passive uptake of the antibiotics, thereby causing cavitation and disruption of cell membranes and biofilm.[56–59] These properties have been leveraged against various in vitro pathogenic models of *S. epidermidis, P. aeruginosa,* and *E. coli*,[60–62] and in vivo killing of *E. coli* biofilms on subcutaneous polyethylene discs containing gentamicin and vancomycin in rabbit models.[60,61,63] Interestingly, similar to EC, low-frequency US (70 kHz) with low acoustic intensity increased the transport of oxygen and nutrients to the cells, thereby killing *S. epidermidis, P. aeruginosa,* and *E. coli* biofilms.[62] To further enhance efficacy, insonation of *E. coli* or *P. aeruginosa* biofilms with microbubbles has been investigated to improve antibiotic efficacy.[63] For example, US (0.08 MHz) targeted microbubble destruction of biofilm in an in vivo rabbit model enhanced the effects of vancomycin.[64] Similarly, biofilm growth in ciprofloxacin-loaded hydrogels with US induced (43 kHz ultrasonic bath for 20 minutes daily) was significantly lower compared controls.[65] Clearly, augmenting antibiotic treatment with ultrasound is a promising device combination for drug delivery to counter biofilms.

### Photodynamic approaches to biofilm management

Light-based technology, termed photodynamic therapy (PDT), uses harmless visible light in combination with nontoxic photosensitizer to control infections.[66] Antimicrobial PDT was discovered more than 100 years ago and is under active investigation for cancer and age-related macular degeneration therapy.[66] In PDT light-sensitive dye, the nontoxic photosensitizer is illuminated with light of the appropriate wavelength to an excited state that causes molecular collisions with oxygen, resulting in formation of reactive oxygen species (ROS) and singlet oxygen by energy or electron transfer.[66,67] The high selectivity of PDT for rapidly growing hyperproliferating malignant cells can also be leveraged for microbial cell destruction.[67–69] Thus, current research with antimicrobial PDT is focusing on: (1) exploring the photophysical and photochemical properties, (2) exploring chemical properties to develop more effective and clinically compatible nontoxic photosensitizers, (3) bypassing the microbial permeability barrier and investing in novel delivery methodologies, and (4) preclinical and clinical investigations of PDT applications. Some examples of their application are in targeting dental plaques,[70] periodontitis,[71] gingivitis, endodontics,[72] osteomyelitis,[73] infections in cystic fibrosis,[74] infections of permanent indwelling devices (eg, joint prostheses and heart valves and implants),[75] and oral candidiasis.[76] Similar applications in biofilm treatment have also been superior to conventional antibiotics. A single photomechanical wave treatment (laser light at 666 nm) in *Actinomyces viscosus* biofilm in the oral cavity enhanced penetration of methylene blue by up to 75%.[77] Similarly, multi-species oral biofilms irradiated with helium/neon laser light in the presence of toluidine blue killed 95% of biofilm bacteria.[78] One major hindrance to biofilm targeting with PDT is slime production and growth phase (both characteristics of biofilm that hinder photodynamic inactivation of many pathogens, including *S. epidermidis* and *S. aureus*). This can be addressed partially through the use of polylysine-based cationic photosensitizers, which are currently being studied.[79]

## Role of nanomaterials in biofilm treatment and prevention

Nanotechnology is a multidisciplinary scientific field focused on materials whose physical and chemical properties can be controlled at the nanoscale range (1–100 nm) by incorporating chemistry, engineering, and manufacturing principles.[80] The convergence of nanotechnology and medicine, termed "nanomedicine", can potentially advance the fight against a range of diseases.[81] In particular, the application of nanomedicine for biofilm therapy can sustain drug release over time, increase solubility and bioavailability, decrease aggregation, and improve efficacy.[82–84] Various nanoparticle drug delivery carriers such as lipid-, polymer-, and nanometal-based carrier systems, have been developed to prevent bacterial colonization and biofilm formation as described below.

### Liposome delivery to biofilms

Among several promising nanoparticle drug-delivery systems, liposomes represent an advanced technology to deliver active molecules to the site of action; several formulations are already in clinical use (Table 5). Liposomes can carry both hydrophobic and hydrophilic drugs, have slow clearance rates,[85,86] and may deliver agents at increased concentrations, both in biofilm interfaces[87,88] or phagocytosed by cells harboring intracellular pathogens.[89–93] These specific liposomal characteristics are especially advantageous for antibiotic treatment to counter biofilm formation on medical devices and interfaces.

### Liposome encapsulation in medical devices

Liposomes encapsulating ciprofloxacin have been sequestered in polyethylene glycol (PEG) with rhGH (PEG-GH) and coated onto the surface of catheters; such coatings can completely inhibit bacterial adhesion for 1 week.[94] Similarly, liposomal ciprofloxacin hydrogel-coated silicone coupons prevented bacterial colonization during *P. aeruginosa* induced peritonitis in male Sprague-Dawley rats.[95] The ciprofloxacin-loaded liposomal hydrogels have also been incorporated in silicone Foley catheters

**Table 6** Nanocarriers for antimicrobial drug delivery

| Nanocarrier type | Composition | Encapsualted antibiotics | Target microorganisms |
|---|---|---|---|
| Liposomes | Phosphatidyl glycerol, phosphatidyl choline and cholesterol | Streptomycin | *Mycibacterium avium* |
| | 1,2-dipalmitoylphosphatidylcholine and cholesterol | Ciprofloxacin | *Salmonella dubli* |
| | Egg phosphatidyl choline, diacetylphosphate and cholesterol | Vancomycin and teicoplanin | *Methicillin-resistant Staphylococcus aureus* |
| | Soybean phosphatidyl choline and cholesterol | Ampicillin | *Micrococcus luteus* and *Salmonella typhimurium* |
| | Hydrogenated soybean phosphatidyl choline; phosphatidyl choline, cholesterol, and distearoyl phosphatidylglycerol | Amikacin | *Gram negative* |
| | Partially hydrogenated egg phosphatidyl choline, cholesterol, and 1-2-disteroyl-sn-glycero-3-phosphoethanolamine-N-(polyethylene glycol-2000) | Gentamycin | *Klebsiella pneumoniae* |
| | 1,2-dipalmitoylphosphatidylcholine and cholesterol | Polymixin B | *Pseudomonas aeruginosa* |
| | 1,2-dipalmitoylphosphatidylcholine, cholesterol, and dimethylammonium ethane carbamoyl cholesterol | Benzyl penicillin | *Staphylococcus aureus* |
| Solid lipid Nanoparticles | Stearic acid, soybean phosphatidyl choline, and sodium taurocholate | Tobramycin | *Pseudomonas aeruginosa* |
| | Glyceryl behenate, and sodium deoxycholate | Ketoconazole | *Fungi* |
| | Stearic acid | Rifampicin, isoniazid, pyrazinamide | Mycobacterium tuberculosis |
| | Glycerol palmitostearate | Econazole nitrate | *Fungi* |
| | Stearic acid, soybean phosphatidyl choline, and sodium taurocholate | Ciprofloxacin hydrochloride | Gram negative and gram positive bacteria, and mycoplasma |
| Solid Nanoparticles | Polyisohexylcyanoacrylate | Ampicillin | *Salmonella typhimurium* |
| | Polyisohexylcyanoacrylate | Ampicillin | *Listeria monocytogenes* |
| | Poly(ε-carprolactone) | Amphotericin B | *Leishmaina donovani* |
| | Polyacrylate | N-methylthiolated β-lactams | *Methicillin-resistant Staphylococcus aureus* |
| | Polyacrylate | Penicillin | *Methicillin-sensitive Staphylococcus aureus and Methicillin-resistant Staphylococcus aureus* |
| | Glycosylated polyacrylate | N-sec-butylthio β-lactam; ciproflaxacin | *Staphylococcus aureus* and *Bacillus anthracis* |
| Dendrimers | Polyamidoamine | Silver salts | *Staphylococcus aureus, Pseudomonas auroginosa, Escherichia coli* |
| | Pegylated lysine based copolymeric dendrimer | Artemether | *Plasmodium falciparum* |
| | Polyamidoamine | Sulfamethoxazole | *Escherichia coli* |
| | Polyamidoamine | Nadifloxacin and prulifloxacin | *Escherichia coli* |

to evaluate catheter-associated nosocomial urinary tract infections.[96] Insertion of these catheter (size 10 F) into New Zealand white rabbits and subsequent challenge with $5 \times 10^6$ virulent *E. coli* at the urethral meatus twice daily for 3 days resulted in a significant delay in average time to positive urine culture (from 3.5 to 5.3 days) and a 30% decrease in the rate of bacteriuria. Thus, this technology can potentially improve patient well-being and reduce health care costs.[96]

## Liposomes as drug delivery carriers to biofilm interfaces

A wide range of liposomes can also directly affect bacterial interactions during biofilm formation without the need for a device.[97–103] For example, pegylated cationic liposomes can inhibit adsorption of bacteria to biofilms, as the polyethylene glycol mole percent of component lipid is increased from 0% to 9%.[101] It is interesting to note that these interactions are generally an interplay of biofilm, liposomal, and surface type. For example, *Streptococcus sanguis* and *S. salivarius* biofilms respond differently to liposomes loaded with triclosan, with superior effects against *S. sanguis*.[102] Similar to biofilm type, the interaction of surface component and liposomes can cause contrasting outcomes. For example, solid supported vesicles enable adsorption of liposomes on the surface of metal nanoparticles (eg, zinc citrate particles), but result in antagonistic action particularly against *Streptococcus oralis*

biofilms.[99] Despite this, due to targeted delivery, a variety of liposomes are effective in inhibiting bacterial biofilm growth (Table 5), at lower drug concentrations, compared to equivalent concentrations of free drug in inhibiting cell growth.

## Polymer drug carrier

In addition to liposomal carriers, the use of biocompatible and biodegradable polymer based drug delivery systems has gained prominence in the medical field. Examples (Table 6) of polymer based carriers include microspheres, micelles, and hydrogel-type materials.[104] Poly(rhylene-glycol)- poly(alpha, beta-asparic acid), carboxylates, and heterobifunctional polyethylene glycol generally serve as important chemical components in biofilm treatment.[105] Efficacy can further be improved by adding pore-forming polymer.[106] For example, an albumin- or polyallylamine-based nanostructured polymer system can cause pores in cells during antibiotic delivery.[107] These pore forming delivery carriers can be useful, especially against mesh-related infection.[108] As an example, coating meshes with an ofloxacin-containing poly(ε-caprolactone) demonstrated prolonged and persistent release (72 hours), against *E. Coli, S. aureus, S. epidermis*,[109] *Enterobacteriaceae,* and some Gram-positive cocci that constitute nosocomial pathogens.[110,111] Efficacy can be improved further by drug combinations to increase the antibacterial spectrum of the anti-infective mesh and reduce the risk of selecting resistant bacteria.[112,113] For example, in clinical studies, a quinolones-rifampicin combination was highly effective in preventing device-associated infections.[109,114]

There are recent technological advances in development of dual drug-release coating around mesh filaments via an airbrush spray system.[115] This coating is made layer by layer and contains ofloxacin and rifampicin dispersed in a degradable polymer reservoir comprised of (poly[ε-caprolactone] [PCL] and poly[DL-lactic acid] [PLA]). This layered approach provided controlled drug release kinetics due to an ability to vary the structure of the degradable polymer in the multilayer coating. These meshes had excellent antibacterial properties against microorganism adhesion, biofilm formation, and peri-device inhibition of bacterial growth. The layer coating technology can also be easily extrapolated to other medical devices and drug combinations, as long as the particle parameters are controlled to achieve sustained drug release, and maintain therapeutic concentrations of antimicrobials combinations.

## Metal nanomaterials

Due to their unique physico-chemical properties, inorganic and metallic-based nanostructured materials have important roles in several biotechnological applications (Table 6).[116–118] An important aspect of these nanoparticles is the requirement of toxicity-free synthesis. These nanomaterials present interesting morphologies, including spheres, tubes, rods, and prisms. Examples include metal oxides (zinc oxide, iron oxide, titanium dioxide, and cerium oxide), metals (gold, silver and iron, copper, and magnesium) and quantum dots (cadmium sulfide and cadmium selenide).[119–125] Additionally, silicon dioxide, aluminum oxide,[96] and alginate nanomaterials can also be used as antimicrobial agents; each has specific properties and spectra of antimicrobial activity.[123–126] One nanoparticle which has demonstrated significant potential is silver. Nano-silver can reduce patient infection, dependence on antibiotic use, and associated costs. One major limitation in clinical translation is suboptimal clearance kinetics and ability to cause inflammation.[127] Other nanoparticles like nitric oxide (NO)-releasing silica nanoparticles have also had significant therapeutic efficacy in killing biofilm-based *P. aeruginosa*, *E. coli*, *S. aureus*, *S. epidermidis*, and *Candida albicans*.[128] In viability experiments, 99% of bacteria from each type of biofilm were killed via NO release. Thus, these nanoparticles have tremendous potential for clinical applications.

## Phage therapy

Phages are proteins that encapsulate a deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) genome (eg, a virus, bacterial surface proteins, etc). Phages can replicate at the site of infection, propagate radially throughout a biofilm, and exert strong bactericidal activity compared to conventional antimicrobial agents.[129] It is estimated that a radial propagation of single dose of a progeny phage can treat a biofilm infection of bacterial origin, infect adjacent cells, and degrade the biofilm matrix.[130] The mechanism of action of phages is through enzyme production (depolymerisation) that hydrolyses and degrades extracellular matrix of a biofilm.[131–134] In addition to mediating direct bacterial killing, phage agents can also be incorporated into a hydrogel coating on a catheter. Phages can significantly reduce adherence and biofilm formation on the catheter surface[135] as demonstrated in a *P. aeruginosa* in vitro.[136] Such a multimodal approach is an excellent example of a biofilm treatment, especially on indwelling devices.

Despite encouraging results, the use of phage therapy in humans is still in infancy. Bacterial resistance to phage, inactivation by the patient's immune system, and the presence of impurities (eg, endotoxins or phage-encoded virulence genes) in scaled-up phage formulation needs to be appropriately addressed prior to clinical use.[137] Inclusion of phage mixtures, engineered phages, controlled

scaled-up preparations to evade the immune system, and specific targeting of the bacterial genome may assist in such a goal.

## Conclusion

Persistent biofilm formations in medical devices have negative consequences for patient wellbeing and increase both the duration of hospitalization and health care costs. One major challenge in biofilm therapy is altered pathogen characteristics and occurrence of antibiotic resistance. Conventional antimicrobials have a restricted range of cellular targets and are mainly active against fast-growing pathogens, with no or reduced activity against biofilms. For desirable outcomes, conventional antimicrobial therapy needs to be complemented with electric current, ultrasound, drug carriers, and surface modifications to deliver a cocktail therapy. As discussed, complementary bacterial killing approaches have been reported by several research groups. Further developments in imaging and surface-analytical techniques allowing quantitative in situ investigation of cell/surface interactions at a submicron scale, providing information on the strength of microbial cell attachment to solid substrata, and the properties of macromolecules involved in this process, can significantly improve clinical outcomes.

## Disclosure

The authors report no conflicts of interest in this work.

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