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REVIEW

Novel Strategies to Combat Bacterial Biofilms

Fatemeh Hemmati[1,2] · Mohammad Ahangarzadeh Rezaee[2] · Saba Ebrahimzadeh[3] · Leila Yousefi[1,4] · Roghayeh Nouri[1,2] · Hossein Samadi Kafil[4] · Pourya Gholizadeh[4]

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Abstract

Biofilms are considered as a severe problem in the treatment of bacterial infections; their development causes some noticeable resistance to antibacterial agents. Biofilms are responsible for at least two-thirds of all infections, displaying promoted resistance to classical antibiotic treatments. Therefore, finding new alternative therapeutic approaches is essential for the treatment and inhibition of biofilm-related infections. Therefore, this review aims to describe the potential therapeutic strategies that can inhibit bacterial biofilm development; these include the usage of antiadhesion agents, AMPs, bacteriophages, QSIs, aptamers, NPs and PNAs, which can prevent or eradicate the formation of biofilms. These antibiofilm agents represent a promising therapeutic target in the treatment of biofilm infections and development of a strong capability to interfere with different phases of the biofilm development, including adherence, polysaccharide intercellular adhesion (PIA), quorum sensing molecules and cell-to-cell connection, bacterial aggregation, planktonic bacteria killing and host-immune response modulation. In addition, these components, in combination with antibiotics, can lead to the development of some kind of powerful combined therapy against bacterial biofilm-related infections.

Keywords Biofilm · Antibiofilm agents · Biofilm-related infections · Therapeutic approaches

Introduction

Most nosocomial infections have been caused by opportunistic pathogens in the recent decades; these are associated with various infections including bacteremia, urinary tract infections (UTIs), wound, meningitis and biofilm-associated infections [1]. Bacteria that are firmly attached to artificial medical devices cause biofilm-associated infections. Treatment of these infections is difficult because of the increased antibiotic resistance in the biofilm, which is commonly due to multidrug-resistant strains [2, 3]. Nowadays, biofilm infections have become a serious concern and a main global healthcare problem [1]. The identification of novel therapeutic targets to fight biofilm-related infections, thus, signifies one of the primary problems in the field of antibiotic therapy [4]. Bacteria are present in the environment in two forms: free-living or biofilm. Bacteria inside a biofilm can demonstrate low levels of sensitivity to some antimicrobial compounds including biocides and antibiotics [2, 3]. Therefore, antibiofilm compounds can be regarded as one of the most encouraging options to combat biofilm-related infections. These compounds have shown a broad range of biological functions including antibacterial properties on free-living cells, as well as antibiofilm properties [5].

Biofilm is known as a bacterial population in which cells adhere to biotic or abiotic surfaces by extracellular polymeric substances (EPS). Biofilm could be considered as a strategy some bacteria could adopt to withstand adverse conditions such as desiccation, host defense system and antibacterial agents [6].

The stages involved in the formation of biofilm in bacteria comply with a generic model consisting of three stages. The features of each stage are characteristics in different species, but the general traits are all alike [7]. The main

✉ Pourya Gholizadeh
poorya.gholizadeh@gmail.com

1 Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran

2 Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

3 Department of Food Science and Technology, Faculty of Agriculture and Natural Resources, Urmia University, Urmia, Iran

4 Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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stages in the biofilm formation are attachment, accumulation and dispersal [8]. In the first stage, the attachment to the surface is reversible and not very strong. Next, a loose bond is established between cells, and the extracellular matrix begins to secrete. In the later stages, the biofilm structure gains shape and other bacteria still in the environment attach themselves to this structure. Following the evolution of the biofilm structure, the cells are released back into the environment, causing new biofilm foci elsewhere [9].

In the biofilm phase, bacteria are surrounded in a self-produced extracellular matrix principally made of EPS [10]. EPS comprises over 90% of the dry weight of the biofilm, facilitating bonding to surfaces, which can cause microcolony formation and resistance to antimicrobial agents [11]. EPS is made up of exopolysaccharides, proteins and extracellular DNA (eDNA) [10]. EPS matrix reduces the permeation of antibiotics to spread bacteria within the biofilm by dispersion restriction or neutralization of antibacterial agents with extracellular polysaccharides [12]. Bacterial biofilms are significant in causing infectious diseases, especially chronic infections, in the host [13, 14]. According to some estimations previously carried out, approximately 80% of bacterial infections in the human body are associated with the formation of biofilms, which can increase the mortality rate in the hospitalized patients [2, 14]. Therefore, managing biofilm-related infections is challenging because of the problems inherent in inhibiting and treating them [3].

Given the specific conditions in the biofilm environment, treating and employing antibiotics to tackle this problem can be regarded as one of the important challenges the medical sciences face. Biofilm-associated infections cannot be treated by classical antibiotics, and they are a challenge worldwide. This has led to a set of studies aiming to find modern treatments for biofilm infections [15]. Therefore, new antibacterial or antibiofilm strategies with various mechanisms of action are urgently needed. Among these strategies, development of new classes of antimicrobial peptides (AMPs) [16], bacteriophages [17], quorum sensing inhibitors (QSIs) [18], aptamers [19], nanoparticles (NPs) [20] and peptide nucleic acids (PNAs) [21] can be considered as the most feasible solution.

In the recent years, several innovative antibiofilm agents have been developed to limit bacterial adhesion to abiotic and biotic surfaces; these are intended to target bacterial signals for removing grown biofilms or displacing cells from the established biofilms [4]. Therefore, this review aims to review and describe the potential therapeutic strategies that could be applied to prevent bacterial biofilm development; these include the usage of antiadhesion agents, AMPs, bacteriophages, QSIs, aptamers, NPs and PNAs, which can prevent or eradicate the biofilm formation.

Properties of Desirable Antibiofilm Agents

Biofilms are bacterial populations demonstrating exclusive properties in comparison with their free-living forms [22]. These properties should be correctly considered when assessing the potential of biofilm inhibition mechanisms. The EPS matrix in the biofilm development plays a significant and critical role in determining antibiotic resistance mechanisms of biofilm [4]. It basically establishes a dispersion barrier which can prevent the interaction of antibacterial agents with bacterial cells [23]. Restricting or preventing EPS accumulation and having the capability to permeate EPS could be regarded as characteristics of an ideal antibiofilm agent [24]. Therefore, characteristics of an ideal antibiofilm substance include antibacterial activities, easy penetration into the cell, unique structure, interference with the machinery of bacterial cells communication and synergism with other antibacterial agents [4]. Many of such properties can be observed in natural and synthetic antibiofilm substances [25–28]. Inhibition of the biofilm development by these compounds may simplify the treatment of biofilm-related infections.

Strategies for the Inhibition and Disruption of Bacterial Biofilms

Biofilms are highly resistant to conventional antibiotics; therefore, new alternative therapeutic approaches are needed to treat biofilm-related infections [3, 29]. There are several strategies to inhibit and eradicate biofilm development; these include antiadhesion agents, AMPs, bacteriophages, QSIs, aptamers, NPs and PNAs.

Antiadhesion Agents

The introduction of bacteria to a surface is influenced by the stochastic process, which is driven by gravitational forces, the surrounding hydrodynamic forces and Brownian motion [30, 31]. To overcome repulsive and hydrodynamic forces, motile bacteria utilize flagella, which can play an important role in the initial attachment in the case of several pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Vibrio cholera* [32–35]. The roles of chemotaxis in directing attachment and biofilm formation in response to nutrient composition have been demonstrated. Schmidt et al. [36], for instance, showed that the CheR1 methyltransferase mutations of *P. aeruginosa* could alter the amino acid response and inhibit biofilm maturation through impairing attachment. In addition, biofilm defects have been revealed in the *tar* gene (encodes

methyl-accepting chemotaxis protein II) in uropathogenic *E. coli* (UPEC) [37]. Type 1 pili is another type of adhesion in UPEC and other *E. coli* that can play a role in the initial attachment, maintaining a steadfast grip on the surface and shear forces [38, 39]. These pili, which are assembled by the chaperon usher pathway (CUP), are multi-subunit adhesive [40]. CUP pili systems facilitate adherence in a niche-specific manner [37, 40, 41]. Adherence in the type 1 pili is mediated by the FimH adhesion, which recognizes mannose-rich regions [42]. Antigen 43 and curli fibers are other types of adhesion mediating interbacterial interactions and attachment on biotic and abiotic surfaces [43]. There are other attachment organelles in bacteria, including type IV pili and numerous CUP fimbriae (such as CupA) in *P. aeruginosa* [44], Ace, Esp and Ebp in *Enterococcus faecalis* [45], and SagA and Acm in *Enterococcus faecium* [46]. Therefore, several studies have focused on the development of compounds interacting and interfering with the first step of attachment, leading to the biofilm formation. Mannosides are molecules that competitively inhibit FimH mannose binding [47]. Among mannosylated proteins, the highest affinity of the monovalent ligands has been displayed in long chain aryl mannosides and alkyl mannosides, which could be due to the increased interactions with Tyr-48, Tyr-137 and Ile-52 of the binding pocket [48]. The development of monomeric biphenyl mannosides could lead to FimH inhibitors [49], which can prevent in vitro UPEC biofilm formation and interfere with the in vivo adherence and invasion of UPEC [49]. Cusumano et al. [49] also demonstrated that the combination of mannosides with TMP-SMZ (trimethoprim-sulfamethoxazole) could enhance antimicrobial treatment in clinical settings. In addition, the bladder bacterial load of UPEC was reduced in orally mannoside treated mice within 6 h, thus serving as an efficient therapeutic strategy for chronic UTIs [49].

In parallel, pilicides have been introduced to inhibit CUPs and type 1 pili, which are components derived from peptodomimetic scaffolds such as C-2 substituted thiazolo and dihydrothiazolo ring-fused 2-pyridones, as well as the bromomethyl substituted scaffold that can interfere and interact with the exportation of the subunits corresponding to the pili structure [50, 51]. Furthermore, curlicides (components derived from ring-fused 2-pyridones, such as FN075 and BibC6) have been introduced to inhibit the curli synthesis followed by the inhibition of the biofilm formation [43]. According to some studies, bacteria utilize a variety of receptors and adhesion types during their adherence process, facilitating adhesion in a niche-specific manner; therefore, interactions of multiple molecules may need to be inhibited to remove a biofilm from surfaces.

Antimicrobial Peptides

Antimicrobial peptides (AMPs) are small cationic molecules displaying a broad range of activity against microorganisms [52]. AMPs are divided into natural and synthetic groups. Table 1 shows the list of AMPs and their properties. Natural AMPs are produced by cellular tissues in a wide range of

Table 1 Peptides with the antibiofilm activity

Peptide	Mode of action	Affected bacteria	Refs.
Nisin A	Disturbing or destruction of the membrane of biofilm cells	Methicillin-resistant *S. aureus* (MRSA)	[61]
Human cathelicidin LL-37	Stimulation of twitching motility mediated by type IV pili, Inhibition of QS signal, reducing the adhesion of bacterial cells on the surface	*P. aeruginosa*	[62]
Hepcidin 20	Decreasing the extracellular matrix mass, targeting PIA to change biofilm structure	*Staphylococcus epidermidis*	[63]
Human -defensin 3	Reducing the expression of the icaA, icaD, and IcaR genes to decrease biofilm development	*S. epidermidis*	[16]
SMAP-29	Inhibit biofilm formation	*Burkholderia thailandensis*	[64]
RT2 and KT2 (Synthetic tryptophan-rich cationic peptides)	Inhibition of biofilm formation (interaction with cytoplasmic membranes and DNA)	*E. coli* O157:H7	[65]
AS10 (CRAMP)	Inhibition of biofilm formation	*P. aeruginosa* and *E. coli*	[66]
CAMA	Inhibition of biofilm formation	MRSA	[67]
IDR-1018	Alterations in the mRNA expressions of the QS related vicR, vicK, comC and comD genes or blocking (p)ppGpp	*S. mutans*	[68]
Pleurocidin (NRC-16)	Inhibition of nucleic acid synthesis and proteins	*P. aeruginosa*	[69]
ZXR-2	Inhibition of biofilm formation	*S. mutans*	[70]
KT2 and ZXR-2.3	Diminish the expression of the comC, vicK and vicR genes and decrease of biofilm development	*S. mutans*	[68]

organisms; they can be a source for synthetic AMPs [53, 54]. AMPs are suggested as a potential method to cope with the biofilm formation. AMPs as antibiofilm agents have received much attention. Many AMPs have a positive charge, enabling them to interact with the phosphate groups of lipopolysaccharides as negatively charged components of the cell membrane in Gram-negative bacteria or lipoteichoic acids in Gram-positive bacteria [55, 56]. Most AMPs exhibit strong antibiofilm properties against antibiotic-resistant bacteria, which can be effective with different mechanisms at various phases of the biofilm development and on different molecular targets [16]. Some peptides can kill bacteria through membrane distraction and/or pore formation, as well as inhibition of the bacterial cell division; further, they can be effective on bacteria through preventing the adherence of the bacterial cells to the substrate surface, downregulating QS signals and removing the pre-formed biofilm [16]. Downregulation of genes involved in the motility and inhibition of a series of cellular biological procedures, such as the synthesis of cell walls, DNA, RNA and proteins, are other instances of AMP-antibiofilm mechanisms of function [57–60]. Figure 1 shows multiple mechanisms of antimicrobial peptides on the bacterial biofilm formation.

Antibiofilm peptides (ABPs) are a type of AMPs with the antibiofilm activity; they prevent the biofilm development at concentrations much lower than those of common antibiotics. The minimum inhibitory concentration (MIC) is, in fact, greater than the minimum biofilm inhibitory concentration (MBIC) [71]. ABPs can be linked to ppGpp molecules (second messenger nucleotide), disrupting them; they are involved in several metabolic pathways like colonization, attachment and aggression in bacteria. Therefore, ABPs prevent the increase of these molecules in the cell and inhibit the biofilm development in bacteria [72, 73]. ABPs commonly interact with signal molecules to apply control or distraction effects on the biofilms development; therefore, they can help other antibacterial agents to cope with the bacterial cells. For instance, Ribeiro et al. [74] described that ABPs promoted the sensitivity of carbapenemase-producing *Klebsiella pneumoniae* to carbapenems.

It has been demonstrated that most AMPs can be in combined with antibiotics to enhance their ability to prevent biofilm development and eliminate mature biofilms. Synergism of AMPs with other antibacterial agents is promised in the cleaning of biofilm-related infections, as they can increase the antibiofilm effect and reduce the drug dose [68]. Tong et al. [75] also reported the synergistic interaction between nisin (a natural AMP) and penicillin or chloramphenicol against the *E. faecalis* biofilm. The synergistic efficacy of nisin with nafcillin on biofilm in *Streptococcus mutans* was evaluated by Tong et al. [76]. Their results showed that nisin in combination with antibiotics considerably decreased the biofilm development. Therefore, AMPs could be regarded as potential antibiofilm agents through different mechanisms

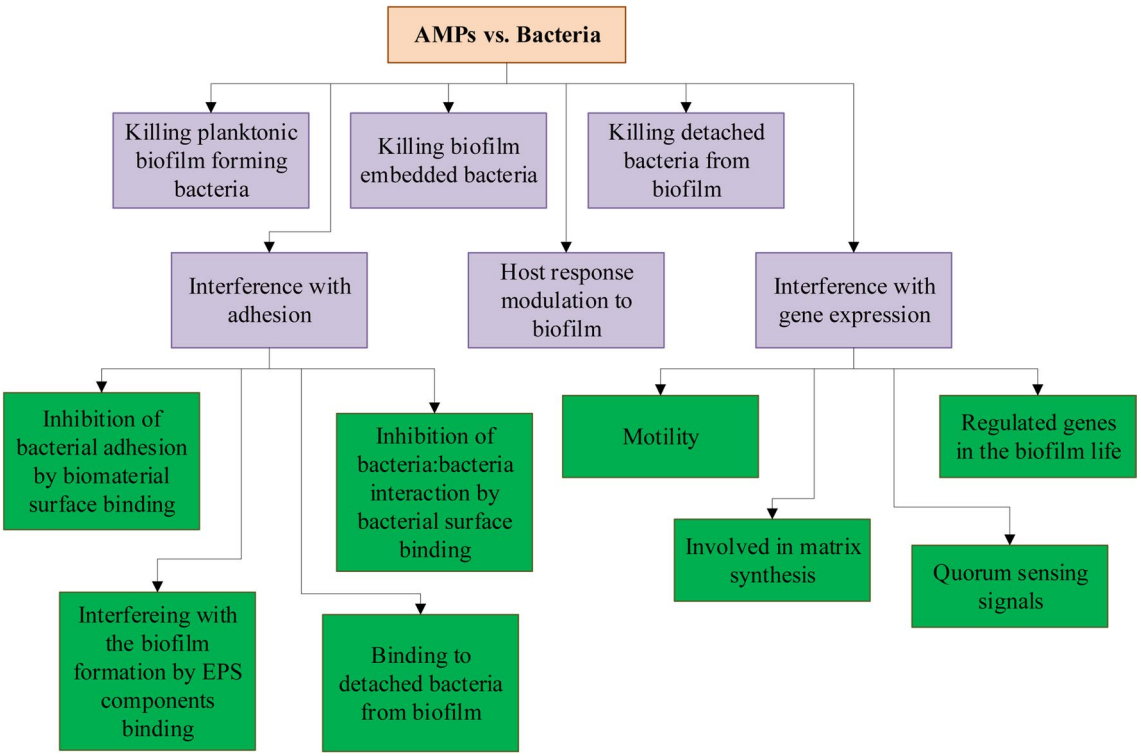

Fig. 1 Multiple mechanisms of antimicrobial peptides on the bacterial biofilm formation

including functional inhibition of proteins, cell-membrane-disrupting action, detoxification of lipoteichoic acid and lipopolysaccharide, and binding with DNA.

Bacteriophages

The viruses that can infect and kill bacteria are known as bacteriophages (phages); they are not able to infect human or animal cells [77]. Therefore, phages are known as the key hunters of bacteria in the environment, and phage therapy has always been of interest for medical goals. Phage therapy emphasizes on lytic phages because they kill their host bacterial cells. In addition, lytic phages lack integrases and/or genes that participate in the horizontal gene transfer. Lytic phages are capable of lysing their host bacterial cells; as well, they can be amplified inside the host bacterial cells. Lysis of the host bacterial cells destroys the bacteria, as well as releasing the progeny phages for the re-infection of more bacteria. Phages are species-specific; thus, they can be used to target special pathogenic bacteria, without any effect on the commensal bacteria [17].

Phage therapy has several advantages in comparison to common antibiotics; these include specificity of function, a narrow range of action, greater safety, greater tolerability, an effect limited to the site of infection and cost effectiveness [78]. The use of phages to inhibit bacterial infections is being followed as an alternative to antibiotics [79]. According to some recent studies, the role of phages in the eradication and/or prevention of bacteria biofilms has been highlighted. Phages are capable of penetrating into the structure of bacterial biofilms and removing them [80, 81]. There are several water channels in the biofilm structure, allowing the phages to easily penetrate into the biofilms' inner structure [82]. Furthermore, most of the phages generate depolymerases, which are capable of hydrolysing the EPS of the bacterial biofilms [83]. For instance, phage phi 15 produces the polysaccharide depolymerase enzyme, which hydrolyses the EPS of *Pseudomonas putida* and inhibits its biofilm formation [84]. As well, it has been observed that T4-like and Φ29-like phages from the family of *Myoviridae* and *Podoviridae*, respectively, suppress the biofilm of *Staphylococcus aureus* [85].

Phage-derived enzymes like lysin enzymes are known as bactericidal components that hydrolase peptidoglycan, the key composition of the cell-wall of both Gram-negative and Gram-positive bacteria. Destruction of peptidoglycan by lysine could induce lysis and degrade the bacterial cell-wall and biofilm structure [86, 87]. Current studies have shown that phage-derived lysin enzymes can potentially act as anti-biofilm development and antibacterial components [81, 88, 89]. It has also been reported that osmotic lysis, independent of bacterial metabolism, can occur upon applying Art-175 lysin against *P. aeruginosa* biofilms. This phenomenon is often important for the elimination of the bacterial biofilms; owing to lysin, even at low metabolic levels, persistent bacteria inside biofilms can be killed [90].

Some phages cannot produce specific enzymes to permeate and diffuse into the EPS matrix for the biofilms inhibition [91]. Nevertheless, phages are often genetically modified to produce enzymes that can damage the EPS matrix and ease the elimination of biofilms. Lu et al. [80] demonstrated that a modified phage T7 of *E. coli* intracellularly produced a hydrolase during infection, which could increase the biofilm removal by being released into the extracellular matrix. An eradication rate more than 99% was observed by testing on *E. coli* biofilms, thus confirming the advantage of using the modified phages.

In phage therapy, individual or cocktail (mixture) phages can be used. The usage of cocktails versus individual phages could considerably increase the host spectrum and decrease the generation of phage-resistant types. The cocktails of phages have been confirmed to be effective in the prevention of the biofilm development and biofilm removal. Antibacterial components such as disinfectants and antibiotics may be applied with phages to increase the efficiency of the biofilm eradication [81].

The combination therapy of phages and antibiotics can increase the treatment effectiveness, as well as inhibiting the resistance to phages without enhancing the toxicity of the antibiotics [79]. A combined usage of phages with antibiotics increases the synergistic activation of these factors in improving the biofilm disruption. Combined therapy of phage T4 and tobramycin on *E. coli* biofilms has also been done; this could drastically decrease tobramycin-resistant *E. coli*. The same experiment was reported against *P. aeruginosa* biofilms by phage PB-1 [92]. Also, the synergistic efficacy of the phage with amoxicillin on the biofilm of *Klebsiella pneumoniae* B5055 was demonstrated by Bedi et al. [79]. The combined therapy of amoxicillin and phages significantly increased the elimination of the biofilm development in the case *K. pneumoniae*, as compared to each of the agents alone [79]. Henriksen et al. [93] also demonstrated that sub-MIC concentrations combined ciprofloxacin and phages have a synergic effect on *P. aeruginosa* biofilm, showing a decrease of about 6 log in the biofilm formation. Depolymerized enzymes in combination with antibiotics could promote the antibacterial efficacy by facilitating the entrance of antibiotics to the biofilm inside. These enzymes could diminish the adherence of EPS matrix and bacteria, thus favoring the function of antibiotics [94].

However, a main limitation of the combined therapy of phages and antibiotics is that antibiotic-resistant biofilms may be increased because phages could favorably infect antibiotic-sensitive bacteria [17]. Furthermore, the interference with bacterial metabolism is needed for the DNA replication and protein synthesis of phages. Since phage therapy

is strictly associated to the growth condition of its bacterial host and common antibiotics potentially act on the log phase of bacterial growth, which have the maximum metabolism, it can be regarded as one of the obstacles in combining antibiotics and phage therapy [81, 95, 96]. Therefore, to prevent incompatibility, the potential adverse effects of the combination therapy of antibiotics should be considered. As some pathogenic bacteria prefer to remove competitors, the use of the combination therapy of antibiotics and phage cocktails is especially interesting for the treatment of mixed infections.

Quorum Sensing Inhibitors

Bacterial cell-to-cell communication is known as Quorum sensing (QS); it directly acts in the biofilm development of various bacterial species. This system can control the expression of various pathogenic and virulence genes in the biofilm phase [97, 98]. In this system, small molecules, known as autoinducers, are responsible for the communication of bacteria with each other. The gene expression levels may display major changes when the bacterial density reaches the concentration threshold of autoinducers [99]. Changes in the gene expression levels could affect (induction or suppression) various virulence factors in bacteria; these also include the biofilm production. The changes in the environment surrounding the microorganism can modify the planktonic state to become a biofilm. The gene expression in the planktonic state undergoes many changes during the transition to the biofilm state. Cell surface molecules, specific metabolic pathways, and the production of various factors can all contribute to the bacterial survival under biofilm conditions [44]. Quorum quenchings (QQ) or quorum sensing inhibitors (QSIs) are described as molecules produced by eukaryotes and/or prokaryotes with the ability to prevent the QS systems, which can lead to decreasing the expression of efflux pump genes and the disruption of bacterial biofilms [99]. Several methods have been applied to disturb QS, such as blocking the production of acyl-homoserine lactones (AHLs), diminishing the activity of the AHL synthase, disturbing and inactivating AHLs, and utilizing numerous competitors compounds as the signaling molecules antagonists [100–102]. Given that QS controls different phases of the biofilm development, including initial colonization/adhesion, bacterial aggregation, biofilm maturation and cell dispersion, inhibiting QS will prevent the biofilm formation [103]. QSIs are, therefore, applied as a therapeutic agent in treating biofilm infection to inhibit the biofilm formation [104]. Antibiotics, synthetic compounds and natural products may influence the QSIs function [99]. Some synthetic and natural compounds that act as QSIs and reduce the biofilm formation in bacteria are shown in Table 2. Drugs like aspirin, piroxicam and meloxicam, which are in the category of nonsteroidal anti-inflammatory drugs, may be applied as potential inhibitors to control the QS signaling molecules of *P. aeruginosa*, as well as biofilm development [105–107]. Antibiotics such as azithromycin, erythromycin, ciprofloxacin, ceftazidime, gentamicin, tobramycin, piperacillin, spectinomycin and streptomycin display good levels of the QSI activity [108, 109]. Bacteria could be resistant to a single synthetic and natural compound, leading to decreasing the effective activity of them. Therefore, a combined therapy of antibiotics and QSIs is recommended. Consequently, this combined therapy can improve the efficiency of treatment without increasing the antibiotics toxicity and inhibiting the resistance to a single QSI [110, 111]. The combination of Aminoglycoside antibiotics with resveratrol significantly diminishes the biofilm production, as compared with each of the compounds alone [112]. The synergistic effectiveness of curcumin with ceftazidime, ciprofloxacin, gentamicin and azithromycin on the *P. aeruginosa* QS signaling molecule showed that the sub-MIC of each of the compounds, both alone and in combination, could significantly decline the biofilm development [28, 113]. Zinc oxide (ZnO) nanoparticles, Chitosan and Chitosan-ZnO nanocomposite, in combination with gentamicin, significantly decreased the biofilm formation of both *S. aureus* and *P. aeruginosa*, when they were treated with MIC and 1/4 MIC of the compounds [114]. Therefore, targeting QS by various anti-QS agents could be a potential application in the treatment of antibiofilm infections.

Aptamers

Aptamers are peptides or single-stranded oligonucleotide molecules produced in an in vitro procedure called Systematic Evolution of Ligands by Exponential Enrichment (SELEX) [141]. Due to their 3-dimensional constructions, they can be linked with great affinity and specificity to select target molecules including small molecules, proteins, drugs, metal ions, and even whole cells [142, 143]. Such properties of aptamers lead to a broad range of activities as antibiofilm and antibacterial agents [144]. Bacterial cell-wall depolarization may be due to the antibacterial effects of aptamer [145]. Although the available research is limited, several studies have suggested that aptamers could be used as an alternative strategy to inhibit the development of biofilms [144, 146, 147]. Figure 2 shows multiple mechanisms of aptamers for the suppression of the bacterial biofilm formation. The aim of these researches has been selection of aptamers against targets involved in the biofilm development. Thevendran et al. [148] developed a quickly growing field of research by scientists from various scientific branches. The flexibility of aptamers as agents of both diagnostics and therapy has introduced them as good candidates for a broad range of uses. Bacterial flagella are responsible for the initial attachment and motility, which are necessary

Table 2 Major recent developments in the inhibition of biofilm formation

Compounds	Biofilm inhibitor molecules	Bacteria affected	Ref
Natural chemicals	Halogenated Furanone	*P. aeruginosa* and *Vibrio harveyi*	[115, 116]
	Penicillium: penicillic acid and patulin	*P. aeruginosa*	[117]
	Ananas comosus	*P. aeruginosa* PAO1	[118]
	Grapefruit juice (furocoumarins)	*E. coli*	[119]
	Manilkara zapota	*P. aeruginosa*	[118]
	Ocimum sanctum	*P. aeruginosa*	[118]
	Medicinal plant extracts such as C. viminalis (leaves), C. erectus (leaves), B. buceras (leaves)	*P. aeruginosa*	[120]
	Combretum albiflorum (bark)	*P. aeruginosa*	[121]
	Eugenol from clove extract	*P. aeruginosa*	[122]
	Curcumin from Curcuma longa (turmeric)	*P. aeruginosa*, *E. coli*, *Serratia marcescens* and *Proteus mirabilis*	[123]
	Carvacrol	*S. aureus* and *S. enterica* subsp. *typhimurium*	[124]
	Diterpene phytol	*P. aeruginosa*	[125]
	6-Gingerol	*P. aeruginosa*	[27]
	Menthol from peppermint (Mentha piperita) oil	*P. aeruginosa* and *Aeromonas hydrophila*	[126]
	Zingerone	*P. aeruginosa*	[127]
	Baicalin	*P. aeruginosa*	[128]
	Berberine	*P. aeruginosa*	[129]
	Flavonoid fraction of Psidium guajava leaves	*P. aeruginosa*	[130]
	Ajoene from garlic	*P. aeruginosa*	[131]
	Diterpene phytol	*P. aeruginosa*	[132]
Synthetic chemicals	2-heptyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxamide	*P. aeruginosa*	[133]
	N-(indole-3-butanoyl)-L-HSL	*P. aeruginosa* PAO1	[134]
	Furanone C-30	*P. aeruginosa*	[135]
	Brominated furanone	*S. anginosus, Staphylococcus intermedius* and *S. mutans*	[136]
	Meta-bromo-thiolactone	*P. aeruginosa*	[137]
	MuPEP1 Inhibitors	*S. mutans*	[138]
	Pyridoxal lactohydrazone	*P. aeruginosa*	[139]
	Lacto hydrazone	*P. aeruginosa*	[140]

for the biofilm development. Therefore, inhibiting flagella represents an efficient potential approach to prevent the biofilm development. Ning et al. [142] demonstrated that a specific aptamer targeting the flagella of *Salmonella choleraesuis* could develop the antibiofilms effect on the inhibition of the bacterial biofilms. Yu et al. [149] also screened the aptamers targeted QS signal molecule that could interfere with the virulence gene expression of *P. aeruginosa*. Furthermore, Mao et al. [146] reported that aptamer-graphene oxide conjugates displayed excellent antibiofilm properties against *Salmonella typhimurium* and could serve as a long-term approach to control the bacterial biofilm formation. In addition, Shatila et al. [144] demonstrated that the DNA aptamer (Apt17) targeted invasion protein A of *Salmonella enteritidis* (SidA) could reduce the biofilm formation, either alone or in combination with ampicillin. Further, Wang et al. [150] revealed that the PA-ap1 aptamer in combination with SWNTs (Single-walled carbon nanotubes) could reduce the biofilm development of *P. aeruginosa* about 36%, in comparison to the SWNTs alone. In addition, the combination of ciprofloxacin with PA-ap1 aptamer–SWNTs had a greater antibiofilm efficacy than any compound alone. As bacteria accumulation represents a significant phase at the initial step of the biofilm development, aptamer-SWNTs could spontaneously catch and link to bacterial cells, quickly accumulating a greater effective concentration of aptamer-SWNTs around bacterial cells that could interfere with the bacteria accumulation. In addition, it was revealed that

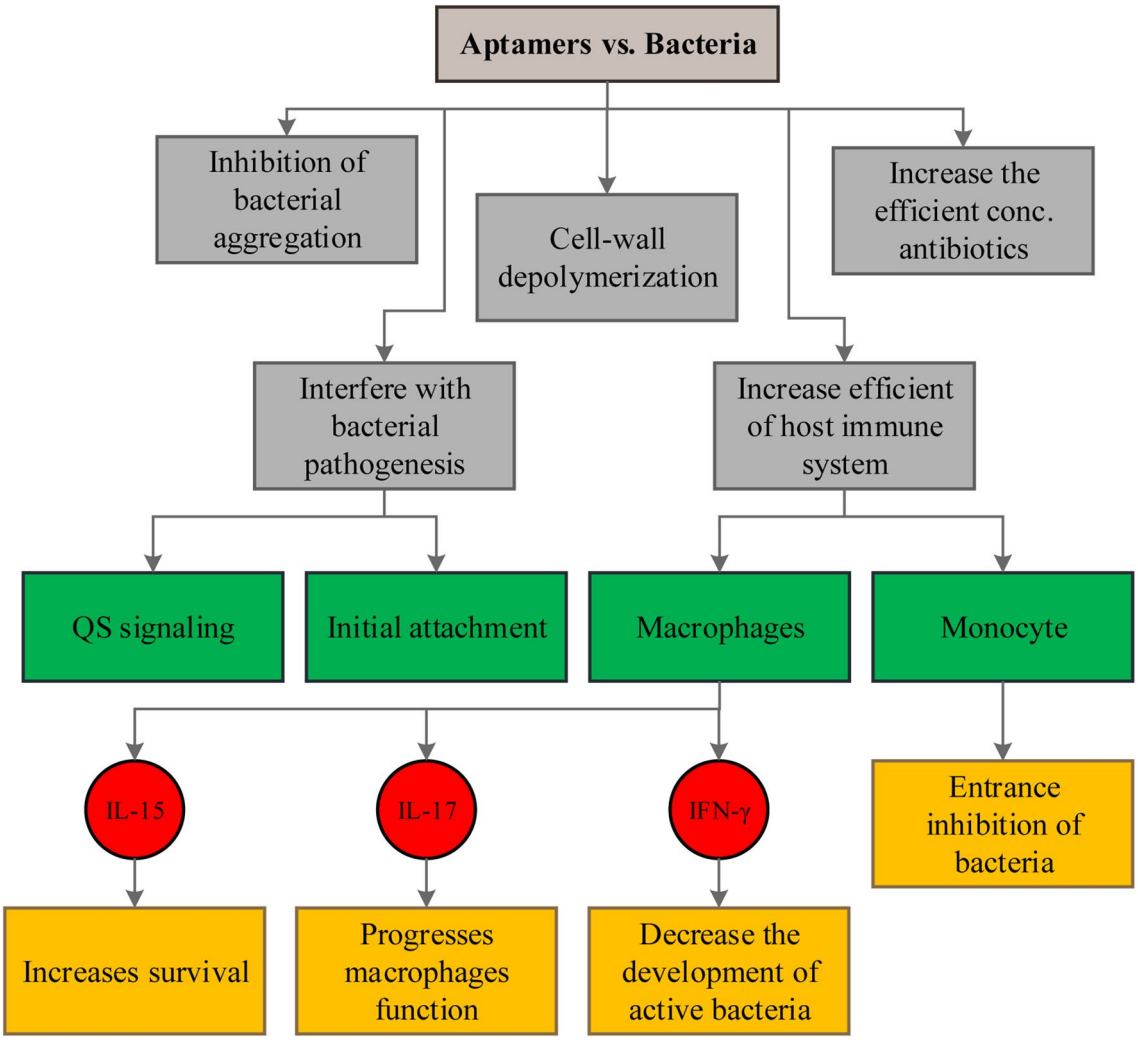

Fig. 2 Multiple mechanisms of aptamers for the suppression of the bacterial biofilm formation

aptamer–SWNTs significantly decreased the biofilm formation, in comparison to the SWNTs alone, thus implying the possibility of using the targeted effective aptamers in the removal of the biofilm. Aptamers, as special targeting agents, can be used for the treatment of bacterial infections, which could increase the efficient concentration of antibiotics and decrease the off-target effects. It was reported that the combination of aptamer with antibiotics could lead to attacking more bacterial cells, in comparison to the use of antibiotics alone [142]. Wang et al. [150] also demonstrated that C4-HSL aptamers could efficiently decrease the biofilm development of *P. aeruginosa* with inhibiting QS, in comparison to that of the untreated groups. In enteropathogenic *E. coli* (EPEC), the biofilm formation is upregulated using some genes such as cell interaction (*lsrA*), motility (*motB*) and curli gene (*csgA*). Aptamer SELEX 10 Colony 5 could reduce the mRNA level of *csgA*, *lsrA* and *motB* gene, consequently decreasing the biofilm formation of the treated group [151]. Sengupta et al. [152] also reported that aptamer-DNA templated Ag-NC (silver-nanocluster) could act as a sensor, thus creating a novel possibility to detect planktonic and biofilm forms. In addition, Ag-NC aptamer could be potentially effective in inhibiting the biofilm development in *P. aeruginosa*. Therefore, aptamer and aptamer coupled with excellent agents could provide high target specificity to inhibit the bacterial growth and biofilm development; thus, they could be regarded as ideal strategies for the development of antibacterial and antibiofilm agents.

Nanoparticles

Recently, nanoparticles have received much attention due to their antibacterial and antibiofilm characteristics. Therefore, NPs can be presented as an alternative therapeutic strategy to inhibit the development of the bacterial biofilm [20]. Figure 3 shows different physicochemical interactions between

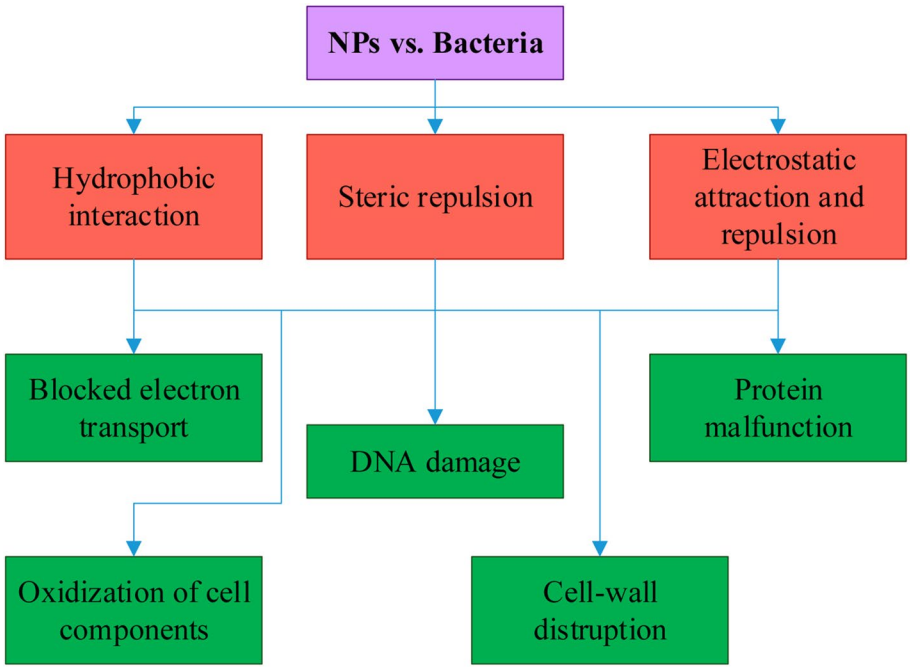

Fig. 3 Different physicochemical interactions between bacterial biofilm and nanoparticles

bacterial biofilms and nanoparticles. One of the advantages of NPs is the high surface-area to volume ratio, which can provide a platform for the development of materials with a wide range of chemical, mechanical, magnetic and electrical characteristics [153]. NPs can easily interact with the bacterial cell because of their shape, small size, surface charge, hydrophobicity and high surface-to-volume ratio [154, 155].

NPs with a positive charge prefer to interact with the negatively charged components of the cell walls and plasma membranes of bacteria (polysaccharide, extracellular DNA (eDNA) and proteins). Furthermore, NPs with a negative charge prefer to interact with the positively charged components of the cell-wall, enhancing the bacterial membrane permeability and flowing out of the cytoplasm contents, which can cause the bacterial cell death. In addition, there are several water channels in the bacterial biofilm structure, which can enable bacteria to transport nutrients through these pores. Therefore, NPs can have an antibacterial effect on the biofilm through spreading from these channels [156].

The inhibition of the efflux pumps of bacteria by NPs is another potential mechanism for the antibiofilm activity [157]. Gupta et al. [157] indicated that efflux pumps could play effective roles in the bacterial biofilm formation; as well, regulation of their expression could directly control the antibiotic resistance and development of biofilms. In addition, Padwal et al. [158] showed the combination of antibiotics with NPs could inhibit the activity of efflux pumps, thus suggesting that these compounds could be applied as efflux pump inhibitors. They proposed that NPs could distract efflux kinetics through linking to the active site of efflux pumps and decreasing their activities, thus leading to inhibition of the extrusion of antibiotics outside cells. Similar results have revealed the ZnO nanoparticles could have an inhibitory effect on such efflux pumps as MexAM-OPrM of *P. aeruginosa* and NorA of *S. aureus* [159, 160]. Therefore, the downregulation or inhibition of efflux pumps with NPs could be regarded as a potential therapeutic approach to inhibit and/or decrease the bacterial biofilm development.

EPS produced by bacteria play critical roles in the primary attachment of the bacterial cells to the host cell surface and the development of a complex biofilm structure, which could protect biofilms against the classical antibiotics and host-immune system [161, 162]. Production of EPS is one of the critical strategies to protect the bacterial biofilm against the antibiotic activity. Therefore, one of the mains restrictive strategies for the biofilm development is also EPS reduction [25]. Inhibition of the EPS development can be one of the antibiofilm mechanisms of NPs; it has been proved that NPs could reduce the biofilm development by the disruption of the EPS production [155, 163]. In *S. aureus* biofilm, the chemical compounds of EPS contain polysaccharide, proteins and eDNA. One of the major compounds of the biofilm matrix is Polysaccharide Intercellular Adhesin (PIA), which is a compound with a positive charge. Cytoplasmic proteins and eDNA are positively and negatively charged, respectively [164]. NPs like chitosan NPs, due to having a positive charge, can bind to eDNA with a negative charge, disrupting the biofilm formation with their penetration into the biofilm structure [163]. In contrast, NPs with a negative charge like ZnO NPs can interact with positively charged PIA and modify the permeability of the biofilm structure; then they can easily penetrate into the biofilm

matrix, inhibiting the biofilm development by inducing ROS [164–167]. The biofilm matrix of *P. aeruginosa* consists of exopolysaccharides and eDNA. In *P. aeruginosa*, there are three types of exopolysaccharides including alginate, Pel (a glucose-rich polysaccharide polymer encoded by *pel* operon including *pelA-F* genes), and Psl (composed of a repeating pentamer containing D-glucose, L-rhamnose, and D-mannose, which is encoded by the *psl* operon including *pslA-L*); they perform different roles in the biofilm development [168, 169]. Alginate is a major compound of the biofilm matrix accounting for the cell surfaces binding and biofilm sustainability. Alginate and Pel are negatively and positively charged polysaccharides, respectively [170]. The positive and negative NPs could be charged by binding to alginate and Pel, respectively, which can prevent the attachment of the bacteria to cell surfaces; this is the early stage of biofilm development. These nanoparticles can disrupt the development of biofilm by preventing the bacterial attachment to the cellular surfaces [163, 170, 171]. Messiaen et al. [172] also demonstrated that the negatively charged components of tobramycin-loaded liposome were placed near the bacterial cell clusters of *Burkholderia cepacia* complex biofilms, while fiber-like structures such as the extracellular DNA became immobilized by the interactions with the positively charged particles of nanospheres in the biofilm matrix.

In addition, owing to the antibacterial activity of NPs, they can reduce the bacterial attachment to surfaces and to themselves, as well as the replication and development of biofilm [154]. Table 3 shows nanoparticles inhibiting the bacterial biofilm formation and development. According to these studies, NPs could be regarded as potential antibiofilm agents as a function of their anti-adhesive activity, bactericidal activity and delivery capability. However, NPs may be toxic, but they could be modified to reduce their toxicity, making them useful for biomedical applications. Therefore, further studies should be directed toward changing the oxidative state and charge density, by applying surface coatings and altering the ability to aggregate. Clinical and nonclinical studies are also needed to determine the safety and tolerance of NPs formulating the potential commercial applications.

Peptide Nucleic Acids

Peptide nucleic acids (PNAs) are structurally similar to DNA or RNA; they are the synthetic analogs of nucleic acid. Their structure is the repetition of *N*-(2-aminoethyl)-glycine units in nucleotide bases instead of sugar phosphate back bones connected by unnatural pseudodipeptide bonds [182]. Properties of PNAs are a combination of both nucleic acids and peptides, which are chemically similar to protein and structurally similar to nucleic acid, which are relatively stable against enzymes degrading proteins and nucleic acids [182, 183]. Antimicrobial and antibiofilm effects of PNAs are related to their small size and the targeted nucleotide

Table 3 Nanoparticles with the antibiofilm properties

Nanoparticles	Mode of action	Bacteria affected	Refs.
FeOOH (iron-based NP)	Initial stage	*P. aeruginosa*	[173]
ZnO	Initial stage	*P. aeruginosa*, *Streptococcus mitis* and *Streptococcus pneumoniae*	[25, 74]
Silver	Initial stage	*P. aeruginosa*, *S. aureus*	[174, 175]
Gold	Initial stage	*P. aeruginosa*	[26]
Gentamicin-loaded gold	Maturation stage	*S. aureus, E. coli, P. aeruginosa* and *Listeria monocytogenes*	[176]
Gentamicin-loaded nanotubes titanium	Attachment disruption of the sessile cells to surfaces (Initial stage)	*S. aureus and S. epidermidis*	[177]
Fucoidan-stabilized gold	Attachment disruption of the sessile cells to surfaces (Initial stage)	*P. aeruginosa*	[178]
Crataeva nurvala	–	*P. aeruginosa*	[179]
Chitosan oligosaccharide-capped gold	Disrupted the attachment of sessile cells to surfaces (Initial stage)	*P. aeruginosa*	[162]
Sulfonated Chitosan	Secretion of exopolysaccharide and decreasing of the metabolic activity	*S. aureus* and *E. coli*	[180]
Titanium dioxide	Initial stage (inhibition EPS production)	*S. mitis*	[181]
Chlorhexidine-loaded mesoporous silica nanoparticles	–	*Streptococcus sobrinus* and *S. mutans*	[182]
Curcumin	Decrease of quorum sensing virulence factors	*S. aureus* and *P. aeruginosa*	[175]
Chitosan	–	*S. aureus* and *P. aeruginosa*	[114]
Chitosan-ZnO nanocomposite	–	*S. aureus* and *P. aeruginosa*	[114]

sequences of genes involved in the biofilm formation, in the absence or presence of a linker between the peptide conjugated to PNAs and its position in the structure of peptide-PNA [21]. The major property of PNA is its great affinity and specificity binding. Therefore, it has a good potential to synthesize PNA-based specific antibacterials for particular genes involved in the biofilm formation of the selected bacteria. PNAs demonstrate an enormous potential to inhibit the increase of the resistant bacteria [184]. However, one of the most widespread negative properties of PNAs is the hydrophobicity of its structure, which could be due to its insolubility in the aqueous solution. The impermeability of bacterial membranes to PNA is another important limitation preventing the development of the antibacterial PNA due to the difficulty in finding the effective carriage and carriers of PNA to the bacterial cells [183]. The lipid bilayer, lipopolysaccharide and peptidoglycan of bacteria serve as main barriers to the entrance of PNAs [185].

Different mechanisms have been suggested to increase the transport of PNAs to bacterial cells: chemical changes of the PNAs structure can increase their hydrophilicity. In addition, covalent conjugation of various cell penetrating peptides (CPPs) with PNA [186], such as photochemical internalization (PCI), chloroquine, cationic lipids and HIV-TAT [187], and the use of the tetrahedral DNA nanostructure (TDN) can act a complementary base pair between PNAs and DNA [188].

PNAs can prevent or overcome the bacterial biofilm development. PNAs treated at the *efaA* gene have a negative influence on the biofilm development in *Enterococcus faecalis*; this gene serves an important role in the bacterial attachment to surfaces [21]. In addition, Otsuka et al. [189] showed that the *acpP* gene targeted by PNA-peptides caused antibacterial and antibiofilm effects on both planktonic and biofilm states of *Haemophilus influenzae*. Binding PNAs to their targeted genes could prevent and interfere with the gene expression. The *motA* gene could contribute to the downstream adhesion events of attachment in the development of biofilm in *P. aeruginosa,* playing a critical role in the initial state of the biofilm formation, which encodes the element of the flagellar motor complex [190]. Xia et al. [191] also revealed that PNA-peptides targeting the *motA* gene could decrease the *motA* expression and inhibit the biofilm development. PNAs have also been used in combination with classical antibiotics to increase their antibacterial and antibiofilm activity. Castillo et al. [192] showed the synergistic activity of mRNA targeted PNAs in *E. coli* O157:H7. The synergistic effect of trimethoprim and polymyxin B antibiotics in combination with anti-*acpP* PNA (encoding acyl carrier protein (AcpP) was determined using the checkerboard titration method. Their results showed that *acpP* mRNA targeted PNAs could improve the antibacterial activity of antibiotics by suppressing the *acpP* expression, which is functionally related with antibiotic resistance. In general, designing PNAs targeting specific genes involved in the development and formation of biofilm, as well as targeting critical genes involved in the living and surviving bacteria, could enhance the susceptibility of bacteria and their biofilm.

Future Directions

In the post-antibiotic era, development of novel antibiofilm strategies and alternative therapeutic agents has been of interest to combat the threat of bacterial biofilm infections. In this era, we need more complex biological approaches to investigate the interactions between single- and multi-species bacterial biofilms and their environments in chronic infections. Some of the introduced strategies could only be used to treat single-species biofilms and may not be applicable to mixed-species biofilms. In addition, it is possible for bacteria to protect themselves during the use of these agents; this is a great challenge that should be addressed in regard to combating bacteria and their biofilm formation. Therefore, combination of these agents and flexibility in the use of them could be great promises for future biofilm infection treatments. The applicability of these strategies could be summarized in three steps including entry, delivery and release into the biofilm matrix. Recruitment of alternative and promising therapeutic approaches or strategies could increase the local concentration of antibiotics or antibiofilm agents improve their local accumulation and decrease their systematic toxicity. Nanomedicine, as the most versatile and flexible strategy, can be combined with other novel therapeutic strategies as the state-of-art concepts. There are several tenuous antibiofilm agents and a few of them could be used in clinical relevant models. Therefore, great efforts should be devoted to the evaluation of the biosafety and efficacy of strategies; effective routs for the entry, delivery and release of the agents to the biofilm matrix should be developed; further, the novel agents-biofilm interactions should be studies, and the complexity of prescription and cost of fabrication should be reduced.

Conclusions

Antibiofilm agents such as antiadhesion agents, antimicrobial peptides, aptamers, nanoparticles and peptide nucleic acids represent a promising therapeutic target to treat biofilm infections and develop a strong capability to interfere with different phases of the biofilm development, including adherence, polysaccharide intercellular adhesion (PIA), quorum sensing molecules and cell-to-cell connection, bacterial aggregation, planktonic bacteria killing and host-immune

response modulation. Therefore, rational and experimental design approaches allow the synthesis of new antibiofilm compounds with improved and varied biological functions. In addition, these novel strategies can be promising methods against bacterial biofilm due to their improved permeation and targeted delivery of antibacterials inside the biofilm. As well, these components in combination with antibiotics can lead to developing a powerful approach for the treatment of bacterial biofilm-related infections.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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