ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Review

Nanoparticle-Based Strategies for Managing Biofilm Infections in Wounds: A Comprehensive Review

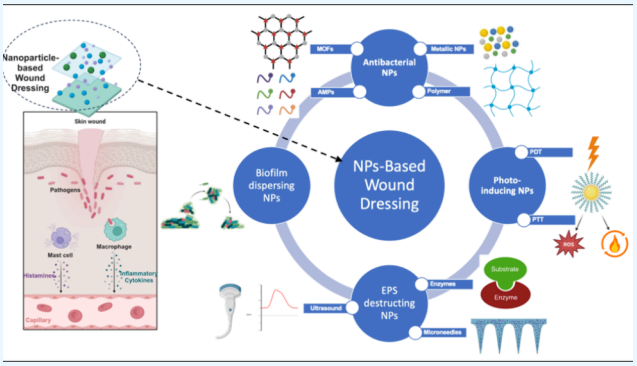
Omid Sedighi, Brooke Bednarke, Hannah Sherriff, and Amber L. Doiron*

Cite This: *ACS Omega* 2024, 9, 27853−27871

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Chronic wounds containing opportunistic bacterial pathogens are a growing problem, as they are the primary cause of morbidity and mortality in developing and developed nations. Bacteria can adhere to almost every surface, forming architecturally complex communities called biofilms that are tolerant to an individual's immune response and traditional treatments. Wound dressings are a primary source and potential treatment avenue for biofilm infections, and research has recently focused on using nanoparticles with antimicrobial activity for infection control. This Review categorizes nanoparticle-based approaches into four main types, each leveraging unique mechanisms against biofilms. Metallic nanoparticles, such as silver and copper, show promising data due to their ability to disrupt bacterial cell membranes and induce oxidative stress, although their effectiveness can vary based on particle size and composition. Phototherapy-based nanoparticles, utilizing either photodynamic or photothermal therapy, offer targeted microbial destruction by generating reactive oxygen species or localized heat, respectively. However, their efficacy depends on the presence of light and oxygen, potentially limiting their use in deeper or more shielded biofilms. Nanoparticles designed to disrupt extracellular polymeric substances directly target the biofilm structure, enhancing the penetration and efficacy of antimicrobial agents. Lastly, nanoparticles that induce biofilm dispersion represent a novel strategy, aiming to weaken the biofilm's defense and restore susceptibility to antimicrobials. While each method has its advantages, the selection of an appropriate nanoparticle-based treatment depends on the specific requirements of the wound environment and the type of biofilm involved. The integration of these nanoparticles into wound dressings not only promises enhanced treatment outcomes but also offers a reduction in the overall use of antibiotics, aligning with the urgent need for innovative solutions in the fight against antibiotic-tolerant infections. The overarching objective of employing these diverse nanoparticle strategies is to replace antibiotics or substantially reduce their required dosages, providing promising avenues for biofilm infection management.



1. INTRODUCTION

Biofilms are sessile complex communities of bacteria cells that adhere to a surface and are formed within a self-generated extracellular matrix (ECM). Bacterial infections, particularly biofilms, present an ongoing challenge in health care. The presence of a biofilm in a wound environment can prolong the healing process and lead to chronic infections. Despite recent advancements, chronic wounds remain a significant burden on healthcare systems. In developed countries, 1−2% of people are affected by chronic wounds in their lifetime. Particularly in the United States, chronic wounds impact 6.5 million patients annually.[1,2] The antibiofilm wound dressing market was valued at $729.7 million in 2022. Based on current trends, the global advanced wound care market is projected to reach $2.3 billion by 2033.[3] Biofilms are found in chronic wounds, with prevalence rates ranging from 20% to 100%.[4] These statistics highlight the importance of developing clinically translatable strategies for the management of wound infections.

As shown in Figure 1, the biofilm growth process is divided into three main steps, each of which has been extensively described in the literature: (1) attachment and growth, (2) maturation and quorum sensing, and (3) dispersal.[5−8] Formation of an ECM and quorum sensing (QS) are known to be two signs of matured biofilms. The ECM mainly includes polysaccharides, proteins, nucleic acids, and lipids, and it assists the planktonic bacteria in adhering to a surface and provides nutrients and water retention.[9,10] The biofilm matrix acts as a protective barrier, making the bacteria more tolerant to harsh environmental conditions such as starvation and desiccation,

Received: March 10, 2024
Revised: May 28, 2024
Accepted: June 4, 2024
Published: June 17, 2024

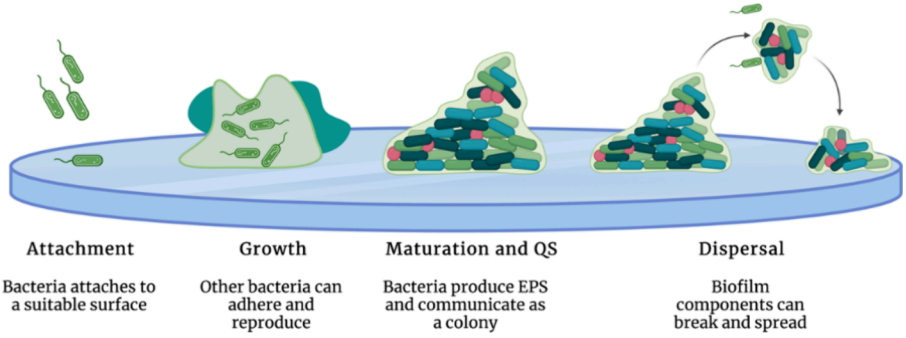


Figure 1. Schematic of biofilm formation (created with BioRender.com).

Table 1. List of Bacteria Capable of Forming a Biofilm

bacteria	Gram stain	description	common site of infection in body
Escherichia coli (*E. coli*)	−	rod-shaped	**urinary tract**: biofilms can be formed on the lining of the bladder and urethra
			intestines: most strains are harmless, some pathogenic strains can cause intestinal infections and form biofilms that contribute to their persistence
			medical devices: In a healthcare setting, *E. coli* can form biofilms on the surfaces of medical devices, such as catheters or implants, which are inserted into the body
			wounds: if *E. coli* contaminates a wound, it can form a biofilm on the wound surface, potentially leading to a more complicated and prolonged healing process
Pseudomonas aeruginosa (*P. aeruginosa*)	−	rod-shaped	**lungs**: in patients with cystic fibrosis or other chronic lung diseases
			wounds: it can infect wounds and enhance the tolerance of bacteria in biofilms to antibiotics
			medical devices: biofilm can be formed on the surfaces of medical devices such as catheters, ventilators, and prosthetic devices
			urinary tract: especially in patients with urinary catheters, where biofilms can form on the catheter surfaces
			ears: biofilm can be formed in the ear canal
			eyes: in contact lens wearers, it can form biofilms on the lenses or in lens cases, leading to eye infections
Staphylococcus aureus (*S. aureus*)	+	round in shape	**skin and soft tissue**: this includes conditions like boils, impetigo, and cellulitis
			wounds
			medical devices
			bone: in cases of osteomyelitis, an infection of the bone, *S. aureus* can form biofilms on the bone tissue, leading to chronic infection
			endocardium: this bacterium can infect the heart valves and the lining of the heart chambers (endocarditis), particularly in people with pre-existing heart conditions or with implanted heart devices
			respiratory tract: in patients with ventilators or those with cystic fibrosis, *S. aureus* can colonize the respiratory tract and form biofilms, contributing to lung infections
			urinary tract
Staphylococcus epidermidis (*S. epidermidis*)	+	cocci	**medical devices**
			surgical sites: *S. epidermidis* can form biofilms in surgical wounds or on implanted surgical materials
			prosthetic joints: In cases of prosthetic joint infections, *S. epidermidis* can form a biofilm on the surface of the artificial joint, leading to chronic infection and possibly requiring surgical intervention to resolve
			central nervous system shunts: this bacterium is a common cause of infections associated with shunts used in the treatment of hydrocephalus (excess fluid in the brain)
			heart valves
			intravenous catheters: it can colonize and form biofilms on intravenous catheters, leading to bloodstream infections
Enterobacter cloacae (*E. cloacae*)	−	rod-shaped	**medical devices and implants**
			respiratory tract
			urinary tract
Klebsiella pneumoniae (*K. pneumoniae*)	−	rod-shaped	**respiratory tract**: *K. pneumoniae* is a common cause of pneumonia
			urinary tract
			wounds: especially surgical site infection
			medical devices
			bloodstream: in cases of bacteremia, *K. pneumoniae* can form biofilms on the internal surfaces of intravenous lines, contributing to persistent bloodstream infections

so the bacteria become less susceptible to removal and eradication.[11−14] Quorum sensing (QS) allows individual cells to communicate and detect the presence of other cells, which can influence the colony's structure, trigger gene expression, and lead to tolerance to antibiotics and the host immune response.[15] The structure of biofilms makes them up to 1000-fold more tolerant to antimicrobial and immunological attacks compared to planktonic cells.[16] This extraordinary tolerance is responsible for their high infection and death toll. Table 1 lists common bacterial species that can form biofilm structures.

https://doi.org/10.1021/acsomega.4c02343
ACS Omega 2024, 9, 27853−27871

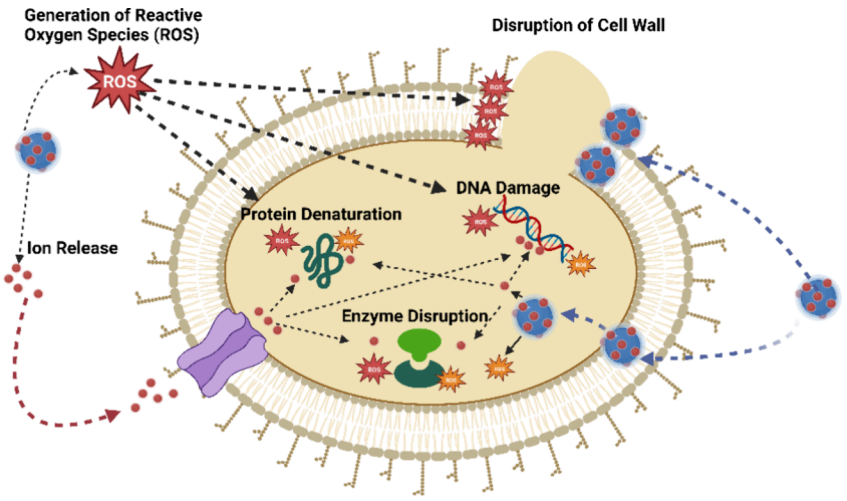


Figure 2. Antibacterial mechanisms of metallic nanoparticles (created with BioRender.com).

Among them, *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) can form polymicrobial biofilms that are known to be the leading cause of chronic wound infections.[17] The immense impact of biofilm infections has sparked various treatment approaches, of which the most prominent include antibiotics.

1.1. Biofilms in Wounds. Biofilms develop on diverse surfaces within the body, including teeth (as dental plaque), the respiratory tract (especially in individuals with chronic conditions), the urinary tract (common in catheterized patients), medical devices (like catheters and prosthetics), biomaterials (like implants), and the gastrointestinal tract; notably, they are highly prevalent in wounds, where their presence poses a significant challenge to effective healing and treatment. The primary focus of this Review is their presence in wound environments. The wound healing process involves several complex, highly regulated, and interdependent mechanisms that include inflammation, cell proliferation, and tissue remodeling.[18−20] Biofilms in wounds can impede the healing process by enabling bacteria to evade immune responses, prolonging inflammation, and restricting skin barrier function. The polysaccharide matrix of biofilms shields them from the host immune system and antimicrobial agents, thereby enabling immune evasion and prolonged inflammation.[21] This environment facilitates the continuous release of toxins and enzymes that damage surrounding tissues and perpetuate inflammation by activating immune cells.[22] Excessive neutrophil recruitment at the site leads to further tissue damage through the release of proteases and reactive oxygen species, impairing crucial healing processes such as epithelialization and granulation.[23,24] Aside from delaying wound healing and triggering inflammation, biofilms can transfer the genes for antibiotic tolerance to neighboring susceptible bacterial cells.[25]

1.2. Wound Dressing. The primary purpose of a wound dressing is to protect the injured area from external contaminants while maintaining appropriate hydration in the wound to support healing and tissue regeneration.[26] As a result, the materials used as wound dressings should be biocompatible, semipermeable to water and oxygen, and hypoallergenic. Traditional wound dressings were designed as passive barriers to protect against external contaminants. However, recently, nanotechnology has helped scientists create wound dressings capable of offering a protective environment while also delivering compounds that aid the wound healing process.[27] Common wound dressing materials that maintain appropriate moisture levels for healing include hydrocolloids, alginates, collagen, and other polymers that can sustain high moisture levels within their environment.

1.3. Nanoparticles: A Solution to Biofilm Antibiotic Tolerance. Employing antibiotics has several drawbacks, like the antibacterial tolerance exhibited by biofilms and issues related to antibiotic overuse, including antibiotic resistance.[28,29] To overcome these challenges, switching from antibiotics to novel nanoparticle-based methods holds great potential for treating biofilm-infected wounds. Recently, nanoparticles have emerged as promising tools in this battle thanks to their exceptional properties. Some have demonstrated considerable potential not only in disrupting established biofilms but also in preventing the formation of new ones. Their effectiveness is attributed to their small size and the ability to tailor their surface characteristics for specific bacterial targets, offering a new and efficient strategy for managing these resilient infections. Therefore, many nanoparticle-embedded wound dressings have been developed to help the treatment of biofilm-infected wounds as alternatives to antibiotics or reduce antibiotic usage. The methodologies are categorized as follows: (1) the most common nanoparticles with promising antibacterial effects, (2) nanoparticles utilized in phototherapy methods to disrupt bacterial infections, (3) nanoparticles designed in systems to disrupt extracellular polymeric substances (EPSs), and (4) nanoparticles that help induce dispersion in biofilms. Each category has its own subgroups. Throughout this Review, we review biocompatible nanoparticles used to demonstrate these specific properties and not the nanoparticles used as carriers for antibiotics.

2. NANOPARTICLES WITH ANTIBACTERIAL PROPERTIES

A distinctive characteristic of nanoparticles is their large surface area/volume ratio, which gives them unique and differing properties compared to their macroscopic counterparts. A hallmark of the potential of biomedical nanotechnology is the ability to design the nanoparticle's surface specifically to control interactions with the surrounding microenvironment. Additionally, certain types of nanoparticles have demonstrated

https://doi.org/10.1021/acsomega.4c02343
ACS Omega 2024, 9, 27853−27871

antimicrobial effects. This section will discuss the application of nanoparticles in wound dressings, highlighting their antibacterial properties and elucidating the underlying mechanisms.

2.1. Metallic Nanoparticles with Antibacterial Properties. Metallic nanoparticles such as silver (Ag), copper (Cu), and gold (Au) have been widely studied in wound treatment for the prevention and treatment of bacterial infections. These nanoparticles can also be utilized as single ions or in biometallic forms, further expanding their versatility in biomedical applications. These nanoparticles demonstrate unique physical and chemical properties, including small size, high surface area, surface energy, and reactivity. There are three main hypotheses for the mechanisms behind the antibacterial properties of these nanoparticles (Figure 2): (1) accumulation of nanoparticles on the bacterial cell membrane leads to increased bacterial permeability, (2) metallic nanoparticles cause oxidative damage via the generation of reactive oxygen species (ROS), and (3) metallic ions released from these particles disrupt DNA replication by depleting intracellular ATP.[30,31] Combining the above antibacterial pathways with an appropriate antibiotic can potentially offer the advantage of effectively disrupting antibiotic-tolerant biofilms with a low dose of antibiotics, although each approach has its shortcomings. The main obstacles hindering the commercialization of metallic nanoparticles include their size, shape, dose-dependent behaviors, concerns about cytotoxicity, and reduced effectiveness against certain specific bacterial species. This section briefly reviews the antibacterial properties of some of the most studied metallic nanoparticles. It should be noted that the bactericidal impact of each of the following nanoparticles depends on various parameters, including size, the shape of the synthesized particles, and the surface charge of the particles. Size can not only impact the properties of nanoparticles, including their antibacterial properties, but also determine if the particle can reach the nuclear content of bacteria. Nanoparticles greater than 10 nm tend to aggregate on the cellular surface and compromise cellular permeability; however, NPs smaller than 10 nm tend to diffuse into the bacteria, impacting DNA and the enzymes leading to cellular lysis.[32] Despite having differences in membrane structure, a majority of the Gram-positive and Gram-negative bacteria carry a negative charge. Therefore, the electrostatic interaction that can be caused by the surface charge of NPs and bacterial cells can play a crucial role in antibacterial properties of these nanoparticles.[32,33] Aggregation and excessive release of ions can negatively impact antibacterial activity and cytotoxicity of nanoparticles. Coating materials, such as polymers, lipids, or inorganic substances, can prevent NPs from aggregating and provide a sustained release of ions, leading to improved antibacterial properties with lower toxicity. Coatings protect the NPs from premature degradation and help target the specific sites within the body or within a microbial colony.[34] For example, polymer-coated nanoparticles can be engineered to be responsive to pH changes,[35] enzymes,[36] or other biochemical cues present in infection sites, which trigger the release of the encapsulated antimicrobial agent. Lipid coatings can enhance the biocompatibility and fusion with microbial membranes.[37]

2.1.1. Silver Nanoparticles with Antibacterial Properties. Silver is widely used in antimicrobial products, some with FDA-approved medical applications, including wound dressings. The atomic form of silver (Ag) must be oxidized to Ag^+ to exert biological activity. When the applied Ag is exposed to wound exudate, it ionizes and becomes biologically active. Silver ions can interact with phosphorus- and sulfur-functionalized groups of proteins and DNA and adhere to them. The adhered ions can enhance the permeability of the cytoplasmic membrane, disrupting the bacterial envelope. After the uptake of free silver ions into cells, these ions generate ROS and interrupt adenosine triphosphate production. This process may lead to problems in DNA replication, resulting in the death of microorganisms.[38]

Recent studies have questioned the cytotoxicity of silver NPs and suggested bacterial tolerance to silver. A recent study proved for the first time that the silver released from a wound dressing could penetrate the intact porcine dermis and induce DNA damage in the residing cells, hindering wound healing.[39] The antibacterial efficiency and toxicity of silver NPs are correlated not only to the concentration of silver but also other factors like the form of silver and the construction of the dressings. Using zebrafish fins as an *in vivo* model, Pang et al. show that using silver-containing wound dressings during the beginning stage of wound healing might have an adverse effect and disrupt wound healing by impairing granulation tissue.[40] Moreover, the appearance of silver-resistance genes in methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (MR-CNS) isolated from wounds has raised concerns about the future of silver-containing wound dressings.[41] Furthermore, recent data show silver to be less effective on Gram-positive bacteria and lacking in the ability to improve healing rates.[42] While FDA-approved silver wound dressings appeared to be highly effective and safe initially, the identified concerns make further research into these technologies necessary. Many articles specifically review silver NPs in more depth.[43−45]

2.1.2. Copper Nanoparticles with Antibacterial Properties. Another metallic nanoparticle widely investigated in wound healing is copper (CuNP) due to its excellent antibacterial properties. The antibacterial mechanism of CuNPs is still a subject of debate. One of the most probable mechanisms is the release of Cu^{2+}, resulting in cell wall and membrane damage.[46] Copper ions also inhibit the production of enzymes and proteins that bind to DNA.[47] The antibacterial efficiency of CuNPs depends on the size and concentration of these NPs. A study conducted by Alizadeh et al. examined the potential therapeutic effects of various CuNP concentrations (1 μM, 10 μM, 100 μM, 1 mM, and 10 mM) and sizes (20, 40, 80 nm) on wound healing.[48] When a 1 μM concentration of 80 nm CuNPs is used, the peak of antibacterial properties is reached without inducing cellular toxicity against endothelial cells; these NPs also accelerate the healing process by promoting endothelial cell migration and proliferation and collagen 1A1 expression. The promotion of angiogenesis and acceleration in the healing of full-thickness skin wounds with no adverse effects were observed after treating rat models with the same size and concentration of CuNPs. Another antibacterial mechanism associated with CuNPs is ROS generation. ROS generation hinders DNA replication by causing membrane lipid peroxidation and disrupting membrane integrity and permeability.[49] In elevated concentrations, copper can be highly toxic; therefore, more comprehensive studies on copper-embedded wound dressing are encouraged.

Copper sulfides can also be functionalized onto surfaces to exploit their potential antimicrobial properties as inorganic nano-objects. Gargioni et al.[50] have synthesized copper sulfide

https://doi.org/10.1021/acsomega.4c02343
ACS Omega 2024, 9, 27853−27871

Table 2. List of Metallic NPs Used in Wound Dressings

metallic NPs	matrix	tested microorganisms	*in vivo*	properties	ref
Ag	chitosan-stabilized silver nanoparticles	biofilm: *S. aureus* and *P. aeruginosa*		Antibiofilm activity against *P. aeruginosa* at sub-MIC levels: reduce biofilm formation by 78% and 69% at 0.5× and 0.25× MIC, respectively. Antibiofilm activity against *S. aureus* at sub-MIC levels: reduce biofilm formation by 48% and 36% at 0.5× and 0.25× MIC, respectively. Potential antioxidant activity is 92%, 90%, and 75% at concentrations of 4000, 2000, and 1000 μg/mL, respectively, compared to ascorbic acid.	57
Ag	chitosan-capped silver	*S. aureus* and *P. aeruginosa*		Biofilm growth of *S. aureus* and *P. aeruginosa* is inhibited by 85% and 95%, respectively, at 100 μg/mL of Ch-AgNPs. Ch-AgNPs promote wound healing by increasing the migration of RAW 264.7 murine macrophages cells at 75 and 100 μg/mL after 24 h.	58
Ag	silver nanoparticles coated with pectin	*E. coli* and *S. epidermidis*		Pectin acts both as a reductant and a coating. For planktonic cells: the 24 h MIC values against *E. coli* are 31.25 and 125 μM for p-AgNp and Ag^+, respectively. the 24 h MIC values against *S. epidermidis* are 500 and 125 μM for p-AgNP and Ag^+, respectively. On preformed *E. coli* biofilms, p-AgNP and Ag^+ show MICs of 500 and 125 μM, respectively, and 80 and 65% viability reduction. p-AgNPs promote fibroblast proliferation and wound healing on model cultures.	59
Ag	colloidal silver nanoparticles (cAgNPs)	methicillin-sensitive and methicillin-resistant *S. aureus* (MSSA, MRSA), *Enterococcus faecalis*, and vancomycin-resistant Enterococci (VRE)	20 patients with chronic infected DFU (≥1 month) without any vascular or other large wounds, aged 18−65 years	The MIC and MBC values of the cAgNPs ranged from 0.5 to 1.0 μg/mL and 1.0 to 8 μg/mL against the tested organisms, respectively. *Enterococcus faecalis* and VRE show higher MBC values as compared to the *S. aureus* strains. The cAgNPs inhibit biofilm formation in the low, medium, and high biofilm producers by 91%, 83%, and 75%, respectively, at the highest concentration (52 ppm).	60
Cu	cotton fabric coated with reduced graphene oxide (rGO) and copper nanoparticles (CuNPs)	*E. coli*, *P. aeruginosa*, *S. epidermidis*, *Corynebacterium xerosis*, and *Micrococcus luteus*		The fabric releases copper ions through an oxidation−reduction process. The fabric inhibited the growth of *E. coli*, *C. xerosis*, and *M. luteus* with a 99% efficiency, while it had no cytotoxic effects against CCD-986Sk, human dermal fibroblast (HDF), human skin cells, and NIH/3T3, a mouse skin cell, up to 24 h of exposure.	61
Cu	copper oxide nanoparticles (CuO-NPs)	*S. aureus*, *Bacillus subtilis*, *P. aeruginosa*, *E. coli*, and *Salmonella typhimurium*		Inhibit bacteria growth in significant diameters around their placement. Biofilm study: only effective against Gram-positive strains of *S. aureus* and not against the tested Gram-negative strain. However, the results show CuO-NPs at 0.3, 0.15, 0.07, 0.03, and 0.01 mg/mL reduce the biofilm formation by 95%, 94.1%, 94.4%, 85.9%, and 68.8%, respectively.	62
Cu	chitosan-based copper nanocomposite (CCNC)		open excision wound model in adult Wistar rats	Significant increase in wound contraction in treated rats. Increased fibroblast proliferation, collagen deposition and intact re-epithelialization in CCNC-treated rats. Chitosan-based copper nanocomposites efficiently enhance cutaneous wound healing by modulating various cells, cytokines, and growth factors during different phases of healing process.	63
Cu	copper incorporated into nanofibers of poly-D,L-lactide (PDLLA) and poly(-ethylene oxide) (PEO)	*P. aeruginosa* PA01 and *S. aureus* (strain Xen 30)		After 48 h in the presence of Cu−F, biofilm formation by *P. aeruginosa* and *S. aureus* is reduced by 41% and 50%, respectively. Only a few breast epithelial cells (3%) survive exposure to copper-containing fibers, suggesting that the levels of copper released from the nanofibers are highly toxic to cells in the tissue culture.	64
Au	Au capped by MBA	MDR *S. aureus* and MDR *S. epidermidis*	female Sprague−Dawley (SD) rats (average weight of 250 g); wound infection models were built by infecting the full-thickness skin wounds (2 cm in diameter) with different pathogenic bacteria (*S. aureus* and MDR *S. aureus*)	Au-MBA NPs show no toxicity even at extremely high concentrations. The final data show that the permeability of the bacterial cell wall for MDR *S. aureus* that are treated with Au_MBA NP increases sharply with the increase in concentration of Au-MBA NPs. When the concentration of Au-MBA NPs increases to 6 μg/mL, the cell wall starts to become unclear. The cell wall becomes invisible when 12 μg/mL NPs is added.	56
Au	4,6-diamino-2-pyrimidinethiol-functionalized gold nanoparticles (DAPT-AuNPs) and a silk fibroin (SF) mixed-matrix membrane	MDR *E. coli*	Eight-week-old SD rats (female) with an average weight of 250 g	*In vitro*: great antibacterial activities against both antibiotic-resistant and antibiotic-susceptible *E. coli*. The superior hydrophilicity of SF allows the effective release of DAPT-AuNPs from the film within minutes. *In vivo*: the healing degree of membranes with a DAPT-AuNP (3 $\mu g/cm^2$) group is distinctly better compared to those treated with DAPT-Au-SF MMMs (0 $\mu g/cm^2$) and gauze. High antibacterial activities of DAPT-Au-SF MMMs (3 $\mu g/cm^2$) allow the acceleration of wound closure.	65
Au	nonspherical AuNPs, including rods, peanuts, stars, and porous spherical-like nanoparticles	*E. coli* and *P. aeruginosa*		These shapes can affect the membrane architecture of the tested microorganisms, *E. coli*, and *P. aeruginosa* strands. The enhanced antibiofilm properties can be attributed to the improved membrane-permeabilizing properties of the varying shaping of AuNPs. Alongside damaging the membrane of the bacteria, ROS formation is also observed, playing a key role in the death of the pathogen and prevention of biofilms.	66

https://doi.org/10.1021/acsomega.4c02343
ACS Omega 2024, 9, 27853−27871

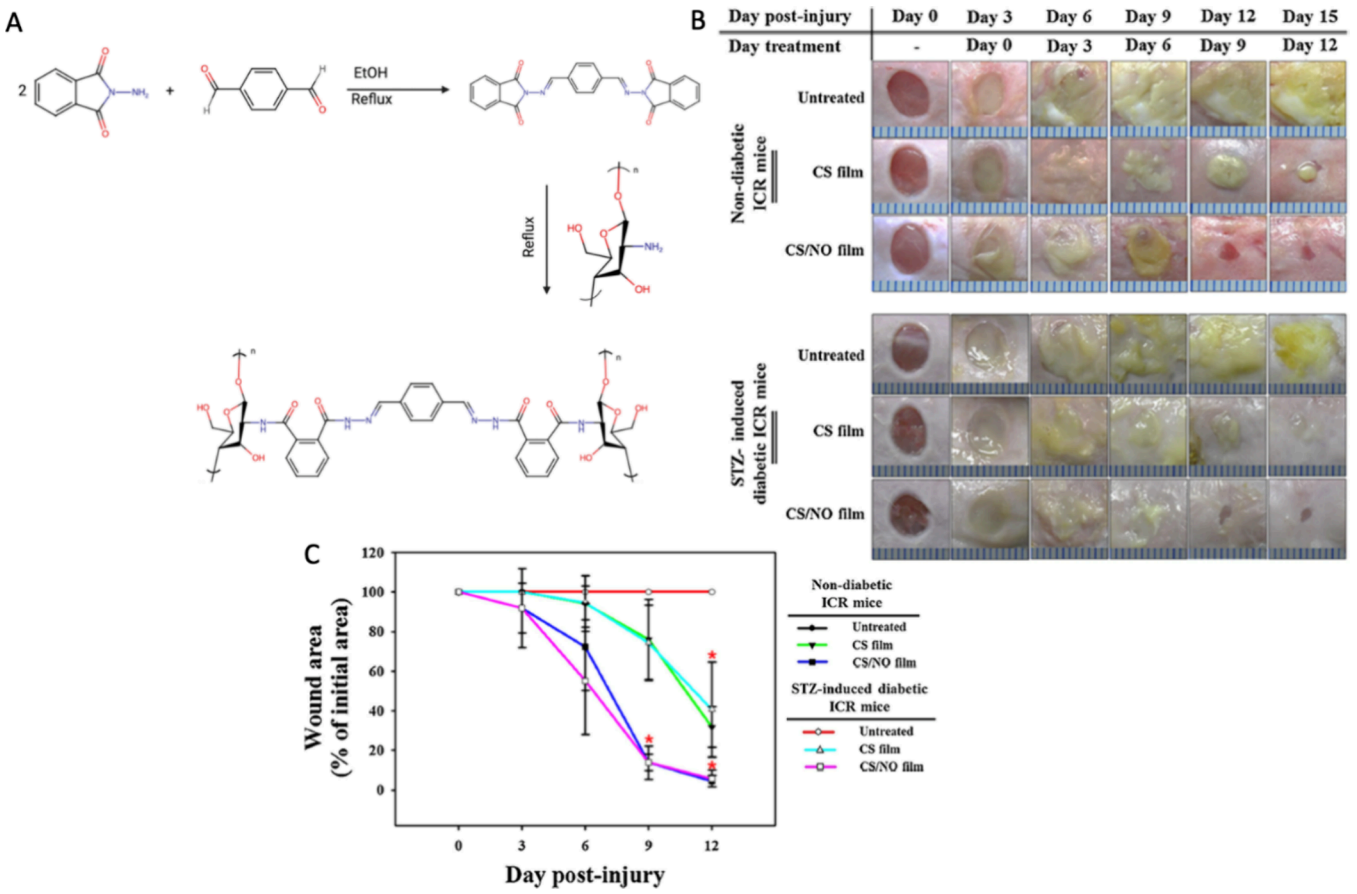


Figure 3. (a) Schematic of chitosan's reaction with with 2-([4-[(1,3-dioxoisoindolin-2-ylimino)methyl]phenyl]methyleneamino)isoindoline-1,3-dione (created with BioRender.com). Reproduced with permission from ref 70. Copyright 2020 Elsevier. (b) MRSA biofilm-infected wounds in nondiabetic and STZ-induced diabetic ICR mice. Representative photographs of healing in nondiabetic (upper panel) and STZ-induced diabetic ICR mice (lower panel) with the MRSA biofilm challenge treated with or without the CS/NO film. (c) Wound area reduction percentage of mice skin lesions relative to the initial 6 mm wound. Data shown are mean ± SD ($n = 6$), different wounds; *$P < 0.05$, compared with untreated group. Reprinted with permission from ref 72. Copyright 2020 Elsevier.

nanoparticles (CuS NPs) and functionalized them with (3-aminopropyl)trimethoxysilane (APTES) such that the nanoparticles adhered to the glass plate. Their results show good microbicidal effects over 24 h against both *S. aureus* and *E. coli*. The nanoparticles eradicate the bacteria and release small amounts of Cu^{2+}, which has also been shown to accelerate the wound-healing process. This combination of effects from the CuS NPs could improve the treatment of biofilm infections. CuS NPs are easy to prepare and relatively inexpensive compared to other noble metal nanoparticles.

2.1.3. Gold Nanoparticles with Antibacterial Properties. Gold nanoparticles (AuNPs) are biocompatible and have been extensively investigated for biomedical applications like wound healing to a range of effects. Kim et al. have shown that a hydrocolloid membrane (HCM) coated with phytochemically stabilized gold nanoparticles (pAuNPs) substantially facilitates dermatological wound healing.[51] They have used Sprague–Dawley (SD) rats to study skin regeneration and observed that in the first 5 days the rate of wound closure is four times quicker in the pAuNP-HCM-treated group than in the gauze (GZ)- or HCM-treated groups. Moreover, in wounds treated with the pAuNP-HCM, an increase in vascular endothelial growth factor (VEGF), angiopoietin 1 (Ang-1), and angiopoietin 2 (Ang-2) expression was reported.

By changing the size, shape, and surface properties of AuNPs combined with their inherent biocompatibility, AuNPs have been exploited as potential alternatives to antibiotics, especially for bacterial biofilm infections. AuNPs are known to show their antibacterial properties through two mechanisms. First, they alter the membrane potential by inhibiting ATPase activities to decrease the ATP level, leading to a reduction in the metabolism of microorganisms. Next, they inhibit tRNA binding of the ribosome subunit, causing a collapse in the biological mechanism.[52,53] Positively charged gold nanoclusters with an average size of 2 nm demonstrated another antibacterial mechanism that undermined the integrity of negatively charged cell membranes, leading to cell rupture.[54]

AuNPs can be incorporated into a wound dressing to inhibit bacteria and facilitate healing. For instance, both *in vitro* and *in vivo* studies show that higher antimicrobial and antifungal properties could be achieved when AuNPs are cross-linked with an aqueous extract of *Gundelia tournefortii* L. leaves (GT).[55] Compared to the other metallic agents, AuNPs show less antibacterial activity, and as a result they usually are modified with functional groups. Adding functional groups can enhance the electrostatic interaction of AuNPs with the membrane cells, leading to a more robust antibacterial activity. Mercaptophenylboronic acid is a functional group containing boronic and mercapto groups. The boronic acid group can

https://doi.org/10.1021/acsomega.4c02343
ACS Omega 2024, 9, 27853−27871

covalently bind to the peptidoglycan layer, which is a protective shell around the cell wall in Gram-positive bacteria, and the mercapto group can connect to AuNPs through a Au−S bond.[56] In addition to the challenges related to the penetration of AuNPs into biofilms and concerns about their biocompatibility, particle shape and size variations can profoundly impact their antibacterial effectiveness. This variability in shape and size may present further hurdles on their path toward industrial-scale production, as discussed in other articles.[54] Besides being antibacterial agents, AuNPs can disrupt bacterial cells by generating heat, which will be addressed in the phototherapy section. Table 2 summarizes recent experimental studies that use these metallic nanoparticles as antibacterial agents for the treatment of bacteria-infected wounds.

2.2. Polymer Nanoparticles with Antibacterial Properties: Chitosan. Polymers have been widely used in biomedical applications because of their intrinsic biocompatibility, lack of toxicity, low immunogenicity, and biodegradability. In most cases, the polymers themselves do not show antibacterial properties, but nanocomposites can incorporate this functionality.[67] Among polymers, chitosan has been widely used since it is antibacterial and biocompatible.[68] When used as a wound dressing, chitosan has also greatly stimulated the natural healing process. Chitosan's mechanism of action against microbial cells is not fully recognized. Numerous studies have classified it as follows: (1) electrostatic interactions between cationic chitosan and anionic molecules at the microbial cell surface, causing cell wall disruption; (2) low molecular weight chitosan can diffuse through the cell membrane, interact with DNA, and interfere with the protein synthesis; and (3) chitosan chelates metals that are fundamental to cell stability.[69]

Chemical and physical modifications can further enhance chitosan's antimicrobial properties. Modified chitosan and modified chitosan nanoparticles can also be used to create hydrogels for antimicrobial treatment, as reported by Ahmed et al.[70] The sustainable antimicrobial hydrogel is synthesized by the reaction of chitosan with 2-([4-[(1, 3-dioxoisoindolin-2-ylimino)methyl]phenyl]methyleneamino)isoindoline-1,3-dione via ring opening of a cyclic imide moiety in a compound (the reaction is shown in Figure 3a). Gels are tested against eight pathogenic strains of Gram-negative and Gram-positive bacteria and two forms of fungi. The modified chitosan and the modified chitosan nanoparticle gels show higher antimicrobial activity than the unmodified chitosan and chitosan nanoparticle gels. The modified chitosan nanoparticle gel has the highest minimal inhibitory concentration and minimal bactericidal concentration, especially with Gram-positive bacteria *Streptococcus pyogenes* at 19.5 and 39 μg/mL compared to the standard antibiotic ciprofloxacin at 19 and 38 μg/mL, respectively. This study shows how modifying antimicrobial materials allows for even more antimicrobial properties, which could be very beneficial for treating infections.

Pairing chitosan nanoparticles with biosurfactants has also proven effective for antimicrobial treatments, as studied by Marangon et al.[71] using rhamnolipid. The biosurfactant rhamnolipid reduces the size and polydispersity index of the chitosan nanoparticles and produces a more positive surface charge by leaving more free amino groups on the surface of chitosan NPs. When tested on planktonic bacteria and biofilms of *Staphylococcus* strains (Gram-positive), the chitosan nanoparticles with rhamnolipid (CRNPs) have more antimicrobial effects than the chitosan or rhamnolipid alone. The observed minimum inhibitory concentration (MIC) of the CRNPs is 0.74 μg/mL for *S. aureus* DSM 1104 and 0.78 μg/mL for *S. aureus* ATCC 29213. The minimum bactericidal concentration (MBC) of the CRNPs is 0.78 μg/mL for *S. aureus* DSM 1104 and 0.77 μg/mL for *S. aureus* ATCC 29213. For *S. epidermidis*, the MIC is 0.78 μg/mL and the MBC is 0.74 μg/mL. When testing the nanoparticles in *S. aureus* DSM 1104 and *S. epidermidis* biofilms, Marangon et al. observed that chitosan alone and bare chitosan nanoparticles eliminate bacteria in the upper parts of the tested biofilms, while the CRNPs are much more effective. When the concentration was 0.11 μg/mL, the CRNPs eradicated most of the sessile bacteria within the biofilms and reduced the number of viable cells below the detection limit. The overall improved antimicrobial activity of the CRNPs is linked to an increase in the local delivery of the chitosan and the new ability to successfully target Gram-positive bacteria due to the presence of the rhamnolipid. These particles have low toxicity and are promising for widespread use in the pharmaceutical and food industries.

In another study, a nitric oxide (NO)-releasing chitosan film was developed and its antibiofilm activity was evaluated, including *in vivo* wound healing efficacy against MRSA biofilm-infected wounds in diabetic mice.[72] The results show a sustained release of NO over 3 days in the simulated wound fluid. Bacterial viability results show that the NO-releasing chitosan film (CS/NO film) substantially augments antibacterial activity against MRSA by >3log reduction. Furthermore, the CS/NO film has threefold higher antibiofilm activity than the chitosan film. *In vivo* results reveal that samples treated with the CS/NO film exhibit faster biofilm dispersal, wound size reduction, and epithelialization rates than untreated and collagen film-treated samples. Figure 3b shows the difference between the MRSA biofilm dispersion of samples treated with the CS/NO film, the CS film, and the untreated sample under nondiabetic and diabetic conditions. To induce insulin-dependent diabetic conditions, the authors administered streptozotocin (STZ) intraperitoneally. Biofilm dispersal can be seen in the CS-treated group after 15 days of injury in both the nondiabetic and diabetic mice groups. Also, a combination of CS and NO results in a faster treatment. For this group, biofilm dispersal happens after 12 days under the nondiabetic and diabetic conditions, followed by substantial wound size reduction. Figure 3c presents the percentage wound area of mouse skin lesions compared to the initial wound size of 6 mm. These data also show significant wound area reduction when samples are treated with the CS/NO film. Chitosan comes with certain limitations as well. One of the primary obstacles associated with chitosan is its limited solubility and its pH-dependent antibacterial efficacy. Notably, chitosan exhibits varying antibacterial performance against Gram-negative and Gram-positive bacteria due to differences in their surface characteristics. While recent research has made strides in overcoming these obstacles, further investigations are necessary to enhance and optimize the use of chitosan as an antibacterial wound dressing. Further details have been reviewed in the literature.[69,73,74]

2.3. Metal−Organic Frameworks with Antibacterial Properties. Metal−organic frameworks (MOFs) are hybrid materials containing organic linkers and inorganic components. MOFs have many interesting characteristics, like a large number of active sites, high specific surface area, high porosity, adjustable uniform pore sizes, excellent thermal stability, and

https://doi.org/10.1021/acsomega.4c02343
ACS Omega 2024, 9, 27853−27871

facile functionalization.[75] The antibacterial properties of MOFs have been studied by Wyszogrodzka et al.,[76] and the following antimicrobial mechanisms have been reported: (1) stable release of metal ions from the structure can permeabilize cell membranes; (2) both polymer linkers and metal ions can damage DNA; (3) metal ions can react with sulfhydryl and amino groups in proteins, damaging the cell's electron transport system; and (4) ROS generation. One of the main obstacles to using metal oxide NPs is that they must be encapsulated in a polymeric matrix and should be released gradually to reach the highest efficiency. MOFs are great candidates for overcoming this obstacle. In the structures of MOFs, metals have been stabilized by chemical bonds that are strong enough to make MOFs durable but weak enough not to constrain their activity. As a result, they can be ideal for the steady release of metal ions, leading to constant and long-term antibacterial activity. Since the physical and chemical properties of MOFs depend on their structure, the release of the metallic ion can be easily tuned by manipulating or replacing the metallic centers or organic linkers.

Compared with metal NPs or metal oxide NPs, MOFs show advantages in preventing metal oxidation and agglomeration. Yuan et al.[77] developed a novel wound dressing by growing zinc-based zeolitic imidazolate framework (ZIF) nanodagger arrays (ZIF-Ls) on cotton gauze. The arrays in the structure help ion release and the physical disruption of bacteria cells. Compared with the commercially available Ag gauze, this wound dressing displays higher biocompatibility, lower cytotoxicity, and improved wound healing performance. Animal studies show that the ZIF-L-coated gauze can effectively kill bacteria in a *S. aureus* wound infection model in mice. The healing process in untreated wounds is significantly slower than those in the samples treated with the ZIF-L-coated gauze or the Ag-coated gauze. The untreated wound is still open with purulent discharge on day 11, while the wounds treated with ZIF-L-coated gauze or Ag-coated gauze are closed and epithelialized. The number of *S. aureus* recovered after 11 days from the wounds treated with the ZIF-L-coated gauze and the Ag-coated gauze is 98.7% and 91.4% lower than that for the wound treated with the uncoated gauze, respectively. Table 3 briefly shows the results of other studies that have used MOFs in wound dressings. Unlike many other nanoparticles, MOFs are still in the early stages of research and demand further investigation. The biocompatibility and toxicity of MOFs remain uncertain, posing challenges due to the significant release of metal ions and organic ligands, the innate characteristics of the material, and the low degradation rate and cellular accumulation of MOFs and their components, which hinders metabolic processes and excretion.[78]

2.4. Antibacterial Peptide-Based Nanoparticles. Antibacterial peptides, also known as antimicrobial peptides (AMPs), are a vital part of natural immune defense mechanisms and can be produced by all multicellular organisms. AMPs include poly(amino acid)s,[85] lipopeptides,[86] and synthetic antibacterial peptides.[87] They have broad biological applications, but here we are focused only on their potential as antibacterial agents. The primary mechanism of microorganism killing for AMPs is disruption of cell membrane integrity. Moreover, there is evidence that AMPs can inhibit vital cellular activities like protein synthesis, nucleic acid synthesis, enzyme activity, and cell wall synthesis.[88] Normally, AMPs display a net positive charge and a high ratio of hydrophobic amino acids, enabling them to selectively attach

Table 3. List of MOFs That Have Been Used in Wound Dressings

material	metal ion	bacteria species	note	ref
film composed of the naturally derived polysaccharide chitosan (CS) and a copper metal−organic framework (HKUST-1)	Cu^{2+}	*S. aureus* and *E. coli*	the release of copper ions, biodegradable, antibacterial activity, reduced cytotoxicity, ability to proliferate new epidermal tissue and cells, promotion of vessel regeneration, low level of inflammation	79
Cu-MOFs (HKUST-1) incorporated in electrospun chitosan/polyvinyl alcohol (HKUST-1/chitosan/PVA) fibers	Cu^{2+}	*S. aureus* and *E. coli*	biocompatible, low cytotoxicity, support cell adhesion and proliferation, 99% antibacterial efficiency, low inflammation	80
hyaluronic acid (HA) based wound dressings blended with functionalized ZIF-8	Zn^{2+}	*S. aureus* and *E. coli*	improved mechanical properties, release of zinc ions and methyl imidazole ligands, biocompatible, biodegradable, induce the migration and proliferation of fibroblasts, high hydrophilic behavior	81
ZIF-8-encapsulated methacrylated hyaluronic acid (MeHA) microneedle (MN) array	Zn^{2+}	*S. aureus* and *E. coli*	sustainable release of zinc ions, high bacterial cell death, promotion of angiogenesis, reduced inflammation	82
a bilayer composite composed of Ag-MOF loaded chitosan nanoparticles (0.1%Ag@MOF/1.5% CSNPs) as the upper layer and polyvinyl alcohol/sodium alginate/chitosan (PACS) as the lower layer	Ag^{+}	*S. aureus* and *E. coli*	strong antibacterial activity, good biocompatibility, promoted blood coagulation and cell proliferation, accelerate the wound healing, more complete re-epithelialization with less inflammatory cells, good swelling, water retention, water vapor permeability	83
carboxymethyl cellulose/tetracycline@UiO-66 nanocomposite hydrogel films	Zr^{2+}	*S. aureus* and *E. coli*	stable release of TC over 72 h, good biocompatibility, good antibacterial properties, inducing fibroblast cell proliferation	84

https://doi.org/10.1021/acsomega.4c02343
ACS Omega 2024, 9, 27853−27871

to negatively charged bacterial membranes. This electrostatic interaction can either destroy the membrane's integrity through pore formation or cause the AMPs to enter the bacterium to inhibit intracellular function.[89] By altering the specific amino acids and functionalized groups on AMPs, it is possible to modify their properties, such as their antimicrobial activity, selectivity, and toxicity. This allows for the design of tailored AMPs with improved therapeutic potential for various applications, including wound dressings. A recently developed pH-switchable antimicrobial supramolecular hydrogel based on the IKFQFHFD peptide sequence shows interesting properties.[90] Under neutral pH, this sequence is electrically neutral and biocompatible, while under acidic conditions (pH 5.5) the sequence is amphipathic and positively charged. The antibacterial activity of this hydrogel comes from the stable release of the peptide in an acidic environment, leading to disruption of the cell wall and membrane. To increase efficiency, this hydrogel is also loaded with the photothermal agent cypate and procollagen component proline, promoting collagen formation, cell proliferation, and angiogenesis. *In vitro* data show an augmentation in the proliferation of fibroblasts and endothelial cells. The integrated hydrogel system significantly improves the *in vivo* healing of MRSA biofilm-infected wounds in diabetic mice. Clinical application of these technologies may be limited due to their vulnerability to environment-related (hydrolysis, oxidation, and photolysis) and wound-related (pH and proteolysis) factors.[91] Another potential setback associated with the use of AMPs in wound dressings is their tendency to cause cytotoxicity and high hemolytic activity, particularly at concentrations approaching therapeutic dosages.[92] Table 4 summarizes some of the peptide sequences used as antibacterial agents in wound dressings. Further details about AMPs can be found in the literature.[93−96]

2.5. 2D Nanosheets. The antibacterial action of 2D nanosheets is primarily achieved through direct physical interactions with bacterial membranes, influenced by factors such as the nanosheet's size, thickness, oxidation level, surface charge, and hydrophilicity/hydrophobicity. These interactions occur either as surface-area-mediated physical contacts or through edge-mediated mechanisms, where nanosheets engage with bacterial membranes either by lying flat against them or penetrating them with their sharp edges, respectively.[101] Larger nanosheets tend to engage in surface-area-mediated interactions, often enveloping and isolating bacterial cells, impeding nutrient transfer, and potentially leading to reversible bacterial inactivation. In contrast, smaller nanosheets utilize their sharp edges to puncture bacterial membranes directly, creating pores that disrupt the membrane integrity and lead to bacterial death.[101−103] This mode of interaction is enhanced by the specific orientation of the nanosheets, with perpendicular alignment facilitating more effective penetration. Furthermore, upon initial contact, nanosheets can induce stress on the bacterial membrane, leading to membrane perturbations through physical stress or chemical interactions, such as electrostatic forces. These interactions can result in the embedding of nanosheets into the lipid bilayer, disrupting membrane integrity through the extraction of phospholipid molecules and ultimately compromising the bacterial cell's viability. This comprehensive interaction mechanism underscores the potent antibacterial capabilities of 2D nanosheets, making them effective agents in combating bacterial infections.

The deployment of 2D nanoparticles to combat bacterial infections faces several challenges that are discussed in the

Table 4. List of AMPs That Have Been Used in Wound Dressings

peptide	matrix	bacterial species	note	ref
NZ2114	hydroxypropyl cellulose (HPC) and sodium alginate (SA)	*S. aureus*	The NZ2114-HPC hydrogel displays favorable mechanical characteristics and a sustained release profile *in vitro*. Moreover, it preserves its biological activity, increasing cell migration and regeneration both *in vitro* and *in vivo*. This hydrogel is applied to treat full-thickness skin wounds infected with the highly virulent *S. aureus* CVCC 546. In comparison to conventional antibiotics like mupirocin ointment and ofloxacin hydrogel, NZ2114-HPC hydrogel exhibits superior antibacterial efficacy, reduced inflammation, and stimulated angiogenesis.	97
melittin		MRSA	The minimum inhibitory concentration of melittin varies from 0.12 to 4 μM. Melittin's ability to form pores in the membrane reverses the resistance of vancomycin-intermediate *Staphylococcus aureus* (VISA) to amoxicillin and vancomycin. *In vivo*, melittin reduces bacterial load and the content of pro-inflammatory cytokines, such as tumor necrosis factor-α, interleukin-6 (IL-6), and IL-1-β.	98
LL-37		*S. epidermidis*	Compared the antibacterial activity of fragments of peptide to the parent peptide. The fragments FK-12, KR-12, and VQ-12 show an increased antimicrobial effect compared with the parent peptide by inhibiting bacterial attachment and biofilm growth and disrupting established biofilms. In addition, fragments KR-12 and VQ-12 increase the migration of HaCaT cells in an *in vitro* wound model.	99
hepcidin 20		*S. epidermidis*	It decreases the amount of extracellular matrix in *Staphylococcus epidermidis* and alters its biofilm structure by targeting the polysaccharide intercellular adhesin (PIA).	100

https://doi.org/10.1021/acsomega.4c02343
ACS Omega 2024, 9, 27853−27871

literature in detail.[104] Briefly, ensuring biosafety and biocompatibility is critical to avoid adverse human effects. Environmental impacts from the disposal and accumulation of these nanoparticles require thorough regulation. Scalability of production remains an important barrier. Overcoming these challenges requires ongoing research and interdisciplinary collaboration on the potential of 2D nanoparticles in combating bacterial infections.

Certainly, the variety of nanoparticles utilized as antibacterial agents for wound dressings extends beyond those mentioned in this discussion. We have aimed to cover the nanoparticles predominantly utilized in studies that have yielded promising outcomes. Table 5 provides an overview of other NPs currently under consideration in research as potential antibacterial agents.

3. NANOPARTICLES FOR PHOTOINDUCED THERAPY

Phototherapy, including photodynamic therapy (PDT) and photothermal therapy (PTT), has attracted the attention of scientists due to the potential for a targeted approach to disease treatment with high selectivity. These methods stand out for being noninvasive and having broad-spectral antibacterial activity. While they generally present fewer side effects and systemic toxicity compared with traditional treatments, it is significant to note that some specific precautions, such as the need for limited exposure to light post-treatment in certain cases, may be needed. Ongoing research is exploring the possibility of bacterial tolerance to these treatments and potential solutions. PDT and PTT each have their strengths and limitations, which will be discussed in the following paragraphs.

3.1. Nanoparticles for Photodynamic Therapy (PDT).

PDT utilizes a photosensitizer, light, and oxygen to generate a phototoxic reaction to kill bacteria. The result of this phototoxic reaction is the generation of highly cytotoxic reactive oxygen species (ROS) or excitable singlet oxygen that can oxidize the biological molecules within or on the cell membrane, causing DNA damage and cell membrane or organelle destruction and eventually leading to cell death.[115] Oxygen diffusion limitations in the biofilm as well as oxygen consumption by both bacteria and the inflammatory cells surrounding the biofilm can lead to hypoxia in the interior of the biofilm microenvironment. Since PDT is an oxygen-dependent process, there is a concern that this condition might restrict the application of PDT for the treatment of biofilms. On the other hand, it has been reported that an excess amount of H_2O_2 can be found in the biofilm tissues. As a result, scientists have been motivated to use agents that can convert H_2O_2 into oxygen. Xiu et al.[116] have designed a novel nanosheet-based material, termed MnO_2-BSA/PEG-Ce6 (MBP-Ce6 NSs), for pH-responsive dual-mode imaging and PDT. These nanosheets release Ce6 and Mn^{2+} in acidic biofilm environments, leading to the activation of magnetic resonance (MR) and fluorescence (FL) signals for dual-mode imaging (Figure 4a). *In vivo* studies show that the fluorescence intensity of Ce6-incubated samples is 2.5× higher than that of mouse tissues at 12 h. Also, the transverse relativity (r1) of MBP-Ce6 NSs after being incubated in acidic buffer (pH = 5.0) for 24 h is about 3× that in neutral buffer (pH = 7.4). *In vivo* studies show that upon the injection of MBP-Ce6 NSs into biofilm-infected tissues of mice, there is a substantial enhancement in fluorescence and T1-weighted MR signals compared to those of the controls. *In vivo* results show that under NIR light

Table 5. List of Antibacterial NPs That Have Been Used in the Design of a Wound Dressing

material	mechanism of action	bacteria species	concerns	ref
carbon-based nanomaterials	inhibition of bacterial adhesion, cell membrane damage, afflicting oxidative stress to bacteria, echanical interaction (+graphene, nanotubes) or unspecific reactivity (fullerenes, nanodiamonds), and ROS generation	*K. oxytoca*, *P. aeruginosa*, and *S. epidermidis* biofilms	the exact antibacterial mechanism is still unknown, and there are doubts about their cytotoxicity	105−107
mesoporous silica NPs	inhibition of bacteria adhesion, physical damage to cell membranes, and ROS production; mainly used as a drug delivery system	*S. aureus*, methicillin-resistant *S. aureus* (MRSA), and *E. coli*	possibility of Protein fouling and immunogenicity	108, 109
zinc oxide NPs	still under debate; however, the most probable ones are ROS generation, cell wall damage due to ZnO-localized interaction, enhanced membrane permeability causing membrane dysfunction, and uptake of toxic dissolved zinc ions	*E. coli* and *S. aureus*	toxicity at the effective antibacterial concentration differs from one study to another according to the test conditions, induces oxidative stress to mammalian cells by generating ROS	110−112
lipid-based NPs	cell membrane lysis, interfering with energy production by uncoupling oxidative phosphorylation, impairing nutrient uptake, and disrupting electron transport chain	*S. aureus*, *E. faecalis*, and *P. aeruginosa*	generally, Gram-positive species are more prone to be killed by lipid-based nanoparticles; due to different lipopolysaccharide (LPS) structures, some Gram-negative bacteria showed susceptibility; poor solubility; susceptibility of lipids to oxidation and degradation	113, 114

https://doi.org/10.1021/acsomega.4c02343
ACS Omega 2024, 9, 27853−27871

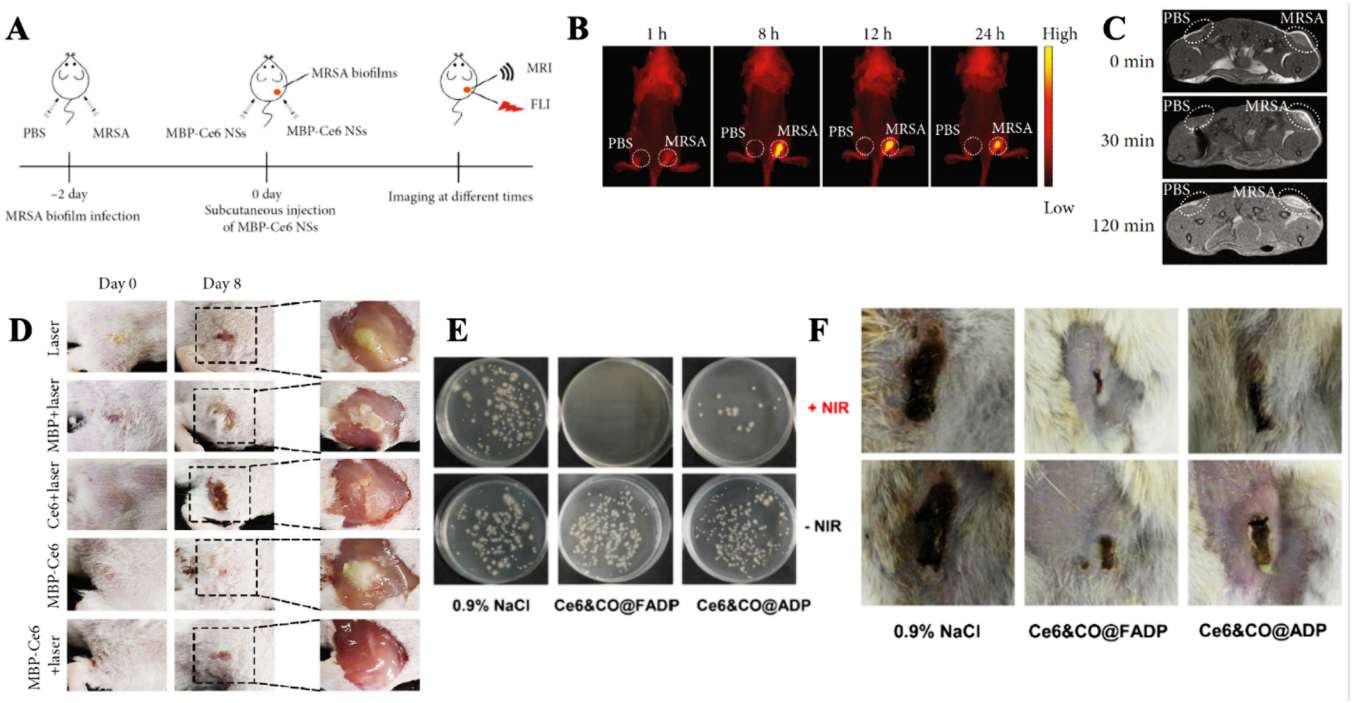


Figure 4. *In vivo* fluorescence imaging (FLI) and magnetic resonance imaging (MRI) of MRSA biofilm infections using MBP-Ce6 NSs. (a) Schematic illustration for the detection of MRSA biofilms by *in situ* injection of MBP-Ce6 NSs (50 μL; 50 μg/mL MnO_2 and 40 μg/mL Ce6) in both the left thigh (normal tissue) and the right thigh (infected tissue). (b) Fluorescence images and (c) T1-weighted MR images of the infected mice at different times. (d) Photographs of the biofilm-infected tissues from the mice treated with various treatments at different times. Reprinted with permission from ref 116. Copyright 2020 American Association for the Advancement of Science (AAAS). (e) *In vivo* antibacterial activity of Ce6&CO@FADP without or with NIR light irradiation. An *E. coli*-infected mouse knife injury model served as an *in vivo* bacterial infection model, while 0.9% NaCl and Ce6&CO@ADP served as controls. Bacterial colonies were isolated from the infection sites of mice after various treatments. (f) Photographs of the skin tissues subcutaneously implanted with catheters after various treatments. Reprinted with permission from ref 118. Copyright 2020 American Chemical Society.

irradiation the inhibitory effect of the Ce6&CO@FADP sample is about 2.5log (99.7%), much higher than others. In Figure 4d, control mice with thigh-implanted *S. aureus* biofilms show swelling and suppuration, suggesting a severe inflammatory response. In contrast, after treatment with Ce6&CO@FADP under NIR light irradiation, wounds heal without signs of inflammation.

CO is a relatively stable gas with antibacterial properties, which can promote phagocytosis and inhibit adenosine triphosphate (ATP) supplies in bacteria.[117] In a similar application, the gas therapy method is used alongside PDT to increase the efficiency of biofilm eradication.[118] The new nanosystem, called Ce6&CO@FADP, is made by conjugating chlorin e6 (Ce6) and encapsulating CORM-401 (as a CO-releasing molecule) into a fluorinated amphiphilic dendritic peptide (FADP). Ce6&CO@FADP can rapidly enter bacteria (*S. aureus* and *E. Coli*); after light irradiation, H_2O_2 is formed, which then is consumed by the nanoparticles to release CO. Interestingly, the release of CO in bacteria does not hinder the production of singlet oxygen. As a result, the presence of these two antibacterial agents can significantly affect biofilm ablation. This nanosystem was biocompatible in *in vitro* (cytotoxicity and hemolysis experiments) and *in vivo* (assessment of HbCO concentration in blood) testing. An *E. coli*-infected subcutaneous wounded mouse model was used and showed that almost no *E. coli* survived after treatment with 1 mg/mL Ce6&CO@FADP for 3 days (Figure 4e). Figure 4f shows the skin tissue with the catheters subcutaneously implanted after treatment. Swelling and suppuration are observed in the 0.9% NaCl group, suggesting the presence of an inflammatory response at the site. Following treatment with nanoparticles without NIR light, the swelling and suppuration were improved, which is associated with the limited antibacterial activities of the nanosystem in the absence of NIR light. After treatment with nanoparticles under NIR light irradiation, all of the wounds healed well without signs of swelling and purulence, and the degree of healing was also better than that of the Ce6&CO@ADP group.

3.2. Nanoparticles for Photothermal Therapy (PTT).

PTT is based on local heat generation under near-infrared (NIR) light, which can effectively ablate biofilms in the infected area.[119] This method can be controlled remotely, is site-specific and minimally invasive, and has low incidence of side effects.[120] The temperature (around 70 °C) required to kill the biofilm may also damage the surrounding tissue, the prevention of which is an active area of research. Another challenge is the production of heat-shock proteins (HSPs) in the bacteria, which help bacteria acquire thermoresistance. A variety of attempts have been made to address this challenge, one of the most recent of which used Pifithrin-μ (PES), a heat-shock protein inhibitor. PES hinders HSP function, lowering bacterial heat tolerance and thus reducing the temperature required for effective PTT. However, PES lacks selectivity, impacting both pathogenic bacteria and normal tissue cells, and can be cytotoxic in high amounts. Therefore, controlled release and targeted delivery of PES are vital for its effective use in PTT. Peng et al.[121] have developed a pH-responsive core−shell nanostructure consisting of zeolite-based imidazole

https://doi.org/10.1021/acsomega.4c02343
ACS Omega 2024, 9, 27853−27871

framework (ZIF-8)-coated mesoporous polydopamine (MPDA) core−shell NPs loaded with Pifithrin-μ (PES). The nanostructure degrades in acidic biofilm environments, releasing PES and zinc ions and disrupting bacterial membranes and metabolic pathways. The MPDA@ZIF-8/PES nanoparticles quickly increased the temperature from 25 to 45 °C, having concentration- and density-dependent photothermal properties and high stability across multiple cycles. Cytotoxicity tests reveal about 80% cell viability in NIH-3T3 cells after 3 days, even during drug release. *In vivo* studies on mice with infected wounds treated with MPDA@ZIF-8/PES+NIR show significant reductions in wound size and bacterial count. Both PDT and PTT can be applied at the same time to increase the effectiveness and avoid high temperatures. This method can be used as a low-temperature (≤45 °C) PTT system and releases ROS. Both *in vivo* and *in vitro* results demonstrate effective biofilm eradication with significantly reduced damage to healthy cells.[122] Another disadvantage associated with using PTT is its specificity. To address this obstacle, Sankari et al.[123] conjugated gold nanorods with two peptides: LL-38, a cationic antimicrobial peptide, and ANGI-2, a neuropeptide, both known for their specificity toward targeted bacterial binding when conjugated with gold nanorods (GNRs) via electrostatic interactions. Further details focusing on the usage of a nanomaterial as a PTT agent to fight against biofilms can be found in literature.[124]

4. NANOPARTICLES THAT DISRUPT EXTRACELLULAR POLYMERIC SUBSTANCES

Biofilms are encased within an extracellular polymeric substance (EPS), a dense, protective matrix that not only facilitates the adhesion of bacteria to surfaces and to each other but also contributes significantly to chronic infections and antibiotic tolerance. The disruption of the EPS through various methods, like microneedle patches, biofilm degradation with enzymes, and ultrasound therapy, has been extensively studied for infected wound management. The main benefits of this approach include facilitating the penetration of antibiotics and antimicrobial agents, making bacteria more susceptible to treatment, and potentially preventing the formation of biofilms in the initial stages of wound infection. However, this strategy also presents disadvantages, such as potential damage to surrounding tissues, development of microbial resistance, variability in biofilm community responses, negative impacts on wound healing, and challenges in complete biofilm eradication. Details regarding each method are discussed in the following sections.

4.1. Nanoparticles Integrated with Microneedle Patches. One limitation of traditional methods of biofilm treatments is believed to be the poor penetration of antimicrobials into the biofilm structure, since matrix components and extracellular polymeric substances (EPSs) form a dense barrier that hinders diffusion.[125,126] In addition, the negative charge of the EPS components can repel the negatively charged antimicrobial agents or sequester positively charged agents.[127] Engineering a wound dressing with microscale needle tips on its surface for transdermal drug delivery addresses this issue[128] by disrupting the integrity of the biofilm. Microneedle patches provide a painless, localized, and low-cost administration method that improves patient compliance. This technology allows the penetration of antimicrobial agents to the stratum corneum by controlling the dimensions of the tips and applied force,[129] as shown in Figure 5a. Yi et al.[130] have developed dissolvable microneedles

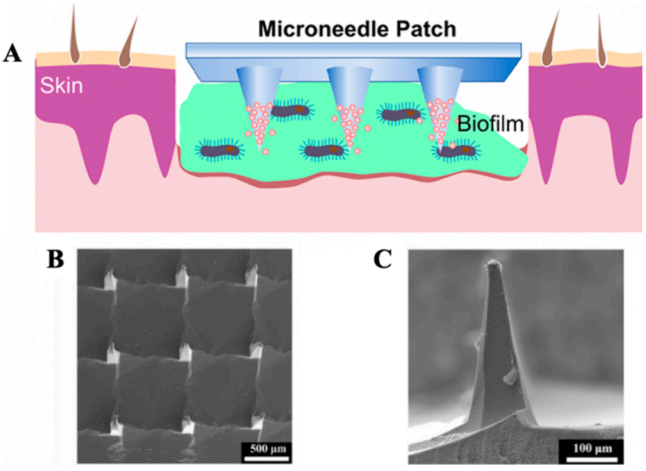


Figure 5. (a) Schematic of microneedle-mediated biofilm treatment. The microneedles penetrate through and dissolve into the biofilm to transdermally release gelatin nanoparticles (GNPs) loaded with antibiotics (chloramphenicol, CAM). Reprinted with permission from ref 127. Copyright 2019 American Chemical Society. (b) Top-view and (c) front-view SEM images of CS-Zn^{2+}MNs. Tips had a diameter of 23 μm and height of 320 μm. Reprinted with permission from ref 130. Copyright 2020 Elsevier.

(MNs) composed of chitosan (CS) and zinc nitrate (CS-Zn^{2+}MNs), which are illustrated in Figure 5b and c. Elemental mapping displayed a uniform distribution of zinc atoms inside the MNs. The final product benefited from both the antimicrobial properties of Zn^{2+} and the structural characteristics of the microneedles. CS-Zn^{2+}MNs have shown approximately 16% and 25% higher antibacterial rates against *E. coli* and *S. aureus*, respectively, compared to CS-MNs. In a similar study, MNs were used to deliver antibiotics to the region of active growth.[127] These dissolvable microneedles (MNs) are loaded with chloramphenicol (CAM) encapsulated in gelatin nanoparticles (CAM@GNPs) for targeted antibiotic delivery. After penetration of these microneedles into the biofilm matrix, the GNPs, responsive to the high levels of gelatinase at infection sites, release CAM *in situ*. The cytotoxicity of CAM is reduced significantly when it is encapsulated in the GNPs, which favors the wound healing process. Significant decreases of 55.6% (for an incubation time of 4 h) and 63.2% (for an incubation time of 8 h) are reported in colony forming units (CFU) per millimeter in the CAM@GNPs patch compared to free drug in solution. Keeping all the mentioned information in mind, the role of the EPS as a barrier against drug penetration is currently under dispute, as the lack of drug penetration through EPS may not fully explain the increased resistance observed in biofilms.[131] As a result, we believe that further in-depth studies are needed to understand more about the role of the EPS in biofilm drug resistance.

4.2. Enzyme-Enhanced Nanoparticles for Biofilm Degradation. Another approach for biofilm treatment is using enzymes with the capacity to selectively degrade different components of the biofilm structure. These enzymes can be recombinantly, naturally, or synthetically produced. Some enzymes attack the components in the EPS, while others degrade the components in the biofilm's structure that are vital for the biofilm to maintain its life. One of the main challenges

https://doi.org/10.1021/acsomega.4c02343
ACS Omega 2024, 9, 27853−27871

Table 6. List of Some Enzymes That Have Been Used for Biofilm Treatment

biofilm-dispersing enzyme	carrier	preserved enzyme activity	comment	ref
alginate lyase	hyaluronan−cholesterol hydrogel	80%	alginate lyase is an enzyme that degrades alginate, a key component of the mucoid biofilm matrix, without any additional antimicrobial agent	143
dispersin B	Fe_3O_4@SiO_2	>50%	hydrolyzes the polysaccharide β-1,6-*N*-acetyl-D-glucosamine (PGA), which is essential for biofilm formation in some species of staphylococci. no *in vivo* studies	144
DNase	nanostructured lipid carrier	unaffected	DNase combined with levofloxacin in NLC drastically reduced biofilm	145
DNase	large pore mesoporous silica nanoparticles	72.4%	silver is also doped into the structure	146
papain	chitosan	95%	damages cell membranes; oth soluble and immobilized papain efficiently destroy biofilms formed by *S. aureus* and *S. epidermidis*	147
ficin	chitosan	90%	ficin is a sulfhydryl protease that can degrade the EPS components of the biofilm matrix; ficin (either soluble or immobilized) could reduce the *S. aureus*-infected skin wound areas in rats twofold after 4 days of treatment instead of 6 days	148

in this method is designing a smart delivery system that stabilizes the enzyme, protects it from the exterior environment, and minimizes unnecessary systematic exposure. Nithya et al.[132] have developed a novel chitosan-based hydrogel containing lysostaphin (LST) as an antibacterial enzyme for *S. aureus* biofilm eradication. The final product shows stability at physiological temperature and flexibility. After 15 min of incubation, 95% of the bacteria are lysed. The cytotoxicity assays suggest a nontoxic behavior for the final hydrogel.

During the past few years, a variety of enzymes, including dispersin B and α-amylase, have been tested as a potential agents to disrupt the structural integrity of the EPS for biofilm dispersion.[133−135] Specific enzymes degrade particular polysaccharides that exist in the EPS. The *P. aeruginosa* biofilms, for instance, rely mainly on the polysaccharides Psl and Pel for their matrix's structural integrity. Glycoside hydrolase (PslG) attacks and degrades the dominant Psl polysaccharide in the EPS matrix of *P. aeruginosa* biofilms.[136−138] Thorn and colleagues[138] combined enzyme degradation with an antibiotic to reach higher efficiency. For that purpose, lipid liquid crystal nanoparticles (LCNPs) are loaded with PslG and tobramycin. The system helps the enzyme remain stable and provides sustainable release, which is sensitive to the presence of bacterial infection. The PslG + tobramycin-LCNPs sample shows a greater than 10-fold reduction in bacteria compared to the antibiotic alone.

Extracellular DNA (eDNA) is a significant structural component in a biofilm's matrix that forms a lattice-like structure and stabilizes the biofilm structure. Therefore, the addition of DNase, as an agent to degrade eDNA, is another approach for the disintegration of biofilms.[139] Liu et al.[140] have used radical polymerization to encapsulate DNase in polyMPC-*co*-polyAPM. Triggered by the biofilm's acidic environment, the polymer degrades, leading to the release of DNase. This process results in a notable biofilm disintegration efficiency of 92.2%. Mesoporous silica nanoparticles (MSNs) are another material that has been loaded by enzymes for biofilm treatment.[141] These NPs are loaded with lysostaphin, serrapeptase, and DNase I enzymes. The first enzyme causes cell lysis of *S. aureus* bacteria, and the other ones degrade the biofilm's matrix. The highest efficiency is achieved when all three enzymes are combined, and the efficacy of all three enzymes is improved by immobilization onto the MSN structure compared to the free enzymes. eDNA is more prevalent in immature biofilms, so the addition of eDNA is more effective when it is applied in the early stages of biofilm formation.[142] Besides the vulnerability of enzymes to denaturation, degradation, and clearance upon administration, there are additional challenges, including their substrate-specific nature. Since enzymes specifically target particular substrates, they may not effectively degrade all components of a biofilm matrix. This issue becomes more complex in clinical scenarios, where wounds often contain various types of biofilms, making it difficult to find a single enzyme effective against all present biofilms. Table 6 presents some of the most commonly used enzymes for degrading the structure of biofilms in wound dressings.

4.3. Nanoparticles for Ultrasound therapy. Ultrasound is used as a method to eradicate biofilms through different approaches such as antimicrobial sonodynamic therapy (aSDT) and microbubbles. In the aSDT method, ultrasound irradiation activates sonosensitizers that secrete toxic agents that kill bacterial cells. Pourhajibagher et al.[149] have used ZnO and TiO_2 because of their good biocompatibility and antimicrobial and photocatalytic properties. Based on FE-SEM analysis, ZnO/TiO_2 NP samples have a strong antibiofilm effect and decrease the metabolic activity of the bacteria to 85.5%. Microbubble oscillation can lead to discrete morphological changes in the *P. aeruginosa* biofilm, so ultrasound stimulation is an innovative method for overcoming the physical barrier of the biofilm matrix.[150] A strategy for effective biofilm eradication was reported through the combination of low-boiling -point phase-change contrast agents (PCCAs) with ultrasound (US-PCCA) and antibiotics that target persister cells.[151] Bharatula et al.[152] have used high-intensity focused ultrasound (HIFU) at various acoustic pressures to better understand the biological response of the bacteria within biofilm after ultrasound treatment. A pressure amplitude of 4.5 MPa, HIFU detaches both living and dead cells from the surface, but complete removal cannot be achieved. Beside the physical impact of HIFU, an increase in biomarkers for biofilm development (cyclic-di-GMP) has been reported. Another study on acoustic pressure variation displays a reduction in colony forming units (CFU) at relatively high temperatures, and higher exposure levels (i.e., pr of 6.2 and 7.6 MPa) show no viable colony forming units.[153] However, a

https://doi.org/10.1021/acsomega.4c02343
ACS Omega 2024, 9, 27853−27871

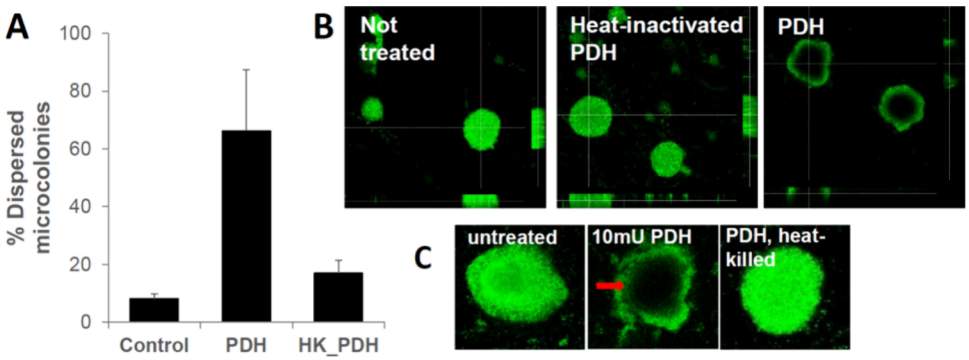


Figure 6. Pyruvate depletion induces dispersion by *P. aeruginosa* biofilms. (A) Number of microcolonies as the percent of the total colonies counted per treatment group. (B) Confocal images of biofilms left untreated or after exposure to 10 mU PDH or heat-inactivated PDH (HK_PDH). (C) Void formation/dispersion indicated by a red arrow. Reprinted with permission from ref 166. Copyright 2019 Nature.

systematic review assessing the effectiveness of ultrasound in the treatment of chronic wounds concluded that there is insufficient clinical evidence to support its efficacy, suggesting more studies are needed before drawing any conclusion.[154]

5. NANOPARTICLES THAT INDUCE BIOFILM DISPERSION

The life cycle development of biofilms is a sequential, highly regulated process ending with a dispersion step (Figure 1). During dispersion, sessile, matrix-encased biofilm cells escape the biofilm, leaving central voids behind. As dispersion happens, biofilm cells convert back to the planktonic mode of growth, making them more susceptible to antibacterial agents. The primary advantage of this phenomenon is that it converts biofilms to a state highly susceptible to antimicrobials and the immune system, thereby avoiding the need for aggressive antibiotic treatments. However, this method faces challenges, including the potential for the spread of infection, especially if an insufficient dose of antibiotics is used, and the risk of incomplete eradication. Hence, the discovery of effective methods to induce dispersion in biofilms has paved the way for innovative approaches in treating biofilm infections. Several other studies have delved into the mechanisms in greater detail.[155,156] This section explores some of the innovative and effective approaches that hold promise for biofilm treatment.

Dispersion does not involve the entire biofilm, and the ratio of the biofilm's population that disperses is associated with the diameter and thickness of the biofilm. *cis*-2-Decenoic acid (*cis*-DA), a small messenger fatty acid molecule, is identified as a dispersion inducer in *P. aeruginosa*.[157] The external introduction of *cis*-DA also triggers dispersion in a wide range of biofilm-forming bacteria, including *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *Proteus mirabilis*, *S. pyogenes*, *Bacillus subtilis*, and *S. aureus*.[158] Oxygen deprivation and carbon source starvation can also be other triggers to induce dispersion.[159,160] Nitric oxide (NO), which is mostly used as a signaling molecule in biological systems, can induce the dispersion of the biofilm at low concentrations. The NO-donor sodium nitroprusside (SNP) has been used to induce dispersion in a *P. aeruginosa* biofilm, and approximately 80% of the biofilm biomass was reduced at the low concentration of 500 nM.[161] Applying SNP alongside antimicrobial compounds like tobramycin, hydrogen peroxide, and sodium dodecyl sulfate would significantly enhance their efficiency for biofilm removal. The impact of NO reduction was also studied on other bacterial species. A wide range of both Gram-positive and Gram-negative biofilm-forming microorganisms including *Serratia marcescens*, *Vibrio cholerae*, *E. coli*, *Fusobacterium nucleatum*, *Bacillus licheniformis*, and *S. epidermidis* have been exposed to SNP at different concentrations and displayed around 60%, 72.5%, 38.1%, 55.6%, 93.2%, and 58.6% biomass reduction, respectively.[162] Additionally, biofilms that receive low NO doses show an augmented vulnerability to antimicrobial treatments compared to untreated biofilms. The effectiveness of standard chlorine treatments in eradicating multispecies biofilms is increased by 20-fold in NO-treated biofilms compared to the untreated biofilms. However, higher concentrations of NO in the millimolar range promote the biofilm mode of growth.

Like NO, pyruvate has shown a concentration-dependent effect on biofilm formation. In the absence of alternative anaerobic respiratory pathways, species such as *P. aeruginosa* initiate fermentative processes, including pyruvate fermentation. In these cases, cells at the core of the biofilm experience reductive stress (abundant electrons/insufficient oxygen).[163] Pyruvate fermentation is an alternative metabolism source for biofilms to survive, and it supports the survival of *P. aeruginosa* for up to 18 days compared to the control group where pyruvate is absent.[164] The addition of 10 mM pyruvate was observed to enhance biofilm formation in *P. aeruginosa*, while continuous depletion of pyruvate from the growth medium (via addition of pyruvate dehydrogenase (PDH)) effectively prevents biofilm formation.[165] Data from our laboratory prove that enzymatic depletion of pyruvate leads to the dispersion of established biofilms by *S. aureus* and laboratory and clinical *P. aeruginosa* isolates and prevents their formation.[166,167] *In vivo* results show that applying pyruvate depletion conditions to second-degree burn wounds infected with *P. aeruginosa* biofilm cells led to biofilm biomass reduction. We have shown that depletion of pyruvate by PDH renders *P. aeruginosa* biofilms in porcine wounds nearly 1000× more susceptible to antibiotics. Burn wounds were infected with tobramycin-tolerant *P. aeruginosa* and left untreated for 24 h to allow biofilms to establish. Similar to tobramycin treatment alone, exposure to PDH alone coincides with up to a ∼2log reduction in the biofilm population present in wounds after 3 and 6 days of infection. Co-treatment with 200 mU PDH and tobramycin coincides with an over 4log reduction in both biofilm bacteria and the planktonic population relative to untreated controls. Furthermore, these *in vivo* data are supported by *in vitro* findings showing that, relative to untreated biofilms, *P. aeruginosa* biofilms exposed to PDH are characterized by

https://doi.org/10.1021/acsomega.4c02343
ACS Omega 2024, 9, 27853−27871

central voids, which are associated with biofilm dispersion (Figure 6b and c). However, given that PDH, like all enzymes, is highly prone to loss of activity, improving the capacity of the enzyme to function in challenging microenvironments, such as the increased temperature or pH range of the wound, is significant. We have successfully encapsulated PDH in poly(lactic-*co*-glycolic) acid nanoparticles with an encapsulation efficiency of 17.9 ± 1.4%.[168] This encapsulation results in improved enzyme stability under different storage conditions. *In vitro* studies show that PDH−PLGA NPs actively disperse mature *P. aeruginosa* biofilms through the action of depleting pyruvate. Biofilm dispersion as a treatment method is a relatively new field compared to the other discussed approaches, and it is evident that there is still a lack of well-structured clinical trials. Additionally, there is a concern regarding the virulence of the dispersed bacterial cells. Although our preliminary *in vivo* data indicated that the use of antibiotics can ensure the death of these dispersed bacterial cells, further investigations are warranted before dredging definitive conclusions.

6. CONCLUSION

This Review highlights novel methods to combat biofilm infection in wounds. The exceptional tolerance of biofilms to both immune defenses and conventional antibiotic therapies prompted the search for novel and more effective treatment methods. The unique properties of nanobiomaterials have made them ideal candidates to address these obstacles. In this Review, we explore the potential of nanobiomaterials incorporated into wound dressings to combat biofilm infections through their antibacterial properties, photoinduced therapies, EPS disruption, and induction of biofilm dispersion. Each approach exhibits unique mechanisms of action, contributing to the overarching goal of replacing or significantly reducing the reliance on antibiotics in wound care. Antibacterial nanoparticles like silver were first developed through nanotechnology as alternatives for antibiotics. Despite the promising results, the application of these nanoparticles has been limited due to toxicity concerns primarily related to ion release and ROS generation. These toxicity issues, along with their reduced effectiveness against Gram-positive bacteria compared to Gram-negative ones, have spurred scientists to develop alternative materials such as nanoparticle complexes including functionalized groups and coatings, MOFs, and 2D nanosheets. Although these alternatives show promise, they are comparatively newer materials, and more studies are required to gather a thorough understanding of their properties and potential applications, especially their long-term safety and environmental impact. Further, nanoparticles employed in photodynamic therapy (PDT) and photothermal therapy (PTT) face similar challenges. For PTT, although it does not rely on ROS, the high temperatures required could damage cells, and the emergence of thermoresistant bacteria complicates its effectiveness. Enzyme-containing complex nanoparticles targeting extracellular polymeric substances (EPSs) have shown promise against specific bacterial species, yet developing enzymes that are effective against a broad range of bacteria remains a formidable challenge. This is crucial, as clinical settings often involve infections with multiple bacterial strains. Another significant focus is on nanoparticles that induce bacterial dispersion, which are particularly intriguing because they can address both Gram-positive and Gram-negative bacteria *in vivo*. However, concerns about the virulence of dispersed bacteria and the dosage of associated antibiotics remain. The Review underscores that while these methods show potential, none are without flaws, and substantial research is still needed. We aim to spotlight these shortcomings to guide future studies. Despite rapid advances in the field, a thorough understanding of these technologies is still emerging. More in-depth studies are essential, especially regarding the biocompatibility and toxicity of materials, and their long-term impacts must be explored. Additionally, this Review highlights a persistent gap in research: the lack of solid data on the storage, administration, mucous interactions, blood clearance, long-term outcomes, safety, and side effects of many of these materials. Furthermore, most studies focus on *in vitro* results and single-target bacteria, pointing to a need for more comprehensive testing.

7. FUTURE PERSPECTIVE

Continued exploration into the specific interactions between nanoparticles and biofilms is essential to enhance the efficacy and minimize the side effects of bacterially infected wound treatments. Combining various types of nanoparticles could synergize their antibacterial effects and overcome existing limitations. One of the main antibacterial mechanisms of nanoparticles is through the generation of oxidation stress in the form of ROS. While effective against bacterial structures, ROS can also damage healthy cells near the infection site. Consequently, it is crucial to develop smart, responsive nanoparticles that can control ROS release. Recent studies indicate that biofilms are increasingly tolerant to ROS and heat, underscoring the potential of nanoparticles that naturally induce biofilm dispersion. When these methods are employed, it is vital to ensure sufficient antibiotic coverage to eradicate dispersed bacterial cells.

Beyond infection control, nanoparticles could be used to promote wound healing and tissue regeneration. Incorporating biocompatible and biodegradable materials that support cell growth and tissue repair into the nanoparticle design will be crucial. We believe that developing *in vivo* models consisting of multispecies bacteria is necessary to better understand the potential of these therapies. Furthermore, the successful translation of these novel wound dressing approaches from the laboratory to clinical practice remains a critical challenge. Comprehensive clinical trials are needed to validate the efficacy and safety of these innovative strategies, ensuring that they can deliver on their promise in real-world scenarios. Still, the clinical translation of nanoparticles faces significant challenges, including ensuring biocompatibility and safety, scalable manufacturing, regulatory compliance, better understanding of biofilm infections, and economic viability. As we move forward, these innovative approaches have the potential to revolutionize wound care, improving the quality of life for countless individuals suffering from chronic wound infections while reducing the risk of antibiotic resistance.

AUTHOR INFORMATION

Corresponding Author

Amber L. Doiron − *Department of Electrical and Biomedical Engineering, University of Vermont, Burlington, Vermont 05405, United States;* orcid.org/0000-0002-6963-0989; Phone: 802-656-3382; Email: amber.doiron@uvm.edu

https://doi.org/10.1021/acsomega.4c02343
ACS Omega 2024, 9, 27853−27871

Authors

Omid Sedighi − *Department of Electrical and Biomedical Engineering, University of Vermont, Burlington, Vermont 05405, United States;* orcid.org/0000-0002-4766-7983

Brooke Bednarke − *Department of Electrical and Biomedical Engineering, University of Vermont, Burlington, Vermont 05405, United States*

Hannah Sherriff − *Department of Electrical and Biomedical Engineering, University of Vermont, Burlington, Vermont 05405, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c02343

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Aeronautics and Space Administration (NASA) under Grant 80NSSC21M0325.

■ REFERENCES

(1) Järbrink, K.; et al. Prevalence and incidence of chronic wounds and related complications: a protocol for a systematic review. *Syst. Rev.* **2016**, *5*, 152.

(2) Sen, C. K. Human wound and its burden: updated 2020 compendium of estimates. *Advances in wound care* **2021**, *10* (5), 281−292.

(3) Ghosh, S. *Anti-Biofilm Wound Dressing Market Outlook (2023 to 2033)*; REP-GB-16474; Future Market Insights: Newark, DE, 2023.

(4) Goswami, A. G.; et al. Biofilm and wound healing: From bench to bedside. *Eur. J. Med. Res.* **2023**, *28*, 157.

(5) Caiazza, N. C.; O'Toole, G. A. SadB is required for the transition from reversible to irreversible attachment during biofilm formation by Pseudomonas aeruginosa PA14. *J. Bacteriol.* **2004**, *186* (14), 4476−4485.

(6) Hinsa, S. M.; et al. Transition from reversible to irreversible attachment during biofilm formation by Pseudomonas fluorescens WCS365 requires an ABC transporter and a large secreted protein. *Molecular microbiology* **2003**, *49* (4), 905−918.

(7) Sauer, K.; et al. The biofilm life cycle: expanding the conceptual model of biofilm formation. *Nature Reviews Microbiology* **2022**, *20* (10), 608−620.

(8) Sahoo, J.; et al. Nanomaterial-based antimicrobial coating for biomedical implants: new age solution for biofilm-associated infections. *ACS omega* **2022**, *7* (50), 45962−45980.

(9) Nwodo, U. U.; Green, E.; Okoh, A. I. Bacterial exopolysaccharides: functionality and prospects. *International journal of molecular sciences* **2012**, *13* (11), 14002−14015.

(10) Percival, S. L.; et al. A review of the scientific evidence for biofilms in wounds. *Wound repair and regeneration* **2012**, *20* (5), 647−657.

(11) Smith, A. W. Biofilms and antibiotic therapy: is there a role for combating bacterial resistance by the use of novel drug delivery systems? *Adv. Drug Deliv. Res.* **2005**, *57* (10), 1539−1550.

(12) Percival, S. L.; et al. Antimicrobial tolerance and the significance of persister cells in recalcitrant chronic wound biofilms. *Wound repair and regeneration* **2011**, *19* (1), 1−9.

(13) Jesaitis, A. J.; et al. Compromised host defense on Pseudomonas aeruginosa biofilms: characterization of neutrophil and biofilm interactions. *J. Immunol.* **2003**, *171* (8), 4329−4339.

(14) Thurlow, L. R.; et al. Staphylococcus aureus biofilms prevent macrophage phagocytosis and attenuate inflammation in vivo. *J. Immunol.* **2011**, *186* (11), 6585−6596.

(15) Anbazhagan, D.; et al. Detection of quorum sensing signal molecules and identification of an autoinducer synthase gene among biofilm forming clinical isolates of Acinetobacter spp. *PloS one* **2012**, *7* (7), No. e36696.

(16) Singh, A.; et al. *Bacterial biofilm infections, their resistance to antibiotics therapy and current treatment strategies. Biomedical Materials* **2022**, *17* (2), No. 022003.

(17) Almutairi, L. A. *Effects of Mild Magnetic Nanoparticle Hyperthermia on the Susceptibility of Biofilm Infections to Antibiotics*. Ph.D. Thesis, Kent State University, Kent, OH, 2020.

(18) Gurtner, G. C.; et al. Wound repair and regeneration. *Nature* **2008**, *453* (7193), 314−321.

(19) Naskar, A.; Kim, K.-s. Recent advances in nanomaterial-based wound-healing therapeutics. *Pharmaceutics* **2020**, *12* (6), 499.

(20) Dhar, Y.; Han, Y. Current developments in biofilm treatments: Wound and implant infections. *Engineered Regeneration* **2020**, *1*, 64−75.

(21) Mendoza, R. A.; Hsieh, J.; Galiano, R.D. The impact of biofilm formation on wound healing. In *Wound healing - Current Perspectives*; Dogan, K. H., Ed.; IntechOpen: London, UK, 2019. DOI: 10.5772/intechopen.85020.

(22) McCarty, S. et al. Wound infection and biofilms. In *Biofilms in Infection Prevention and Control*; Elsevier, 2014; p 339−358.

(23) Metcalf, D. G.; Bowler, P. G. Biofilm delays wound healing: A review of the evidence. *Burn Trauma* **2013**, *1* (1), 2321−3868.

(24) Fazli, M.; et al. Quantitative analysis of the cellular inflammatory response against biofilm bacteria in chronic wounds. *Wound repair and regeneration* **2011**, *19* (3), 387−391.

(25) Percival, S. L.; Bowler, P. G. Biofilms and their potential role in wound healing. *Wounds* **2004**, *16*, 234−240.

(26) Dreifke, M. B.; Jayasuriya, A. A.; Jayasuriya, A. C. Current wound healing procedures and potential care. *Materials Science and Engineering: C* **2015**, *48*, 651−662.

(27) Mihai, M. M.; et al. Nanomaterials for wound healing and infection control. *Materials* **2019**, *12* (13), 2176.

(28) Rossiter, S. E.; Fletcher, M. H.; Wuest, W. M. Natural products as platforms to overcome antibiotic resistance. *Chem. Rev.* **2017**, *117* (19), 12415−12474.

(29) McCullough, A.; et al. A systematic review of the public's knowledge and beliefs about antibiotic resistance. *J. Antimicrob. Chemother.* **2016**, *71* (1), 27−33.

(30) Godoy-Gallardo, M.; et al. Antibacterial approaches in tissue engineering using metal ions and nanoparticles: From mechanisms to applications. *Bioactive Materials* **2021**, *6* (12), 4470−4490.

(31) Frei, A.; et al. Metals to combat antimicrobial resistance. *Nature Reviews Chemistry* **2023**, *7* (3), 202−224.

(32) Abbaszadegan, A.; et al. The effect of charge at the surface of silver nanoparticles on antimicrobial activity against gram-positive and gram-negative bacteria: A preliminary study. *J. Nanomater.* **2015**, *2015*, 720654.

(33) Huang, T.; et al. Enhanced antibacterial activity of se nanoparticles upon coating with recombinant spider silk protein eADF4 (κ16). *Int. J. Nanomed.* **2020**, *15*, 4275−4288.

(34) Guo, L.; et al. Polymer/nanosilver composite coatings for antibacterial applications. *Colloids Surf., A* **2013**, *439*, 69−83.

(35) Radovic-Moreno, A. F.; et al. Surface charge-switching polymeric nanoparticles for bacterial cell wall-targeted delivery of antibiotics. *ACS Nano* **2012**, *6* (5), 4279−4287.

(36) Hibbard, H. A.; Reynolds, M. M. Enzyme-activated nitric oxide-releasing composite material for antibacterial activity against Escherichia coli. *ACS Applied Bio Materials* **2020**, *3* (8), 5367−5374.

(37) Jiang, L.; Lee, H. W.; Loo, S. C. J. Therapeutic lipid-coated hybrid nanoparticles against bacterial infections. *RSC Adv.* **2020**, *10* (14), 8497−8517.

(38) Yin, I. X.; et al. The antibacterial mechanism of silver nanoparticles and its application in dentistry. *Int. J. Nanomed.* **2020**, *15*, 2555−2562.

(39) Nešporová, K.; et al. Effects of wound dressings containing silver on skin and immune cells. *Sci. Rep.* **2020**, *10*, 15216.

(40) Pang, S.; et al. Toxicity of silver nanoparticles on wound healing: A case study of zebrafish fin regeneration model. *Science of the total environment* **2020**, *717*, No. 137178.
(41) Loh, J. V.; et al. Silver resistance in MRSA isolated from wound and nasal sources in humans and animals. *International Wound Journal* **2009**, *6* (1), 32−38.
(42) *Classification of Wound Dressings Combined with Drugs*; U.S. Food and Drug Administration: White Oak, MD, 2016.
(43) de Lacerda Coriolano, D.; et al. Antibacterial and antibiofilm potential of silver nanoparticles against antibiotic-sensitive and multidrug-resistant Pseudomonas aeruginosa strains. *Brazilian Journal of Microbiology* **2021**, *52*, 267−278.
(44) Paladini, F.; Pollini, M. Antimicrobial silver nanoparticles for wound healing application: progress and future trends. *Materials* **2019**, *12* (16), 2540.
(45) Shanmuganathan, R.; et al. Synthesis of silver nanoparticles and their biomedical applications-a comprehensive review. *Current pharmaceutical design* **2019**, *25* (24), 2650−2660.
(46) Xu, C.; et al. Nanoparticle-based wound dressing: recent progress in the detection and therapy of bacterial infections. *Bioconjugate Chem.* **2020**, *31* (7), 1708−1723.
(47) Chatterjee, A. K.; Chakraborty, R.; Basu, T. Mechanism of antibacterial activity of copper nanoparticles. *Nanotechnology* **2014**, *25* (13), No. 135101.
(48) Alizadeh, S.; et al. *Copper nanoparticles promote rapid wound healing in acute full thickness defect via acceleration of skin cell migration, proliferation, and neovascularization*. *Biochemical and biophysical research communications* **2019**, *517* (4), 684−690.
(49) Majumdar, T. D.; et al. *Size-dependent antibacterial activity of copper nanoparticles against Xanthomonas oryzae pv. oryzae−A synthetic and mechanistic approach*. *Colloid and Interface Science Communications* **2019**, *32*, No. 100190.
(50) Gargioni, C.; et al. Self-assembled monolayers of copper sulfide nanoparticles on glass as antibacterial coatings. *Nanomaterials* **2020**, *10* (2), 352.
(51) Kim, J. E.; et al. Accelerated healing of cutaneous wounds using phytochemically stabilized gold nanoparticle deposited hydrocolloid membranes. *Biomaterials science* **2015**, *3* (3), 509−519.
(52) Shamaila, S.; et al. Gold nanoparticles: an efficient antimicrobial agent against enteric bacterial human pathogen. *Nanomaterials* **2016**, *6* (4), 71.
(53) Cui, Y.; et al. The molecular mechanism of action of bactericidal gold nanoparticles on Escherichia coli. *Biomaterials* **2012**, *33* (7), 2327−2333.
(54) Okkeh, M.; et al. Gold nanoparticles: can they be the next magic bullet for multidrug-resistant bacteria? *Nanomaterials* **2021**, *11* (2), 312.
(55) Zhaleh, M.; et al. *In vitro and in vivo evaluation of cytotoxicity, antioxidant, antibacterial, antifungal, and cutaneous wound healing properties of gold nanoparticles produced via a green chemistry synthesis using Gundelia tournefortii L. as a capping and reducing agent*. *Appl. Organomet. Chem.* **2019**, *33* (9), No. e5015.
(56) Wang, L.; et al. Mercaptophenylboronic acid-activated gold nanoparticles as nanoantibiotics against multidrug-resistant bacteria. *ACS Appl. Mater. Interfaces* **2020**, *12* (46), 51148−51159.
(57) Shehabeldine, A. M.; et al. *Multifunctional silver nanoparticles based on chitosan: Antibacterial, antibiofilm, antifungal, antioxidant, and wound-healing activities*. *Journal of Fungi* **2022**, *8* (6), 612.
(58) Parthasarathy, A.; et al. *Chitosan-coated silver nanoparticles promoted antibacterial, antibiofilm, wound-healing of murine macrophages and antiproliferation of human breast cancer MCF 7 cells*. *Polym. Test.* **2020**, *90*, No. 106675.
(59) Pallavicini, P.; et al. Silver nanoparticles synthesized and coated with pectin: An ideal compromise for anti-bacterial and anti-biofilm action combined with wound-healing properties. *J. Colloid Interface Sci.* **2017**, *498*, 271−281.
(60) Singh, A.; et al. Significant in-Vitro and in-Vivo Antimicrobial and Antibiofilm Activity of Colloidal Silver Nanoparticles (cAgNPs) in Chronic Diabetic Foot Ulcers. *Int. J. Lower Extremity Wounds* **2022**, DOI: 10.1177/15347346221088690.
(61) Kim, J.; et al. Antibacterial and biofilm-inhibiting cotton fabrics decorated with copper nanoparticles grown on graphene nanosheets. *Sci. Rep.* **2023**, *13*, No. 11947.
(62) Mohamed, A. A.; et al. *Eco-friendly mycogenic synthesis of ZnO and CuO nanoparticles for in vitro antibacterial, antibiofilm, and antifungal applications*. *Biological trace element research* **2021**, *199* (7), 2788−2799.
(63) Gopal, A.; et al. Chitosan-based copper nanocomposite accelerates healing in excision wound model in rats. *European journal of pharmacology* **2014**, *731*, 8−19.
(64) Ahire, J. J.; et al. Copper-containing anti-biofilm nanofiber scaffolds as a wound dressing material. *PloS one* **2016**, *11* (3), No. e0152755.
(65) Zhu, G.; et al. *Composite film with antibacterial gold nanoparticles and silk fibroin for treating multidrug-resistant E. coli-infected wounds*. *ACS biomaterials science & engineering* **2021**, *7* (5), 1827−1835.
(66) Piktel, E.; et al. Varied-shaped gold nanoparticles with nanogram killing efficiency as potential antimicrobial surface coatings for the medical devices. *Sci. Rep.* **2021**, *11*, 12546.
(67) Gobi, R.; et al. Biopolymer and synthetic polymer-based nanocomposites in wound dressing applications: a review. *Polymers* **2021**, *13* (12), 1962.
(68) Ma, Z.; Garrido-Maestu, A.; Jeong, K. C. *Application, mode of action, and in vivo activity of chitosan and its micro-and nanoparticles as antimicrobial agents: A review*. *Carbohydr. Polym.* **2017**, *176*, 257−265.
(69) Matica, M. A.; et al. Chitosan as a wound dressing starting material: Antimicrobial properties and mode of action. *International journal of molecular sciences* **2019**, *20* (23), 5889.
(70) Ahmed, M. E.; et al. Sustainable antimicrobial modified chitosan and its nanoparticles hydrogels: Synthesis and characterization. *Int. J. Biol. Macromol.* **2020**, *162*, 1388−1397.
(71) Marangon, C. A.; et al. Combination of rhamnolipid and chitosan in nanoparticles boosts their antimicrobial efficacy. *ACS Appl. Mater. Interfaces* **2020**, *12* (5), 5488−5499.
(72) Choi, M.; et al. Chitosan-based nitric oxide-releasing dressing for anti-biofilm and in vivo healing activities in MRSA biofilm-infected wounds. *Int. J. Biol. Macromol.* **2020**, *142*, 680−692.
(73) Ahmed, S.; Ikram, S. Chitosan based scaffolds and their applications in wound healing. *Achievements in the life sciences* **2016**, *10* (1), 27−37.
(74) Ardean, C.; et al. Factors influencing the antibacterial activity of chitosan and chitosan modified by functionalization. *International Journal of Molecular Sciences* **2021**, *22* (14), 7449.
(75) Furukawa, H.; et al. The chemistry and applications of metal-organic frameworks. *Science* **2013**, *341* (6149), No. 1230444.
(76) Wyszogrodzka, G.; et al. Metal-organic frameworks: mechanisms of antibacterial action and potential applications. *Drug Discovery Today* **2016**, *21* (6), 1009−1018.
(77) Yuan, Y.; et al. ZIF nano-dagger coated gauze for antibiotic-free wound dressing. *Chem. Commun.* **2019**, *55* (5), 699−702.
(78) Xing, F.; et al. Multifunctional metal-organic frameworks for wound healing and skin regeneration. *Materials & Design* **2023**, *233*, No. 112252.
(79) Ren, X.; et al. Copper metal−organic frameworks loaded on chitosan film for the efficient inhibition of bacteria and local infection therapy. *Nanoscale* **2019**, *11* (24), 11830−11838.
(80) Wang, S.; et al. Incorporation of metal-organic frameworks into electrospun chitosan/poly (vinyl alcohol) nanofibrous membrane with enhanced antibacterial activity for wound dressing application. *Int. J. Biol. Macromol.* **2020**, *158*, 9−17.
(81) Abednejad, A.; et al. Hyaluronic acid/carboxylated Zeolitic Imidazolate Framework film with improved mechanical and antibacterial properties. *Carbohydr. Polym.* **2019**, *222*, No. 115033.
(82) Yao, S.; et al. Zn-MOF encapsulated antibacterial and degradable microneedles array for promoting wound healing. *Adv. Healthcare Mater.* **2021**, *10* (12), No. 2100056.

https://doi.org/10.1021/acsomega.4c02343
ACS Omega 2024, 9, 27853−27871

(83) Zhang, M.; et al. Ag@ MOF-loaded chitosan nanoparticle and polyvinyl alcohol/sodium alginate/chitosan bilayer dressing for wound healing applications. *Int. J. Biol. Macromol.* **2021**, *175*, 481−494.
(84) Javanbakht, S.; et al. Carboxymethyl cellulose/tetracycline@ UiO-66 nanocomposite hydrogel films as a potential antibacterial wound dressing. *Int. J. Biol. Macromol.* **2021**, *188*, 811−819.
(85) Mayandi, V.; et al. Multifunctional antimicrobial nanofiber dressings containing ε-polylysine for the eradication of bacterial bioburden and promotion of wound healing in critically colonized wounds. *ACS Appl. Mater. Interfaces* **2020**, *12* (14), 15989−16005.
(86) Stachurski, O.; et al. Effect of self-assembly on antimicrobial activity of double-chain short cationic lipopeptides. *Bioorg. Med. Chem.* **2019**, *27* (23), No. 115129.
(87) Wang, Y.; et al. Design of novel analogues of short antimicrobial peptide anoplin with improved antimicrobial activity. *Journal of Peptide Science* **2014**, *20* (12), 945−951.
(88) Moghaddam, M. M.; et al. Investigation of the antibacterial activity of a short cationic peptide against multidrug-resistant Klebsiella pneumoniae and Salmonella typhimurium strains and its cytotoxicity on eukaryotic cells. *World J. Microbiol. Biotechnol.* **2014**, *30*, 1533−1540.
(89) Zhang, L.-j.; Gallo, R. L. Antimicrobial peptides. *Current biology* **2016**, *26* (1), R14−R19.
(90) Wang, J.; et al. pH-switchable antimicrobial nanofiber networks of hydrogel eradicate biofilm and rescue stalled healing in chronic wounds. *ACS Nano* **2019**, *13* (10), 11686−11697.
(91) Miao, F.; et al. Antimicrobial peptides: the promising therapeutics for cutaneous wound healing. *Macromol. Biosci.* **2021**, *21* (10), No. 2100103.
(92) Roque-Borda, C. A.; et al. Challenge in the discovery of new drugs: antimicrobial peptides against WHO-list of critical and high-priority bacteria. *Pharmaceutics* **2021**, *13* (6), 773.
(93) Di Somma, A.; et al. Antimicrobial and antibiofilm peptides. *Biomolecules* **2020**, *10* (4), 652.
(94) Otvos, L., Jr; Ostorhazi, E. Therapeutic utility of antibacterial peptides in wound healing. *Expert review of anti-infective therapy* **2015**, *13* (7), 871−881.
(95) Nasseri, S.; Sharifi, M. Therapeutic potential of antimicrobial peptides for wound healing. *Int. J. Pept. Res. Ther.* **2022**, *28*, 38.
(96) Moravej, H.; et al. *Antimicrobial peptides: features, action, and their resistance mechanisms in bacteria. Microbial Drug Resistance* **2018**, *24* (6), 747−767.
(97) Woodburn, K. W.; Jaynes, J. M.; Clemens, L. E. *Evaluation of the antimicrobial peptide, RP557, for the broad-spectrum treatment of wound pathogens and biofilm. Front. Microbiol.* **2019**, *10*, No. 1688.
(98) Lima, W. G.; et al. In-depth characterization of antibacterial activity of melittin against Staphylococcus aureus and use in a model of non-surgical MRSA-infected skin wounds. *European Journal of Pharmaceutical Sciences* **2021**, *156*, No. 105592.
(99) Saporito, P.; et al. LL-37 fragments have antimicrobial activity against Staphylococcus epidermidis biofilms and wound healing potential in HaCaT cell line. *J. Pept. Sci.* **2018**, *24* (7), No. e3080.
(100) Brancatisano, F. L.; et al. Inhibitory effect of the human liver-derived antimicrobial peptide hepcidin 20 on biofilms of poly-saccharide intercellular adhesin (PIA)-positive and PIA-negative strains of Staphylococcus epidermidis. *Biofouling* **2014**, *30* (4), 435−446.
(101) Roy, S.; et al. *Mechanistic insight into the antibacterial activity of chitosan exfoliated MoS2 nanosheets: membrane damage, metabolic inactivation, and oxidative stress. ACS applied bio materials* **2019**, *2* (7), 2738−2755.
(102) Roy, S.; et al. Antimicrobial mechanisms of biomaterials: from macro to nano. *Biomaterials Science* **2022**, *10* (16), 4392−4423.
(103) Roy, S.; et al. Quaternary Pullulan-Functionalized 2D MoS2 Glycosheets: A Potent Bactericidal Nanoplatform for Efficient Wound Disinfection and Healing. *ACS Appl. Mater. Interfaces* **2023**, *15* (20), 24209−24227.
(104) Hameed, S.; et al. Emerging trends and future challenges of advanced 2D nanomaterials for combating bacterial resistance. *Bioactive Materials* **2024**, *38*, 225−257.
(105) Liu, Y.; et al. Research progress on the use of micro/nano carbon materials for antibacterial dressings. *New Carbon Materials* **2020**, *35* (4), 323−335.
(106) Malek, I.; et al. Vertically aligned multi walled carbon nanotubes prevent biofilm formation of medically relevant bacteria. *J. Mater. Chem. B* **2016**, *4* (31), 5228−5235.
(107) Nowak, M.; Barańska-Rybak, W. *Nanomaterials as a successor of antibiotics in antibiotic-resistant, biofilm infected wounds? Antibiotics* **2021**, *10* (8), 941.
(108) Mosselhy, D. A.; et al. Nanosilver−silica composite: Prolonged antibacterial effects and bacterial interaction mechanisms for wound dressings. *Nanomaterials* **2017**, *7* (9), 261.
(109) Jia, Y.; et al. Electrospun PLGA membrane incorporated with andrographolide-loaded mesoporous silica nanoparticles for sustained antibacterial wound dressing. *Nanomedicine* **2018**, *13* (22), 2881−2899.
(110) Sirelkhatim, A.; et al. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. *Nano-micro letters* **2015**, *7*, 219−242.
(111) Batool, M.; et al. *Adsorption, antimicrobial and wound healing activities of biosynthesised zinc oxide nanoparticles. Chemical Papers* **2021**, *75*, 893−907.
(112) Wang, Y.-W.; et al. Superior antibacterial activity of zinc oxide/graphene oxide composites originating from high zinc concentration localized around bacteria. *ACS Appl. Mater. Interfaces* **2014**, *6* (4), 2791−2798.
(113) Zhang, Q.; et al. Antimicrobial lipids in nano-carriers for antibacterial delivery. *J. Drug Targeting* **2020**, *28* (3), 271−281.
(114) Ceresa, C.; et al. *Production of mannosylerythritol lipids (MELs) to be used as antimicrobial agents against S. aureus ATCC 6538. Current microbiology* **2020**, *77* (8), 1373−1380.
(115) Hu, X.; et al. Antimicrobial photodynamic therapy to control clinically relevant biofilm infections. *Front. Microbiol.* **2018**, *9*, No. 1299.
(116) Xiu, W. Biofilm microenvironment-responsive nanotheranostics for dual-mode imaging and hypoxia-relief-enhanced photodynamic therapy of bacterial infections. *Research* **2020**, *2020*, 9426453.
(117) Wang, T.-Y.; Zhu, X.-Y.; Wu, F.-G. *Antibacterial gas therapy: strategies, advances, and prospects. Bioactive Materials* **2023**, *23*, 129−155.
(118) Ma, W.; et al. Ultra-efficient antibacterial system based on photodynamic therapy and CO gas therapy for synergistic antibacterial and ablation biofilms. *ACS Appl. Mater. Interfaces* **2020**, *12* (20), 22479−22491.
(119) Cui, T.; et al. Self-propelled active photothermal nano-swimmer for deep-layered elimination of biofilm in vivo. *Nano Lett.* **2020**, *20* (10), 7350−7358.
(120) Mei, Z.; et al. Activatable NIR-II photoacoustic imaging and photochemical synergistic therapy of MRSA infections using miniature Au/Ag nanorods. *Biomaterials* **2020**, *251*, No. 120092.
(121) Peng, D.; et al. Fabrication of a pH-responsive core−shell nanosystem with a low-temperature photothermal therapy effect for treating bacterial biofilm infection. *Biomaterials Science* **2021**, *9* (22), 7483−7491.
(122) Yuan, Z.; et al. Near-infrared light-triggered nitric-oxide-enhanced photodynamic therapy and low-temperature photothermal therapy for biofilm elimination. *ACS Nano* **2020**, *14* (3), 3546−3562.
(123) Sankari, S. S.; et al. Comparative study of an antimicrobial peptide and a neuropeptide conjugated with gold nanorods for the targeted photothermal killing of bacteria. *Colloids Surf., B* **2021**, *208*, No. 112117.
(124) Liu, H.; et al. Nanomaterials-based photothermal therapies for antibacterial applications. *Materials & Design* **2023**, *233*, No. 112231.

https://doi.org/10.1021/acsomega.4c02343
ACS Omega 2024, 9, 27853−27871

(125) Luo, Y.; et al. Mechanisms and control strategies of antibiotic resistance in pathological biofilms. *Journal of Microbiology and Biotechnology* **2021**, *31* (1), 1.
(126) Szomolay, B.; et al. Adaptive responses to antimicrobial agents in biofilms. *Environmental microbiology* **2005**, *7* (8), 1186−1191.
(127) Xu, J.; et al. Microneedle patch-mediated treatment of bacterial biofilms. *ACS Appl. Mater. Interfaces* **2019**, *11* (16), 14640−14646.
(128) Prausnitz, M. R.; Langer, R. Transdermal drug delivery. *Nature biotechnology* **2008**, *26* (11), 1261−1268.
(129) Blicharz, T. M.; et al. Microneedle-based device for the one-step painless collection of capillary blood samples. *Nature biomedical engineering* **2018**, *2* (3), 151−157.
(130) Yi, X.; et al. A novel bacterial biofilms eradication strategy based on the microneedles with antibacterial properties. *Procedia CIRP* **2020**, *89*, 159−163.
(131) Dincer, S.; Uslu, F.M.; Delik, A. Antibiotic resistance in biofilm. In *Bacterial biofilms*; Dincer, S., Özdenefe, M. S., Arkut, A., Eds.; IntechOpen: London, UK, 2020. DOI: 10.5772/intechopen.92388.
(132) Nithya, S.; et al. *Preparation, characterization and efficacy of lysostaphin-chitosan gel against Staphylococcus aureus. Int. J. Biol. Macromol.* **2018**, *110*, 157−166.
(133) Ramasubbu, N.; et al. *Structural analysis of dispersin B, a biofilm-releasing glycoside hydrolase from the periodontopathogen Actinobacillus actinomycetemcomitans. Journal of molecular biology* **2005**, *349* (3), 475−486.
(134) Fleming, D.; Chahin, L.; Rumbaugh, K. Glycoside hydrolases degrade polymicrobial bacterial biofilms in wounds. *Antimicrob. Agents Chemother.* **2017**, DOI: 10.1128/AAC.01998-16.
(135) Abdelkader, J.; et al. Modification of Dispersin B with Cyclodextrin-Ciprofloxacin Derivatives for Treating Staphylococcal. *Molecules* **2023**, *28* (14), 5311.
(136) Howell, L., et al. *Soluble bacterial and fungal proteins and methods and uses thereof in inhibiting and dispersing biofilm*. US 11672846 A12021.
(137) Colvin, K. M.; et al. The Pel and Psl polysaccharides provide Pseudomonas aeruginosa structural redundancy within the biofilm matrix. *Environmental microbiology* **2012**, *14* (8), 1913−1928.
(138) Thorn, C. R.; et al. Protective liquid crystal nanoparticles for targeted delivery of PslG: a biofilm dispersing enzyme. *ACS Infectious Diseases* **2021**, *7* (8), 2102−2115.
(139) Thiyagarajan, D.; Das, G.; Ramesh, A. Extracellular-DNA-Targeting Nanomaterial for Effective Elimination of Biofilm. *ChemNanoMat* **2016**, *2* (9), 879−887.
(140) Liu, C.; et al. Encapsulated DNase improving the killing efficiency of antibiotics in staphylococcal biofilms. *J. Mater. Chem. B* **2020**, *8* (20), 4395−4401.
(141) Devlin, H.; et al. Enzyme-functionalized mesoporous silica nanoparticles to target Staphylococcus aureus and disperse biofilms. *Int. J. Nanomed.* **2021**, *16*, 1929−1942.
(142) Whitchurch, C. B.; et al. Extracellular DNA required for bacterial biofilm formation. *Science* **2002**, *295* (5559), 1487.
(143) Montanari, E.; et al. Hyaluronan-cholesterol nanohydrogels: Characterisation and effectiveness in carrying alginate lyase. *New biotechnology* **2017**, *37*, 80−89.
(144) Liu, Z.; et al. Functional immobilization of a biofilm-releasing glycoside hydrolase dispersin B on magnetic nanoparticles. *Appl. Biochem. Biotechnol.* **2022**, *194*, 737−747.
(145) Islan, G. A.; et al. *Smart lipid nanoparticles containing levofloxacin and DNase for lung delivery. Design and characterization. Colloids Surf., B* **2016**, *143*, 168−176.
(146) Tasia, W.; et al. Enhanced eradication of bacterial biofilms with DNase I-loaded silver-doped mesoporous silica nanoparticles. *Nanoscale* **2020**, *12* (4), 2328−2332.
(147) Baidamshina, D. R.; et al. Biochemical properties and anti-biofilm activity of chitosan-immobilized papain. *Marine Drugs* **2021**, *19* (4), 197.
(148) Baidamshina, D. R.; et al. Anti-biofilm and wound-healing activity of chitosan-immobilized Ficin. *Int. J. Biol. Macromol.* **2020**, *164*, 4205−4217.
(149) Pourhajibagher, M.; Bahador, A. Synergistic biocidal effects of metal oxide nanoparticles-assisted ultrasound irradiation: Antimicrobial sonodynamic therapy against Streptococcus mutans biofilms. *Photodiagnosis and Photodynamic therapy* **2021**, *35*, No. 102432.
(150) Ronan, E.; et al. USMB-induced synergistic enhancement of aminoglycoside antibiotics in biofilms. *Ultrasonics* **2016**, *69*, 182−190.
(151) Durham, P. G.; et al. Harnessing ultrasound-stimulated phase change contrast agents to improve antibiotic efficacy against methicillin-resistant Staphylococcus aureus biofilms. *Biofilm* **2021**, *3*, No. 100049.
(152) Bharatula, L. D.; et al. Influence of high intensity focused ultrasound on the microstructure and c-di-GMP signaling of Pseudomonas aeruginosa biofilms. *Frontiers in microbiology* **2020**, *11*, No. 599407.
(153) Bigelow, T. A.; et al. The destruction of Escherichia coli biofilms using high-intensity focused ultrasound. *Ultrasound in medicine & biology* **2009**, *35* (6), 1026−1031.
(154) Alkahtani, S. A. Ultrasound-based techniques as alternative treatments for chronic wounds: a comprehensive review of clinical applications. *Cureus* **2017**, *9* (12), e1952.
(155) Rumbaugh, K. P.; Sauer, K. Biofilm dispersion. *Nature Reviews Microbiology* **2020**, *18* (10), 571−586.
(156) Wille, J.; Coenye, T. Biofilm dispersion: The key to biofilm eradication or opening Pandora's box? *Biofilm* **2020**, *2*, No. 100027.
(157) Amari, D. T.; Marques, C. N.; Davies, D. G. The putative enoyl-coenzyme A hydratase DspI is required for production of the Pseudomonas aeruginosa biofilm dispersion autoinducer cis-2-decenoic acid. *Journal of bacteriology* **2013**, *195* (20), 4600−4610.
(158) Davies, D. G.; Marques, C. N. A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. *Journal of bacteriology* **2009**, *191* (5), 1393−1403.
(159) Sauer, K.; et al. Pseudomonas aeruginosa displays multiple phenotypes during development as a biofilm. *J. Bacteriol.* **2002**, *184* (4), 1140−1154.
(160) Huynh, T. T.; et al. Glucose starvation-induced dispersal of Pseudomonas aeruginosa biofilms is cAMP and energy dependent. *PLOS One* **2012**, *7* (8), No. e42874.
(161) Barraud, N.; et al. Involvement of nitric oxide in biofilm dispersal of Pseudomonas aeruginosa. *Journal of bacteriology* **2006**, *188* (21), 7344−7353.
(162) Barraud, N.; et al. Nitric oxide-mediated dispersal in single- and multi-species biofilms of clinically and industrially relevant microorganisms. *Microbial biotechnology* **2009**, *2* (3), 370−378.
(163) Schobert, M.; Jahn, D. Anaerobic physiology of Pseudomonas aeruginosa in the cystic fibrosis lung. *International Journal of Medical Microbiology* **2010**, *300* (8), 549−556.
(164) Eschbach, M.; et al. Long-term anaerobic survival of the opportunistic pathogen Pseudomonas aeruginosa via pyruvate fermentation. *Journal of bacteriology* **2004**, *186* (14), 4596−4604.
(165) Petrova, O. E.; et al. Microcolony formation by the opportunistic pathogen P seudomonas aeruginosa requires pyruvate and pyruvate fermentation. *Molecular microbiology* **2012**, *86* (4), 819−835.
(166) Goodwine, J.; et al. Pyruvate-depleting conditions induce biofilm dispersion and enhance the efficacy of antibiotics in killing biofilms in vitr o and in vivo. *Sci. Rep.* **2019**, *9*, 3763.
(167) Sauer, K.; Doiron, A.L. *Compositions and methods for disrupting biofilm formation and maintenance*. US 11541105 B2, 2023.
(168) Han, C.; et al. Enzyme-encapsulating polymeric nanoparticles: A potential adjunctive therapy in Pseudomonas aeruginosa biofilm-associated infection treatment. *Colloids Surf., B* **2019**, *184*, No. 110512.

https://doi.org/10.1021/acsomega.4c02343
ACS Omega 2024, 9, 27853−27871