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Phage therapy as an alternative or complementary strategy to prevent and control biofilm-related infections

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The complex heterogeneous structure of biofilms confers to bacteria an important survival strategy. Biofilms are frequently involved in many chronic infections in consequence of their low susceptibility to antibiotics as well as resistance to host defences. The increasing need of novel and effective treatments to target these complex structures has led to a growing interest on bacteriophages (phages) as a strategy for biofilm control and prevention. Phages can be used alone, as a cocktail to broaden the spectra of activity, or in combination with other antimicrobials to improve their efficacy. Here, we summarize the studies involving the use of phages for the treatment or prevention of bacterial biofilms, highlighting the biofilm features that can be tackled with phages or combined therapy approaches.

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Introduction

Biofilms are microbial communities adhered to surfaces, or formed on air–liquid interfaces, and are composed by cells embedded in a self-produced polymeric matrix of polysaccharides, proteins, nucleic acids and lipids [1]. These microbial structures are involved in many difficult to treat chronic infections on account of their tolerance to antibiotics and host immune defences. Accordingly, due to the failures of antibiotic therapy toward bacterial biofilms and also the emergence of multidrug-resistant bacterial strains, which currently constitutes one of the most widespread public health concerns, there is today a renewed interest in alternative therapeutic modalities to treat biofilm-associated infections, such as phage therapy. Bacteriophages (phages) are natural predators of bacteria and have been considered as a promising strategy against bacterial biofilms. Several studies have shown that phages can effectively infect and lyse cells present in single and polymicrobial species biofilms [2,3], which supports the concept of phage therapy as an alternative or complementary strategy to prevent and control biofilm-related infections. The interaction between phages and biofilms is a rather complex process [4]. Theoretically, the close proximity of cells within the biofilm structure could enhance phage-host interaction and facilitate phage infection [5,6]. Conversely, the biofilm structure and composition as well as the physiological state of the biofilm cells may be an obstacle to phage infection [4]. It must be stressed that phage/biofilm interactions are highly dependent on the bacterial host strain, the phage characteristics, and the biofilm structure and its composition. The biofilm formation set-up has a great impact on the biofilm characteristics making it difficult if not impossible to withdraw clear conclusions based on the state of art, regarding how phages interact with biofilms and what phage characteristics are in need to make them good biofilm controllers. Moreover, most of the studies do not mimic real clinical biofilms, usually formed by polymicrobial populations and human cells. Besides, measurements of phage's effectiveness against biofilms is based on the overall outcome of phage infection. There is a limited knowledge of how phages interact with the different population of bacterial cells that compose the biofilm. So, this review is restricted to the current knowledge of how phages interact with biofilms alone or combined with other antibiofilm approaches highlighting the limitations as well as the strategies that can be used to overcome biofilm barriers and enhance phage therapy.

Limitations of bacteriophages when used as anti-biofilm agents

It is well known that phages are currently considered as promising antimicrobial agents for biofilm prevention and control [7,8]. Nonetheless, although several studies have reported significant reductions in mono and dual-species biofilms after phage treatment [2,3,9–12], the complete eradication of biofilms is an almost impossible task [13•]. Phages need to reach their host bacteria and attach to specific receptors located at cell surfaces before infection

and replication inside their hosts is initiated. The access to host receptors in planktonic cultures is easier, since the receptors are fully available for phage docking and not surrounded by a matrix as found in biofilm cells. The biofilm matrix, the reduced metabolic activity of biofilm cells and the proliferation of phage-resistant phenotypes within the biofilm are some of the major challenges to the application of phage therapy for biofilm control.

Diffusional limitation through the biofilm matrix

It is estimated that only 10% of biofilm dry biomass are cells. The biofilm matrix comprises the other 90% and it is composed by different types of polymers, also known as extracellular polymeric substances (EPS), which are responsible for forming complex three-dimensional structures involved in the adhesion and cohesion of the biofilms [14]. The EPS matrix has been considered as one of the main obstacles that limits the diffusion of antimicrobial agents, including phages, to the bacterial cells embedded in the biofilm [14].

Biofilm matrix is a reservoir of microbial enzymes secreted or released upon cell lysis (such as amidases and peptidases) that can lead to phage inactivation [14,15]. Biofilms are also composed by dead cells that can anchor phages and, in case of adsorbing to dead cells receptors, phages will not be able to infect living cells. Phages may also interact with some components of the matrix, namely with proteins, lipopolysaccharides, polysaccharides and teichoic acids [16,17], which ultimately may limit phage access to biofilm cells.

Phage diffusion through the biofilm matrix can be influenced by both phage morphology and biofilm density. Generally, the diffusion of phages through denser biofilms will be more difficult than through less dense biofilms [18]. Phage diffusion is also dependent on the biofilm architecture, and it is known that biofilms can exhibit different 3D shapes (mushroom-like, pillar-like, hilly, or flat multicellular structures) [19] that can be bathed by water channels making these biofilms, in theory, more prone to phage attack than dense biofilm structures. A recent study using LNA-FISH probes to image phage-biofilm interaction showed that phage infected cells were primarily located on the edges of water channels [20•].

Reduced metabolic activity of biofilm bacterial cells

The physiological state of cells has a great influence on phage replication since exponential-phase cells are more rapidly lysed than stationary-phase bacteria [21]. When a bacterial culture is under nutrient limitation, there is a transition between exponential to slow or no growth, which is commonly also observed in mature biofilms [22]. In biofilms, cells inhabiting the deeper layers and under oxygen and nutrient depletion conditions [5], have reduced metabolic activity constituting one of the main reasons why phages replicate faster in planktonic than biofilm cells. This physiological change is linked to the increased resistance or tolerance of biofilms to antibiotics, since the majority of antibiotics have no activity or impaired activity against non-dividing cells [22].

Although phages have activity against some bacterial host cells growing under nutrient limitation, their growth parameters are strongly influenced by the physiological state of the host cells [23,24••]. For instance, nutrient limitations of *Escherichia coli* cells resulted in severe inhibition of lytic development of phages λ, P1 and T4 [23]. More recently, Bryan *et al.* showed that the infection of stationary *E. coli* cells by the T4 phage at low MOIs did not progress until nutrient addition. These authors hypothesised that T4 responds to the starvation state of *E. coli* by entering what, the authors call a 'hibernation' mode, in which T4 initiates protein synthesis but arrests further phage development [24••]. The impaired activity of phages against stationary-phase cells has also been reported for phages infecting other bacterial species, such as *Pseudomonas fluorescens* [25], *Klebsiella pneumoniae* [26] and *Staphylococcus epidermidis* [27]. The amount of cells with reduced metabolic activity within biofilms are expected to increase throughout biofilm maturation, consequently older biofilms will be more difficult to tackle that young biofilms.

In literature there is a misconception about dormancy, persistence and stationary cells. It is often referred that part of the biofilm population is composed by dormant cells, which are cells that persist without division for extended periods [28]. This type of population is also part of suspended cultures and the number of dormant cells increases with the age of population, being very high in stationary growth cultures. Bacterial cells that persist after antibiotic treatment without undergoing genetic changes and revert to sensitive when cultured, are called persisters [29]. To the authors knowledge, there are no phage studies against persisters or dormant cells, therefore it would be important to fulfil this gap in order to better understand the interaction between phages and biofilms.

Development of phage resistant sub-population within the biofilm

Phage resistance mechanisms are crucial for bacterial survival in a set of different ecological niches. The four main resistance mechanisms described so far are: (i) prevention of phage DNA integration by superinfection exclusion systems; (ii) degradation of phage DNA by restriction-modification systems or by CRISPR-Cas systems; (iii) use of abortive infection systems that block phage replication, transcription or translation; and (iv) prevention of phage adsorption by loss or structural modifications of bacterial receptors, and/or masking phage receptor molecules by physical barriers such as

the EPS matrix [30]. These mechanisms enable a quick adaptation of bacteria to the presence of phages and consequently, the emergence of phage-resistant mutants is almost inevitable.

Several studies have reported that the initial reduction of biofilm cells caused by phage treatment is usually followed by a fast proliferation of phage-resistant sub-populations within a short period of time [10,31–34]. For instance, the arising of phage-resistant bacterial variants resulting from *P. aeruginosa* biofilm treatment with phages was already reported [10,31]. Fu *et al.* studied the effect of lytic phages in the prevention of *P. aeruginosa* biofilm formation in hydrogel-coated catheters [10]. Although a reduction in biofilm formation was observed in the first 24 h comparatively to untreated catheters, a regrow of biofilms was observed between 24 and 48 h, and phage-resistant biofilm isolates were recovered [10]. Phage-resistant bacteria was also observed in *Serratia marcescens* biofilms after 24 h of contact with phage [35]. In a study developed by Oechslin *et al.*, the genomic profile of two *P. aeruginosa* phage-resistant strains was analysed and revealed mutations in genes encoding phage receptors, namely *pilT* and *galU*, when compared to the wild-type strain [36]. The same was reported by Pires *et al.* in which the authors described that mutations affecting the *galU* gene and the *pil* genes were responsible for bacterial resistance to phages [13•]. This fact can be a consequence of the endogenous oxidative stress suffered by biofilm cells that leads to DNA damage within biofilms resulting in the development of genetic variants with high adaptability to external conditions [37,38].

Quorum sensing inhibits phage infection of biofilms

Quorum sensing (QS) is a chemical communication process that bacteria use to regulate collective behaviours. Accordingly, QS molecules secreted by individual bacteria accumulates in dense bacterial populations enabling bacterial cells to recognise the population density and activate the corresponding response. QS is particularly important to regulate biofilm physiology and therefore believed to have a great impact in a biofilm population regulating virulence, growth and dispersion [39]. QS can be involved in the anti-phage process by reducing the phage receptor numbers on the cell surface as described for *E. coli* and *Vibrio anguillarum* [40,41]. In the case of *E. coli*, the number of receptors displayed at the bacterial surface for λ phage docking is reduced in response to N-acyl-l-homoserine lactone (AHL) quorum-sensing signals, causing a 2-fold reduction in the phage adsorption rate [40]. For *V. anguillarum* a similar mechanism was described. In this case, in high cell culture densities, a QS mediated down regulation of *ompK*, the KVP40 receptor, was observed and rendered individual cells almost unsusceptible to phage infection [41]. Qin *et al.* observed an increased infection efficiency of the *P. aeruginosa* phage K5 in the presence of penicillic acid, a QS inhibitor [42]. In this work the authors did not find any alterations in phage adsorption rateand expression of cell receptors. According to the authors, this enhanced activity was probably due to an improved metabolic state of the cells [42]. QS mediated defensive mechanism can be a consequence of the biofilm phenotype itself, in which part of the cells exhibits a low metabolic state as a means to ensure efficient energy and resources to the biofilm population, which is controlled by QS mechanisms. Nevertheless, QS mediated mechanisms of defence to phages need to be further elucidated and described also for other species.

Strategies to enhance biofilm control by phages

In order to circumvent phage limitations and improve their performance for an efficient biofilm control, different approaches, such as synergistic combinations with other phages or antimicrobials, mechanical debridement of biofilms, and genetic engineering of phage genomes have been addressed (summarized in Figure 1). Some of the most relevant studies performed *in vitro* are indicated in Table 1.

Mechanical debridement

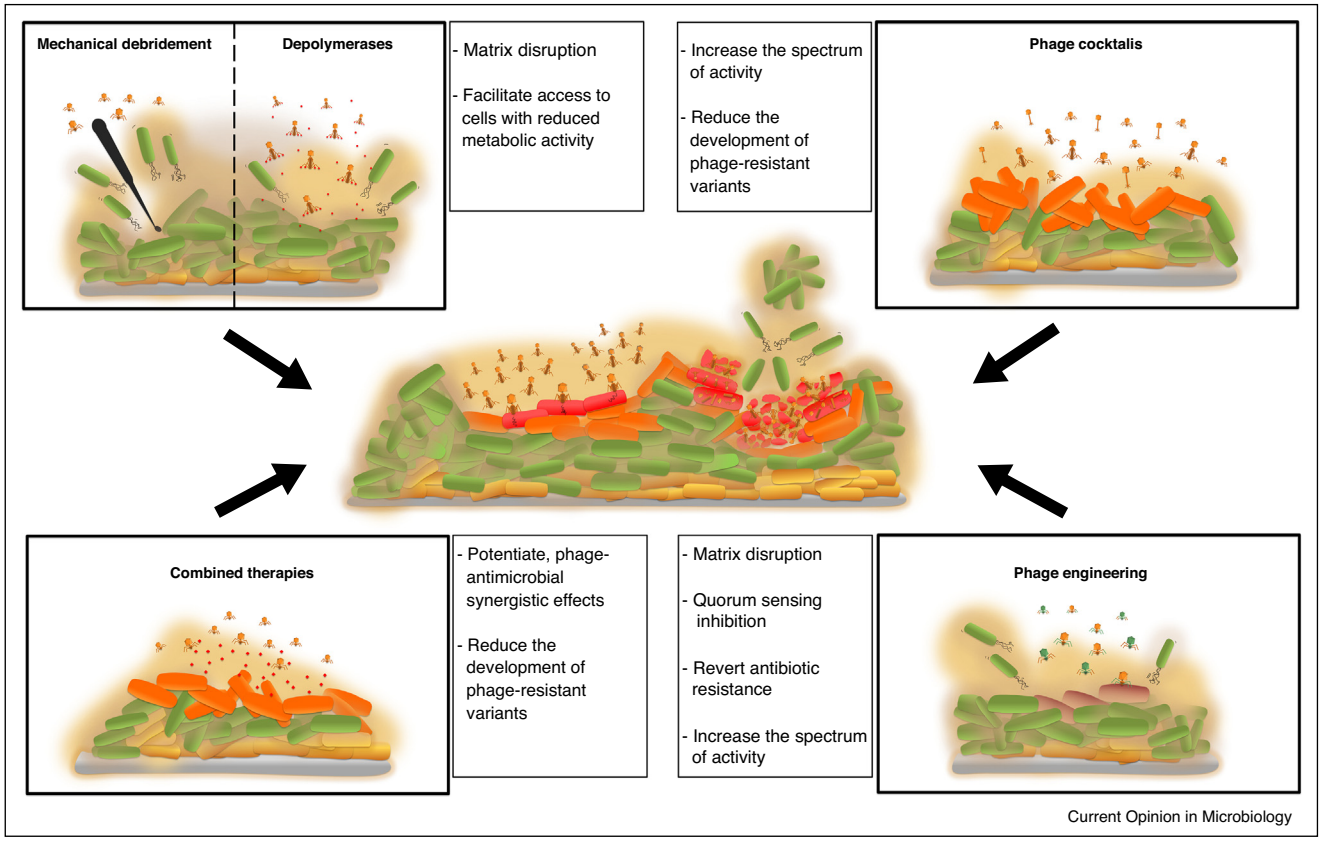
The hypothesis that a mechanical disruption of biofilms can facilitate phage infection has been studied in *in vivo* wound models. Seth *et al.*, found that the tested phage was not active against *Staphylococcus aureus* intact biofilms [55]; however, when the phage was administered after sharp debridement, wound healing parameters assessed by histological analysis improved significantly and bacterial counts diminished [55]. In another study, using two different diabetes mellitus animal models (rodent and porcine), the combination of phages and debridement decreased bacterial counts of *P. aeruginosa* and *S. aureus*, and improved wound healing, particularly in the rodent model [56].

Overall, it can be assumed that debridement enhances phage infection as a consequence of a better phage accessibility to the biofilm cells. Moreover, cells released due to debridement also become more susceptible to phage infection [8], suggesting that this type of approach can be valuable therapeutically.

Combined therapy with antibiotics and antiseptics

The combination of phages with other antimicrobial agents, such as antibiotics or antiseptics, has been widely studied. Previous studies have suggested that sublethal concentrations of antibiotics can improve the production and activity of virulent phages, a phenomenon called phage-antibiotic synergy (PAS) [43,57]. The synergistic effect of phages and antibiotics has been studied in planktonic cultures (reviewed in [58•]) and expanded to biofilms [43,46]. For instance, Ryan *et al.* evaluated the effect of a combined treatment of T4 phage and cefotaxime in the eradication of *E. coli* ATCC 11303 biofilms. The combination of phage and antibiotic

Figure 1



Schematic representation of strategies to overcome biofilm barriers using phages and combined therapies. The biofilm is represented by cells under different metabolic conditions and susceptibility to phages (red cells: phage infected cells; green cells: metabolically active cells; orange cells: phage-resistant variants; yellow cells: low metabolic activity).

significantly enhanced the biofilm eradication compared with antibiotic alone; for example, the use of phages (titres of 10^4 and 10^7 PFU mL^{-1}) reduced the minimum biofilm eradication concentration (MBEC) of cefotaxime against *E. coli* biofilms by 2 and 8 folds, respectively [43]. Combinations of phages and antibiotics were also tested against *S. aureus* [46] and *K. pneumoniae* [44] biofilms resulting in an improved efficacy of biofilm control compared to what was obtained after the use of each antimicrobial alone.

Nonetheless, the combination of phages with antibiotics has not always resulted in improved biofilm removal efficacy. For example, the lytic *K. pneumonia*e KPO1K2 phage was used alone or combined with ciprofloxacin to treat *K. pneumonia*e biofilms and no significant differences in biofilm removal efficacies between both treatments were observed [45]. However, the combined therapy significantly restricted the formation of resistant variants compared with each treatment alone [45]. It has been described that the order of treatment might have a great impact on the outcome of biofilm control. Chaudhry *et al.* concluded that in most cases the use of phages before antibiotics resulted in maximum killing of *P. aeruginosa* biofilms *in vitro*. The combined treatment was particularly effective against biofilms grown on layers of cultured epithelial cells [59•].

Besides antibiotics, the synergistic effect between phages and antiseptics has also been studied. Using a continuous flow system or microtiter plates, a combined therapy of chlorine and phages revealed to be more effective in controlling *P. aeruginosa* biofilm growth and removing pre-formed biofilms than the separate use of each therapy [48].

Combined therapy with enzymes

It was believed that depolymerases, which are polysaccharide-degrading enzymes encoded in phage genomes, could be potentially used by phages to circumvent the biofilm barrier and facilitate the phage access to the host cells [60,61]. However, the main function of depolymerases consists in the degradation of capsular polysaccharides to facilitate phage adsorption [62–64,65•] rather

Table 1

Some of the most relevant studies about the *in vitro* efficacy of phages against biofilms

Bacteria	Phage(s)	Experimental approach	Results	Reference
Combined therapy				
E. coli	T4	The antimicrobial synergy between T4 phage and cefotaxime in the eradication of *E. coli* biofilms was evaluated	The use of phages (titres of 10^4 and 10^7 PFU mL^{-1}) reduced the MBEC of cefotaxime against *E. coli* biofilms by 2 and 8 folds, respectively	[43]
K. pneumoniae	Uncharacterized	*K. pneumoniae* biofilms of different ages were subjected to phage treatment (MOI of 0.01) in combination with amoxicillin (512 μg/mL)	A significant reduction of the biofilm bacterial counts was observed after combined therapy application	[44]
K. pneumoniae	KPO1K2	12 h old *K. pneumoniae* biofilms were subjected to the combined treatment of phage (MOI of 1) and ciprofloxacin (1 mg/L)	No significant differences in biofilm removal efficacies between phage treatment alone or combined with ciprofloxacin were observed. However, the combined treatment significantly prevented the emergence of resistant variants	[45]
S. aureus	SAP-26	Phage (10^8 PFU) was applied together with azithromycin (80 mg/L), vancomycin (10 mg/L), and rifampicin (0.6 mg/L) against 24 h old *S. aureus* biofilms	Phage alone was able to kill approximately 28% of the biofilm bacteria after 24 h. Azithromycin and vancomycin could kill ~25% and 17%, respectively and when biofilms were treated with phage and rifampicin ~35% of the live cells remained after this treatment. Phage/azithromycin and phage/vancomycin treatments showed ~40% and 60% cells alive after 24 h, respectively	[46]
E. coli and P. aeruginosa	T4 and PB-1	*E. coli* and *P. aeruginosa* 48 h biofilms were exposed to a combination of tobramycin (2 μg/mL) and T4 phage (MOI of 0.01) or tobramycin (0.5 μg/mL) and PB-1 phage (MOI of 0.01) for 24 h, respectively	The combination of phage and antibiotic led to ~99.99% decrease on the survival of *E. coli* biofilms compared to the use of tobramycin alone, while the combination of tobramycin and PB-1 on *P. aeruginosa* biofilms was just as effective as tobramycin alone in decreasing biofilm cells. However, phage infection in combination with tobramycin reduced the emergence of antibiotic and phage resistant cells	[47]
P. aeruginosa	Cocktail of RNA phages	A mixture of phages and chlorine with different concentrations was tested to control and remove *P. aeruginosa* biofilms	The phage cocktail (3×10^7 PFU mL^{-1}) and chlorine (210 mg/L) reduced biofilm growth by ~94% and removed ~88% of existing biofilms	[48]
Phage cocktails				
P. mirabilis	Cocktail of two phages	Catheters were pre-treated with the phage cocktail before bacterial inoculation	A significant reduction in the number of *P. mirabilis* biofilm cells was observed after 96 and 168 h of biofilm formation in phage-coated catheters	[49]
P. mirabilis	Cocktail of three phages	Models mimicking either an established infection, or early colonization the catheters, were treated with a single dose of phage cocktail	In models simulating established infection, a single dose of phage cocktail significantly extended the time taken for catheters to block (~3 fold), compared to untreated control In models simulating an early stage infection, the phage cocktail completely prevented catheter blockage and eradicated infection	[50]
P. aeruginosa	Cocktail of five phages	Catheters were pre-treated with a cocktail of five phages prior to bacterial inoculation	The pretreatment of catheters with the phage cocktail resulted in a 3-log reduction of biofilm cell populations after 48 h, compared with untreated catheters	[10]
P. aeruginosa and P. mirabilis	Cocktail of six *P. aeruginosa* phages and cocktail of four *P. mirabilis* phages	Hydrogel-coated catheters were pre-treated with one or both phage cocktails before bacterial challenge	Phage pretreatment reduced *P. aeruginosa* biofilm counts by 4 $\log_{10}$ CFU/cm^2 and *P. mirabilis* biofilm counts by >2 $\log_{10}$ CFU/cm^2 over 48 h	[2]
S. aureus	Mixture of phage K and six of its derivatives	The phage cocktail was used to prevent biofilm formation and to remove established biofilms	Crystal violet staining assays revealed that the main reduction of biofilm biomass occurred between 15 and 20 h after phage treatment. Furthermore, a complete inhibition of *S. aureus* biofilm formation over a period of 48 h was observed	[51]

Table 1 (*Continued*)

Bacteria	Phage(s)	Experimental approach	Results	Reference
S. aureus	K and DRA88	The efficacy of phage mixture (MOI 1 and 10) was evaluated against three different biofilm-producing *S. aureus* isolates	A significant reduction of biofilm biomass over 48 h of treatment was observed in all cases	[9]
P. aeruginosa	Cocktail of six phages (DL52, DL54, DL60, DL62, DL64 and DL68)	48 h biofilms were treated with the phage cocktail (MOI 1 and 10) and their biomass density was evaluated post phage infection	Under static conditions, more than 95% biofilm biomass was eliminated within 4 h using a MOI of 10. In the flow biofilm model, 48 h after contact with phage cocktail, >4 logs of biofilm cells were eliminated	[11]
Genetic manipulated phages				
E. coli	M13mp18	The lysogenic phage M13mp18 was modified to overexpress *lexA3*, a repressor of the SOS DNA repair system, to enhance antibiotic-induced killing of *E. coli*	The *in vitro* simultaneous application of *lexA3*-producing phage and ofloxacin improved killing of biofilm cells by about 1.5 and 2 orders of magnitude compared with unmodified phage plus ofloxacin and no phage plus ofloxacin, respectively	[52]
E. coli	T7select415-1	A T7 phage was engineered to express the biofilm-degrading enzyme dispersin B during phage infection	After 24 h of treatment, the engineered phage was able to reduce *E. coli* biofilm cell counts by 4.5 orders of magnitude, which was about 2 orders of magnitude better than the reduction caused by the non-enzymatic wild type phage	[53]
E. coli and *P. aeruginosa*	T7select415-1	A T7 phage was engineered to express a quorum-quenching enzyme (AHL lactonase) during phage infection	The engineered phage was added to the mixture of *P. aeruginosa* PAO1, *E. coli* TG1, and *E. coli* BL21 and caused significant reductions of the biofilm compared to the no-phage control: 74.9% and 65.9% reduction, at 4 and 8 h of biofilm formation, while wild-type phage caused reductions of only 23.8% and 31.7% at 4 and 8 h, respectively	[54]

than cleavage of EPS present in biofilm matrix. These enzymes encoded by phages can be heterologously expressed, purified and added to planktonic bacteria and biofilms, to degrade the capsular polysaccharides and enhance phage killing [66–68]. Studies performed by Cornelissen *et al.* showed that although *Pseudomonas putida* phages encoding polysaccharide depolymerases revealed biofilm degradation properties, the application of the recombinantly expressed phage depolymerases or non-infectious phage particles (UV inactivated) to biofilms did not exhibit biofilm-degrading properties [66,67]. These studies showed that depolymerases alone were not capable of degrading *P. putida* biofilms and highlighted the role of phage amplification in biofilm degradation [66,67]. Nonetheless, in a study developed by Gutiérrez *et al.*, a depolymerase derived from a *S. epidermidis* phage was expressed and applied to biofilms and, although dose-dependent, it revealed biofilm removal properties, which were evaluated by viable cell counting and crystal violet staining of total biofilm biomass [69].

Phage cocktails

Multiple phages with different host ranges and targeting different receptors can be combined in a single phage preparation (phage cocktails) to expand their spectrum of activity and prevent the development of phage-resistant bacterial variants [70]. Several studies have reported the use of this strategy to treat bacterial biofilms [2,10,11,51,71]. For example, Fu *et al.* studied the prevention of *P. aeruginosa* biofilm formation on hydrogel-coated catheters pre-treated with phages [10]. In that work, a cocktail of 5 phages was developed after evaluating their efficacy against phage-resistant biofilm variants recovered from single phage treated catheters. The pre-treatment of catheters with phage cocktail resulted in a reduction of 3 orders-of-magnitude in the number of biofilm cells after 48 h, compared with untreated catheters [10]. According to the authors, the use of phage cocktails could be potentially applied in indwelling medical devices to prevent bacterial colonization and biofilm formation, which might inhibit the development of bacterial infections. Similarly, a recent study evaluated the potential of phage cocktails (a cocktail of six *P. aeruginosa* phages and a cocktail of four *Proteus mirabilis* phages) to prevent single and mixed species *P. aeruginosa* and *P. mirabilis* biofilm formation [2]. Hydrogel-coated catheters were pre-treated with one or both phage cocktails and significant reductions in biofilm cell counts were observed in both cases [2].

Phage cocktails have also been successfully used to target *S. aureus* biofilms. In a biofilm prevention study, crystal violet staining assays revealed a complete inhibition of *S. aureus* biofilm formation over a period of 48 h [51]. Other studies have used phage cocktails to treat pre-formed *S. aureus* biofilms and significant biofilm biomass reductions were also observed [9,72].

Genetic manipulation of phages

Recent advances in biotechnology and synthetic biology fields have enabled the development of a new generation of phages designed for specific purposes. For instance, Lu and Collins genetically engineered the T7 phage to express a biofilm-degrading enzyme dispersin B during phage infection [53]. Thus, the engineered phage was able to simultaneously kill the biofilm cells and degrade the biofilm matrix resulting in an improved efficacy of biofilm removal than the wild type phage [53]. After 24 h of treatment, the engineered phage was able to reduce *E. coli* TG1 biofilms by 4.5 orders-of-magnitude, which was about 2 orders-of-magnitude better than the reduction caused by the non-enzymatic wild type phage [53].

To understand the stability of such mutation in engineered phages, Schmerer *et al.* studied whether the transgene will be lost or maintained during phage replication [73]. Although the engineered enzymatic phage was better than wild type phage in clearing short-term biofilms, no significant differences were observed in clearing long-term biofilms (7 days), indicating that dispersin B has no effect in old biofilms. However, it was further observed that the frequency of the dispersin transgene increased in both short-term and long-term biofilms at least temporarily [73].

In another study reported by Lu and Collins, a filamentous phage was engineered to enhance the efficacy of antibiotic therapy [52]. The engineered phage overexpressed a repressor of the SOS DNA repair system in *E. coli* and its application resulted in an improved activity of antibiotics against antibiotic-resistant bacteria, persister cells and biofilm cells [52].

Pei *et al.* engineered T7 phage to encode a quorum-quenching enzyme (acyl homoserine lactonase (AHL)) that has broad-range specificity for cleaving the lactone rings of diverse AHLs. The engineered phage was able to degrade the AHLs from many bacteria and to inhibit the formation of mixed-species biofilms composed by *P. aeruginosa* and *E. coli* [54].

Conclusions

Phage therapy is an attractive option to prevent and control biofilm related infections. Apparently, due to the close proximity of cells, phage infection of biofilms seems to be very efficient. However, in a closer look, the biofilm phenotype also offers protection to cells against phage attack. The dense biofilm matrix, the low metabolic state of biofilm cells and the rapid proliferation of phage resistant variants are some of the features that hitch biofilm/phage interactions. Consequently, it is difficult to efficiently control a biofilm population with only one phage. Therefore we propose combined therapies to overcome biofilm barriers to phage infection and genetically engineered phages with new functions to turn them more effective biofilm controllers. Further progress is also required to understanding of how bacterial cells respond to different therapeutic approaches, preferentially at a single-cell level. We consider also important to normalize biofilm studies, using biofilm formation set-up that best mimic real biofilm environments.

Conflict of interest

None.

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