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Nanogel-based composites for bacterial antibiofilm activity: advances, challenges, and prospects

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Nano-based approaches, particularly nanogels, have recently emerged as a potential strategy for combating biofilm-related infections. Their exceptional characteristics including biocompatibility, biodegradability, stability, high water content, stimuli-responsiveness, and their nano size (which enables their penetration into biofilms) make nanogels a promising technology in the biomedical field. However, exploring nanogels for biofilm treatment remains in its early stages. This review examined the status of nanogels application for the treatment of bacterial biofilms. Recent investigations studied nanogels derived from natural polymers like chitosan (CS), hyaluronic acid (HA), and alginate, among others, for eliminating and inhibiting biofilms. These nanogels were utilized as carriers for diverse antibiofilm agents, encompassing antibiotics, antimicrobial peptides, natural extracts, and nanoparticles. Utilizing mechanisms like conventional antibody-mediated pathways, photodynamic therapy, photothermal therapy, chemodynamic therapy, and EPS degradation, these nanogels effectively administered antibiofilm drugs, exhibiting efficacy across several bacterial strains, notably *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Escherichia coli* (*E. coli*), among others. Despite showing promise, nanogels remain relatively underexplored in biofilm treatment. This review concludes that research gaps are still present in biofilm treatment processes including (i) a better understanding of the stimuli-responsive behaviors of nanogels, (ii) active targeting strategies, and (iii) the narrow spectrum of antibiofilm agents loaded into nanogels. Hence, future studies could be directed towards the following elements: the exploration of multi-strain biofilms rather than single-strain biofilms, other endogenous and exogenous stimuli to trigger drug release, active targeting mechanisms, a broader range of antibiofilm agents when employing nanogels, and fostering more comprehensive and reliable biofilm treatment strategies. This review found that there are currently several research gaps as well in the use of nanogels for biofilm therapy, and these include: (i) very limited exogenous and endogenous stimuli were explored to trigger drug release from nanogels, (ii) the active targeting strategies were not explored, (iii) a very narrow spectrum of antibiofilm agents was loaded into nanogels, and (iv) only biofilms of single strains were investigated.

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1. Introduction

To adapt to their surrounding environment, many microorganisms have developed the ability to evolve a range of survival strategies. One strategy utilized by microorganisms to survive harsh surrounding conditions such as immune responses and treatment with antimicrobial therapeutics is the development of biofilms. Biofilms are aggregates of microorganisms formed within a self-generated matrix called the extracellular polymeric substance (EPS). Unlike their freely suspended planktonic counterparts, biofilms form on surfaces that can be of living or non-living nature. Such surfaces onto which biofilms can attach and form include dental and implantable device surfaces, tissues, and wounds. Furthermore, biofilm-associated microorganisms differ from planktonic cells in their altered metabolic activity, genetic evolution, and even communication between microorganisms. Importantly, biofilms are typically resistant to antimicrobial therapies due to the biofilm blocking drugs and host immune responses. This, in turn, makes annihilating infections associated with biofilms more complicated and challenging compared to planktonic bacteria and reduces the chances of survival, and increases possible relapse post-treatment.[1,2] Overall, biofilm-forming microorganisms account for approximately 65% of clinically encountered microbial infections mainly due to their antimicrobial resistance and evading the immune system.[3] It is also expected that by 2050, infections caused by microorganisms resistant to antibiotics will become the primary reason for mortality with biofilms being responsible for most of the long-lasting infections in humans.[2]

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The challenge of biofilm treatment stems from several factors of which one of the most important is their self-produced, polysaccharide-based EPS. In addition to polysaccharides, the EPS matrix is also composed of proteins, extracellular DNA, and lipids. The EPS, also defined as "the house of biofilm cells", accounts for 90% of the biofilm matrix serving as a shelter for the remaining 10% of microbial cells found within the biofilm. The EPS plays a vital role in protecting the biofilm-residing microorganisms from damage by acting as a chemical and physical barrier. Furthermore, in addition to sheltering the microorganisms, the EPS also provides the biofilm with mechanical and structural stability while also keeping the microorganisms close to each other hence facilitating communication between them. Moreover, the EPS also aids in the spread of the microorganisms within the biofilm by enabling oxygen to diffuse and releasing extracellular enzymes to obtain nutrition.[4,5] When it comes to treating biofilms, the EPS plays an important role in protecting the biofilm from damage such as that induced by drugs. Due to its small pores, the EPS restricts the penetration of drugs, such as antibiotics, thereby blocking their access to the inside of the biofilm.[2] Additionally, the EPS can mitigate the effect of antibiotics by neutralizing them or restricting their diffusion with the aid of extracellular polysaccharides.[5] Such limiting of the ability of a drug to penetrate the EPS and reach the embedded bacterial cells increases the dose sufficient to yield an antimicrobial response by up to 1000 times compared to that required for planktonic cells.[2] Importantly, although a higher dose can have the desired curative effect, it also increases the risk of drug toxicity and resistance as well as the cost of the treatment.[6] Moreover, even if it penetrates the biofilm, the drug could be enzymatically inactivated within the biofilm. Furthermore, microbial cells within the EPS usually exist in a dormant state due to the low levels of oxygen and nutrients present. This can negatively affect drugs that rely on the active growth of microorganisms to exert their effects. These factors, in turn, complicate the annihilation of biofilm-residing microorganisms and increase the risks of re-infection post-treatment.[2]

One type of microbial biofilm posing a major clinical challenge is bacterial biofilms, shown in Fig. 1. Almost all types of bacterial strains can develop biofilms that can be of a single bacterial strain (monospecies biofilm) or different strains (multispecies biofilm).[4,5] Importantly, once mature, biofilms break releasing mobile bacteria that can move on to colonize new surfaces.[4] Although the discovery and introduction of antibiotics represent a major medical breakthrough, controlling infections and saving millions of lives, it has also increased the development of bacteria that are antimicrobial resistant (AMR). Importantly, these AMR bacterial strains can develop biofilms.[7] Typically, bacteria residing with biofilms are AMR bacteria possessing between 10 and 1000 times more resistance to antibiotics than their planktonic equivalents. This further expands the challenges inherently associated with the treatment of biofilms as it necessitates finding alternative antimicrobial therapeutics to replace antibiotics.[8] Therefore, finding innovative and efficient treatments for bacterial biofilms has become a topic of increased research interest. One field receiving significant research attention for the treatment of numerous medical conditions including biofilm-associated infections is nanotechnology. Nanotechnology involves the use of nanomaterials (particles with at least one dimension in the nanoscale) for various purposes including medical therapeutics and diagnostics.[1] Numerous nanomaterials have been studied to combat biofilms whether by therapy, imaging, or dual imaging and therapy.[9–13] Nanomaterials can exert their antibiofilm effects either *via* their intrinsic abilities such as heat generated from nanoparticles like iron oxide nanoparticles[14] or by acting as carriers for antibiofilm agents.[15] Due to their very small size, nano-sized therapeutic agents are especially advantageous to penetrate the biofilm's EPS and kill the biofilm-residing cells.[1] One type of nano-sized material recently being investigated as a promising candidate for the treatment of bacterial biofilms is a types of hydrogels called nanogels.[11,12,16,17]

Hydrogels are soft networks of hydrophilic polymer chains. Due to their hydrophilicity, hydrogels contain high water contents which contributes to their characteristic biocompatibility and softness. Due to their benefits, hydrogels have reached the clinic in several forms such as contact lenses, dermal fillers, and cancer drug delivery vehicles. However, hydrogels are macroscopic and hence unsuitable for applications where effects at the cellular level, such as bacterial cells for instance, are needed. Therefore, for such applications, nano-sized hydrogels (nanogels) are preferable to interact with cells and possibly be internalized by them.[18] Nanogels are hydrogels typically smaller than 100 nm in size that combine the advantageous features of hydrogels with those of the small nano size.[19,20] Like hydrogels, nanogels are usually soft, biodegradable, have a high surface area, biocompatible due to their high content of water, and porous which allows them to carry and release materials such as drugs. Furthermore, due to their softness, biocompatibility, deformability, and small size, nanogels usually have good circulation and penetration features.[18,21] When it comes to delivering drugs, nanogels have the ability to carry and deliver both hydrophilic and hydrophobic drugs and can be internalized by cells for intracellular drug delivery. Moreover, unlike hydrogels which are macro-sized, nanogels are stimuli-responsive responding rapidly to their surrounding changes.[21] Hence, nanogels are considered "smart" biomaterials responding to both endogenous and exogenous stimuli.[22,23] Furthermore, in the area of drug delivery, nanogels are more advantageous than hydrogels due to their high drug-loading capacity and stability as well as site-specific targeting.[6,16,21,24] Due to their many favorable features, nanogels have very recently been explored for biofilm therapy.

Nanogel's stimuli-responsiveness has been especially beneficial for pathologies having unique microenvironments such as cancer.[21] As with cancer, biofilms have a distinctive microenvironment due to their encapsulation within the EPS, with features different from those present outside the biofilm. One of the important features of biofilms is their acidic pH. Due to the blockage of nutrients and oxygen (hypoxia) by EPS, bacteria within the biofilm are forced to undergo anaerobic metabolism thereby resulting in acidic metabolites and making the biofilm microenvironment acidic. Other features unique to the biofilm

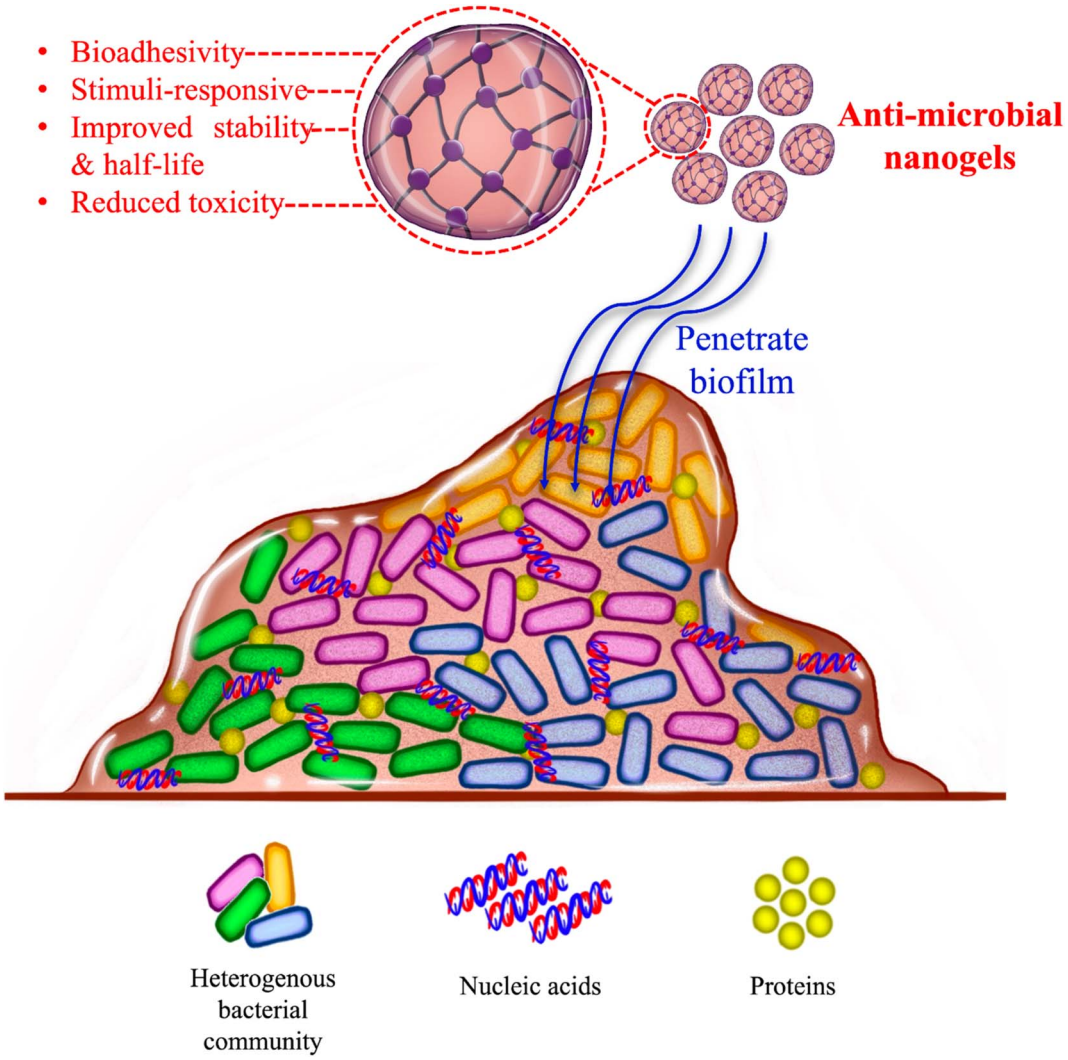

Fig. 1 Antimicrobial nanogels penetrating the EPS of bacterial biofilms.

microenvironment include high H_2O_2, enzyme overexpression, and redox conditions. Due to their absence in normal tissues, these biofilm-specific attributes can be utilized to achieve a biofilm-specific therapeutic strategy.[9,25] Of the endogenous biofilm-specific stimuli, low pH has been the most utilized by nanogels for biofilm treatment.[11,13,26] Furthermore, in addition to endogenous stimuli like low pH, exogenous stimuli can be used to improve the specificity of drug delivery and hence the treatment. Exogenous stimuli can include triggers such as near-infrared (NIR) light, magnetic field, ultrasound, and temperature.[21,27] As with endogenous stimuli, the exogenous stimuli studied for biofilm therapy using nanogels have been limited to NIR light.[13,28–30]

Despite the attractive features of nanogels for biofilm therapy, nanogels have only been very briefly and recently studied for the treatment of biofilms. This is in contrast with other medical applications such as cancer theragnostic where nanogels have been more investigated and their potential has been clearly highlighted. To the knowledge of the authors, there are currently no review articles in the literature highlighting the role and status of nanogels in biofilm treatment. Therefore, to address this void in the literature, this review critically summarizes the status of nanogels in the treatment of biofilms, particularly bacterial biofilms. Based on the search of the authors, the earliest study utilizing nanogels for biofilm treatment was reported in 2018 indicating the very recent interest and exploration of nanogels as potential biofilm therapeutic strategies. Moreover, based on our search, three natural polymers have been commonly explored as materials to synthesize antibiofilm nanogels. These polymers are chitosan, hyaluronic acid, and alginate. Other polymers were also briefly investigated for biofilm eradication. Therefore, this review discusses the recent progress in the use of chitosan-, hyaluronic acid-, and alginate-based nanogels as well as other nanogels for biofilm inhibition and eradication. Particularly, it critically analyzes and compares the performance of these nanogels for biofilm treatment in terms of: (i) the ability to eliminate pre-formed biofilms and stop the growth of forming biofilms, (ii) the ability to respond to internal and/or external stimuli, (iii) the different antibiofilm agents and mechanisms used to treat the

biofilm, and (iv) and the ability of the nanogel to target the biofilm. From this point on, the manuscript proceeds by first discussing the different types of nanogels used for biofilm therapy, critically analyzing them in terms of the type of nanogel and loaded drug, the antibiofilm activity (killing formed or inhibiting forming biofilm), and the mechanism used by the nanogel to exert its antibiofilm effects. Following that, some gaps in the literature are discussed and potential prospects for nanogels in biofilm treatment are proposed.

2. Nanogels for biofilm therapy

To combat biofilms, nanogels based on natural polymers such as chitosan, hyaluronic acid, and alginate alongside other polymers have been developed and studied as antibiofilm agents. The nanogels have been loaded with a range of biofilm-killing agents (Fig. 2) utilizing different mechanisms to efficiently eradicate formed biofilms and inhibit the growth of forming biofilms. The different nanogels loaded with antibiofilm drugs and mechanisms utilized against bacterial biofilms are discussed in the sections below and summarized in Table 1.

2.1 Chitosan-based nanogels for biofilm therapy

Until now, natural polymers have exhibited remarkably efficient performances in advancing biomedical fields, particularly drug delivery, tissue engineering, and wound healing.[31–33] To date, natural polymers' effectiveness and significant potential have been validated in producing various nanostructures such as micelles, polymersomes, and nanogels.[34] Among the various natural polymers, chitosan (CS) stands out as a cationic natural linear polyaminosaccharide acquired through the deacetylation process of chitin, which is derived from the exoskeleton of crustaceans like shrimp. CS is comprised of β-(1,4)-D-glucosamine and *N*-acetyl-D-glucosamine units and possesses distinctive characteristics such as non-toxicity, biocompatibility, and biodegradability, along with antibacterial, antifungal, mucoadhesive, and analgesic properties.[31,35,36] Due to its resemblance to glycosaminoglycans (GAGs), CS is extensively employed in tissue engineering and stands as one of the most utilized natural biopolymers in biomedical applications.[35,37,38]

A rising trend in antibacterial research involves exploring natural antimicrobial compounds, like CS, as substitutes for synthetic antibiotics. These studies aim to address critical issues, notably the escalating emergence of antibiotic-resistant bacterial infections, largely attributed to the excessive use of antibiotics.[18,39] Recent advancements have involved incorporating nanogels to facilitate the transportation and conservation of these natural substances, aiming to enhance their efficacy.[40–42] This approach presents a promising potential in tackling multidrug-resistant bacteria.[18,43] Therefore, CS nanogels have been recently studied for the treatment of bacterial biofilms.[11,17,44]

2.1.1 Chitosan-based nanogels encapsulating synthetic antibiotics. Bacteria commonly possess negatively charged cell membranes, which are typically comprised of lipid layers and peptidoglycan.[45] Consequently, nanogel carriers exhibiting a positive surface charge may intensify the interaction between the nanogel and the bacteria's surface. Accordingly, Palaniraj *et al.*[17] addressed the potential of preventing periodontal diseases and dental caries, particularly in reducing bacterial biofilms that persist on teeth. The research aimed to create an antimicrobial agent, chlorogenic acid (CGA), within a porous nanogel structure composed of calcium phosphate-CS (CaPNP@CS). CGA, a secondary phenolic metabolite in plants, is recognized for its various beneficial properties, including its preventive impact on dental caries and numerous health benefits.[46] The nanogel, formed *via* ionic gelation of calcium phosphate nanoparticles (as crosslinkers) and chitosan at a 1.25 : 1 ratio, utilized CGA due to its known antibacterial properties and potential to disrupt bacterial cell membranes.[47] This structure, with negatively charged phosphate ions and positively charged CS, neutralized bacterial growth with both CS and CGA demonstrating the ability to disrupt bacterial cell membranes thereby hindering biofilm formation. The CaPNP@CS@CGA nanogel exhibited a significant 68% increase

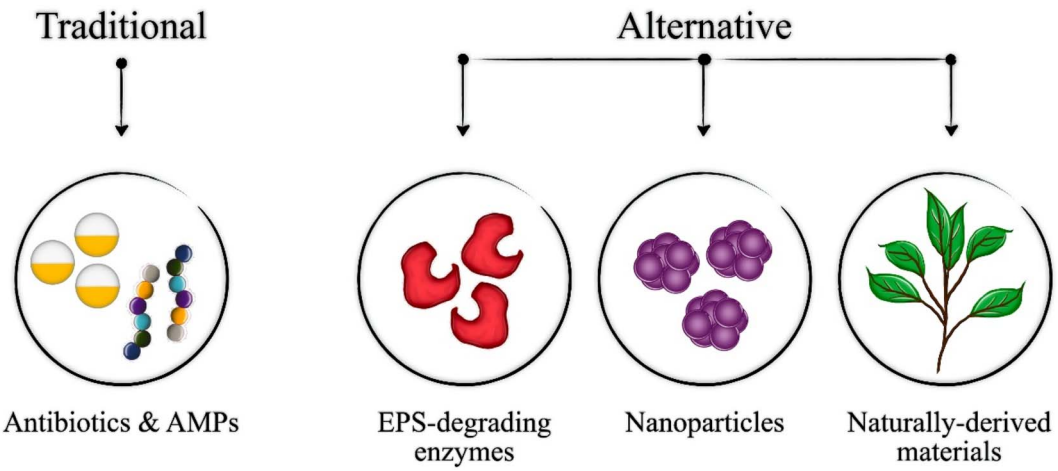

Fig. 2 Types of antibiofilm agents loaded into nanogels.

Table 1 A summary of the different types of nanogels investigated for biofilm annihilation and inhibition along with their mechanisms of action and target bacterial strains

Incorporated molecules	Eradication mechanism	Bacterial strain	Ref.
Chitosan-based nanogels			
AgNPs	Slow release of Ag^+ ions	*S. aureus*	11
Chlorogenic acid	Disruption of the bacterial cell membrane due to the nanogel-membrane electrostatic interaction	*S. aureus*, *K. pneumoniae*, and *P. aeruginosa*	17
Thymol	Reduction of OmpA and PgaB biofilm gene expression	*Staphylococcus*, *Acinetobacter*, and *Pseudomonas*	44
Mentha piperita essential oils	Down-regulation and inhibition of some glycosyltransferase genes (gtfB, C and D)	*S. mutans*	55
Gymnema sylvestre essential oils	Down-regulation of hypha-specific gene ALS3 expression	*C. albicans*	56
Tanshinone IIA	Penetration of the biofilm barriers with negatively charged surfaces	*S. mutans*	26
Hyaluronic-based nanogels			
SNAP and AMP	NO and AMP combination therapy to eradicate mature biofilm	MRSA and *P. aeruginosa*	16
Azithromycin	Azithromycin-induced prevention of biofilm formation and eradication of pre-formed biofilm	*P. aeruginosa*	12
Ab-Cath	Ab-Cath-mediated antimicrobial activity	Biofilm-residing AMR *S. aureus* and *A. baumannii*	8
SAAP-148	SAAP-148-mediated antimicrobial activity	Biofilm-residing *S. aureus* and *A. baumannii*	65
Alginate-based nanogels			
Fe^{3+}, and tannic acid	Dual PTT and enhanced CDT	*S. aureus* and *E.coli*	13
Enrofloxacin	Enrofloxacin-induced antimicrobial activity	*S. aureus* small colony variants	6
S-Benzyl-L-cysteine	Inhibition of bacterial growth by destruction of their cell walls	*P. aeruginosa*	76
Other nanogels			
Clindamycin	Adhering to the bacterial cells and direct administration of the antibiotic onto the bacterial biofilm cell walls	*S. aureus*	77
Cy3-AMP	Gelatinase degrades GNPs to release Cy3-AMP which destroys bacterial cells	*S. aureus*	30
Ciprofloxacin	Disruption of the bacterial biofilms EPS matrix by protease Alcalase 2.4 L FG	*S. aureus*, *P. aeruginosa*, *S. epidermidis*, *Klebsiella pneumoniae*, *E. coli*, and *Enterococcus faecalis*	78
Tetracycline (Tc)	Direct administration of the antibiotic onto the bacterial biofilm cell walls	*S. epidermidis*	79
Copper sulfide (CuS) nanoclusters	Suppression of the production of pro-inflammatory cytokines and modulation of the expression of anti-inflammatory factors	*S. aureus*	29
Triclosan	Disruption of the bacterial cell membrane and direct administration of the antibiotic onto the bacterial biofilm	*S. aureus*	80
Luliconazole	—[a]	*Candida albicans*	82
Peptidomimetic (A lysine-based α-peptide/β-peptoid hybrid)	—[a]	*P. aeruginosa*	81
Indocyanine green (ICG) and manganese pentacarbonyl bromide ($MnBr(CO)_5$)	Penetration and ablation of the biofilm by combined CO, PTT, and PDT	*S. aureus* and MRSA	28
ICS-Ag nanocomposite	Damage to the bacterial cytomembrane and the death of the bacterium after the release of its intracellular contents	*S. aureus*, *E. coli*, and *P. aeruginosa*	83
S-Benzyl-L-cysteine	Inhibition of bacterial growth by destruction of their cell walls	*P. aeruginosa*	76

[a] The exact mechanism needs further studies.

in biofilm degradation compared to the untreated group. Furthermore, the positively charged nanogel interacted effectively with bacterial cell membranes, disrupting their integrity. Moreover, toxicity studies revealed that CaPNP@CS@CGA nanogels remained non-toxic up to 40 μg mL^{-1} concentrations for HaCaT cells (immortalized human keratinocytes). These findings highlight the potential of CaPNP@CS@CGA nanogels as a viable solution for biofilm degradation, suggesting its applicability as a restorative dental material.[17]

Additionally, Fan *et al.*[11] aimed to leverage electrostatic interactions between sulfonated chitosan (SCS), silver ions (Ag^+), and chitosan (CS) and created a versatile antibacterial CS-based nanogel, AgNPs@CS/SCS. The developed nanogel demonstrated several key properties, such as stability in physiological conditions, slow and sustained release of Ag^+ due to the pH-dependent behavior of silver nanoparticles (AgNPs), and remarkable short- and long-term antibacterial efficacy against *S. aureus* and *E. coli*, particularly in addressing implant infections.[11] AgNPs exhibit antibacterial activities through a sequential cascade of actions contributing to the eventual elimination of bacteria. Initially, AgNPs can dissolve and release Ag^+ ions; subsequently, the released Ag^+ ions enhance the permeability of the bacterial cell membrane and disrupt bacterial DNA, ultimately leading to the eradication of the bacteria. This multifaceted approach highlights the efficacy of AgNPs in combating bacterial infections by targeting crucial cellular structures and functions, thereby hindering bacterial growth and survival.[11,48] The effectiveness of AgNPs@CS/SCS against *S. aureus* and *E. coli* was evaluated for short-term antibacterial activity. The findings reveal that the survival of *E. coli* and *S. aureus* relied on the concentration of AgNPs@CS/SCS and the duration of incubation. Higher concentrations and longer incubation periods resulted in stronger antibacterial effects. However, the effectiveness of AgNPs@CS/SCS against *E. coli* surpassed its impact on *S. aureus*. While nearly all *E. coli* were eliminated by AgNPs@CS/SCS, eradicating all *S. aureus* required more than double the incubation time and concentration compared to *E. coli*. Furthermore, AgNPs@CS/SCS displayed long-term antibacterial and inhibitory effects on the growth of both *E. coli* and *S. aureus*. Importantly, AgNPs@CS/SCS displayed excellent biofilm ablation abilities while maintaining good biocompatibility, hence, showing promise for the effective clinical treatment of implant-related biofilm infections.[11]

As previously discussed, the intricate and diverse nature of EPS, which encapsulates biofilms, presents significant challenges for therapies aimed at targeting EPS and eradicating biofilms. Within biofilms, an acidic microenvironment can develop, often decreasing below a pH of 4. This acidity primarily results from by-products generated during bacterial carbohydrate metabolism, including acetic and lactic acids, alongside extracellular DNA (eDNA) found within the EPS.[49–51] Consequently, these unique characteristics of EPS present formidable obstacles to biofilm-based therapies. Therefore, Wang *et al.*[26] focused on developing CS-based nanogels as carriers for Tanshinone IIA (TA) to heighten their effectiveness against *Streptococcus mutans* (*S. mutans*) in terms of antibacterial and antibiofilm activities. The developed nanogels (TA@CS) exhibited remarkable features, including high encapsulation efficiency and stability, even under challenging conditions such as exposure to light and other harsh environments. Notably, TA@CS demonstrated a pH-responsive behavior, allowing the selective release of higher TA amounts in acidic conditions, which could be beneficial for a more targeted, biofilm-specific delivery. Moreover, owing to their positive charge, TA@CS displayed an affinity for negatively charged biofilm surfaces, facilitating their efficient penetration through biofilm barriers and exhibiting promising antibiofilm activity. Crucially, the encapsulation of TA within CS nanogels notably boosted its antibacterial efficacy by at least fourfold. Simultaneously, TA@CS effectively inhibited 72% of biofilm formation. These outcomes highlighted the synergistic enhancement of antibacterial and antibiofilm properties when TA is encapsulated within CS-based nanogels. These advancements hold considerable promise for various applications in pharmaceuticals, food industries, and other relevant domains.[26]

2.1.2 Chitosan-based nanogels encapsulating natural compounds. Natural compounds present another group of promising antibacterial agents. However, inherent challenges associated with natural compounds, such as limited potency, restricted accessibility, and instability, have impeded their widespread applications. Thus, selecting a suitable vehicle carrying an appropriate natural compound with antibiofilm and antibacterial activities could be used to mitigate risks associated with multi-drug resistant strains. For instance, Gharaghie *et al.*[44] explored the antibacterial and antibiofilm impacts of thymol-encapsulating CS nanogels. Thymol, a beneficial compound from the Thyme (*Thymus vulgaris* L., Lamiaceae) plant, has well-established antimicrobial properties throughout its growth cycle.[52,53] Despite its effective antimicrobial properties, thymol faces limitations such as low water solubility, susceptibility to degradation, chemical reactivity, and volatility-induced chemical and biological instability, restraining its effectiveness as an antimicrobial and preservative agent.[54] Consequently, Gharaghie *et al.*[44] encapsulated thymol within CS nanogels with this encapsulation not only enhancing thymol's antibacterial and antibiofilm qualities but also mitigating its cytotoxic attributes. Moreover, this encapsulation enhanced thymol's efficacy by enabling the thymol-loaded CS nanogels to adhere to the surface of bacterial biofilms and employ controlled drug release mechanisms. In this study, the antibacterial properties of CS nanogels, thymol-free drug, and thymol-loaded CS nanogels were examined using the well diffusion technique. The findings showed that the thymol-loaded CS nanogels, with the greatest diameter of growth inhibition, were the most effective in inhibiting the growth against multi-drug resistant strains, including *Staphylococcus*, *Acinetobacter*, and *Pseudomonas*, which are recognized as the most challenging bacteria to date. On the other hand, thymol-free drug exhibited the least amount of inhibitory activity. Additionally, the results of the *in vitro* biological studies demonstrated that thymol-infused CS nanogels exhibited notably minimal cytotoxic effects toward human embryonic kidney 293 (HEK-293) cells compared to those observed in CS and free drug thymol. Particularly, these results highlighted the

robust antibacterial efficacy of thymol-based CS nanogels against all three types of multi-drug resistant organisms. Hence, it can be inferred that encapsulating thymol in CS nanogels enhances its antibacterial properties, along with providing targeted drug delivery and controlled drug release.[44]

2.1.3 Chitosan-based nanogels encapsulating essential oils. Several investigations have highlighted the versatile nature of CS-based nanogels, showcasing their capability to address multifaceted microbial challenges by encapsulating essential oils within the nanogel. Essential oils are derived from plants and comprise a complex blend of various components, thereby possessing multiple antimicrobial properties. For example, essential oils contain several phenylpropanoids, serving as natural antibacterial and antifungal agents. However, antimicrobial essential oils possess inherent hydrophobic properties and tend to evaporate quickly.[18,54] To address these challenges, researchers have encapsulated these oils within CS nanogels. For instance, Ashrafi *et al.*[55] investigated *Mentha piperita* essential oils (MPEO) loaded into CS nanogels as antibiofilm agents against *S. mutans*, addressing dental plaque issues. The study highlighted the down-regulation of key biofilm synthesis genes in the presence of both unloaded and MPEO-loaded nanogels. MPEO-chitosan nanogels notably inhibited glycosyltransferase genes responsible for extracellular polymers, essential for biofilm formation. Therefore, MPEO-CS nanogels demonstrated potent antibiofilm efficacy against *S. mutans*, emphasizing their potential application in dental care formulations to combat plaque formation.[55] CS-based nanogels have shown promise not only in reducing bacterial infections but also in addressing fungal issues. Studies have investigated their potential beyond antibacterial activities, focusing on their efficacy in controlling fungal growth. For instance, Akbari *et al.*[56] created a nanogel by combining CS and myristic acid and incorporating *Gymnema sylvestre* oil. The aim was to enhance the stability and antifungal efficacy against *Candida albicans* strain (ATCC 10231). The findings demonstrated that this CS nanogel exhibited fungicidal properties against the *Candida albicans* strain while also inhibiting its formation of biofilms.[56] Moreover, further studies have shown that CS-based nanogels encapsulating essential oils enhanced both the antimicrobial effectiveness, specifically leading to higher antifungal activity, and stability of the oils, facilitating their utilization within the food industry.[57–59]

Therefore, CS-based nanogels have remarkable potential in various biomedical applications including antibiofilm therapeutics. This is due to CS nanogels' unique characteristics and wide-range properties, including antibacterial, antifungal, and mucoadhesive traits. Currently, the emerging research is focusing on utilizing natural antimicrobial compounds, like CS, to reduce rising antibiotic resistance, leveraging recent advancements in nanogel technologies to enhance their efficacy against multidrug-resistant bacteria and bacterial biofilms.

2.2 Hyaluronic acid-based nanogels for biofilm therapy

Like CS, hyaluronic acid (HA), a vital component of cartilage, is another naturally available polysaccharide that has been explored for biomedical purposes including drug delivery.[60] HA is highly advantageous for drug delivery applications due to its biocompatibility, biodegradability, and presence of multifunctional groups.[61] These benefits make HA an attractive biocompatible and easily functionalized starting material for the synthesis of drug delivery vehicles like nanogels.[62] Moreover, in the area of microbiology, HA has been shown to possess bactericidal activity against bacterial biofilms thereby further adding to its suitability as a nanogel material for antibiofilm applications.[12] Additionally, the FDA approval of HA gels makes them even more promising candidates to be explored for biofilm treatment.[16] Therefore, HA-based nanogels have recently been investigated to eliminate and inhibit the growth of bacterial biofilms. HA-based nanogels studied for bacterial biofilm treatment are discussed below.

2.2.1 HA-based nanogels as carriers for AMPs and antibiotics. Several HA-based nanogels have been studied to eradicate biofilms *via* the encapsulation and delivery of different antibiofilm agents.[16] For instance, Fasiku *et al.*[16] co-delivered the antibiofilm agent nitric oxide (NO) and an antimicrobial peptide *via* HA-based nanogels for the eradication of both Gram-positive and Gram-negative bacterial biofilms. NO is a diatomic gas reported to possess antibacterial and antibiofilm activities. However, despite its promising performance, NO's short half-life, instability, and lack of targeting have limited its transition to the clinic. Likewise, AMPs are antibacterial and antibiofilm amino acid residues that are highly advantageous due to their ability to penetrate bacteria and reach intracellular targets.[16] However, AMPs are limited by their cytotoxicity to mammalian cells.[7,8,62] Encapsulation of NO and AMP solves the issues associated with NO and AMPs while also enhancing their antibiofilm effects *via* combination therapy. HA nanogels encapsulating the NO donor *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP) and AMP slowly released NO from the nanogel *in vitro*. Such slow release is highly advantageous as it makes the drug available for the target tissues for a prolonged period while also reducing toxicity, dosage, and administration frequency of the drug. In terms of antibiofilm activity, the loaded HA-based nanogels well-eradicated matured MRSA and *P. aeruginosa* biofilms *in vitro* with efficacies of 80% and 82%, respectively. This eradication efficacy surpassed that of SNAP only and SNAP-incorporated nanogels thereby indicating the high biofilm eradication stemmed from the synergistic effect of SNAP and the AMP. Furthermore, the loaded HA nanogel significantly reduced MRSA and *P. aeruginosa* biofilms colonized within catheters by a degree notably exceeding that induced by SNAP alone or SNAP-loaded HA nanogels. This, again, indicates the improved antibiofilm activity is induced by the SNAP/AMP combination therapy.[16] Importantly, MRSA and *P. aeruginosa* explored in this study are strains of special clinical significance due to the multi-drug resistance that can be exhibited by both strains. This in turn complicates the treatment of these bacterial strains by rendering traditional therapies such as antibiotics ineffective.[12,63,64]

HA-based nanogels have also been investigated as carriers for several other AMPs such as the synthetic antimicrobial and antibiofilm peptide (SAAP)-148 (ref. 7 and 65), the snake

cathelicidin Ab-Cath,[7,8] and DJK-5.[62] Peptides with antimicrobial and antibiofilm activities are promising alternatives to antibiotics for the treatment of AMR bacterial infections. However, despite their advantageous activity, these peptides are limited by their *in vivo* toxicity. The cytotoxic effects of these peptides can be reduced by encapsulating them within biocompatible and biodegradable drug delivery systems such as nanogels. Therefore, several studies explored the encapsulation of antimicrobial and antibiofilm peptides within HA-based nanogels for biofilm treatment.[7,8,62] For instance, a HA-based nanogel exploited to deliver the AMP Ab-Cath achieved a slow and sustained release of Ab-Cath and exhibited an efficient antimicrobial activity against the AMR *S. aureus* and *A. baumannii* strains residing within biofilms. Importantly, the Ab-Cath-loaded nanogel reduced the toxicity typically associated with free Ab-Cath while still preserving the peptide's antimicrobial activity.[8] In a similar manner, HA nanogels loaded with SAAP-148 achieved a sustained release of SAAP-148 from the nanogel while also retaining an antimicrobial activity similar to that of free SAAP-148 against the biofilm-inhabiting AMR *S. aureus* and *A. baumannii*. The encapsulation within the nanogel also prevented the typical unwanted SAAP-associated toxicity.[65] While the nanogels demonstrated impressive results in *in vitro* models, the proteolytic stability of SAAP-148 post-encapsulation in HA nanogels still needs to be examined with proteases. A more comprehensive evaluation using *in vivo* models could provide a deeper understanding of the pharmacokinetic and pharmacodynamic properties of the drug delivery system. Furthermore, creating a larger scale delivery system, like a gel, cream, or ointment, that contains the SAAP-148-loaded OSA-HA nanogels could aid in evaluating the potential of this nanoscale delivery system for clinical use as an antimicrobial treatment.[65] Another study by Van Gent *et al.*[7] encapsulated both Ab-Cath and SAAP-148 within HA-based nanogels and assessed their antimicrobial activity against AMR *S. aureus* and *A. baumannii*. Although this study showed reduced peptide-associated toxicity and maintained peptide-associated antimicrobial activity in AMR *S. aureus* and *A. baumannii*, this study did evaluate the performance of the loaded nanogels on the AMR strains residing within biofilms.[7] Likewise, HA-based nanogels delivered the potent antibiofilm peptide DJK-5 showing its maintained antimicrobial activity against *P. aeruginosa* and reduced toxicity typically associated with the peptide *in vivo*. However, this study also did not relate the results in any way to biofilms.[62] Nevertheless, the obtained results could motivate future testing of this peptide-loaded nanogel for biofilm residing bacteria since DJK-5 is one of the most potent antibiofilm peptides available.

HA-based nanogels have also been explored to improve the performance of some antibiotics such as Azithromycin. Although effective against some resistant strains such as *P. aeruginosa*, Azithromycin is limited by its *in vivo* widespread tissue distribution which reduces its concentration at the target site. This, in turn, necessitates the use of a suitable delivery vehicle to achieve a targeted delivery of the antibiotic Azithromycin.[12] HA-based nanogels have been reported by Kłodzińska *et al.*[12] for the delivery of Azithromycin to *P. aeruginosa* biofilms for improved antibiofilm activity. Azithromycin-loaded HA nanogels which were found to have a lower minimum inhibitory concentration than bare Azithromycin, effectively penetrated *P. aeruginosa* biofilms in a time-dependent manner while also significantly attenuating the formation of *P. aeruginosa* biofilms and eradicating its preformed (2 day old) biofilms. Importantly, these effects of the Azithromycin-loaded HA nanogels against forming and preformed *P. aeruginosa* biofilms were significantly greater than those explored with bare Azithromycin treatments. Moreover, bare nanogels had no effect on the biofilms indicating that the observed antibiofilm activity originated from the delivery of Azithromycin by the HA nanogels. In terms of safety, the Azithromycin HA nanogels showed no toxicity to normal cells *in vitro*.[12]

2.2.2 Challenges of HA-based nanogels. Although HA-based nanogels were reported to deliver peptides with activities against AMR biofilm-residing bacteria with promising results, the available studies do not discuss the performance of the loaded HA nanogels in eradicating pre-formed biofilms and/or preventing the formation of a biofilm. Furthermore, all studies discussed utilizing HA nanogels to deliver antimicrobial/antibiofilm agents, whether antibiotics, peptides, or NO did not utilize any biofilm active targeting agents. HA nanogels can potentially be targeted to biofilms for a more specific and efficient biofilm elimination. For instance, drug delivery systems can be targeted to biofilms *via* modification with antibodies such as immunoliposomes for instance.[66] Moreover, HA-based nanogels failed to utilize any external or internal stimuli to trigger the release of the nanogel-loaded cargo. This is in contrast to the CS-based nanogels discussed in the previous section which in addition to being more studied than HA-based nanogels, explored endogenous stimuli-responsive release for a more effective and promising biofilm eradication and growth inhibition.[11,26] Moreover, combined imaging and therapy of biofilms have recently become an important topic of interest. Several nano-based therapeutic systems have been reported for dual imaging and therapy of biofilms.[9,67,68] However, based on our search, biofilm treatment *via* HA-based NGs has not been coupled with imaging yet.

Overall, despite not being heavily explored, the promise of HA-based nanogels for biofilm treatment is evident through the delivery of antibiofilm agents such as NO, antibiotics, and antimicrobial peptides. Further progress with HA-based nanogels for biofilm therapy could include the encapsulation of other biofilm-eradicating agents such as iron oxide nanoparticles,[69] silver nanoparticles,[70] and copper sulfide nanoparticles.[67] These nanoparticles can eliminate biofilms *via* mechanisms different from those exploited by antibiotics and AMPs. For instance, SPIONs can annihilate biofilms *via* heat generation (hyperthermia) while copper sulfide nanoparticles can eradicate biofilms *via* photothermal and photodynamic therapies.[14,67] Furthermore, HA nanogels can be targeted to biofilms and triggered *via* stimuli to release their cargo for more efficient elimination and inhibition of biofilms. These modifications could potentially further enhance the performance of drug-loaded HA-based nanogels for biofilm therapy.

2.3 Alginate-based nanogels for biofilm therapy

Another less explored yet very promising polymer explored as a nanogel material for biofilm treatment is alginate. Alginate is a natural polymer known for its favorable features including accessibility, biocompatibility, biodegradability, non-toxicity, and low cost, all of which make it a promising candidate for a range of applications including the medical field. Particularly, the bio-adhesive nature of alginates makes them especially interesting for biomedical and pharmaceutical applications. Furthermore, alginate can be easily functionalized due to its available free hydroxyl and carboxyl functional groups. Sodium alginate (SA), the salt form of alginate, is usually used in literature due to its pH-induced gel-forming ability. Further adding to its advantages, alginate received its FDA approval for use in the medical field as materials for wound dressings and as additives to food.[71,72]

2.3.1 Stimuli responsive alginate-based nanogels. Nanogels based on alginates have been extensively studied in the literature for several biomedical applications such as cancer treatment,[21,73] wound dressing,[74] and even treating microbial infections.[74] Likewise, recently, alginate-based nanogels have been explored for the treatment of bacterial biofilms, however, only briefly. Zhao *et al.*[13] utilized pH and NIR-responsive SA-based nanogels for dual chemodynamic therapy (CDT) and photothermal therapy (PTT). CDT benefits from the high H_2O_2 content present in the bacterial microenvironment to produce toxic hydroxyl radicals ($^{\bullet}OH$) *via* Fenton or Fenton-like reactions. Although this strategy is highly advantageous as it avoids the use of antibiotics and, in turn, avoids the development of antibiotic resistance, it is limited by the body temperature and low Fe^{2+} content at the infection site both of which are needed to fuel the Fenton reaction. Therefore, the efficiency of CDT can be boosted by: (1) heat, and (2) the presence of Fe^{2+} ions. PTT, which can be induced upon exposure to NIR and converting it to thermal energy, can not only be used to boost CDT but also to kill bacteria *via* the destruction of their cell walls and denaturation of their proteins. Furthermore, to supply Fe^{2+} and even further boost CDT, Fe^{2+} ions can be supplied by metal-phenolic networks (MNPs) such as Fe^{3+}/Tannic acid which not only possess a high photothermal capacity but can generate $^{\bullet}OH$ *via* Fenton or Fenton-like reactions, but also reduce Fe^{3+} to Fe^{2+} which can further enhance CDT. Therefore, Zhao *et al.*[13] utilized a pH and NIR-responsive nanogel composed of SA, tannic acid, and Fe^{3+} to eradicate *S. aureus* and *E. coli* biofilms *via* stimuli-responsive, dual CDT and PTT. The tannic acid/Fe^{3+} containing SA nanogels had a photothermal ability surpassing that of bare SA nanogels and a pH- and NIR-responsive release of Fe^{2+}. In terms of antibiofilm activity, the tannic acid/Fe^{3+}/SA nanogels did not show any bactericidal activity in the absence of NIR and H_2O_2 while showing only slight effects in the presence of H_2O_2 alone due to CDT. However, under the influence of NIR, only the antibiofilm effects of the loaded nanogel were significantly boosted eliminating 97.23 and 99% of *S. aureus* and *E. coli* biofilms, respectively, *in vitro*. The biofilms were completely eradicated in the presence of dual NIR and H_2O_2. This complete eradication could be attributed to dual enhanced CDT and PTT. Moreover, this study also showed the loaded nanogel had a good safety profile *in vitro*.[13]

2.3.2 Alginate-based nanogels encapsulating antibiotics. Nanogels based on SA were also studied for the delivery of the antibiotic enrofloxacin for biofilm treatment. Enrofloxacin is a drug typically used in veterinary medicine that is limited by its low intracellular concentrations.[6] Luo *et al.*[6] combined SA with gelatin to fabricate nanogels loaded with the antibiotic enrofloxacin for the treatment of *S. aureus* small colony variants.[6] Gelatin is a natural biocompatible and biodegradable protein obtained from collagen that has been widely studied as a delivery vehicle for applications like cancer treatment.[75] Combined with gelatin, the enrofloxacin-loaded SA-based nanogels enhanced the intracellular concentrations of enrofloxacin. After 2 hours of incubation in the RAW 264.7 cells, the quantity of enrofloxacin composite nanogels accumulated in the macrophages was 5 times greater than that of the free enrofloxacin. Additionally, enrofloxacin-loaded gelatin-SA composite nanogels achieved a concentration-dependent antibacterial effect with minimum biofilm inhibitory and minimum biofilm eradication concentrations as low as 4 and 8 μg mL^{-1}, respectively. However, this study did not utilize any stimuli to trigger the release of enrofloxacin.[6]

A new generation of antibiotics studied includes the combination of antibacterial positively charged peptides with antibacterial aromatic amino acids. However, the positive charge of the antibacterial composite could exert some cytotoxicity to mammalian cells. The amino acid cysteine and some of its derivatives such as *S*-benzyl-L-cysteine have been reported to enhance antimicrobial activity. However, some cytotoxicity can still result from positively charged molecules. Therefore, *S*-benzyl-L-cysteine was crosslinked with the negatively charged alginate to inhibit the formation of *P. aeruginosa* biofilms. The *S*-benzyl-L-cysteine-modified alginate-based nanogels effectively inhibited the formation of *P. aeruginosa* biofilms by destroying the walls of bacteria and inhibiting their growth. Moreover, the bare and *S*-benzyl-L-cysteine nanogels were safe to normal organoids.[76]

To the knowledge of the authors, no more studies using alginate-based nanogels were reported for the purpose of biofilm treatment. However, alginate nanogels were more studied for the treatment of microbial infections that did not form biofilms.[74] Moreover, alginate has been extensively studied as a highly promising drug delivery material due to its advantageous properties. Therefore, although only very briefly studied for the ablation and inhibition of bacterial biofilms, the promise of alginate-based nanogels for this application is evident. Hence, further study is required to explore the full potential of alginate-based nanogels in this field. As with HA-based nanogels, alginate nanogels were not targeted to biofilms. However, they did benefit from stimuli-responsiveness.

2.4 Other nanogels for biofilm therapy

In addition to CS, HA, and alginate-based nanogels, nanogels based on other polymers have been reported for biofilm treatment although to a much lower extent. These studies

collectively showcase the efficacy of various other nanogel formulations in combating bacterial biofilms, which pose significant challenges in wound treatments due to their protective EPS matrix. The use of surface-functionalized nanogel particles in several studies proved promising for eradicating biofilms and overcoming antibacterial resistance mechanisms. For instance, Weldrick *et al.*[77] demonstrated the development of a clindamycin-loaded nanogel functionalized with Alcalase 2.4 L FG, targeting biofilms of Gram-positive bacteria, such as *S. aureus*. The Alcalase-coated clindamycin-loaded acrylic copolymer nanogels effectively broke down the EPS matrix of biofilms, adhered to bacterial cells, and delivered the antibiotic directly to their cell walls. Notably, these functionalized nanogels exhibited superior biofilm mass reduction against *S. aureus* compared to conventional antibacterial agents.[77] A similar study proposed the same Alcalase-coated nanogel carriers encapsulating ciprofloxacin to be used to disrupt the EPS matrix of biofilms and deliver antibiotics to the embedded bacteria. These nanogels demonstrated effectiveness against multiple biofilm-forming bacteria, including *S. aureus*, *P. aeruginosa*, *Staphylococcus epidermidis* (*S. epidermidis*), *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterococcus faecalis*, resulting in reduced biofilm mass and bacterial cell density. Co-treatment with ciprofloxacin-loaded Alcalase-coated nanogels showed a significant reduction in viable biofilm-forming cells compared to ciprofloxacin alone, displaying a potential approach for treating chronically infected wounds with biofilm-forming bacteria.[78] On the other hand, another study conducted by Li *et al.*[30] focused on treating *S. aureus* bacterial biofilms using an AMP release nanogel loaded with sulfo-cyanine3 carboxylic acid (Cy3) (cypate-GNPs@Cy3-AMP, CGCA), constructed with gelatinase nanoparticles (GNPs)—the nanogel aimed to control toxicity and facilitate bacterial clearance. Upon degradation of GNPs by gelatinase in the infection site, Cy3-AMP was released to destroy bacterial cells. Adding cypate on GNPs, combined with AMPs and NIR laser irradiation, induced irreversible damage to bacteria, effectively addressing toxicity concerns.[30]

Several other studies showcased innovative approaches using nanogels for eradicating biofilms, combating antimicrobial resistance, and advancing medical treatments. Each investigation employed distinct strategies and various nanogel formulations, demonstrating the versatile applications of these nanoparticles in addressing diverse microbial challenges, from specific biofilm eradication to enhancing antimicrobial efficacy and reducing cytotoxicity. One study targeted *Staphylococcus epidermidis* biofilms using enzyme-coated tetracycline (Tc)-loaded poly(acrylic acid) copolymer nanogel particles. These formulations effectively penetrated biofilms, delivering antibiotics to bacterial cells and outperforming free antibiotics.[79] The study examined the effects of nanogel formulations loaded with Tc and their individual components on the HeLa cell line, a human cell model, in an *in vitro* setting. The group found that while free Tc could significantly disrupt the biofilm, its effectivity was reduced against the bacteria within the biofilm. This suggested that the necessary therapeutic concentration of free Tc needed to kill the bacteria within the biofilm was not achieved, resulting in a lower killing ability compared to Tc delivered by the nanocarrier. The enzymes on the surface of the nanogel broke down the components of the EPS matrix, enabling them to penetrate deeper into the EPS and effectively deliver and release the encapsulated Tc to the bacteria within the biofilm. When an equivalent concentration of Tc was encapsulated in Carbopol nanogel particles and separately coated with the enzymes, there was a greater disruption of the biofilm and killing of the bacteria within it. The nano-formulated antibiotic overcame the EPS matrix barrier by effectively breaking down its components through enzymatic digestion. This allowed the enzyme-coated nanogel particle to deliver the required therapeutic dose of Tc, effectively killing the bacteria within the biofilm. This study highlights the potential of nanotechnology as a powerful tool for controlling pathogenic bacterial biofilms, with possible applications in treating chronic wounds and other hospital-acquired infections. The study also indicates the potential for conducting *in vivo* studies with pig skin and other more advanced animal models to fully assess the impact of these nano-formulations in a wound environment.[79] Another research focused on developing antimicrobial coatings for medical implants, utilizing non-quaternized poly(*N*-isopropylacrylamide-*co*-*N*-[3(dimethylamino)propyl]methacrylamide)(P(NIPAM-*co*-DMAPMA) nanogels modified with quaternary ammonium compounds and triclosan. This approach showcased strong antibacterial properties against *S. aureus* biofilms while maintaining antifouling behavior.[80] Additionally, quercetin-based carbonized nanogels embedded with copper sulfide nanoclusters displayed NIR responsiveness and effectively eradicated MRSA biofilms in diabetic wounds, demonstrating anti-inflammatory properties that promoted wound healing.[29] Another significant advancement involved a carbon monoxide (CO)-enhanced multi-mode antibacterial nanoplatform. The combination of CO, photothermal therapy (PTT), and photodynamic therapy (PDT) exhibited a notable efficacy in biofilm penetration, antibacterial action, and anti-inflammatory effects.[28] Additionally, biopolymer nanogels incorporating antibacterial peptidomimetics reduced cytotoxicity while maintaining antibacterial efficacy against *P. aeruginosa*.[81] DNA was also explored as a nanogel material for antibiofilm activity. *S*-Benzyl-L-cysteine was crosslinked with the negatively charged DNA to inhibit *P. aeruginosa* biofilms. The *S*-benzyl-L-cysteine-modified DNA-based nanogel was reported to inhibit the formation of *P. aeruginosa* biofilms by destroying the walls of bacteria and inhibiting their growth. Importantly, the *S*-benzyl-L-cysteine-modified DNA nanogel was found to be safe to normal organoids.[76]

Furthermore, as with CS, other nanogels were also explored for fungi biofilms. In addressing vulvovaginal candidiasis, a nanostructured lipid carrier-based transvaginal gel loaded with luliconazole showed promising results against *Candida albicans* biofilms.[82] Moreover, biofunctionalized nanosilver (ICS-Ag) using itaconyl-chondroitin sulfate nanogel (ICSNG) effectively combated microbial infections on medical devices. ICS-Ag demonstrated that it damaged the bacterial cell membrane, resulting in the release of internal cell contents and causing bacterial death. Hence, this nanosilver formulation

exhibited excellent antibacterial and antifungal properties alongside high biocompatibility.[83]

Therefore, although CS, HA, and alginate-based nanogels are the most commonly studied for biofilm elimination and growth inhibition, several other nanogels formulations hold promise for this application. However, additional investigation is needed on these formulations to explore their full potential in the treatment of bacterial biofilms. Table 1 summarizes the types of nanogels investigated for biofilm annihilation and inhibition along with their mechanisms of action and target bacterial strains. The various mechanisms utilized by the nanogels are summarized in Fig. 3.

In this regard, it is interesting and noteworthy to report here that the literature seemed to be greatly lacking in the area of utilization of stimuli responsiveness and targeting agents. The obtained results for all the studies report similar improved antibiofilm activity of nanogel encapsulated antimicrobial agent compared to unencapsulated antimicrobial agents. Stimuli-responsiveness and targeted therapy are concepts that have become increasingly investigated in recent years for several diseases such as cancer. Specifically, nanogels have been utilized to respond to various stimuli such as pH and NIR and targeting agents such as aptamers and homotypic targeting.[84–87] Therefore, despite the promising results that are in line with each other, several more investigations are yet to be conducted such as equipping the nanogels with stimuli responsiveness and targeting.

3. Future directions

Although relatively limited, the immense potential of nanogel-based composites as antibiofilm agents for advancements in combating complex biofilm infections is evident in the available literature. However, despite their promising antibiofilm activity, several key aspects are required for further exploration to effectively treat biofilms. The first aspect includes the need to transition from single-strain biofilm studies to complex multi-bacterial strains co-cultured within a single dish. Presently, the literature predominantly examines biofilms with a single bacterial strain. Yet, real-life biofilms often encompass diverse bacterial species, forming poly-microbial biofilms with combinations of Gram-positive, Gram-negative bacteria, and even fungal species sometimes. Understanding and treating such polymicrobial biofilms presents a critical challenge, demanding antimicrobial agents effective against all pathogenic microorganisms present within these biofilms.[88] Therefore, it is not guaranteed that promising results obtained with single-strain biofilms will be duplicated in multi-strain biofilms. Hence, antibiofilm nanogels should be investigated against multi-strain biofilms mimicking real-life biofilms to ensure the effectiveness of the nanogels in antibiofilm therapy. A further path for future exploration involves diversifying the types of antimicrobial components carried by the nanogels. This could include loading nanoparticles that can exert antibiofilm effects into nanogels. Such nanoparticles could include iron oxide

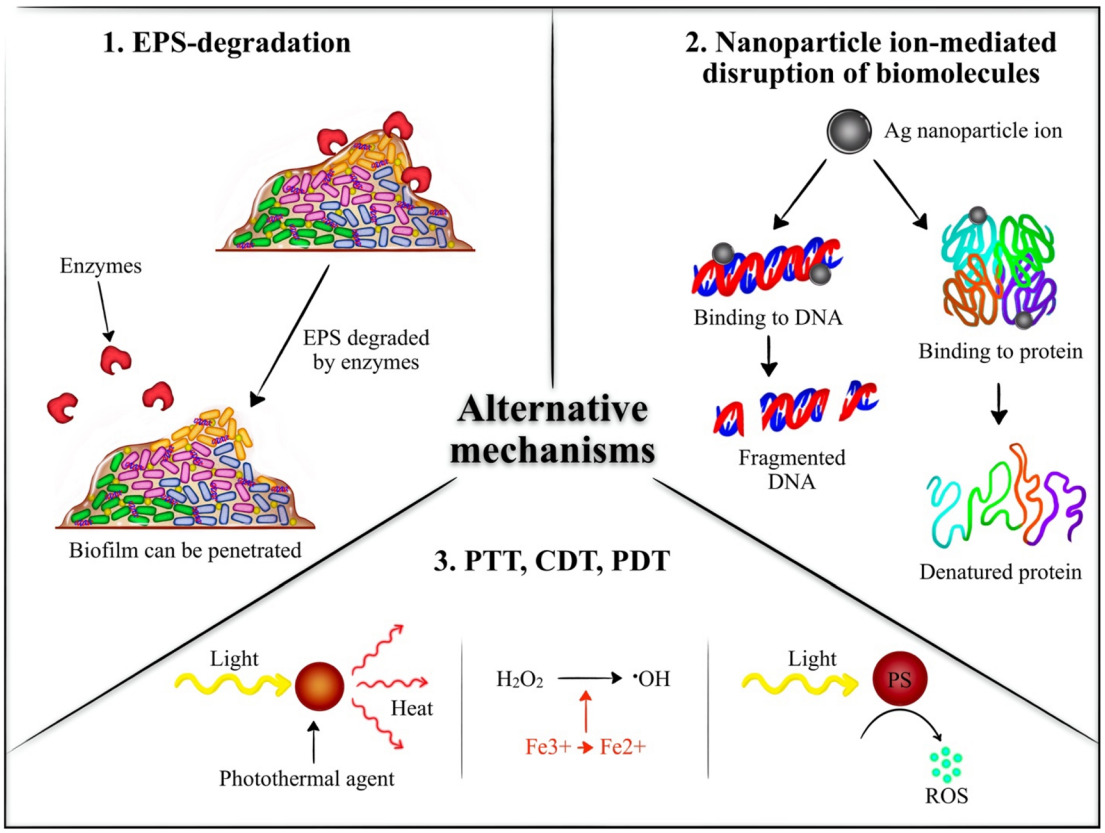

Fig. 3 Alternative antibiofilm mechanisms exploited by nanogels; PS = photosensitizer.

nanoparticles,[69] silver nanoparticles,[70] and copper sulfide nanoparticles[67] which can eliminate biofilms *via* mechanisms different from the conventional ones exploited by antibiotics. For instance, superparamagnetic iron oxide nanoparticles can kill biofilms *via* hyperthermia while copper sulfide nanoparticles can annihilate biofilms *via* photothermal and photodynamic therapies.[14,67] Furthermore, the co-delivery of antimicrobial drugs such as antibiotics or nanoparticles could be explored to benefit from the synergistic effect of the drugs for better therapeutic effects against biofilm infections.[89]

Another crucial aspect for future research involves using targeting moieties to target nanogels specifically to biofilms. However, currently, there is insufficient knowledge about overexpressed proteins on bacterial surfaces within biofilms. Therefore, it is essential to explore and identify novel moieties to achieve targeted interventions against biofilms. Moreover, leveraging the enhanced permeation and retention effect (EPR) observed in biofilm-infected sites, similar to that seen in tumors, can enable passive targeting of nanoparticles within bacteria-infected regions. This passive accumulation can be achieved due to the permeable nature of blood vessels in inflammatory areas, aiding the efficient accumulation of polymeric therapeutic nanoparticles within the biofilm microenvironment.[90] Additionally, stimuli-responsive nanogels represent another promising avenue. While some studies have employed stimuli, such as pH and NIR, the exploration of various other endogenous and exogenous stimuli remains largely untapped. Investigating additional stimuli, both endogenous and exogenous, holds the potential for enhancing the specificity and efficacy of nanogel-based treatments for biofilms.[21]

Furthermore, the range of bacterial strains studied with nanogel-based composites remains limited. While strains like *E. coli* and *S. aureus* have received the most attention, other Gram-positive and Gram-negative bacterial strains need exploration. This includes less prominent strains like *Acinetobacter baumannii*, *Klebsiella*, *Salmonella*, and *Streptococcus* strains, among others. Diversifying the study to encompass a broader spectrum of bacterial strains will provide a more comprehensive understanding of the efficacy and potential limitations of nanogel-based interventions against a wider array of biofilm infections. Therefore, focusing on more bacterial strains from both Gram-positive and Gram-negative categories will allow for a more nuanced exploration, shedding light on potential strain-specific responses and aiding in the development of more universally effective treatments against biofilms.

In conclusion, despite the promising results on antibiofilm nanogels, the available data remains limited in several aspects. Therefore, further research is imperative in the field of biofilm treatment using nanogels-based composites. While existing studies have provided valuable insights, the need persists for more comprehensive investigations involving multi-strain biofilms, targeting agents, diverse stimuli, and a wider spectrum of bacterial strains. Multiple formulations have demonstrated immense potential in exhibiting robust antibiofilm activity, emphasizing the promising trajectory of nanogel-based interventions in combating complex biofilm infections and motivating further studies to explore their full potential.

4. Conclusions

The treatment of bacterial infections has become significantly complicated by the emergence of biofilms to which conventional antibiotics are ineffective. Consequently, several nano-based strategies have been developed and studied to combat biofilm-associated infections. Among these strategies, nanogels have shown promise as a therapeutic strategy for a range of medical conditions including biofilms. This is due to nanogels' small size which enables their penetration into biofilms, along with their biocompatibility, biodegradability, good circulation and stability, and their stimuli-responsiveness. However, despite their promising features, nanogels have only very recently begun to be investigated for biofilm treatment.

Nanogels derived from natural polymers such as CS, HA, and alginate, alongside other polymers, have been recently studied for biofilm eradication and growth inhibition. These nanogels delivered diverse antibiofilm agents ranging from antibiotics and antimicrobial peptides to natural extracts and nanoparticles. Employing various mechanisms namely conventional antibody-mediated mechanisms, alternative photodynamic therapy, photothermal therapy, chemodynamic therapy, and EPS degradation, the studied nanogels effectively delivered the carried antibiofilm drugs and exerted their antibiofilm effects in several bacterial strains including *S. aureus*, *P. aeruginosa*, *S. epidermidis*, *Klebsiella pneumoniae*, *E. coli*, and *Enterococcus faecalis*, and *A. baumannii*. However, the most investigated strains were *S. aureus*, *P. aeruginosa*, and *E. coli*.

This review concluded that nanogel encapsulation of antimicrobial agents has shown to be more effective than free antimicrobials. The encapsulation exhibited excellent antibacterial and *anti*-biofilm activities, as well as controlled release. These results, demonstrating improved treatment of various biofilm infections and combating resistant bacteria, are of great significance. The strategy of combining nanogels with various antibacterial agents could be recommended as a novel approach for applications to treat the hazardous biofilm infection. A current challenge nanogels face is the formulation of compositions and surface coatings used to possess antibiofilm and antibacterial properties, in both *in vitro* and *in vivo* conditions. To tackle this issue, the integration of various strategies, such as the loading of AMPs, antibiotics, essential oils, and nanomaterials into nanogels, serves as a crucial example of potential ideas that could progress the development of efficient nanoparticles to inhibit infections.

Despite their promising performance, nanogels remain relatively underexplored for biofilm treatment. Limited studies have focused on the stimuli-responsiveness of nanogels for biofilm treatment with the studied stimuli restricted to only pH and NIR. Moreover, active targeting strategies to specifically target nanogels to the biofilm have not been explored yet. Additionally, limited antibiofilm agents were loaded into nanogels. Several other antibiofilm agents such as nanomaterials like iron oxide nanoparticles can be investigated with nanogels for better antibiofilm treatment. Most importantly, biofilms typically contain two or more bacterial strains. However, the currently available literature investigates single-

strain biofilms neglecting multi-strain ones. This presents a major limitation on the reliability of the antibiofilm activity observed with the studied nanogels.

Conflicts of interest

There are no conflicts to declare.

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