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Invited Review

Recent advances and future challenges in the use of nanoparticles for the dispersal of infectious biofilms

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ABSTRACT

Increasing occurrence of intrinsically antimicrobial-resistant, human pathogens and the protective biofilm-mode in which they grow, dictates a need for the alternative control of infectious biofilms. Biofilm bacteria utilize dispersal mechanisms to detach parts of a biofilm as part of the biofilm life-cycle during times of nutrient scarcity or overpopulation. We here identify recent advances and future challenges in the development of dispersants as a new infection-control strategy. Deoxyribonuclease (DNase) and other extracellular enzymes can disrupt the extracellular matrix of a biofilm to cause dispersal. Also, a variety of small molecules, reactive oxygen species, nitric oxide releasing compounds, peptides and molecules regulating signaling pathways in biofilms have been described as dispersants. On their own, dispersants do not inhibit bacterial growth or kill bacterial pathogens. Both natural, as well as artificial dispersants, are unstable and hydrophobic which necessitate their encapsulation in smart nanocarriers, like pH-responsive micelles, liposomes or hydrogels. Depending on their composition, nanoparticles can also possess intrinsic dispersant properties. Bacteria dispersed from an infectious biofilm end up in the blood circulation where they are cleared by host immune cells. However, this sudden increase in bacterial concentration can also cause sepsis. Simultaneous antibiotic loading of nanoparticles with dispersant properties or combined administration of dispersants and antibiotics can counter this threat. Importantly, biofilm remaining after dispersant administration appears more susceptible to existing antibiotics. Being part of the natural biofilm life-cycle, no signs of "dispersant-resistance" have been observed. Dispersants are therewith promising for the control of infectious biofilms.

Contents

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1. Introduction

Antimicrobial-resistant bacterial infections pose a growing threat to human health and cause a tremendous economic burden particularly to the health care system, arousing worldwide public concerns [1,2]. Antimicrobial-resistance should be considered as a result of the intrinsic-resistance of bacteria and resistance due to the biofilm-mode of growth in which most bacterial infections present themselves.

Biofilms are composed of bacteria embedded in a self-produced matrix of extracellular polymeric substances (EPS), mainly including extracellular deoxyribonucleic acid (eDNA), proteins and polysaccharides [3]. Therewith, the biofilm matrix acts as a barrier that prevents antimicrobial penetration [4] and as a glue that holds its bacterial inhabitants together. Due to the barrier posed by EPS, killing of bacterial pathogens in a biofilm-mode of growth requires up to 1000 times higher antibiotic concentrations than needed to kill planktonic bacteria, suspended in a fluid phase [5]. In a biofilm community, bacteria can communicate with each other through secretion of signaling molecules that induce varying events ranging from dormancy [6], apoptosis of a part of the community, additional production of EPS or detachment of parts of the biofilm into its surrounding fluid phase ("dispersal").

The intrinsic antimicrobial-resistance of bacteria is equally exhibited by bacteria in a biofilm-mode of growth as well as by planktonic bacteria. Long-term exposure of bacteria, especially to low concentrations of antimicrobials [7] can lead to intrinsic mechanisms of resistance [8], such as increased expression of antimicrobial efflux pumps [9], immunity and bypass [10,11], target modification [12] and enzymatic inactivation of the antimicrobial [13]. Intrinsic antimicrobial-resistance has been attributed mostly to indiscriminate and overly use of antimicrobials not only for the treatment of human infection but also agricultural overuse has introduced high concentrations of antimicrobials in the environment, including the human food chain. Due to this, the time from market introduction and clinical use of a new antimicrobial till the first signs of intrinsic antimicrobial-resistance is becoming shorter and shorter [14]. Whereas this may underline the need to develop new antimicrobials, the speed at which resistance develops makes this commercially little attractive. Rather, new infection-control strategies should focus on designing suitable combinations of existing antibiotics with new nano-antimicrobials in a way that induction of new antimicrobial-resistance mechanisms is avoided [8].

The use of dispersants might meet the above criteria. To the best of our knowledge, the development of bacterial resistance against dispersants has never even been mentioned in the literature, probably because dispersal is an essential part of the biofilm life-cycle and needed for bacterial survival and colonization of new niches once the biofilm has become overpopulated or during nutrient scarcity [15,16]. Accordingly, biofilm dispersants are usually not growth-inhibiting or bactericidal. Once bacteria have escaped from their biofilm community into a planktonic state underway to a new niche, they become more susceptible to environmental threats (see Fig. 1), including the host immune system and antimicrobials [17]. Biofilm dispersal implies that the EPS matrix loses its function as a glue. This can be caused by the degradation of EPS components, modulation of signaling molecules by binding of quorum-sensing inhibitors [18] or environmental changes, as for example nutrient limitation [19,20] or oxygen depletion [21]. Upon dispersal, a biofilm remains that is not only thinner than before, but that may also contain parts with a partially disrupted EPS matrix and lower bacterial density (see also Fig. 1), which leads to better options for antimicrobial treatment of remaining biofilm.

The application of dispersants is hampered by their lack of stability and hydrophobic nature. Conventional intravenous administration makes most natural dispersants prone to enzymatic degradation and clearance by the host immune system. In addition, small molecule dispersants are poorly soluble due to their high hydrophobicity.

Nanoparticle carriers can conceal the hydrophobic nature of dispersants, improving their solubility and providing protection against clearance by host immune cells. Particularly nanoparticle carriers equipped with an outer surface composed of poly(ethylene glycol) (PEG) and poly(β-amino ester) (PAE) are ideally suited for stealth transport through the blood circulation and become positively-charged in the more acid environment of a biofilm to target themselves to negatively charged bacterial pathogens in the biofilm [22]. Recently, nanoparticles with pH-responsive, zwitterionic [23] or dextran [24] shells have been described to possess dispersant properties by themselves.

Molecular dispersants of biofilms have been amply reviewed [25–27], but the use of nanoparticles as a dispersant has not. Therefore, in this review, we focus on the current status of the direct use of unloaded nanoparticles as dispersants or the use of nanoparticles as a dispersant carrier. For completeness, we will first describe different types of molecular dispersants, after which nanoparticles with dispersant properties will be reviewed, including their dispersal mechanisms. Finally, the potential of dispersants for clinical use in infection-control is highlighted and future challenges are identified.

2. Molecular biofilm dispersants

Molecular biofilm dispersants can disrupt a biofilm through different mechanisms and we will here discuss different types of molecules that have the ability to disrupt biofilms, with their possible advantages and disadvantages.

2.1. Enzymatic dispersants

There is a large number of enzymatic dispersants with the ability to disrupt a biofilm matrix, of which DNA is arguably the most important, because of the well-defined role of eDNA as a biofilm

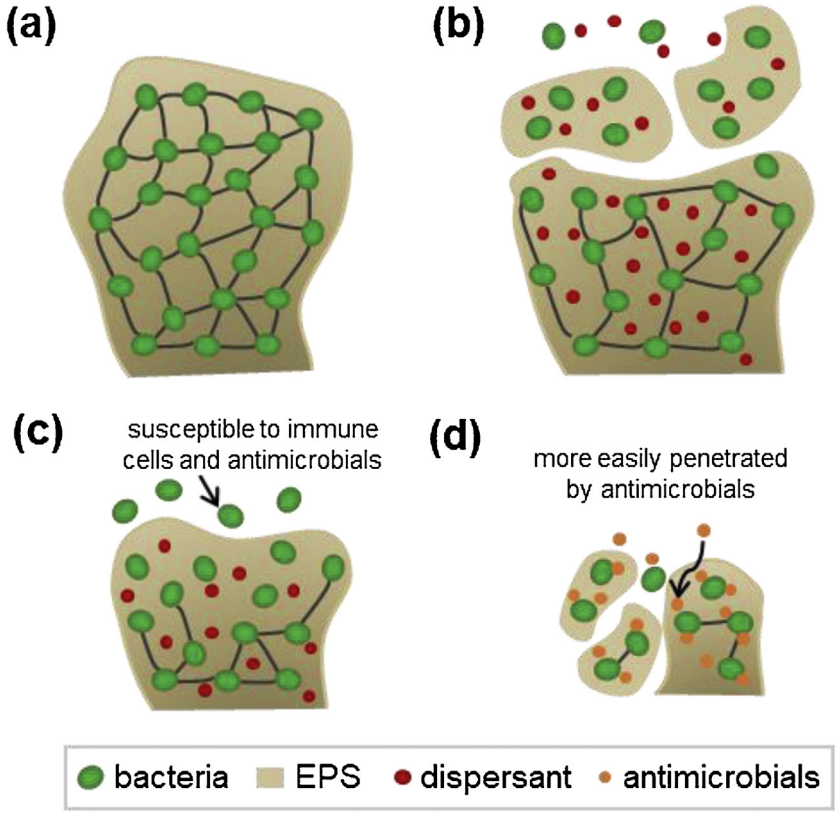

Fig. 1. Sequential steps and consequences of biofilm dispersal. (a) Undisturbed biofilm, in which bacteria are glued together by their self-produced EPS matrix. (b) Dispersant action, continuing from the outside of the biofilm, disrupting the EPS matrix and leading to less dense regions in a biofilm. (c) Ongoing dispersant action into deeper layers and detachment of biofilm inhabitants. (d) Biofilm remaining after dispersal, with lower bacterial density than undisturbed biofilm in which antimicrobials can more readily penetrate.

glue. Therefore, we will discuss enzymatic dispersants with special emphasis to deoxyribonuclease (DNase).

2.1.1. DNase

eDNA as a matrix component plays an important role as a glue holding a biofilm together (Fig. 2(a)). DNase can be used to specifically target eDNA, degrading both single strand and double stranded DNA at phosphodiester bonds adjacent to pyrimidine nucleotides [28] in Gram-positive and Gram-negative biofilms, such as *Staphylococcus aureus* [29,30], *Escherichia coli* [31], *Pseudomonas aeruginosa* [30,32] and *Klebsiella pneumoniae* [33] biofilms. DNase is secreted by biofilm inhabitants to disperse (part of) a biofilm, when conditions require so [15,34]. DNase therewith not only prevents the build-up of biofilms (Fig. 2(b)), but also disperses existing biofilms to leave a biofilm with reduced thickness, as demonstrated *e.g.* for *P. aeruginosa* or *S. aureus* biofilms (Fig. 2(c)) [30]. Remaining biofilms after exposure to dispersants can also be more susceptible to subsequent antimicrobial treatment. Recombinant human DNase I *e.g.*, efficiently detached *S. aureus* biofilms, while pre-exposure of biofilms to DNase increased the susceptibility of *S. aureus* biofilms to different antimicrobials (Fig. 2(d)) [35]. These qualities of DNase, have stimulated the use of DNase I for the dispersal of biofilms.

2.1.2. Other extracellular enzymatic dispersants

Besides DNase, bacteria secrete several other extracellular enzymes that can degrade a biofilm matrix [36]. Dispersin B, a 42-kDa glycoside hydrolase that hydrolyzes polymeric β-1,6-*N*-acetyl-D-glucosamine (PNAG) may be regarded as the most frequently studied hydrolase and inhibited *in vitro* biofilm formation and dispersal by degrading poly-*N*-acetylglucosamine in *E. coli*, *Staphylococcus epidermidis*, *Pseudomonas fluorescens* and *Yersinia pestis* biofilm matrices [37]. Other glycoside hydrolases demonstrated similar features against *S. aureus* and *P. aeruginosa* biofilms [38]. A self-generated bacterial endoglycosidase PslG inhibited biofilm formation, dispersed existing biofilms of a wide range of *Pseudomonas* strains and enhanced susceptibility to antibiotics and phagocytosis *in vitro* [39]. *In vitro* exposure of *Streptococcus intermedius* biofilms to hyaluronidase reduced biofilm mass and promoted its dispersal [40]. Proteases found in *P. aeruginosa* [41] and *Actinomycetes* [42] culture supernatant inhibited *S. aureus* biofilm formation and detached existing biofilms, presumably by inducing protease expression in *S. aureus*. Phages with polysaccharide depolymerase activity can also disperse bacterial biofilms [43]. For example, a depolymerase from a lytic *P. aeruginosa* bacteriophage has been demonstrated to degrade *P. aeruginosa* exopolysaccharides and disperse its biofilm [44].

Bacterially secreted, naturally occurring enzymes can be easily inactivated under the influence of pH, ionic strength or temperature (Fig. 3(a)), which has led to the development of a variety of artificial enzymes [45–49]. Artificial enzymes are usually inexpensive, have a high turnover and stability [45,46]. For example, a DNase-mimetic artificial enzyme exhibited high cleavage of eDNA (Fig. 3(b)) [50] and was more effective in dispersing exiting biofilms than natural DNase (Fig. 3(c) and (d)).

2.2. Reactive oxygen species

Different reactive oxygen species (ROS) [51–53] have the ability to disperse biofilms by disrupting EPS matrix components. Common to all types of ROS is the disruption of the EPS matrix through DNA damage, polysaccharides degradation and protein inactiva-

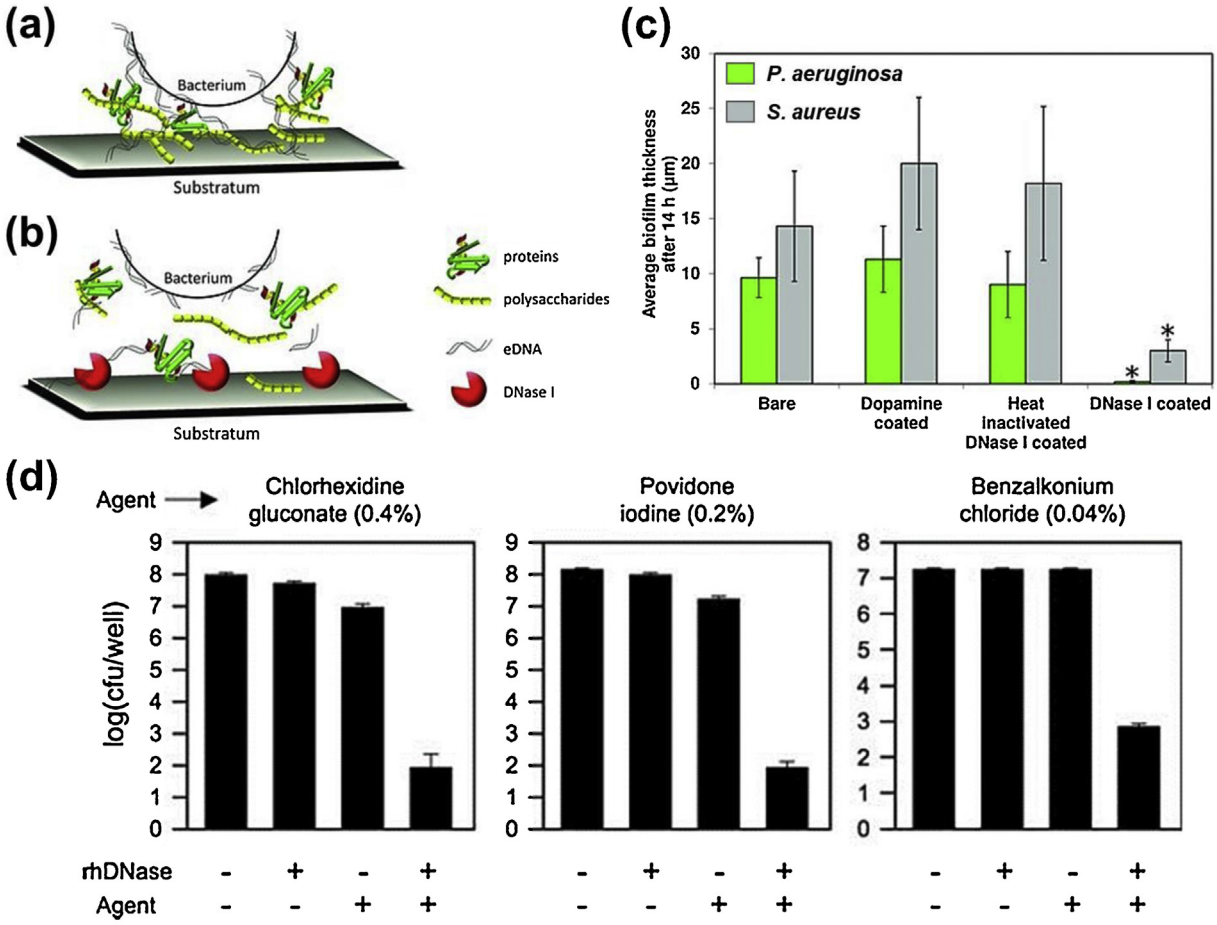

Fig. 2. eDNA and DNase and their roles in biofilm dispersal. (a) eDNA acting as a biofilm glue. (b) Disruption of EPS by DNase I coating attacking the eDNA component of the EPS matrix to prevent bacterial adhesion to a substratum surface. (c) Average thickness of *P. aeruginosa* and *S. aureus* biofilms on DNase I coated surfaces. Panels (a–c) are reprinted with permission [30]. Copyright 2013, Wiley-VCH. (d) Pre-exposure of existing *S. aureus* biofilms to DNase rendered staphylococcal susceptibility to killing by different antimicrobials. Reprinted with permission [35]. Copyright 2011, Springer Nature.

tion. ROS are oxygen-containing reactive species converted from molecular oxygen, mainly including hydrogen peroxide (H_2O_2), superoxide anions ($^{\bullet}O_2^-$), hydroxyl radicals ($^{\bullet}OH$), and singlet oxygen ($^1O_2$), all possessing strong oxidation abilities (Fig. 4(a)). ROS can directly or indirectly participate in the degradation processes of organic compounds through breaking down covalent bonds, including degradation of all EPS components, *i.e.* not limited to degradation of specific EPS components (Fig. 4(b)). Accordingly, ROS have been demonstrated to degrade EPS in Gram-positive *Streptococcus mutans* biofilms (Fig. 4(c)) [54] and to disrupt Gram-negative *P. aeruginosa*, *E. coli* and *Serratia marcescens* biofilms as well as Gram-positive *Listeria monocytogenes* biofilms (Fig. 4(d)) [55].

2.3. Nitric oxide

Nitric oxide (NO) is synthesized as part of the host immune response through arginine-dependent synthesis of NO by activated macrophages [8]. Oxidative and nitrosative stress generated by reactive intermediate species of NO can cause damage to DNA, proteins, and cell membranes [9]. NO is rapidly metabolized by the human body to prevent the build-up of harmful concentrations [10,11]. At micromolar concentrations, it is bactericidal [56], but at lower, picomolar to nanomolar concentrations, NO-induced biofilm dispersal by decreasing c-di-GMP concentration through activation of phosphodiesterase [57]. A variety of NO donors/precursors, like *N*-diazeniumdiolates, *S*-nitrosothiols, metal nitrosyls and organic nitrates, are capable of generating NO. These donors/precursors are used to store and release NO, which can occur upon different environmental stimuli. However, whereas NO-releasing diazeniumdiolates coatings on polypropylene surgical meshes [58] killed *S. aureus*, *E. coli*, *P. aeruginosa* and coagulase-negative staphylococci (CNS) in a biofilm-mode of growth *in vitro*, no beneficial effects on subcutaneous implants in mice could be observed. This is probably due to NO consumption by metabolic processes *in vivo* and the gaseous nature of NO. This calls for encapsulation of NO-donor/precursors in smart, self-targeting nanoparticles carriers [59], as we summarize below.

2.4. Peptides

Naturally occurring antimicrobial peptides exhibit bacterial killing while seldom inducing resistance, but their penetration in biofilms is hampered due to their positive charge causing attraction to negatively charged biofilm components [60]. This makes naturally occurring antimicrobial peptides unsuitable as a dispersant. Moreover, their isolation is expensive. Synthetic antimicrobial peptides are easy to synthesize cost-effectively. Synthetic peptide IDR-1018 killed ESKAPE panel pathogens in a biofilm-mode of growth at 10 $\mu g\ mL^{-1}$, while ten-fold lower concentrations of IDR-1018 led to biofilm dispersal [61]. Stable, monomeric α-sheet structure peptides inhibited phenol soluble modulin formation and therewith reduced the amyloid content of biofilm matrices in *S. aureus* [62] and *S. mutans* biofilms [63]. However, both naturally

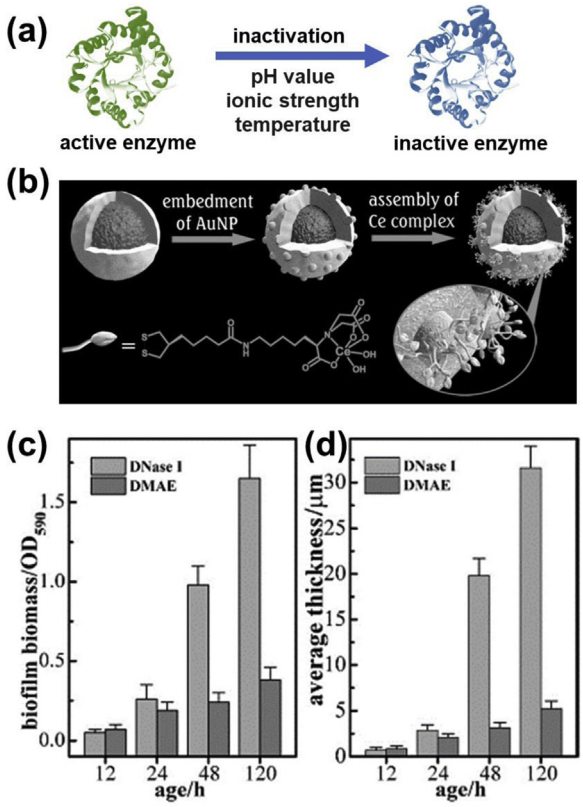

Fig. 3. Enzyme inactivation and artificial enzymes. (a) Factors controlling inactivation of biofilm dispersing enzymes. (b) Preparation scheme of a DNase-mimetic artificial enzyme by confining multiple multinuclear AuNPs covered with Ce complexes on the surface of Fe_3O_4/SiO_2 nanoparticles. (c) Effects of exposing an existing *S. aureus* biofilm grown for different periods to natural DNase I and DNase-mimetic artificial enzyme (DMAE) on biofilm mass. (d) Same as panel (c), now for biofilm thickness. Panels (b-d) are reprinted with permission [50]. Copyright 2016, Wiley-VCH.

occurring as well as synthetic antimicrobial peptides remain prone to proteolysis *in vivo*, which limits the potential role of peptides as a dispersant.

2.5. Molecules regulating signaling pathways

Since dispersal is part of the natural biofilm life-cycle [25], dispersal is regulated by different signaling pathways in a biofilm. Quorum sensing is a cell to cell signaling process which allows bacteria to respond to environmental conditions such as overpopulation [15,16] by producing and detecting extracellular signaling molecules ("autoinducers") [64,65] that stimulate dispersal. This offers the possibility to synthesize autoinducers or inhibitors that influence the natural dispersal process in biofilms. Synthetic 2-aminobenzimidazole based derivatives dispersed *P. aeruginosa* biofilms by reducing its ability to detect autoinducers [66]. Another phenolic compound, hordenine, can inhibit the development of high levels of acyl-homoserine lactones, an autoinducer mediating Gram-negative bacterial quorum sensing at concentrations from 0.5 mg mL^{-1} to 1.0 mg mL^{-1}. Hordenine at sub-minimal inhibitory concentrations disrupted existing *P. aeruginosa* biofilms and enhanced bacterial susceptibility to netilmicin. Additionally, certain quorum sensing-related genes were suppressed in the presence of hordenine [67]. A group of *cis*-2-alkenoic acids as produced by several bacterial strains including *Xanthomonas campestris*, *Stenotrophomonas maltophilia*, and *Burkholderia* species, dispersed biofilms by interfering with quorum sensing signals [68]. Exogenous addition of *cis*-2-decenoic acid produced by *P. aeruginosa*, induced dispersal of *E. coli*, *K. pneumoniae*, *Proteus mirabilis*, *Streptococcus pyogenes*, *B. subtilis* and *S. aureus* biofilms [69]. Gram-positive bacteria use another quorum sensing system and addition of autoinducing peptides, could activate the *agr* system in *S. aureus* biofilms to cause its detachment [70]. Molecules regulating signaling pathway have only been extensively studied in a relatively small number of strains. However, it has become clear that different bacterial strains used widely different quorum sensing systems [64] from which it can be concluded that molecules regulating signaling pathways are unlikely to become broad-spectrum biofilm dispersants.

2.6. Miscellaneous small molecules: amino acids, biosurfactants and polyamines

Several types of small molecules can act as a dispersant. D-amino acids produced by bacteria for instance, prevented bacterial biofilm formation and disrupted existing biofilms, while a mixture of D-leucine, D-methionine, D-tyrosine, and D-tryptophan caused the release of amyloid fibers from *Bacillus subtilis* biofilms [71]. Parthenolide is a plant extract that can also target amyloid fibers formed by TasA proteins in *B. subtilis* biofilms [72]. The application of D-amino acids and parthenolide is limited by their hydrophobicity, suggesting their application in suitable nanocarriers.

Biosurfactants are microbially produced, amphiphilic compounds [73] interfering with biofilm development and causing its dispersal [74,75]. Rhamnolipids, biosurfactants produced by *P. aeruginosa*, dispersed biofilms by decreasing cell-to-cell adhesiveness, cell to matrix and cell to substratum surface interactions [76]. Rhamnolipids not only acted as a dispersant of biofilms formed by their source strain but also dispersed biofilms formed by other bacterial strains [77,78]. Biosurfactants isolated from *lactobacilli* exhibited dispersal abilities against multidrug-resistant strains of *Acinetobacter baumannii*, *E. coli* and *S. aureus* [79].

Norspermidine, a polyamine, and a variety of guanidine and biguanide compounds mimicking norspermidine have emerged as potent biofilm dispersants inhibiting the formation and stimulating dispersal of *B. subtilis* and *S. aureus* biofilms [80,81]. However, the absence of cytotoxicity of these polyamines has to be determined.

3. Nanoparticles for the dispersal of biofilms

Nanoparticles are mostly used as a nanocarrier of molecular dispersants to enhance their solubility and protect against clearance by host immune cells, but can also be used directly as a dispersant with or without an appropriate surface coating. These different modes of use of nanoparticle for disrupting biofilms are summarized in Table 1.

3.1. Nanoparticles with intrinsic dispersant properties

The intrinsic ability of nanoparticles to disperse biofilms is mainly due to ROS generation by metal-based nanoparticles, carbon dots, cationic micelles or peptides (see Table 1). Demonstration of dispersant properties is not trivial and often relies on qualitative assessment of micrographs (Fig. 5(a–c)). Complete dispersal over the entire depth of a biofilm manifests itself as detachment, but often remnants of biofilm remain after dispersal and biofilm detachment is only partial. However, also remaining biofilm can be dispersed which manifests itself by a lower volumetric-biofilm-density, *i.e.* fewer bacteria per unit biofilm volume than prior to exposure to a dispersant (Fig. 5(d)).

Several of the nanoparticles possessing intrinsic dispersant properties listed in Table 1 rely on cationic groups. Cationic nanoparticles easily attach to negatively charged bacterial cell surfaces [108], but *in vivo* exhibit cytotoxicity and

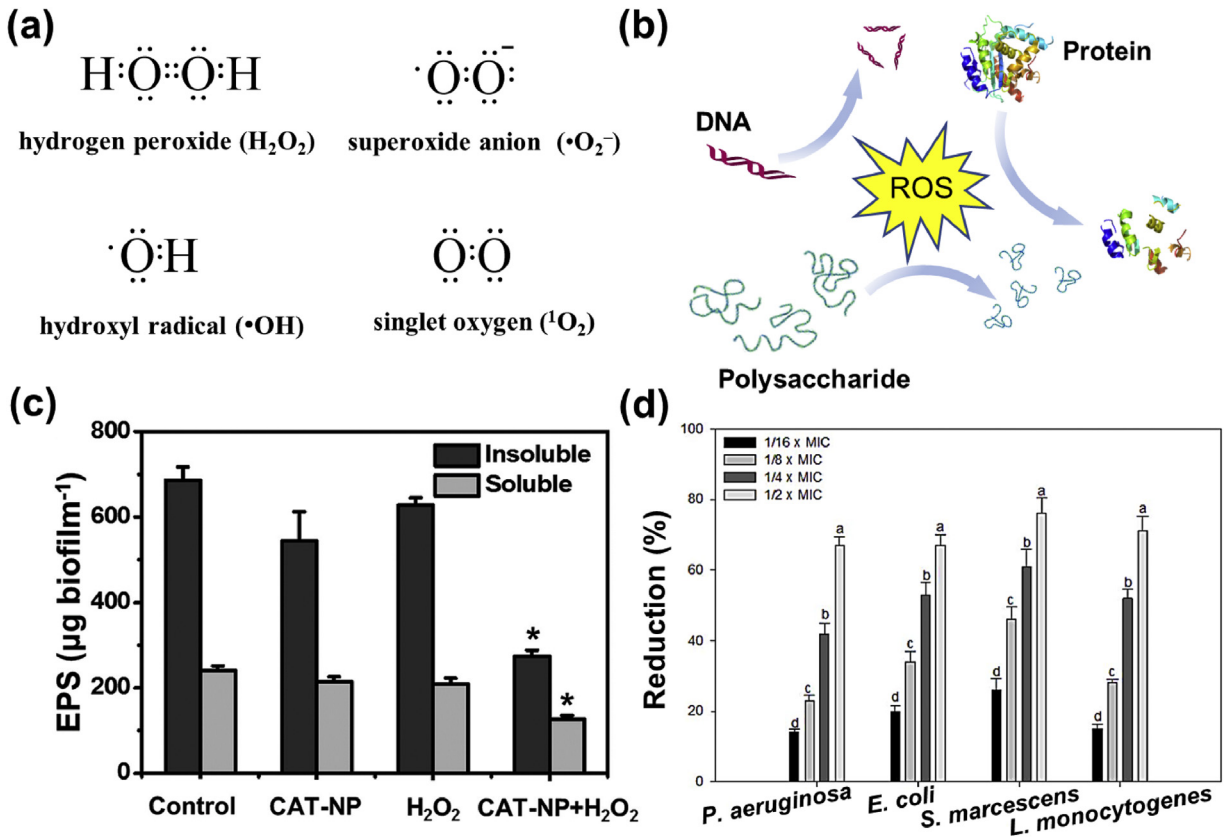

Fig. 4. Biofilm disruption and dispersal by ROS. (a) Different types of ROS. (b) EPS biofilm matrix components oxidized by ROS. (c) EPS degradation within *S. mutans* biofilms and glucan breakdown by a combination of catalytic nanoparticles (CAT-NP) and H_2O_2 yielding ROS generation. Reprinted with permission [54]. Copyright 2016, Elsevier. (d) Dispersal of existing *P. aeruginosa*, *E. coli*, *S. marcescens* and *L. monocytogenes* biofilms by ROS generating iron oxide (Fe_3O_4) nanoparticles at different concentrations in suspension. Reprinted with permission [55]. Copyright 2018, Frontiers Media S.A.

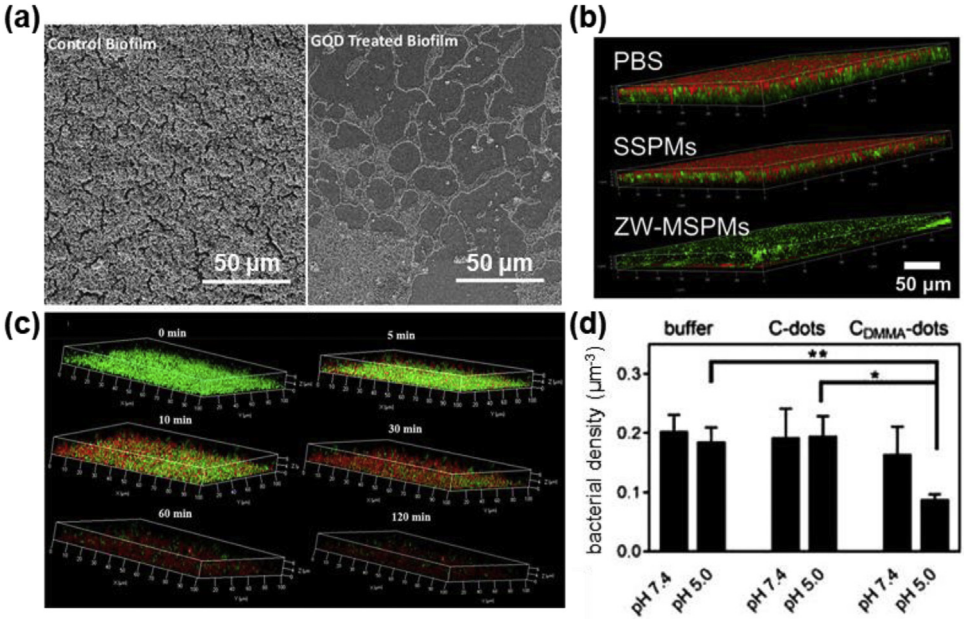

Fig. 5. Examples of the use of nanoparticles for biofilm dispersal. (a) Scanning electron micrographs of graphene quantum dots (GQD)-mediated staphylococcal biofilm dispersal, showing patches that are entirely devoid of biofilm after GQD exposure and remaining biofilms arranged in a pattern that presumably reflects drying artifacts due to low cohesivity of the EPS matrix after exposure to GQD. Reprinted with permission [86]. Copyright 2019, American Chemical Society. (b) Zwitterionic micelles dispersed existing *S. aureus* biofilms *in vitro* (green-fluorescence: bacteria, red-fluorescence: EPS). Reprinted with permission [23]. Copyright 2020, Science Publishing Group. (c) Confocal laser scanning microscopy images of methicillin-resistant *S. aureus* (MRSA) biofilms exposed to cationic dextran-block copolymeric nanoparticles, showing biofilm dispersal over time (Green fluorescence: bacteria, red fluorescence: DA95B5 dispersant). Reprinted with permission [24]. Copyright 2018, American Chemical Society. (d) Volumetric bacterial densities of *S. epidermidis* biofilms after 4 h exposure to buffer or suspensions with 125 μg mL^{-1} carbon-dots without 2,3-dimethylmaleic-anhydride (DMMA) (C-dots) or CDMMA-dots (C_{DMMA}-dots) at pH 7.4 and pH 5.0. Reprinted with permission [87]. Copyright 2020, Elsevier.

Table 1
Summary of uncoated and surface-coated nanoparticles as dispersant and dispersant loaded nanoparticles for use against biofilms of different bacterial strains.

Nanoparticles with intrinsic dispersant properties

Type of nanoparticle	Mechanism	Bacterial strain	Refs.
Citrate-capped gold nanospheres	Induce structural changes in the biofilm, leading to dispersal	*Legionella pneumophila*	[82]
Citrate-coated platinum nanoparticles	Reduce biovolume to induce biofilm dispersal	*L. pneumophila*	[83]
PEG-coated gold nanoparticles	Reduce biovolume to induce biofilm dispersal	*L. pneumophila*	[83]
PEG-coated iron oxide nanoparticles	Reduce biovolume to induce biofilm dispersal	*L. pneumophila*	[83]
Iron oxide nanoparticles	Decrease intracellular c-di-GMP levels to disperse biofilms	*P. aeruginosa*	[84]
Iron oxide nanoparticles	Generate ROS by interaction with bacteria to disperse biofilms	*S. marcescens*, *E. coli*, *P. aeruginosa* and *L. monocytogenes*	[55]
Ferumoxytol nanoparticles	EPS matrix degradation by generating free radicals from H_2O_2	*S. mutans*	[85]
Graphene quantum dots	Disrupt amyloid fibrils to disperse biofilms	*S. aureus*	[86]
2,3-dimethylmaleic-anhydride modified carbon-dots	Reduce volumetric-bacterial-density to disperse non-EPS-producing biofilms	*S. epidermidis*	[87]
Cationic dextran-block copolymer nanoparticles	Interpose in between bacteria and the biofilm matrix to cause dispersal	*S. aureus*, vancomycin-resistant *Enterococci*, and *Enterococcus faecalis*	[24]
Cationic, poly(2-(dimethylamino)ethyl methacrylate) based micelles	Penetrate biofilms to disrupt the EPS matrix	*E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus*	[88]
Cationic polymer micelles bearing silver ions	Interact with acidic moieties of EPS to solubilize the matrix	*P. aeruginosa*	[89]
Zwitterionic, mixed-shell polymeric micelles	Interact with major EPS components to disperse biofilms	*S. aureus*	[23]
DNase-mimetic artificial enzymes based on multinuclear metal complexes	Cleave eDNA to disperse biofilms	*S. aureus*	[50]
A silver-binding peptide fused to Dispersin B	Hydrolyze PNAG in the matrix to disperse biofilms	*S. epidermidis*	[90]

Dispersant-loaded nanoparticle carriers

Type of nanoparticle	Nanoparticle loading	Mechanism	Bacterial strain	Refs.
Nanostructured lipid carriers	DNase	Degrade eDNA to disrupt the biofilm matrix	*P. aeruginosa* and *S. aureus*	[91]
Nanocapsules	DNase	Degrade eDNA to disperse biofilms	*S. aureus*	[92]
Chitosan hydrogel	Dispersin B	Dispersin B degrades PNAG to detach biofilm	*S. aureus, S. epidermidis* and *Aggregatibacter actinomycetemcomitans*	[93]
Alginate	CaO_2 and hemin-loading graphene	Convert H_2O into ROS to degrade matrix components in biofilms	*S. aureus*	[51]
Graphene-mesoporous silica nanosheets	Ascorbic acid and ferromagnetic nanoparticles	Catalyze ascorbic acid forming $^{\bullet}OH$ to disperse biofilms	*S. aureus* and *E. coli*	[52]
Hybrid micelles	D-tyrosine	Affect amyloid fibers to disperse biofilms	*P. aeruginosa*	[94]
Chitin-based nanocomposite containing D-amino acids and iron oxide nanoparticles	D-tyrosine, D-tryptophan, and D-phenylalanine	D-amino acids disrupt biofilms	*S. aureus*	[95]
Polymeric nanoparticle	NO	Sustained release of NO to disperse biofilms	*P. aeruginosa*	[96]
Polydopamine-coated iron oxide nanoparticles	NO	Localized NO release to disperse biofilms	*P. aeruginosa*	[97]
Polymer/gold hybrid nanoparticles	NO	NO triggered dispersal of biofilms	*P. aeruginosa*	[98]
Polyethylenimine/ diazeniumdiolate -doped PLGA nanoparticles	NO	NO triggered dispersal of MRSA biofilms *in vitro* and *in vivo*	methicillin-resistant *S. aureus*	[99]
Micelles	NO	Visible light triggered release of NO to disperse biofilms	*P. aeruginosa*	[100]
Liposomes	Biosurfactants isolated from *Lactobacillus gasseri*	Biosurfactants dispersed biofilms	*S. aureus*	[101]

Dispersant-coated nanoparticles

Type of nanoparticle	Nanoparticle coating	Mechanism	Bacterial strain	Refs.
Poly(L-lysine) coated PLGA nanoparticles	DNase	Disperse biofilm by degrading eDNA	*P. aeruginosa*	[102]
Chitosan nanoparticles	DNase	Disperse biofilm by degrading eDNA	*P. aeruginosa*	[103]
Gold nanoparticles	DNase	Disperse biofilm by degrading eDNA	*S. aureus, S. epidermidis, E. coli*, and *P. aeruginosa*	[104]
Silica nanobeads	Proteinase K	Degrade proteins in biofilm matrix	*P. fluorescens*	[105]
Gold nanoparticles	Proteinase K	Degrade proteins in biofilm matrix	*P. fluorescens*	[106]
Silver nanoparticles	D-cysteine	"Disperse-then-kill"	*E. coli*, *P. aeruginosa* and *S. aureus*	[107]

rapid clearance from the blood circulation by phagocytes [109]. Smart, self-targeting zwitterionic, mixed-shell polymeric micelles self-assembled from poly(ethylene glycol)-*block*-poly(ε-caprolactone) and poly(ε-caprolactone)-*block*-poly(quaternary-amino-ester) overcome this problem [23]. Zwitterionic micelles become positively charged in the acidic environment of a bacterial biofilm and are negatively charged at pH 7.4. Since their cationic nature only becomes evident in the acidic environment of a biofilm, this allows *in vivo* targeting, penetration, and accumulation in biofilms and subsequent dispersal. Real-time imaging of

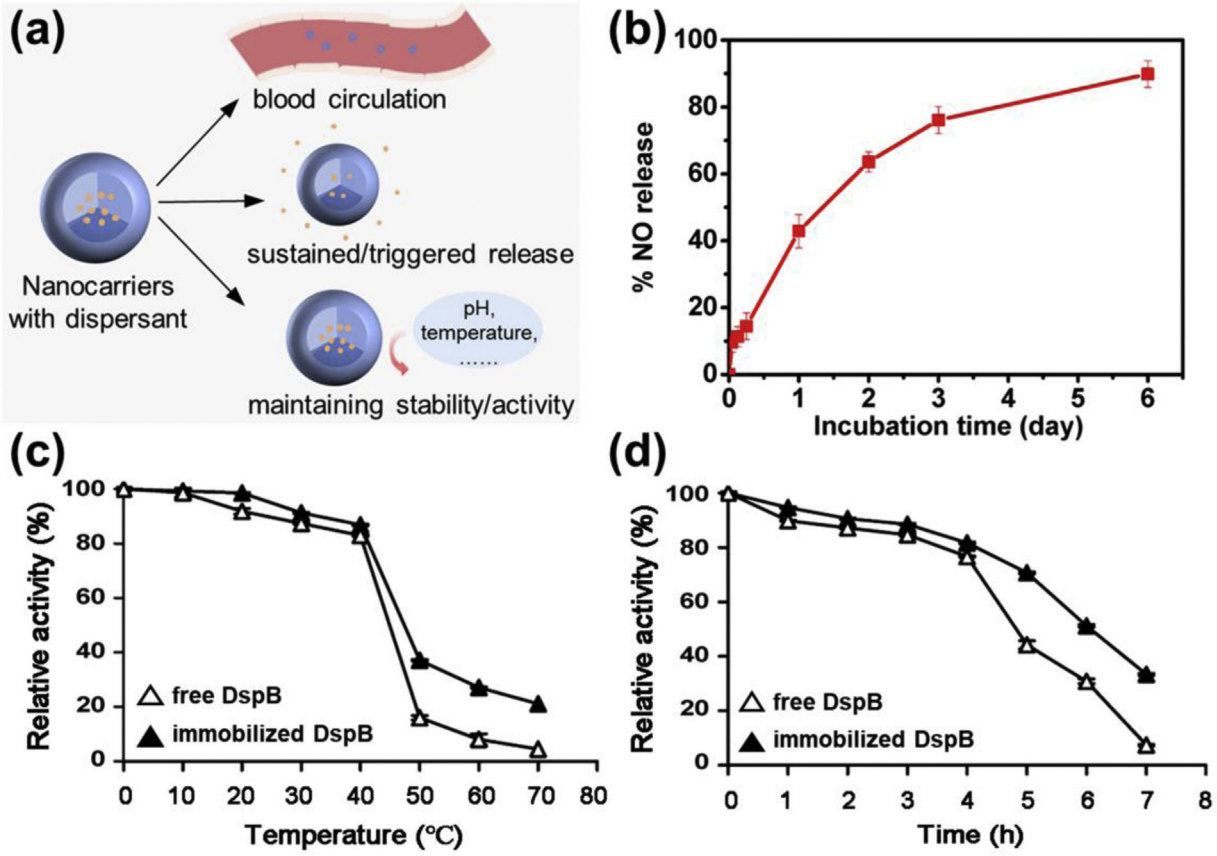

Fig. 6. Protective encapsulation of dispersants in nanoparticle carriers. (a) Advantages of the use of dispersant loaded nanocarriers above non-encapsulated dispersants. (b) Sustained NO release from polymer/gold hybrid nanoparticles in a phosphate buffer (pH 6.8). Reprinted with permission [98]. Copyright 2014, Royal Society of Chemistry. (c) Relative activity of free recombinant Dispersin B (DspB) and DspB loaded in chitosan nanoparticles as a function of temperature. Activity was assessed from the ability of DspB to enzymatically release of p-nitrophenolate from 4-nitrophenyl-N-acetyl β-D-glucosaminide. (d) Relative activity upon storage at 37 °C of free Dispersin B and Dispersin B loaded in chitosan nanoparticles as a function of time. (c and d) Reprinted with permission [93]. Copyright 2015, Elsevier.

a staphylococcal biofilm formed underneath an intravital abdominal window in living mice, demonstrated biofilm dispersal after tail-injection of zwitterionic micelles.

3.2. Dispersant-loaded nanoparticle carriers

Dispersants can be loaded into nanoparticles (see Table 1 for an overview) either to allow transportation through the blood circulation [94], sustained or triggered release from nanocarriers [95–99] or improve thermal stability and activity [93] and therewith storage times [93] (Fig. 6(a)). NO loaded polymer/gold hybrid nanoparticles showed sustained release (Fig. 6(b)), demonstrating stability in biological media [98]. Loading of Dispersin B in chitosan nanoparticles greatly enhanced the thermal stability and storage time of recombinant Dispersin B [93]. Whereas Dispersin B free in solution lost almost its entire activity after 60 min at 70 °C, Dispersin B loaded in nanoparticles retained 21.0% activity (Fig. 6(c)). Moreover, Dispersin B loaded in nanoparticles could be stored longer than Dispersin B free in solution (Fig. 6(d)).

3.3. Nanoparticles coated with dispersant molecules

Instead of loading a nanocarrier with a dispersant, nanoparticles can also be coated with chemical functionalities conveying dispersant properties (see also Table 1). DNase I is most frequently used as a dispersant coating for nanoparticles. Particularly chitosan nanoparticles are suitable for DNase I coating due to their abundant amino groups that can be covalently coupled to DNase I [103]. In addition to DNase coating, DNase I coated chitosan nanoparticles have been loaded with ciprofloxacin to disperse *P. aeruginosa* biofilms by degrading eDNA and kill detached bacteria [103]. The simultaneous release of ciprofloxacin counters the risk of causing acute sepsis due to the detachment of infectious pathogens from a biofilm in the blood circulation.

4. Clinical advantages and disadvantages of biofilm dispersal

Biofilm dispersal is a natural phenomenon in the biofilm life-cycle and is more and more regarded as a potential approach to combat infectious biofilms [110]. In the following sections, we will discuss the advantages and disadvantages of biofilm dispersants, their therapeutic role in addition to antibiotics and their potential for clinical application.

4.1. Advantages and disadvantages of biofilm dispersal

Advantages of dispersants are obvious and generally acknowledged. With dispersal being part of the natural life-cycle of biofilms, there are no indications of any bacterial resistance mechanisms developing against dispersants [25]. Bacteria dispersed from a biofilm become suspended in the blood circulation and are therewith more vulnerable to host immune cells and antibiotics [111]. Moreover, since the remaining biofilm usually has a lower volumetric bacterial density [87], the remaining biofilm is easier to penetrate by antimicrobials and bacteria in the remaining biofilm are more easily killed by antimicrobials [23,107] (see below).

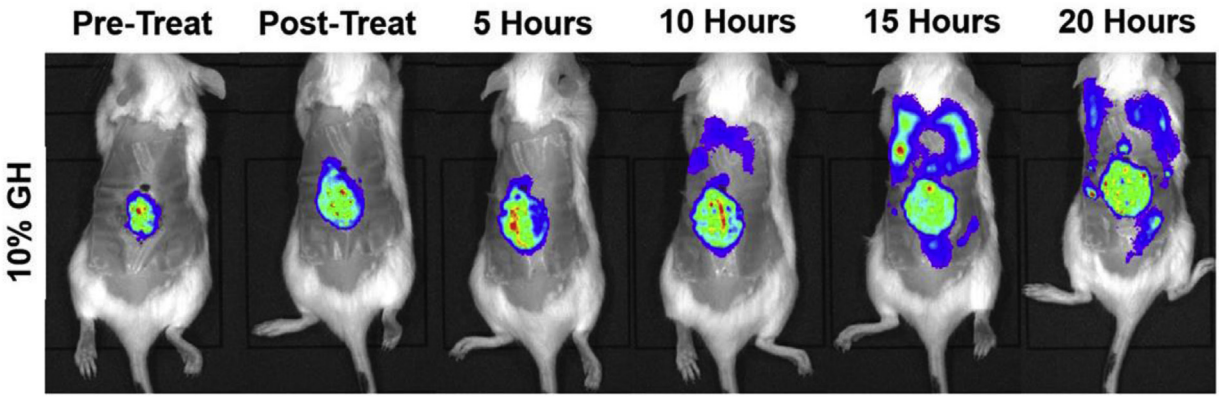

Fig. 7. Spreading of infection in a murine model after dispersal of a subcutaneous, bioluminescent *P. aeruginosa* biofilm using glycoside hydrolase (GH) as a dispersant in absence of simultaneously administered antibiotics. Infection is visualized using bioluminescent imaging. Reprinted with permission [112]. Copyright 2018, Springer Nature.

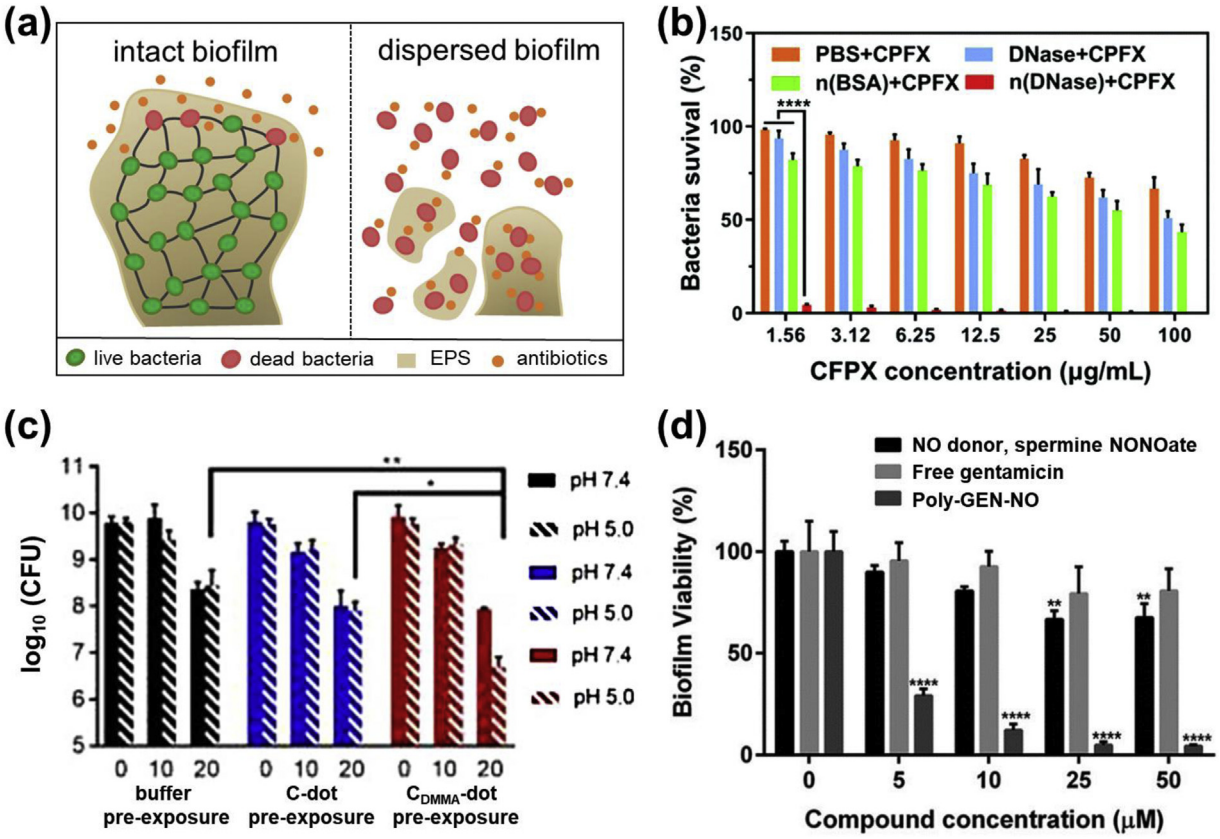

Fig. 8. Biofilm dispersants enhance efficacy of existing antibiotics. (a) Breaking the biofilm barrier by dispersant action makes remaining biofilms more penetrable to antibiotics. (b) Survival of *S. aureus* in biofilms pre-exposed DNase loaded nanocapsules (nDNase) and subsequently exposed to ciprofloxacin (CPFX). For control, similarly encapsulated bovine serum albumin (nBSA) was employed for pre-exposure. Reprinted with permission [92]. Copyright 2020, Royal Society of Chemistry. (c) Log_{10} CFU (colony forming units) of *S. epidermidis* in biofilms after pre-exposure to buffer or carbon dots with or without DMMA (C-dots and C_{DMMA}-dots, respectively) at different pH followed by 72 h growth in medium supplemented with vancomycin at different multiple concentrations of its minimal bactericidal concentration (MBC). Reprinted with permission [87]. Copyright 2020, Elsevier. (d) Effect of gentamicin-NO (GEN-NO) nanoparticles on *P. aeruginosa* biofilm viability after combined release of NO and gentamicin. Reprinted with permission [96]. Copyright 2016, Royal Society of Chemistry.

As a potential disadvantage, the sudden increase in the number of bacteria suspended in the blood circulation after dispersal from a biofilm may cause sepsis in the absence of accompanying antibiotic therapy with the potential of spreading of the infection to other sites of the body (Fig. 7) [112], despite their increased susceptibility to host immune cells. In addition, although it is common to assume that bacteria dispersed from a biofilm are similar to planktonic bacteria, recent studies pointed out that the physiology of dispersed bacteria can be different from the physiology of planktonic bacteria [113,114], with possible consequences for antibiotic susceptibility and clearance by immune cells.

Clinically, a bacterial infection of the blood, *i.e.* sepsis commences already at a low concentration of 1–30 viable bacteria per mL [115,116], while symptoms can become stronger due to a cytokine storm associated with the bacterial presence [117]. Since clinical treatment of infection should start as soon as possible after the first symptoms arise [118], it is necessary to use a combination

therapy of dispersants and antibiotics to avoid the risk of dispersal induced sepsis.

4.2. Biofilm dispersants and antibiotics

Dispersants offer two different ways in which existing antibiotics can be used more efficient for infection control. Whereas dispersants do not solve any of the problems associated with intrinsic antibiotic-resistance of bacteria [25], they allow more efficient use of existing antibiotic by breaking the biofilm-barrier [119] posed by most infections (Fig. 8(a)), either through direct dispersal or decreasing bacterial densities in remaining biofilm. Pre-exposure of existing *S. aureus* biofilms to DNase encapsulated in nanocarriers [92] (Fig. 8(b)), artificial enzymes [50] or surface-modified carbon dots [87] (Fig. 8(c)) yielded high killing efficacy by antibiotics. Also, combinations of gentamicin and NO release (Fig. 8(d)) have been found to enhance *P. aeruginosa* killing through biofilm dispersal [96].

4.3. Clinical application of dispersants and future prospects

Despite potential disadvantages and risks, impediments for the clinical use of dispersants seem to be little. Recombinant human DNase (Pulmozyme®) has been approved for the treatment of cystic fibrosis to reduce the viscosity of mucous but can also be used to disperse infectious *P. aeruginosa* biofilms in the lungs [120] in combination with antibiotics to treat respiratory tract infections [121]. Levofloxacin, a hydrophilic broad-spectrum antibiotic, encapsulated with DNase I in lipid carriers decreased mucous viscoelasticity in the lungs of cystic fibrosis patients, disrupted *P. aeruginosa* biofilm and improved the antibiotic diffusion [91]. Also, inhalation of NO caused dispersal of *P. aeruginosa* biofilm in cystic fibrosis patients, suggesting its use to enhance the efficacy of antibiotics [122]. Dispersal induced sepsis and spreading of infection can be prevented by the simultaneous use of dispersants and antibiotics [112].

Collectively, there appear to be no major hurdles associated the clinical use of DNase or other dispersants like NO. However, so far this conclusion is based on application towards cystic fibrosis patients, and confirmation for other clinical types of infection remains to be demonstrated, for which the occurrence of sudden sepsis may be an important point of attention.

5. Conclusions and future prospects

Biofilm dispersal is part of the natural biofilm life-cycle. A wide variety of highly different molecules exist with the ability to disperse biofilm that can be used for the control of infectious biofilms. Several small molecules, reactive oxygen species, nitric oxide releasing compounds, peptides and molecules regulating signaling pathways in biofilms have been described as dispersants. In order to improve the stability of dispersants and allow their transportation through the blood circulation, dispersants can be encapsulated in nanoparticle carriers. Nanoparticles can also possess dispersant properties of their own.

The clinical application of dispersants forms a challenge because the sudden increase of bacterial concentration in the blood circulation after the dispersal of an infectious biofilm yields the risk of sepsis and spreading of the infection to other organs. To avoid the risk of sepsis, antibiotics must be applied simultaneously with dispersants. Yet, this requires critical timing of establishing the right antibiotic concentration in blood viz a viz the development of a high bacterial concentration in the blood after dispersal administration. This problem in the clinical application can be avoided by designing dispersant nanoparticles that can also act as antimicrobials.

Since dispersal breaks the EPS matrix of biofilms, dispersants eliminate a significant factor in the recalcitrance of infectious biofilms to antibiotic treatment. Importantly, dispersants can work synergistically with antibiotics in eradicating infectious biofilms.

In summary, the use of nanoparticles as dispersants or dispersants carriers has not yet been studied in depth despite their obvious potential, while the clinical use of dispersants in general still poses challenges. More research is therefore needed into understanding mechanisms of biofilm dispersal and enhancing the synergy between dispersants and antibiotics in order to avoid the risk of sepsis and infection spreading, making dispersants a safe and effective tool in the control of infectious biofilms.

Declaration of Competing Interest

H.J.B. is director-owner of a consulting company, SASA BV. The authors declare no potential conflicts of interest with respect to authorship and/or publication of this article.

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