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Bacteriophage interactions with mammalian tissue: Therapeutic applications

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ABSTRACT

The human body is a large reservoir for bacterial viruses known as bacteriophages (phages), which participate in dynamic interactions with their bacterial and human hosts that ultimately affect human health. The current growing interest in human resident phages is paralleled by new uses of phages, including the design of engineered phages for therapeutic applications. Despite the increasing number of clinical trials being conducted, the understanding of the interaction of phages and mammalian cells and tissues is still largely unknown. The presence of phages in compartments within the body previously considered purely sterile, suggests that phages possess a unique capability of bypassing anatomical and physiological barriers characterized by varying degrees of selectivity and permeability. This review will discuss the direct evidence of the accumulation of bacteriophages in various tissues, focusing on the unique capability of phages to traverse relatively impermeable barriers in mammals and its relevance to its current applications in therapy.

Contents

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1. Introduction

Numbering over 10^{31} units [1], viruses are the most abundant entities in our biosphere and contribute the largest reservoir of genetic material. Bacteriophages (phages) are the natural predators of bacteria and are prevalent across the globe, including within the human microbiome, where at least 10^{12} viral particles have been found per gram feces in humans [2–5]. In addition to their lytic life cycle where phage infect, replicate, and lyse host bacterial cells, temperate phages may also harbor their genome within the host genome as stable prophages, replicating passively [6]. Ongoing investigations of the human virome have characterized distinct viral communities that are deeply intertwined with their microbial host cells and human cells.

Interest in the application of phage as safe and efficient therapeutic tools continues to grow due to (i) their lack of tropism for mammalian cells, and (ii) an innate capability to penetrate and traverse tissues and barriers. The restriction to infection and propagation in prokaryotic hosts renders human hosts safe from unintentional phage infection, although immunomodulatory phage-mammalian interactions should still be considered. There is a need for prevention of genetic transfer between administered phage and host resident bacteria via transduction, or lysogenic conversion. Undesired or uncontrolled gene transfer could confer virulence or antibacterial resistance among bacterial populations. It has therefore been suggested to use therapeutic phages without transducing potential and to profile the genomes of the phages prior to their application in phage therapies [7].

Phage translocation throughout the body occurs rapidly upon administration [8], and while the route of administration and initial phage titer can greatly influence the pattern of phage distribution [8–10], phage virion particles demonstrate an astonishingly rapid ability to penetrate the vascular endothelium as well as other mammalian tissue barriers. Phage display technology has further expanded the potential therapeutic capacity of phage; conjugation of targeting peptides or antibodies to phage capsid proteins can extend tropism against previously untargeted bacteria, or to specifically bind eukaryotic cells. Such targeting could facilitate sufficient therapeutic titers at the site of disease with low systemic circulation of phage [11]. In this review, we discuss the capacity of phage to penetrate and interact with human tissues and the resulting applications.

2. Phages in the circulatory system

Phages were first isolated in feces in the 20th century [12] and have since been detected in many biomes of the human body including: the skin [13], oral cavities [14], urine [15], respiratory tracts [16], and the digestive tract [17]. Although the mechanism behind the effect has not yet been clearly elucidated, phages are able to rapidly bypass multiple endothelial, mucosal, and/or epithelial cell barriers to achieve systemic distribution [18,19].

While phages are inherently absent in the blood of healthy humans [15], they can been detected in the blood quickly following intravenous administration. Dissemination to other tissues appears to be fairly rapid, with nearly complete recovery of administered phage in the liver, spleen, kidneys, and lungs following just a few minutes of circulation [20,21]. Plasma half-life is dependent on the type of phage and the specific biochemistry of the capsid, but has been observed to range from 60 min (phage T7) to 6 h (phage λ) [22]. The appearance of phages in the blood despite varied routes of administration can be attributed to their ability to translocate across endothelial cell barriers. In a study by Weber-Dabrowska et al., orally administered phages against *Staphylococcus*, *Escherichia*, *Pseudomonas* and *Proteus* could be detected in the blood samples of most patients with septicaemia and urinary tract infections [23]. In another study, fluorescently labeled M13 particles were able to accumulate at sites of bacterial infection within 24 h of retro-orbital injection in mice [33]. Patients with AIDS or cardiovascular diseases have been characterized with a higher percentage of phage DNA compared to normal adult plasma [34,35]. Additionally, the associated bacterial host DNA is often co-detected, which may suggest the translocation of bacteria harboring intracellular phage particles or lysogenized prophages into the bloodstream, as opposed to the independent translocation of phage particles (see Table 1).

2.1. Passage across the endothelial barrier

Phages that have entered the circulatory system will require infiltration of the endothelium to reach the organ of interest and impart its therapeutic effects. The endothelium forms a semi-permeable barrier between blood and tissue where endothelial cells are typically organized in thin layers joined by tight junctions and thus separate blood from tissue interstitial space. Arteries and veins may be surrounded by multiple thick layers of endothelial cells, smooth muscle, and connective tissue; in contrast, capillaries are typically comprised of a single monolayer of endothelial cells as they are the sites of material exchange. Under homoeostatic conditions, only small molecules (less than 70 kDa) extravasate spontaneously across the endothelial barrier into interstitial space [36]; under inflammatory conditions, larger molecules up to 2000 kDa could extravasate spontaneously. Inflammatory conditions may contribute to the increased permeability of the endothelial barriers, as a higher abundance of phages or their DNA have been detected in the blood of diseased patients [9]. The dissolution of cell junctions has been observed in response to inflammatory cytokines, which may contribute to increased paracellular movement and may explain heightened phage accumulation in patients fighting bacterial infections [37].

Molecular weight alone, however, does not fully account for penetration of molecules across the endothelial barrier. Filament or cylindrical nanoparticles demonstrate greater and prolonged penetration in comparison with spherical counterparts [38,39]. For example, filamentous phage fd measures 14,600 kDa [40] but quickly permeates across the endothelium barrier in many vertebrates [8]. The long rod-shaped structure most likely enhances phage penetration across the endothelium. During this process however, extracellular matrix molecules including fibronectin, gelatin, heparin may bind to phage capsids, impeding their infiltration to target sites [41].

It has also long been recognized that endothelial cells making up this barrier are highly heterogeneous in structure [37,42]. In particular, the presence of fenestrae further modulates exchange of materials between the vasculature and the tissue. Fenestral pores (62-68 nm in diameter) enable transport in the fenestrated endothelium surrounding the kidneys, digestive tract, and endocrine glands [42]. Larger fenestrate of up to 200 nm in diameter occur in the discontinuous endothelium, which lines the liver and bone marrow. Although there is a wide range in size and shape across all phage families [43], many phages investigated for use in humans could pass through fenestrae, enabling their rapid accumulation within highly vascularized organs. The ability of phage to passage through the endothelium barrier remains of great interest as it enables penetration into even very inaccessible areas of the body, such as the brain.

Table 1
Tissues and barriers penetrated by phages and their sites of recovery/detection.

Infiltrated tissue/barrier	Route of administration and site of detection	Organism	Phage type	References
Gastrointestinal barrier	Oral, detected in the blood	Mice	T4 phage	(Majewska et al. 2015)
Gastrointestinal barrier Endothelium Glomerular filtration barrier	Oral, detected in blood and urine	Humans	Phages against *Staphylococcus, Pseudomonas, Klebsiella,* and *Escherichia coli*	Weber-Dabrowska, Dabrowski, and Slopek 1987 [23]
Gastrointestinal barrier Dermal tissue	Topical and oral, cleared dermal infections	Humans	Phages against *Psuedomonas, Staphylococcus, Klebsiella, Proteus* and *Escherichia*	Cislo et al. 1987 [25]
Gastrointestinal barrier	Oral, detected in blood (with the inactivation of gastric juice)	Rats	Phages against *Staphylococcus* – A5/80	Międzybrodzki et al. 2017 [26]
Blood-brain barrier –	Intranasal, detected in olfactory bulb and hippocampus region	Mice	Filamentous phage	Frenkel and Solomon 2002 [27]
Blood-brain barrier –	Intraperitoneal, detected in the brain	Mice	*Shigella dysenteriae* phage	Dubos, Straus, and Pierce 1943 [28]
Dermal tissue	Topical	Mice	Phages against *Klebsiella*, Kpn5	Kumari, Harjai, and Chhibber 2011 [29]
Dermal tissue	Topical	Guinea pigs	Phages against *Pseudomonas,* BS24	Soothill 1994 [30]
Dermal tissue	Topical	Rodent and porcine	*Staphylococcus*, *Pseudomonas* and *Acinetobacter* phage cocktails	Mendes et al. 2013 [31]
Lung tissue	Intranasal application, reached the lung	Mice	Phages against *Pseudomonas,* P1	Debarbieux et al. 2010 [32]
Gastrointestinal barrier Lung tissue Kidney tissue Blood-brain barrier	Intraperitoneal, recovered in the lung, kidney, brain, intestines, blood, spleen and liver	Mice	Phages against *Bacillus megatherium*	Keller & Engley, 1958 [21]
Gastrointestinal barrier Renal filter	Oral, recovered in urine samples and the blood.	Mice	Phages against *Bacillus megatherium*	Keller & Engley, 1958 [21]

2.2. Passage across the epithelial barrier

Following extravasation of phage particles across the endothelium, phages can accumulate in other organs, such as the liver and spleen [8,21]. Epithelial cells line the cavities and surfaces of all tissues in the body and serve as a barrier connected by tight junctions. Transport of material occurs primarily through transcellular pathways [44]. In order to transcytose, a particle must first be internalized, then transported through the intracellular trafficking network, and finally released through exocytosis [45]. In general, the epithelium transports materials across through caveolae-mediated endocytosis (CavME) transcytotic pathways, but other pathways are always possible.

Numerous studies have investigated the transcytosis of engineered M13 phages employing phage display library screening, and their uptake via receptor-mediated endocytosis. Non-specific endocytosis has also been suggested to occur as phages or their DNA are often detected in the bloodstream of healthy humans; however, this mechanism is still unclear [46–48]. Nguyen et al. recently described a generalized mechanism by which phages could transcytose in an apical-to-basal direction [49]. Transcytosis of diverse phage types, including T3, T5, T7, SP01, SPP1, and P22 phages in intact epithelial and endothelial cell layers were investigated in vitro. 0.1% of total phages applied to the apical side of epithelial cells were observed to transcytose, as opposed to 0.0008% traversing from the basal side. Phage particles were found in endosomal compartments and associated with the Golgi apparatus, implicating this organelle in the transcytosis mechanism [50].

2.2.1. Cellular internalization of phages

Cellular internalization can occur through multiple pathways, which vary depending on the ligand and the internalizing cell type: phagocytosis, macropinocytosis, clathrin-mediated endocytosis (CME) or other energy-independent endocytic mechanisms [51]. Tian et al. (2015) followed the uptake of fluorescent M13 in both epithelial (MCF-7 and HeLa) and endothelial (HDMEC) cell lines that were subjected to endocytic pharmacological inhibitors [52]. M13 phage particles were found to internalize primarily through CME and macropinocytosis in epithelial cells, but internalized primarily through caveolae in the endothelial cells.

Phages are readily internalized through receptor-mediated endocytosis if conjugated with a corresponding internalizing ligand [53–59]. One study similarly tracked the internalization of M13 that displayed cell-penetrating peptides (CPPs) 3D8 VL transbody or TAT in HeLa cells [60]. Each CPP interacted with distinct surface receptors to initiate endocytosis. Evidently, uptake also proceeded through different endocytic pathways: TAT-decorated M13 was primarily through CME while 3D8 VL-decorated M13 was primarily through CavME. After endocytosis, internalized phage particles localized into endosomal compartments, the endoplasmic reticulum, the Golgi apparatus within 6 h. This may again point to a Golgi-mediated route of transcytosis although the majority of phages were eventually routed to the lysosome for degradation.

A growing body of evidence does suggest that phages are actually inherently capable of binding specific cell-surface receptors, enabling internalization by endocytic vesicles without the engineered display of targeting ligands [61]. Recently, specific binding and uptake of *Escherichia coli* phage PK1A2 into eukaryotic neuroblastoma cells were observed in vitro [62]. This was mediated through adherence to polysialic acid, a nine-carbon monosaccharide bearing structural similarity to capsular polysaccharides present on many bacterial host - a common route of entry for eukaryotic viruses [63]. The internalized phages were directed to the endosomal route and localized in lysosomes, remaining infective for up to 24 h without impacting cell viability. The authors raised the possibility of other eukaryotic cell surface epitopes for phages that bear structural similarity to polysaccharides present on bacterial hosts, which certainly warrants further investigation [62]. β3 integrins present on cell surfaces are another set of possible

receptors enabling phage uptake, as several studies have supported β3-specific interaction with the KGD (Lys-Gly-Asp) motif on the T4 phage capsid [64,65].

Finally, the presence of homologs in phages and eukaryotic cells has been suggested as indirect evidence of phage internalization into eukaryotic cells. Homologs of the major capsid protein gene for *Chlamydia*-infecting phages and of aerolysin-like genes have been found in the genome of eukaryotes [66]. Conversely, eukaryotic-like domains have been uncovered in bacteriophage genomes, which may be indicative of bi-directional lateral gene transfers [67]. This could be particularly worrisome for future therapeutic phage applications as unintended horizontal gene transfers can have significantly detrimental consequences and must be avoided as much as possible.

2.3. Immune responses in the blood

In the bloodstream, phage particles rapidly activate the immune system and modulate its activity, although the specifics of these interactions are still unclear. Multiple studies agree that clearance of phages by nonspecific defences poses a major problem in maintaining sufficient phage titers for therapeutic activity [7,68].

2.3.1. Phage clearance

The reticuloendothelial system (RES) of the spleen and liver is the primary immune compartment responsible for the clearance of phage, reducing phage concentrations below clinically useful levels. Kupffer cells, which are mononuclear phagocytes from the liver, have been shown to phagocytose T4 phages following intravenous administration [69]. Newer reports have shown that B cells are also implicated in phage clearance, although this may be mostly phage-specific [68]. It is also hypothesized that phages have prolonged circulation in immunodeficient or immunosuppressed individuals due to lack of clearance by immune pathways [70,71]. T7 phage circulation was prolonged in B cell-deficient mice, although mice characterized by other types of immunodeficiency exhibited comparable levels of phage clearance to wild-type mice [68]. Rituximab, an antibody against the B cell CD20 antigen, was implicated in the inhibition of the antibody response against phage φX174 [70], a phage commonly used in the assessment of the humoral response. Modifications to the phage capsid could improve phage half-life, as phages with mutations in their capsid proteins were capable of evading RES, prolonging circulation and thereby prolonging their downstream therapeutic effects [72–74].

Despite the challenges of bioavailability presented by the phage-host defence interactions, phages have achieved success in eradicating various blood-related pathogenesis. 85% of 94 sepsis patients with either one or mixed infections of *Pseudomonas aeruginosa, Escherichia. coli* and/or *Klebsiella pneumoniae* who were not responsive to antibiotic therapy achieved complete recovery after oral administration of phage therapy three times daily [75]. These patients experienced significant reduction in temperature, leukocytosis, and sedimentation rates.

Constant exposure to phages in the environment has resulted in the presence of anti-phage antibodies in human blood. Anti-T4 antibodies have been detected in 81% of healthy humans never subjected to phage therapy, likely a result of natural contact with phages and from infections by their relevant bacterial hosts [76]. Application of large phage titers will induce the production of neutralizing antibodies against the phages, which will undoubtedly deactivate and reduce their therapeutic efficacy [77]. Different routes of administration appear to generate different outcomes in antiphage antibody production. One study showed that oral *Staphylococcal* phage preparations resulted in significantly lower levels of antiphage antibodies in sera compared to local administration [78]. This many not pose a great problem, as Sulakvelidze et al. (2001) argued that neutralizing antibodies should not significantly impede phage activities, as phage kinetics are much faster than the hosts' production of antibodies [7,79]. In any case, reduced rate of neutralization can be achieved by increasing the frequency of administration or by selecting for phages possessing different antigenic profiles while maintaining their host range [79]. While the loss of phage activity is not a safety concern, the repercussions of phage-antibody complexes should be carefully assessed, as their deposition in the renal glomerulus can cause acute and chronic glomerulonephritis in the patient [11,80]. To date, clinical trials have not shown evidence of immunological complication in patients subjected to phages [71], and the use of phage therapy continues to be prevalent in Eastern Europe, particularly the Eliava Institute in Georgia [81–83].

2.3.2. Phage immunomodulation

To date, there have been conflicting reports on the potential anti-inflammatory action of administered bacteriophages. Bacterial lipopolysaccharide (LPS) elicits a strong inflammatory response in animals and humans and could alter the immunomodulatory effects of phage therapy preparations. Tetz et al. (2017) demonstrated that the oral administration of a phage cocktail in mice resulted in an increased gut permeability and endotoxemia [84]. This study also noted increased serum LPS levels, but whether this originated from gut barrier disruption or the phage cocktail itself remains unclear Past reports of anti-inflammatory responses following phage administration have attributed it to a direct phage modulation on cytokine activity; however, most of these remarks pertain solely to studies measuring cytokine levels in vitro [85]. Van Belleghem et al. (2017) demonstrated overall anti-inflammatory (up-regulation of IL-1RN, SOCS3) but also some pro-inflammatory effects (up-regulation of IL-1α, IL-1β, TNFα) after administration of highly purified phages with and without endotoxins [86]. Zhang et al. (2018) demonstrated that phages could reduce the levels of LPS-induced inflammatory cytokines, such as TNF-α, IL1β, IL-6, IL-8 and IL-10 in vitro, but the sole administration of purified phages did not induce changes in these cytokine levels [87]. Most in vivo studies to date describing phage-induced anti-inflammatory effects often report the simultaneous clearance of bacterial infections, which may in fact be the driving force in relieving inflammation [88,89].

Interestingly, purified phage T4 administration appears to exert anti-inflammatory activity to some levels as a recent in vivo study revealed that T4 purified phages were able to significantly slow the course of murine collagen-induced arthritis [90]. This may be induced by the binding of T4 gp12 to LPS, which has been previously shown to reduce inflammatory cytokine levels, including IL-1α and IL-6, in mice [91]. Intraperitoneal application of purified T4 phages has also been shown to inhibit reactive oxygen species (ROS) formation by cells exposed to endotoxin, which may be useful in patients with conditions such as sepsis who are particularly sensitive to excessive ROS levels and oxidative cell death [92]. Other reported outcomes of phage therapy include accelerated turnover rate of neutrophils [88,93], increased numbers of myelocytes [88], reduction of cytotoxicity and bacterial invasion [89], and reduction of T-cell proliferation in vitro [94].

Phages overall possess inherent immunomodulatory that can also be "weaponized" against specific cancers via display of tumour-specific targeting ligands. Tumour-specific phages developed by Eriksson et al. demonstrated significant tumour regression when delivered intratumorally [95]. In this approach, hybrid adeno-associated virus (AAV)/phage particles encoding *TNF*-α and displaying an integrin binding motif (RGD) were targeted to tumour vasculature and were able to successfully reduce the tumour burden in a melanoma xenograft model [96]. In a pre-clinical trial in dogs, these targeted vectors (RGD-A-*TNF*) were found to be well-tolerated even at doses of 5×10^{12} units and found to specifically localize within tumour vasculature [97]. Follow-up in vivo studies and the associated immune responses and role in human health is warranted to further the potential uses of phages in therapy.

3. Phages in the gut

The ever-growing number therapeutic applications for phages have warranted pharmacokinetic studies of phages following various routes of administration. Fortunately, the previously unknown realm of phage safety and efficacy is gradually being strengthened by randomized, double-blind and placebo-controlled phase I/II clinical trials of phage therapy [83,98,99]. Oral phage administration is generally regarded as safe and often the most convenient for patients, and has been the primary route for eradicating both local infections in the gastrointestinal (GI) tract and in other extraintestinal tissues [81,83,100–105]. In addition to phage therapy, phage preparations for biocontrol to improve food safety has been approved by the FDA as "generally considered as safe," and are available commercially [106,107].

Phage applications targeting extraintestinal tissues are more challenging due to the multitude of cellular barriers that have the potential to degrade exogenous phage particles. To target these sites via the oral route, all phage particles that enter alongside or independent of host bacteria must pass through the stomach into the intestines then cross the gut mucosa prior to systemic circulation. Therefore, the gastrointestinal system could be considered as the first and primary barrier against phage tissue penetration.

3.1. Passage across the gut

3.1.1. Passage across the gut mucosal barrier

Bioavailability is significantly compromised in oral administration relative to intravenous administration [26]. Oral administration requires phage preparations to be able to withstand the harsh conditions of the stomach, which is highly acidic, anaerobic, and proteolytically active [108,109]. The combined effects of the unfavourable conditions with active immune clearance can drastically reduce phage titers to be pharmacologically ineffective. Phages are most stable at neutral pH of 6-8, and titers are generally impacted with decreasing pH; this can pose a major problem in the stomach, where the pH is estimated to be about 1-2 unless a neutralizing agent, such as $CaCO_3$, is applied simultaneously [100,109]. Suggested modifications to increase stability in harsh GI-like conditions include phage encapsulation in whey alginate [110], liposomes [111], and other biopolymeric matrices [112]. Miedzybrodzki et al. (2017) demonstrated that phage T4 and antistaphylococcal phage A5/80 titers decreased by almost 10-fold at pH 5 over the course of an hour, but pre-treatment with Alugastrin, ranitidine, or yogurt was able to significantly enhance phage transit from the stomach to other sites [26]. These phages not only retained their activity, but were also able to reach further parts of the gastrointestinal tract.

In extraintestinal applications, penetration of the gut mucosal barrier is a prerequisite for effective oral delivery. The mucosa in the GI tract consists of the epithelium, lamina propria, and the muscularis and compartmentalizes gut bacteria to the lumen [113]. The intestinal epithelium is a relatively impermeable physical barrier that lines the small and large intestines and serves as a protective barrier between the lumen and tissues to prevent direct contact between foreign particles and pathogens with the underlying epithelium [114]. It is composed of a single layer of columnar epithelial cells joined by tight junctions, containing absorptive enterocytes that constitute approximately 80% of all epithelial cells, and embedded by both M cells and goblet cells [115]. The epithelial tissues secrete heavily glycosylated mucin molecules, which combined with the gut commensal bacteria, make up the "mucus". While most mucins are secreted, 10% are membrane-tethered [116]. Ingested phages in mice were found to be strongly associated with the mucosa in the lumen of the large intestine [117]. Hypervariable Ig-like capsid proteins exposed on T4 phage have been shown to mediate the interaction with the glycans of glycoproteins in the mucosa, resulting in a high concentration of phages in the mucus layer [118]. The ability of phages to adhere and pass the mucosal barrier may be dependent on the specific protein sequences on the capsid [9,119].

The integrity of the mucosal barrier is also substantiated by the flora in the lumen, as inoculation of commensal bacteria in germ-free mice have shown to improve host nutrient absorption and processing [120]. The gut microbiota is therefore thought to play an important role in restricting the passage of exogenous phages and can control the permeability of the intestinal barrier. A previous study has shown that colonization of germ-free mice with commensal bacteria can also lead to hyperplasia of the epithelial and goblet cells that can increase the overall surface area of the intestinal barrier [121]. Following interactions with the mucosal barrier, majority of the adherent phages will be degraded or excreted from the body [10].

There are few studies that have evidenced detection of phages in the blood after oral administration, including *Staphylococcal* phage A5/80 [23,24,26]. The mechanism of this passage remains unclear and appears to be both phage- and host species-specific [26,122]. Phages A5/80 and T4 are similar in size and morphology, but despite the initial 1000-fold higher dosage of T4 administered to mice, only trace amounts were detected in blood compared to A5/80 [26]. Additionally, rats were not susceptible to passage of phages through the intestinal barrier, with neither phages recovered in the bloodstream, whereas a contrasting result was observed in mice. A proposed explanation for this phenomenon was the possible adsorption of phages to dead bacteria leading to irreversible phage inactivation, and the inherent difference in microbial composition between the two species,ultimately resulting in different interactions and outcomes for the two phage types [26,123].

Passage of foreign molecules into the bloodstream can be paracellular, transcellular or mediated by M-cells. Tight junctions in the apical side of the epithelial cells restrict the paracellular flux of foreign particles, restricting the size to less than 10 nm [124]. Most phage particles are too large to penetrate through the gut epithelium through this method. While epithelial cells carefully control the passage of substances between the lumen and the blood epithelial cells in healthy individuals, damaged guts have been described as "leaky", whereby phages may paracellularly pass through the spaces between the epithelial cells, a process known as translocation [21,125–127]. This can result in the presence of bacteria and phages in blood [126]. Transcellular transportation is an alternative pathway for the passage of bacteria and associated phage from the intestinal mucosa into systemic circulation; however, this method requires specific receptors for transport, and is an unlikely method of phage transit. It is possible that phages are able to cross the barrier via the M cell-mediated pathway [128–131]. M cells are specialized epithelial cells that act as the antigen sampling system and possess a high capacity for transcytosis of microorganisms for the induction of immune responses [132,133]. M-cell-mediated entry from the intestinal lumen to lymphoid tissues of HIV [134], influenza virus [135], polio virus [136] and reovirus [137] have been described. However, this method of transcytosis is likely to occur less frequently due to the low (<1%) proportion of M cells in the epithelium, and because the overall number of transported phage can be limited by the possible uptake by the macrophages or dendritic cells [26,44].

3.1.2. Passive transport down the gut lumen

Although passage across the intestinal barrier is indeed possible, most evidence suggests that oral application of phage leads to passive transport through the gut and subsequent recovery in the feces. Overall, penetration into the bloodstream is not clearly defined; there exist examples that show recovery of phages in the blood [21,23], as well as studies that do not [72,81,83,117,138], which make it difficult to compile these results into a final discernment.

In a study by Bruttin and Brussow (2005), phage was recovered in the feces of all fifteen healthy human volunteers receiving 10^5 PFU/ml of T4, one day following administration [139]. Interestingly, the administered phage count was very close to the fecal phage count and the fecal

E. coli counts remained the same, evidencing that the administered phage did not replicate within the commensal *E. coli* population. The absence of any anti-T4 immune response in this study suggested that the phages did not enter the bloodstream, which is in agreement with another study involving 120 patients with ETEC or EPEC bacterial infections [81]. This particular study also reported correlating fecal titers to the applied phage dosage, further supporting the theory of passive transit of orally applied phages. It appears that the presence of phage-susceptible bacteria does not directly enable self-propagation of phages in situ [140]. It has been hypothesized that in vivo phage replication requires the target bacterial population to be metabolically-competent to permit phage replication, regardless of their high susceptibility to phage infection in vitro. A large majority of bacterial species in the gut are inactive due to nutritional deprivation and/or do not meet the minimum bacterial density to support phage replication [141]. The minimum threshold of *E. coli* density to support the replication of T4 phages is about 10^4 CFU/ml in laboratory conditions, but this will be higher in vivo due to a heterogenous microbial environment [10,142]. Therefore, the lack of interactions with the intestinal mucosa may be one of the explanations for the low/absence of phage titers in the bloodstream following oral administration, resulting in direct transit through the intestinal lumen to be recovered in the feces.

A high repeated dosage can, however, permit the translocation of phages to circulation followed by an increase in antiphage antibodies [24]. Majewska et al. (2015) reported that 240 days of daily treatment with high doses (approximately 2×10^{10} PFU/mouse) of phage T4 in mice resulted in relatively high phage recovery (10^3 PFU/ml) in the blood, whereas a lower dosage of 10^9 PFU/mouse did not [24].

3.2. Immune responses in the gut

The gut is home to one of the largest lymphoid tissues in the body, collectively known as the gut-associated lymphoid tissue (GALT). Phage interactions with immune cells in the gut may limit the viability of the applied phages and consequently the bioavailability of phages. Epithelial cells have constitutive expression of CD41a and CD61, which may be relevant to phage T4 binding [9]. IgA is one of the hallmarks of the humoral immune system, produced by plasma cells in the lamina propria. The epithelial cells also possess TLRs and NOD-like receptors that can recognize pathogen associated molecular patterns (PAMPs). Upon recognition of foreign molecules by epithelial cells, cytokines, such as IL-6 and TGF-β can enhance IgA secretion and suppress IgM secretion by B cells, which are in close proximity to intestinal epithelial cells in the lamina propria [143]. Intestinal barrier disruption by foreign particles can elicit immune responses such as IgA production, which can upregulate T and B cell production. Oral phage application has only shown weak immune responses in several studies, suggesting that the slight increase of antiphage antibodies does not actually impede the outcome of phage therapy, unless administered in high doses for a long period of time [78,83,144]. One study investigating the production of antiphage antibodies in 122 patients showed that phage inactivation was very low in patients treated with phages orally [145]. However, long-term oral administration of a Staphylococcal phage cocktail induced high production of IgG and IgM [144]. Similarly, Majewska et al. (2015) demonstrated that when a high oral dosage of phage T4 was administered over a long period of time, IgG and IgA were induced after 36 and 79 days, respectively, with increased IgA levels hindering the gut transit of T4 [24]. Antiphage antibody response appears to be phage type-dependent, as phages will harbour different surface proteins conferring various levels of immunogenicity [24].

The resident gut phage community in the gut play a specific role in health and disease, even providing defence to the human host from bacterial pathogens. Barr et al. (2015) describe a model whereby the adherence of phage to the mucosa (BAM model) to confer immunity and antimicrobial defense to the underlying human epithelium from pathogens [80]. It has later been proposed that mucus-adherent phages exhibit a reduced diffusive motion that may increase the chance of encountering a bacterial host before it leaves the mucus. This subdiffusive motion may be beneficial in the gut environment where there are lower bacterial concentrations and enable a more "thorough" search of the local area for the host [146]. The aforementioned coliphages such as T4, that appear to exert immunosuppression, may also play a role in downregulating gut immune cells and prevent inflammation [147]. Phages can also contribute in our defences against invaders not only by lysis of pathogens, but by remaining integrated in the commensal bacteria as prophages and conferring immunity to the host from related phage types, as well as through horizontal gene transfer of beneficial genes between the bacterial hosts [148,149]. The balance between lysis and lysogeny of phages must be maintained for the healthy gut and the disruption of the gut phage population has been correlated to disease [6,17,150,151]

4. Phages in the brain

The blood brain barrier (BBB) is a group of endothelial cells that present perhaps the greatest endothelial barrier in general drug delivery. Typically, molecules capable of passing through this barrier have at least one of the following characteristics: they are less than 500 Da; they are very lipophilic; or they are structurally similar to compounds that enter the brain through active transport [152,153]. This tightly regulated system poses a serious obstacle in drug delivery to the central nervous system (CNS), and prevents reliable treatment for many CNS disorders [152]. Phages have long been recognized for their ability accumulate in the brain, bypassing this barrier with apparent ease [8,10]. Their use as CNS drug delivery vehicles is therefore of particular interest.

4.1. Passage across the blood brain barrier

Overcoming the BBB hurdle has been attempted in many ways, with many novel approaches utilizing phages [28,152,154]. Evidence of phage transport across the BBB was first described in 1943 by Dubos et al. [28]. They noticed that when phages were injected intraperitoneally into mice, phage populations could be isolated from the brain as early as 1 h after administration. As might be expected, phages persisted for longer periods of time and in higher concentrations in the presence of their bacterial host (*Shigella dysenteriae* in this study).

Frenkel and Solomon first reported the surprising ability of filamentous phage M13 to penetrate the brain in 2002 [27]. Despite its large size (900 nm in length), the phage could accumulate in the brain shortly after nasal administration. Wildtype M13 were detectable in the hippocampus and olfactory bulb of BALB/C mice after intranasal administration of 10^{11} phage particles. Frenkel and Solomon attributed the ability to penetrate the BBB to the linear structure of the phage, a conclusion that has since held as phage M13 most likely penetrates the endothelium elsewhere by the same mechanism. They observed that spheroid phages were unable to exhibit the same level of penetration. Ksendzovsky et al. (2012) more recently improved transport of M13 to the brain by using convection-enhanced delivery (CED), which had previously showed success in improving brain transport with much smaller viruses and nanoparticles [155]. While phage titers were not reported, phage M13 were found to successfully distribute across grey and white matter including frontal white matter, suggesting that active axonal transport mechanisms such as axonal transcytosis may play a role in moving phage within the brain.

Phage display of BBB-penetrating peptides can further increase accumulation and prolong phage half-life in the brain, resulting in more effective concentrations [11]. Conjugation of an N-terminal transferrin motif (CRTISGPSVC) to the capsid of an M13 phage derivative enabled receptor-mediated transcytosis through the BBB, thereby improving phage penetration by a factor of 100 [156,157]. Infiltration was greater in orthotopic mouse models of glioblastoma, which is characterized by increased expression of transferrin receptors [157]. Biopanning [158]

efforts have led to numerous potential BBB-penetrating peptides [159]. Urich et al. used phage T7 for in vivo functional screening of cerebrospinal fluid (CSF) homing peptides, observing that while wildtype phage T7 half-life had a half-life of approximately 25 min in CSF, four rounds of panning yielded decorated phage that could persist in CSF for over 40 h.

4.2. Therapeutic use of phages in the brain

In addition to its BBB-penetrating capacity, phage M13 has furthermore demonstrated intrinsic characteristics that are ideal in the treatment of neurodegenerative diseases, including Parkinson's disease (PD) and Alzheimer's disease (AD). Dimant et al. (2009, 2010) observed the inhibitory effects of phage M13 on α-synuclein aggregation, a hallmark of PD, in an SH-SY5Y cell line model [160,161]. Although the mechanisms behind these disaggregation effects were not deeply investigated, the authors postulated preferential interactions between the fibrils and the phage. In a separate study, M13 was shown to remodel and repair amyloid β plaques through a general amyloid interaction motif (GAIM) present on its pIII capsid protein [162]. This holds great promise for novel phage-based GAIM therapeutics as they can also target and dissociate misfolded proteins in AD, PD, and prion diseases [163].

The inherent natural specificity of phages toward their bacterial prey enables their employment as detectors of bacterial infection in the CNS, such as for *Tuberclulous meningitis* [164–171]. Bacterial detection can mediated through the use of reporter phages carrying the firefly luciferase gene (*luc*) [164,167–174]. This technique has also been used to detect *Listeria monocytogenes*, *Yersinia pestis*, *Bacillus anthracis* and *Mycobacterium tuberculosis* [164–171]. Alternatively, reporter genes in phage could also be used for imaging cellular distribution within the CNS in vivo, providing an inexpensive alternative to MRI and PET scans [175].

Interestingly, phage fd was used in a proof of concept in the treatment of chronic addictions [176]. Carrera et al. conjugated anticocaine antibodies to the major coat protein of phage fd. When delivered intranasally, the phage-antibody conjugates were able to pass through the BBB and block the psychoactive effects of cocaine in a murine model. This technique could potentially be extended to other addiction treatments, such as opioid use, which has been a growing issue over the last two decades [176,177].

This same technique can be applied to deliver other genes directly to the CNS for the treatment of various neurological disorders, including mood and anxiety disorders, which are among the most common mental disorders [59,178]. Preliminary results of gene transfers utilizing herpes simplex virus vector have been positive in mood disorders; however, phage-based therapies have yet to be investigated [179]. Phage-BBB interaction is still a hugely lacking research area with tremendous potential in the future of neurological research both in terms of treatment and diagnosis [28,162,176,180]. Increasing the penetrative ability of phage is still an ongoing challenge and will be very necessary in order to increase their own therapeutic activity and/or their therapeutic ferrying ability in the CNS [157,181].

5. Phages on the skin

The two major routes of phage delivery under study are oral and intravenous administration. Direct delivery of phages directly to an organ of interest – bypassing vascular and endothelial barrier obstacles – could prevent loss of phage titer to immune and RES clearance and could therefore yield a greater therapeutic effect. However, localized injection to certain inaccessible sites is not favourable as this tends to be invasive and damaging to the surrounding tissue [182]. In contrast, localized, non-invasive application of phage to readily accessible organs, including the skin, holds much greater promise.

5.1. Passage across the skin

Human skin serves as the first line of defence against pathogens as a physical barrier, and it is composed of various layers. While literature on how phage might traverse these layers is scarce, it is now very well known that phage therapy can be highly effective against skin bacterial infections [104]. Bacterial infections can arise from the epidermis, dermis, subcutaneous and adipose tissues and muscle fascia and multiply in the regions of disruption in the integumentary barrier [183]. While applications of phage in treating dermal wounds and infections have been well documented in the past decade, the mechanisms behind their ability to infiltrate past layers of dermal tissue has not been well investigated. Topical applications of phages in hydrogel formulations to treat *Klebsiella* infections in mice have also effectively reduced mortality, once again confirming phage ability to penetrate layers of dermis [29]. Full-thickness burn wound infections were induced in mice, and *K. pneumoniae* was applied topically to the defective site. Klebsiella phage Kpn5 provided protection on the first day (100%) and declined overtime, but still provided the highest level of survival by day 7 (63%), as compared to the untreated group (0%). Once released from the hydrogel formulation, the phage were able to penetrate the infected area and infect the bacterial host to effectively prevent bacteraemia.

5.2. Therapeutic use of phages on the skin

Phages are ubiquitous to the surface of the skin as are their host bacteria, such as *Propionibacterium* or *Staphylococcus* bacteriophage. [13]. Burn wounds commonly result in bacteremia and septic shock, as opportunistic bacteria colonize and rapidly multiply in the damaged tissue rendered immunosuppressed and eventually disseminate systemically [184,185]. Phage therapy in wound-healing applications initially began in the former Soviet Union and has since been gaining popularity in the last decade. In addition to burn wound experimental models, phages have shown to effectively combat dermal bacterial infections caused by chronic illnesses, such as diabetes mellitus. Mendes et al. (2013) observed smaller epithelial and dermal gaps as a result of phage therapy in *S. aureus* and *P. aeruginosa* infections [31]. Wounds were inflicted in rats with chemically-induced diabetes mellitus, and were inoculated with *S. aureus, P. aeruginosa*, or *A. baumannii*. 4, 5, and 8 days post-wounding. Debridement was performed, and phage cocktails were administered topically twice-daily. The dissemination of phages to the sites of bacterial hosts resulted in the significant decline of colony counts in all three test groups by day 4, in addition to the overall reduction in the wound surface areas caused by *S. aureus* and *P. aeruginosa*. Again, interspecies differences played a role in the outcome of phage therapy was apparent, as the results were not as pronounced in porcine models as compared to rodents.

Phage therapy in experimental animal models holds an impressive record, but the penetrative and other pharmacokinetic parameters in human skin infections have not been extensively studied. Soothill et al. (1994) demonstrated the ability of lytic phages to permeate dead dermis to prevent skin graft destruction by *P. aeruginosa* [30]. Skin grafts of guinea pigs extending down to the subcutaneous fat was excised, and was inoculated with bacteria (10^6 CFU), followed by the replacement of the graft back to its initial position. Co-administration of 10^7 PFU phage permitted graft survival, otherwise failure of graft uptake was observed uniformly in all the guinea pigs. Phage therapy in clearing human wound infections is an emerging area of research in North America. As an early example of topical applications of phage in humans, Cislo et al. (1987) reported the use of phage therapy for faster healing of chronic suppurative skin infections caused by *Pseudomonas, Staphylococcus, Klebsiella, Proteus* and *Escherichia* [25]. Improvements – ranging from suppression of local inflammation, fully negative bacteriologic tests, and faster healing of ulcers – were observed in 25 out of the 31 participating patients. The follow-up study published in 2000 expanded on this and reported full recovery of 85.9% cases in 1307

patients with suppurative bacterial infections [186]. A phase I clinical trial was set out in 2009 by Rhoads et al. to examine the safety profile of *S. aureus*, *P. aeruginosa*, and *E. coli*-targeting phages in 42 patients with chronic venous leg ulcers, and found no adverse events associated with the therapy [187].

While phage therapy is widely acclaimed in Eastern Europe, there are no phage products commercially available for human therapy in the West, mainly due to the lack of controlled, randomized trials, with little English translation of the existing documentation [188]. The Eliava Institute in Georgia developed a commercially available, topical phage application called the PhageBioDerm: a biopolymer bandage consisting of a cocktail of lytic phages, antibiotics, and analgesic to treat burn wound victims with a sustained release following its application [182,189]. This particular formulation of phages is referred to as "pyophage," which is comprised of phages that target *Staphylococcus, Streptococcus, Pseudomonas, Proteus* and *E. coli*, and is continuously updated every 6 months to offset any form of resistance that may arise [189,190]. There is a small, but growing, number of companies established in North America that have begun controlled trials for products for phage therapy under various stages of development; including a phage preparation for venous leg ulcers (Intralytix, n.d.). Currently, there is an ongoing phase I/II clinical trial called Phagoburn – a collaborative project in Europe that was initially launched in 2015 involving 220 patients that evaluates the safety and effectiveness of phage cocktails to target *E. coli* or *P. aeruginosa* in burn wound infections (http://www.phagoburn.eu).

6. Phages in the respiratory system

Another relatively accessible organ for phage delivery is the lungs. Phage delivery into the lungs can occur through multiple routes. After oral or intravenous administration, phage can enter the lungs via the circulatory system. Alternatively, phage may be delivered directly to lung tissue either nasally or by injection. While there is a growing body of evidence demonstrating successful phage accumulation in the lungs after oral exposure via the bloodstream, presentation of sufficient numbers of phages to respiratory epithelial tissue is probably better achieved through nasal or tracheal delivery [8,119,191].

6.1. Passage into the lungs

Aerosol preparations of phage T-2r have been reported as early as 1956 [192,193]. Liquid aerosol preparations have long been studied, generally against *P. aeruginosa* lung infection models [32]. Intranasal instillation of liquid phage suspensions greatly improved survival in mice infected with *P. aeruginosa* [32]. Phage lysis of bacteria began as early as 6 h after intranasal administration [32]. The same group later reported that a single dose intranasal treatment of phage could fully treat or prevent *P. aeruginosa* infection in mice [194]. However, controlled intranasal instillation is easier to carry out in animal models so it may not necessarily reflect the success of aerosol drug delivery in the clinical setting.

Nebulization of phages holds great promise, particularly as nebulized antibacterials have been previously met with positive results in cystic fibrosis patients [192,195,196]. Golshahi et al (2008) investigated nebulization of *Burkholderia cepacia* complex (BCC) bacteriophage, assessing two nebulizers (Pari LC star and eFlow) and their effect on resultant active phage titers [195]. They found that the BCC phages were able to survive nebulization and neither nebulizer substantially decreased the active phage titer. Another study investigated nebulization of a phage cocktail against *P. aeruginosa* using a Porta-neb nebulizer [197]. Again, no significant difference was found in the total number of infective phages before and after the nebulization process and delivery.

In contrast to liquid aerosols, formulation of dry inhalable powders has great advantages for long-term stability [198], storage, and transport [196]. Golshahi et al. (2010) reported an aerolizing dry powder formulation of phages KS4-M and ΦKZ [199]. Lyophilization with lactose and lactoferrin resulted in a titer loss around 1 log PFU/mL, but aerosol delivery did not result in significant decrease in predicted lung deposition of phage. Matinkhoo et al. (2011) formulated dry powders of individual phage suspensions and phage cocktails including phages KS4-M, KS14, ΦKZ/D3, ΦKZ/D3/KS4-M by spray drying, with excipients trehalose and leucine [200]. Similarly, they showed a titer loss less than 1 log PFU/mL after formulation. Vandenheuvel et al. (2013) explored formulations of dry powders across a range of phages by spray drying, with multiple saccharide excipients [201]. Leung et al. (2016) compared spray freeze drying against spray drying to produce phage powders, observing a higher retention of phage particles through spray drying [196]. More recently, Chang et al. (2017) investigated seven spray dried phages against *P. aeruginosa*, comparing excipients trehalose, lactose, and leucine [202]. Excipient composition greatly influenced the degree of titer loss [201,202] after spray drying and the resultant stability of the phage formulation [198,203]. While direct delivery of phages to the lungs through inhalation therapy could improve accessibility of phages to sites of infection, one major limitation is the possibility of heterogeneous placement of the phages, rendering treatment less effective [119,191].

6.2. Therapeutic uses of phage in the lungs

Perhaps the earliest reported use of phages against in the lung may be in a 1936 case where infection by a "colon bacillus" was treated by directly applying a "colon lytic filtrate" to the infected area [204]. Notably in this case, the patient experienced high fever during the course of treatment, possibly due to LPS presence in the phage preparation. Since then, studies have reported overall high success rates in treating lung infections with phage therapy (reviewed more thoroughly elsewhere [191]).

The use of phages appears to be particularly effective in the treatment of *P. aeruginosa*, an opportunistic bacteria commonly found in the lungs of patients with cystic fibrosis [205]. It has been shown that phages are able to serve as both treatment and prophylactic for these infections with high success rates. *Burkholderia cepacia*, another opportunistic bacteria seen in the lungs of cystic fibrosis patients, was also effectively treated using a phage therapy approach. In addition to cystic fibrosis, phages have been able to successfully treat *Pseudomonas fluorescens* and *Staphylococcus lentus* respiratory infections [206].

Phage S13 has also been shown to reduce septicemia from opportunistic *Staphylococcus aureus* infection in the lungs of patients with cystic fibrosis [207]. Intraperitoneal treatment with S13 was shown to decrease lethality and increase phage count in the lungs, spleen and other organs. Interestingly, even though the phage count increased in the lungs, the bacterial count did not change significantly. This was not the case in other tissues and a possible explanation may be related to an overall poor distribution of phage in the lungs due to the administration route (intraperitoneal, rather than nasal or tracheal).

More recently, a group investigated intranasal lytic phage therapy against carbapenem-resistant *Acinetobacter baumannii*, a major causative agent in hospital-acquired infections, in mice [208]. They observed greater therapeutic effect against *A. baumannii*-mediated pneumonia when the phage was delivered earlier than later (1 h post-bacterial challenge compared to 4 or 24 h post-challenge), underscoring the importance of early treatment. Interestingly, they found that the delivered phage persisted at high titers even after 24 h, suggesting that most phage clearance occurs in systemic delivery.

As is often the case, phage therapy can be highly effective in clearing some respiratory infections while being completely ineffective for others, illustrating a knowledge gap that requires extensive investigation before phages can be used clinically as an antibacterial treatment in the respiratory system.

7. Targeted distribution of phage

Phages are exceptionally simple genetically and therefore, exceptionally easy to re-engineer for infinitely many functions. Of particular interest here is the ability to engineer phages for targeted cellular uptake. Phage display can theoretically enable receptor-mediated internalization into any cell depending on the conjugated peptide, while the phage genome can be modified to carry any gene of interest [11,209]. While many of these engineered phages have only been examined in vitro, they hold much promise for future therapeutic phage applications.

Since the majority of phage applications have concentrated on modifications of the phage capsid, the genetic material is simply unnecessary. Phages gutted of their genome are not only non-replicative, but can instead encode a gene of interest for transduction as gene delivery vehicles. These phage vectors can exploit the penetrative ability of phages for targeted gene delivery. Incorporation of eukaryotic viral components can furthermore confer mammalian cell tropism upon phages and has seen much success. Filamentous phage display of the adenoviral penton base was observed to improve cell attachment, internalization, and nuclear entry for successful gene transduction [210]. The adenoviral penton base facilitates escape from the endosomal compartment, improving intracellular release of phage [211]. Extending this further, Hajitou et al. developed hybrid phage/viral vectors that employed both peptide and genetic elements derived from adenoviral associated virus (AAV) [212–213]. Display of an AAV-derived peptide sequence stimulated endocytosis of the chimeric phage particles and greatly improved internalization efficiency, although the majority of internalized hybrid phage particles remained trapped in the endolysosomal compartment [214].

Phage T4 was also engineered for targeted cellular uptake. Modified T4 particles were decorated with cell-penetrating peptides (CPP) or targeting ligands and packaged with a luciferase expressing plasmid for transgene delivery both in vitro and in vivo [215]. T4 heads decorated with CPPs TAT or Antp were efficiently endocytosed by HEK293T cells with efficient transgene expression. T4 particles targeted to dendritic cells were internalized specifically by in vitro dendritic cells and in a murine model. Their gene delivery capacity was demonstrated with the delivery of multiple reporter genes. Phage T4 heads simultaneously packaged with luciferase and GFP plasmids and decorated with β-galactosidase molecules were able to successfully deliver all three markers to cells in vitro. Phage T4 is a very promising targetable high-capacity gene and protein delivery vehicle, particularly in cancer.

Phage T4 anticancer activity was observed in vivo as early as 1940. Most notably, the KGD motif capsid protein gp24 of phage T4 has integrin targeting capabilities. A substrain of phage T4, HAP1, was investigated for its affinity for melanoma cells [65], mediated through interactions between phage capsid protein gp24 and β3 integrin. Intraperitoneal or intravenous administration of purified T4 reduced tumour burden in a melanoma mouse model in a dose-dependent manner [216] while non-purified lysates stimulated tumour growth, an effect attributed to the presence of bacterial cell components. In contrast, oral administration of both purified and non-purified HAP1 lysates both reduced tumour burden similarly [217]. The anticancer effect most likely arises from localized recruitment of tumour-infiltrating neutrophils [95], largely through tumour-localized stimulation of Toll-like receptor (TLR) signalling [218].

Although the ability of therapeutic phages to reach the epithelial cell layer and bypass this barrier to reach various sites throughout the body can be highly beneficial in enhancing its therapeutic potential, this unique penetrative capacity of phages can also be a double-edged sword. Resident phages in the gut can alter the microbiota, and even increase the permeability of the epithelial cells – creating similar conditions of guts of diseased patients, in which inflammation and immune activation can occur. Defects in the intestinal barrier function has been associated with celiac disease, colorectal cancer, irritable bowel syndrome and inflammatory bowel disease [219]. It bears repeating that much remains unknown regarding exogenous phage interaction with the host body immune system and gut microbiota so we must remain cautious as we proceed.

8. Summary

Phages possess a wide range of therapeutic potential. They are: (i) highly specific against bacterial targets, (ii) highly penetrative in various mammalian tissues, bypassing anatomical barriers that are relatively impermeable, (iii) modifiable to meet the needs of the therapeutic purpose, and (iv) safe, as demonstrated by the majority of clinical trials. Phage display can also further increase the penetrative ability and add other activities depending on the conjugated peptide. It has been suggested that with the rapid advancements in synthetic biology, phages targeting against virtually any bacteria will soon be possible [11,209]. Phages also have immunomodulating properties, which could be highly exploitable in the future although the conflicting reports on the nature of this activity necessitates future investigation before that can happen.

Therapeutic use of phages needs to overcome one very major hurdle: achieving sufficient numbers of phage for therapeutic activity. In eradicating bacterial infections, attaining a “killing titer” is difficult if bacteria are able to infiltrate into organs that are less accessible to phage; there may therefore be a need to administer high or repeated phage doses, instead of depending on phages to replicate *in situ* utilizing the bacterial pathogen [190]. Fortunately, high titers of phage have not yet shown any negative impact in healthy humans. Unfortunately, most proof-of-principle demonstrations of phage therapeutic applications use very high titers of phage to achieve efficacy, an unlikely situation in practice, especially after immune clearance.

Many challenges exist in employing phage as therapeutics since there are many variables to be considered, several of which have come up again and again over the course of this review: routes of administration, phage type, frequency of administration, dosage, modifications of the phage capsid, interspecies differences in the mammalian host, and more. Phages require thorough investigation of the immunological response they may elicit, as well as extensive purification steps prior to their administration, as bacterial contaminants can be detrimental and elicit highly pathophysiological effects. The growing number of publications emerging in the last decade along with increasing industry interest in phage therapy marks very encouraging progress in repairing the knowledge gap necessary for phage therapeutic application.

References

[1] M. Breitbart, F. Rohwer, Here a virus, there a virus, everywhere the same virus? Trends Microbiol. 13 (2005) 278–284, https://doi.org/10.1016/j.tim.2005.04.003.
[2] J.L. Castro-Mejía, M.K. Muhammed, W. Kot, H. Neve, C.M.A.P. Franz, L.H. Hansen, et al., Optimizing protocols for extraction of bacteriophages prior to metagenomic analyses of phage communities in the human gut, Microbiome. BioMed. Central 3 (2015) 64, https://doi.org/10.1186/s40168-015-0131-4.
[3] T. Schoenfeld, M. Liles, K.E. Wommack, S.W. Polson, R. Godiska, D. Mead, Functional viral metagenomics and the next generation of molecular tools, Trends Microbiol. 18 (2010) 20–29, https://doi.org/10.1016/J.TIM.2009.10.001 Elsevier Current Trends.
[4] F. Rohwer, Global phage diversity, Cell 113 (2003) 141, https://doi.org/10.1016/S0092-8674(03)00276-9 Cell Press.
[5] L. Hoyles, A.L. McCartney, H. Neve, G.R. Gibson, J.D. Sanderson, K.J. Heller, et al., Characterization of virus-like particles associated with the human faecal and caecal microbiota, Res. Microbiol. 165 (2014) 803–812, https://doi.org/10.1016/j.resmic.2014.10.006.
[6] P. Manrique, M. Dills, M.J. Young, The human gut phage community and its implications for health and disease, Viruses 9 (2017)https://doi.org/10.3390/v9060141 Multidisciplinary Digital Publishing Institute (MDPI).
[7] D.H. Duckworth, P.A. Gulig, Bacteriophages, BioDrugs 16 (2002) 57–62, https://doi.org/10.2165/00063030-200216010-00006 Springer International Publishing.
[8] K. Dabrowska, K. Switała-Jelen, A. Opolski, B. Weber-Dabrowska, A. Gorski, A review: bacteriophage penetration in vertebrates, J. Appl. Microbiol. (2005) 7–13, https://doi.org/10.1111/j.1365-2672.2004.02422.x.

[9] A. Gorski, E. Wazna, B.-W. Dabrowska, K. Dabrowska, K. Switała-Jelen, R. Miedzybrodzki, et al., Bacteriophage translocation, FEMS Immunol. Med. Microbiol. 46 (2006) 313–319, https://doi.org/10.1111/j.1574-695X.2006.00044.x.

[10] J.J. Barr, A bacteriophages journey through the human body, Immunol. Rev. 279 (2017) 106–122, https://doi.org/10.1111/imr.12565.

[11] A. Górski, K. Dąbrowska, K. Hodyra-Stefaniak, J. Borysowski, R. Międzybrodzki, B. Weber-Dąbrowska, Phages targeting infected tissues: novel approach to phage therapy, Future Microbiol 10 (2015) 199–204, https://doi.org/10.2217/fmb.14.126.

[12] F. D'Hérelle, Sur un microbe invisible antagoniste des bacilles dysentérique, Comptes rendus l'Académie des Sci. 165 (1917) 373–375, https://doi.org/10.4161/bact.1.1.14941.

[13] J. Oh, A.L. Byrd, C. Deming, S. Conlan, H.H. Kong, J.A. Segre, et al., Biogeography and individuality shape function in the human skin metagenome, Nature 514 (2014) 59–64, https://doi.org/10.1038/nature13786.

[14] T.M. Santiago-Rodriguez, M. Naidu, S.R. Abeles, T.K. Boehm, M. Ly, D.T. Pride, Transcriptome analysis of bacteriophage communities in periodontal health and disease, BMC Genomics 16 (2015) 549, https://doi.org/10.1186/s12864-015-1781-0.

[15] M. Brown-Jaque, M. Muniesa, F. Navarro, Bacteriophages in clinical samples can interfere with microbiological diagnostic tools, Sci Rep. 6 (2016) 33000, https://doi.org/10.1038/srep33000 Nat. Publ. Group.

[16] D. Willner, M. Furlan, M. Haynes, R. Schmieder, F.E. Angly, J. Silva, et al., Metagenomic analysis of respiratory tract DNA viral communities in cystic fibrosis and non-cystic fibrosis individuals, PLoS One 4 (2009)https://doi.org/10.1371/journal.pone.0007370.

[17] A. Reyes, M. Haynes, N. Hanson, F.E. Angly, A.C. Heath, F. Rohwer, et al., Viruses in the faecal microbiota of monozygotic twins and their mothers, Nature 466 (2010) 334–338, https://doi.org/10.1038/nature09199 NIH Public Access.

[18] S.G. Bradley, Y.B. Kim, D.W. Watson, Immune response by the mouse to orally administered actinophage, Proc. Soc. Exp. Biol. Med. 113 (1963) 686–688.

[19] N. Rascovan, R. Duraisamy, C. Desnues, Metagenomics and the human virome in asymptomatic individuals, Annu. Rev. Microbiol. 70 (2016) 125–141, https://doi.org/10.1146/annurev-micro-102215-095431 Annual Reviews.

[20] Y.L. Yip, N.J. Hawkins, G. Smith, R.L. Ward, Biodistribution of filamentous phage-Fab in nude mice, J. Immunol. Methods 225 (1999) 171–178, https://doi.org/10.1016/S0022-1759(99)00044-7.

[21] R.E.F.J. Keller, Fate of bacteriophage particles introduced into mice by various routes, Proc. Soc. Exp. Biol. Med. 3 (1958) 577–580.

[22] E. Duane, Jr Prasuhn, Pratik Singh, Erica Strable, Steven Brown, Marianne Manchester, M.G. Finn, Plasma clearance of bacteriophage Qβ particles as a function of surface charge, J. Am. Chem. Soc. 130 (2008) 1328–1334, https://doi.org/10.1021/JA075937F Am. Chem. Soc.

[23] B. Weber-Dabrowska, M. Dabrowski, S. Slopek, Studies on bacteriophage penetration in patients subjected to phage therapy, Arch. Immunol. Ther. Exp. (Warsz) 35 (1987) 563–568 , Available: http://www.scopus.com/inward/record.url?eid=2-s2.0-0023492827&partnerID=tZOtx3y1.

[24] J. Majewska, W. Beta, D. Lecion, K. Hodyra-Stefaniak, A. Kłopot, Z. Kazmierczak, et al., Oral application of T4 phage induces weak antibody production in the gut and in the blood, Viruses 7 (2015) 4783–4799, https://doi.org/10.3390/v7082845.

[25] M. Cisło, M. Dabrowski, B. Weber-Dabrowska, A. Woytoń, Bacteriophage treatment of suppurative skin infections, Archivum immunologiae et therapiae experimentalis (1987) 175–183.

[26] R. Międzybrodzki, M. Kłak, E. Jończyk-Matysiak, B. Bubak, A. Wójcik, M. Kaszowska, et al., Means to facilitate the overcoming of gastric juice barrier by a therapeutic staphylococcal bacteriophage A5/80, Front. Microbiol. 08 (2017) 467, https://doi.org/10.3389/fmicb.2017.00467.

[27] D. Frenkel, B. Solomon, Filamentous phage as vector-mediated antibody delivery to the brain, Proc. Natl. Acad. Sci U. S. A. 99 (2002) 5675–5679, https://doi.org/10.1073/pnas.072027199 National Academy of Sciences.

[28] R.J. Dubos, J.H. Straus, C. Pierce, The multiplication of bacteriophage in vivo and its protective effect against an experimental infection with shigella dysenteriae, J. Exp. Med. 78 (1943) 161–168 , Available: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2135327&tool=pmcentrez&rendertype=abstract.

[29] S. Kumari, K. Harjai, S. Chhibber, Bacteriophage versus antimicrobial agents for the treatment of murine burn wound infection caused by Klebsiella pneumoniae B5055, J. Med. Microbiol. 60 (2011) 205–210, https://doi.org/10.1099/jmm.0.018580-0.

[30] J.S. Soothill, Bacteriophage prevents destruction of skin grafts by Pseudomonas aeruginosa, Burns 20 (1994) 209–211, https://doi.org/10.1016/0305-4179(94)90184-8.

[31] J.J. Mendes, C. Leandro, S. Corte-Real, R. Barbosa, P. Cavaco-Silva, J. Melo-Cristino, et al., Wound healing potential of topical bacteriophage therapy on diabetic cutaneous wounds, Wound Repair Regen. 21 (2013) 595–603, https://doi.org/10.1111/wrr.12056.

[32] L. Debarbieux, D. Leduc, D. Maura, E. Morello, A. Criscuolo, O. Grossi, et al., Bacteriophages Can Treat and Prevent Pseudomonas aeruginosa Lung Infections, J. Infect. Dis. 201 (2010) 1096–1104, https://doi.org/10.1086/651135 Oxford University Press.

[33] N.M. Bardhan, D. Ghosh, A.M. Belcher, M13 Virus based detection of bacterial infections in living hosts, J. Biophotonics. 7 (2014) 617–623, https://doi.org/10.1002/jbio.201300010 WILEY-VCH Verlag.

[34] V. Dinakaran, A. Rathinavel, M. Pushpanathan, R. Sivakumar, P. Gunasekaran, J. Rajendhran, Elevated levels of circulating DNA in cardiovascular disease patients: metagenomic profiling of microbiome in the circulation, Bereswill S, editor PLoS One 9 (2014) e105221, https://doi.org/10.1371/journal.pone.0105221 Public Libr. Sci.

[35] S.-K. Li, R.K.-K. Leung, H.-X. Guo, J.-F. Wei, J.-H. Wang, K.-T. Kwong, et al., Detection and identification of plasma bacterial and viral elements in HIV/AIDS patients in comparison to healthy adults, Clin. Microbiol. Infect. 18 (2012) 1126–1133, https://doi.org/10.1111/j.1469-0691.2011.03690.x.

[36] G. Egawa, S. Nakamizo, Y. Natsuaki, H. Doi, Y. Miyachi, K. Kabashima, Intravital analysis of vascular permeability in mice using two-photon microscopy, Sci. Rep. 3 (2013) 1932, https://doi.org/10.1038/srep01932.

[37] L. Claesson-Welsh, Vascular permeability—the essentials, Ups. J. Med. Sci. 120 (2015) 135–143, https://doi.org/10.3109/03009734.2015.1064501 Taylor & Francis.

[38] Y. Geng, P. Dalhaimer, S. Cai, R. Tsai, M. Tewari, T. Minko, et al., Shape effects of filaments versus spherical particles in flow and drug delivery, Nat. Nanotechnol. 2 (2007) 249–255, https://doi.org/10.1038/nnano.2007.70.

[39] L. Tao, W. Hu, Y. Liu, G. Huang, B.D. Sumer, J. Gao, Shape-specific polymeric nanomedicine: emerging opportunities and challenges, Exp. Biol. Med. 236 (2011) 20–29, https://doi.org/10.1258/ebm.2010.010243.

[40] S.A. Berkowitz, L.A. Day, Mass, length, composition and structure of the filamentous bacterial virus fd, J. Mol. Biol. 102 (1976) 531–547, https://doi.org/10.1016/0022-2836(76)90332-6 Academic Press.

[41] C. Porayath, A. Salim, A. Palillam Veedu, P. Babu, B. Nair, A. Madhavan, et al., Characterization of the bacteriophages binding to human matrix molecules, Int. J. Biol. Macromol. (2017)https://doi.org/10.1016/J.IJBIOMAC.2017.12.052 Elsevier.

[42] D. Tse, R.V. Stan, D. Ph, R.V. Stan, Morphological heterogeneity of endothelium, Semin. Thromb. Hemost. 36 (2010) 236–245, https://doi.org/10.1055/s-0030-1253447.

[43] R. Calendar, S.T. Abedon (Eds.), The Bacteriophages, 2nd ed.Oxford University Press, New York, 2006.

[44] M. Yu, Y. Yang, C. Zhu, S. Guo, Y. Gan, Advances in the transepithelial transport of nanoparticles, Drug. Discov. Today (2016) 1155–1161, https://doi.org/10.1016/j.drudis.2016.05.007.

[45] M. Alliegro, Endocytosis. xPharm: The Comprehensive Pharmacology Reference, Elsevier, 2007 1–5, https://doi.org/10.1016/B978-008055232-3.62916-2.

[46] K. Parent, I.D. Wilson, Mycobacteriophage in Crohn's disease, Gut 12 (1971) 1019–1020 , Available: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1411989&tool=pmcentrez&rendertype=abstract.

[47] M. Breitbart, F. Rohwer, Method for discovering novel DNA viruses in blood using viral particle selection and shotgun sequencing, Biotechniques 39 (2005) 729–736, https://doi.org/10.2144/000112019.

[48] A. Moustafa, C. Xie, E. Kirkness, W. Biggs, E. Wong, Y. Turpaz, et al., The blood DNA virome in 8,000 humans, in: R. Belshaw (Ed.), PLOS Pathog, 13, 2017, p. e1006292, https://doi.org/10.1371/journal.ppat.1006292 , Public Libr. Sci.

[49] S. Nguyen, K. Baker, B.S. Padman, R. Patwa, R.A. Dunstan, T.A. Weston, et al., in: V.R. Racaniello (Ed.), Bacteriophage Transcytosis Provides a Mechanism To Cross Epithelial Cell Layers, 8, MBio. Am Soc. Microbiol., 2017https://doi.org/10.1128/mBio.01874-17 , e01874-17.

[50] V.V. Ivanenkov, A.G. Menon, Peptide-mediated transcytosis of phage display vectors in MDCK cells, Biochem. Biophys. Res. Commun. 276 (2000) 251–257, https://doi.org/10.1006/bbrc.2000.3358.

[51] G.J. Doherty, H.T. McMahon, Mechanisms of endocytosis, Annu. Rev. Biochem. 78 (2009) 857–902, https://doi.org/10.1146/annurev.biochem.78.081307.110540.

[52] Y. Tian, M. Wu, X. Liu, Z. Liu, Q. Zhou, Z. Niu, et al., Probing the endocytic pathways of the filamentous bacteriophage in live cells using ratiometric pH fluorescent indicator, Adv. Healthc. Mater. 4 (2015) 413–419, https://doi.org/10.1002/adhm.201400508.

[53] S.L. Hart, a M. Knight, R.P. Harbottle, A. Mistry, H.D. Hunger, D.F. Cutler, et al., Cell binding and internalization by filamentous phage displaying a cyclic Arg-Gly-Asp-containing peptide, J. Biol. Chem. 269 (1994) 12468–12474.

[54] P.D. Kassner, M.A. Burg, A. Baird, D. Larocca, Genetic selection of phage engineered for receptor-mediated gene transfer to mammalian cells, Biochem. Biophys. Res. Commun. 264 (1999) 921–928, https://doi.org/10.1006/bbrc.1999.1603.

[55] D. Larocca, K. Jensen-Pergakes, M.A. Burg, A. Baird, Receptor-targeted gene delivery using multivalent phagemid particles, Mol. Ther. 3 (2001) 476–484, https://doi.org/10.1006/mthe.2001.0284.

[56] D. Larocca, A. Baird, Receptor-mediated gene transfer by phage-display vectors: applications in functional genomics and gene therapy, Drug Discov. Today 6 (2001) 793–801.

[57] D. Larocca, M.A. Burg, K. Jensen-Pergakes, E.P. Ravey, A.M. Gonzalez, A. Baird, Evolving phage vectors for cell targeted gene delivery, Curr. Pharm. Biotechnol. 3 (2002) 45–57, https://doi.org/10.2174/1567203053587026.

[58] . D.M. Duerr, S.J. White, H.J. Schluesener, Identification of peptide sequences that induce the transport of phage across the gastrointestinal mucosal barrier, J. Virol. Methods 116 (2004) 177–180, https://doi.org/10.1016/j.jviromet.2003.11.012.

[59] D. Larocca, A. Witte, W. Johnson, G.F. Pierce, A. Baird, Targeting bacteriophage to mammalian cell surface receptors for gene delivery, Hum. Gene Ther. 9 (1998) 2393–2399, https://doi.org/10.1089/hum.1998.9.16-2393.

[60] A. Kim, T.H. Shin, S.M. Shin, C.D. Pham, D.K. Choi, M.H. Kwon, et al., Cellular internalization mechanism and intracellular trafficking of filamentous M13 phages displaying a cell-penetrating transbody and TAT peptide, PLoS One 7 (2012) https://doi.org/10.1371/journal.pone.0051813.

[61] N. Talago, Bacteriophag Interactions Within the Human Mucosal Immune System, San Diego State University, 2015.

[62] T.A. Lehti, M.I. Pajunen, M.S. Skog, J. Finne, Internalization of a polysialic acid-binding Escherichia coli bacteriophage into eukaryotic neuroblastoma cells, Nat. Commun. 8 (2017) 1915, https://doi.org/10.1038/s41467-017-02057-3 Nat. Publ. Group.

[63] S. Pelkonen, J. Pelkonen, J. Finne, Common cleavage pattern of polysialic acid by bacteriophage endosialidases of different properties and origins, J Virol. 63 (1989) 4409–4416 , American Society for Microbiology Journals. Available http://www.ncbi.nlm.nih.gov/pubmed/2778882.

[64] K. Dabrowska, M. Zembala, J. Boratynski, K. Switala-Jelen, J. Wietrzyk, A. Opolski, et al., Hoc protein regulates the biological effects of T4 phage in mammals, Arch. Microbiol. 187 (2007) 489–498, https://doi.org/10.1007/s00203-007-0216-y.

[65] K. Dabrowska, A. Opolski, J. Wietrzyk, K. Switala-Jelen, J. Boratynski, A. Nasulewicz, et al., Antitumor activity of bacteriophages in murine experimental cancer models caused possibly by inhibition of ??3 integrin signaling pathway, Acta Virol. 48 (2004) 241–248.

[66] A.G. Rosenwald, B. Murray, T. Toth, R. Madupu, A. Kyrillos, G. Arora, Evidence for horizontal gene transfer between Chlamydophila pneumoniae and Chlamydia phage, Bacteriophage 4 (2014) e965076, https://doi.org/10.4161/21597073.2014.965076 Taylor & Francis.

[67] S.R. Bordenstein, S.R. Bordenstein, Eukaryotic association module in phage WO genomes from Wolbachia, Nat. Commun. 7 (2016) 13155, https://doi.org/10.1038/ncomms13155 Nat. Publ. Group.

[68] A.S. Srivastava, T. Kaido, E. Carrier, Immunological factors that affect the in vivo fate of T7 phage in the mouse, J. Virol. Methods. 115 (2004) 99–104, https://doi.org/10.1016/J.JVIROMET.2003.09.009 Elsevier.

[69] C.J. Inchley, The actvity of mouse Kupffer cells following intravenous injection of T4 bacteriophage, Clin. Exp. Immunol. 5 (1969) 173–187 , Wiley-Blackwell. Available http://www.ncbi.nlm.nih.gov/pubmed/5370053.

[70] C.M. Bearden, A. Agarwal, B.K. Book, C.A. Vieira, R.A. Sidner, H.D. Ochs, et al., Rituximab inhibits the in vivo primary and secondary antibody response to a neoantigen, bacteriophage phix174, Am. J. Transplant. 5 (2005) 50–57, https://doi.org/10.1111/j.1600-6143.2003.00646.x Wiley/Blackwell (10.1111).

[71] J. Borysowski, A. Górski, Is phage therapy acceptable in the immunocompromised host? Int. J. Infect. Dis. (2008) 466–471, https://doi.org/10.1016/j.ijid.2008.01.006.

[72] C.R. Merril, B. Biswas, R. Carlton, N.C. Jensen, G.J. Creed, S. Zullo, et al., Long-circulating bacteriophage as antibacterial agents, Proc. Natl. Acad. Sci. 93 (1996) 3188–3192, https://doi.org/10.1073/pnas.93.8.3188.

[73] R. Capparelli, M. Parlato, G. Borriello, P. Salvatore, D. Iannelli, Experimental phage therapy against Staphylococcus aureus in mice, Antimicrob. Agents Chemother. 51 (2007) 2765–2773, https://doi.org/10.1128/AAC.01513-06 American Society for Microbiology (ASM).

[74] R. Capparelli, I. Ventimiglia, S. Roperto, D. Fenizia, D. Iannelli, Selection of an Escherichia coli O157:H7 bacteriophage for persistence in the circulatory system of mice infected experimentally, Clin. Microbiol. Infect. 12 (2006) 248–253, https://doi.org/10.1111/j.1469-0691.2005.01340.x.

[75] Weber-Dąbrowska B, M. Mulczyk, A. Górski, Bacteriophages as an efficient therapy for antibiotic-resistant septicemia in man, Transplant. Proc. 35 (2003) 1385–1386, https://doi.org/10.1016/S0041-1345(03)00525-6 Elsevier.

[76] K. Dąbrowska, P. Miernikiewicz, A. Piotrowicz, K. Hodyra, B. Owczarek, D. Lecion, et al., Immunogenicity studies of proteins forming the T4 phage head surface, J. Virol. 88 (2014) 12551–12557, https://doi.org/10.1128/JVI.02043-14 American Society for Microbiology.

[77] A. Kucharewicz-Krukowska, S. Slopek, Immunogenic effect of bacteriophage in patients subjected to phage therapy, Arch. Immunol. Ther. Exp. (Warsz) 35 (1987) 553–561 , Available http://www.ncbi.nlm.nih.gov/pubmed/3455646.

[78] M. Łusiak-Szelachowska, M. Zaczek, B. Weber-Dąbrowska, R. Międzybrodzki, M. Kłak, W. Fortuna, et al., Phage neutralization by sera of patients receiving phage therapy, Viral Immunol. 27 (2014) 295–304, https://doi.org/10.1089/vim.2013.0128 Mary Ann Liebert, Inc.

[79] A. Sulakvelidze, Z. Alavidze, J. Glenn, M. Jr, Bacteriophage therapy, Antimicrob. Agents Chemother. 45 (2001) 649–659, https://doi.org/10.1128/AAC.45.3.649.

[80] G.L. Plaa, J.B. Hook, Target organ toxicity: liver and kidney, Environ. Health Perspect. 15 (1976) 1 , National Institute of Environmental Health Science. Available http://www.ncbi.nlm.nih.gov/pubmed/17539116.

[81] S.A. Sarker, S. Sultana, G. Reuteler, D. Moine, P. Descombes, F. Charton, et al., Oral phage therapy of acute bacterial diarrhea with two coliphage preparations: a randomized trial in children from Bangladesh, EBioMedicine 4 (2016) 124–137, https://doi.org/10.1016/j.ebiom.2015.12.023.

[82] B. Weber-Dabrowska, M. Mulczyk, A. Górski, Bacteriophage therapy for infections in cancer patients, Clin. Appl. Immunol. Rev. 1 (2001) 131–134, https://doi.org/10.1016/S1529-1049(01)00015-0 Elsevier.

[83] A. Bruttin, H. Brüssow, Human volunteers receiving Escherichia coli phage T4 orally: a safety test of phage therapy, Antimicrob. Agents Chemother. 49 (2005) 2874–2878, https://doi.org/10.1128/AAC.49.7.2874-2878.2005.

[84] G.V. Tetz, K.V. Ruggles, H. Zhou, A. Heguy, A. Tsirigos, V. Tetz, Bacteriophages as potential new mammalian pathogens, Sci. Rep. 7 (2017) 7043, https://doi.org/10.1038/s41598-017-07278-6 Nat. Publ. Group.

[85] E. Jończyk-Matysiak, B. Weber-Dąbrowska, B. Owczarek, R. Międzybrodzki, M. Łusiak-Szelachowska, N. Łodej, et al., Phage-phagocyte interactions and their implications for phage application as therapeutics, Viruses 9 (2017)https://doi.org/10.3390/v9060150 Multidisciplinary Digital Publishing Institute (MDPI).

[86] J.D. Van Belleghem, F. Clement, M. Merabishvili, R. Lavigne, M. Vaneechoutte, Pro- and anti-inflammatory responses of peripheral blood mononuclear cells induced by Staphylococcus aureus and Pseudomonas aeruginosa phages, Sci. Rep. 7 (2017) 8004, https://doi.org/10.1038/s41598-017-08336-9 Nat. Publ. Group.

[87] L. Zhang, X. Hou, L. Sun, T. He, R. Wei, M. Pang, et al., Staphylococcus aureus bacteriophage suppresses LPS-induced inflammation in MAC-T bovine mammary epithelial cells, Front. Microbiol. 9 (2018) 1614, https://doi.org/10.3389/fmicb.2018.01614 Frontiers.

[88] M. Zimecki, J. Artym, M. Kocięba, B. Weber-Dąbrowska, J. Borysowski, A. Górski, Effects of prophylactic administration of bacteriophages to immunosuppressed mice infected with Staphylococcus aureus, BMC Microbiol. 9 (2009) 169, https://doi.org/10.1186/1471-2180-9-169 BioMed Central.

[89] P.R. Secor, L.A. Michaels, K.S. Smigiel, M.G. Rohani, L.K. Jennings, K.B. Hisert, et al., Filamentous bacteriophage produced by pseudomonas aeruginosa alters the inflammatory response and promotes noninvasive infection in vivo, Infect. Immun. 85 (2017)https://doi.org/10.1128/IAI.00648-16 American Society for Microbiology Journals. e00648-16.

[90] R. Międzybrodzki, J. Borysowski, M. Kłak, E. Jończyk-Matysiak, B. Obmińska-Mrukowicz, A. Suszko-Pawłowska, et al., *In vivo* studies on the influence of bacteriophage preparations on the autoimmune inflammatory process, Biomed. Res. Int. Hindawi 2017 (2017) 1–9, https://doi.org/10.1155/2017/3612015.

[91] P. Miernikiewicz, A. Kłopot, R. Soluch, P. Szkuta, W. Kęska, K. Hodyra-Stefaniak, et al., T4 phage tail adhesin Gp12 counteracts LPS-induced inflammation in vivo, Front. Microbiol. 7 (2016) 1112, https://doi.org/10.3389/fmicb.2016.01112 Frontiers Media SA.

[92] R. Miedzybrodzki, K. Switala-Jelen, W. Fortuna, B. Weber-Dabrowska, A. Przerwa, M. Lusiak-Szelachowska, et al., Bacteriophage preparation inhibition of reactive oxygen species generation by endotoxin-stimulated polymorphonuclear leukocytes, Virus Res. 131 (2008) 233–242, https://doi.org/10.1016/j.virusres.2007.09.013.

[93] B. Weber-Dabrowska, M. Zimecki, M. Mulczyk, GÃ³rski A. Effect of phage therapy on the turnover and function of peripheral neutrophils, FEMS Immunol. Med. Microbiol. 34 (2002) 135–138, https://doi.org/10.1111/j.1574-695X.2002.tb00614.x Oxford University Press.

[94] A. Górski, M. Kniotek, A. Perkowska-Ptasińska, A. Mróz, A. Przerwa, W. Gorczyca, et al., Bacteriophages and transplantation tolerance, Transplant Proc. 38 (2005) 331–333, https://doi.org/10.1016/j.transproceed.2005.12.073.

[95] F. Eriksson, W.D. Culp, R. Massey, L. Egevad, D. Garland, M.A.A. Persson, et al., Tumor specific phage particles promote tumor regression in a mouse melanoma model, Cancer Immunol. Immunother. 56 (2007) 677–687, https://doi.org/10.1007/s00262-006-0227-6 Springer-Verlag.

[96] A. Tandle, E. Hanna, D. Lorang, A. Hajitou, C.A. Moya, R. Pasqualini, et al., Tumor vasculature-targeted delivery of tumor necrosis factor-α*, Cancer 115 (2009) 128–139, https://doi.org/10.1002/cncr.24001 Wiley Subscription Services, Inc., A Wiley Company.

[97] M.C. Paoloni, A. Tandle, C. Mazcko, E. Hanna, S. Kachala, A. LeBlanc, et al., Launching a novel preclinical infrastructure: comparative oncology trials consortium directed therapeutic targeting of TNFα to cancer vasculature, Lewin A, editor PLoS One 4 (2009) e4972, https://doi.org/10.1371/journal.pone.0004972 Public Libr. Sci.

[98] M. Merabishvili, J.-P. Pirnay, G. Verbeken, N. Chanishvili, M. Tediashvili, N. Lashkhi, et al., Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials, PLoS One 4 (2009) e4944, https://doi.org/10.1371/journal.pone.0004944 Public Libr. Sci.

[99] A. Wright, C.H. Hawkins, E.E. Änggård, D.R. Harper, A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa* ; a preliminary report of efficacy, Clin. Otolaryngol. 34 (2009) 349–357, https://doi.org/10.1111/j.1749-4486.2009.01973.x Blackwell Publishing Ltd.

[100] Y. Tanji, T. Shimada, H. Fukudomi, K. Miyanaga, Y. Nakai, H. Unno, Therapeutic use of phage cocktail for controlling Escherichia coli O157:H7 in gastrointestinal tract of mice, J. Biosci. Bioeng. 100 (2005) 280–287, https://doi.org/10.1263/jbb.100.280.

[101] H.W. Smith, M.B. Huggins, Successful treatment of experimental Escherichia coli infections in mice using phage: its general superiority over antibiotics, Microbiology 128 (1982) 307–318, https://doi.org/10.1099/00221287-128-2-307.

[102] H.W. Smith, M.B. Huggins, K.M. Shaw, The control of experimental Escherichia coli diarrhoea in calves by means of bacteriophages, J. Gen. Microbiol. 133 (1987) 1111–1126, https://doi.org/10.1099/00221287-133-5-1111.

[103] M. Galtier, L. De Sordi, D. Maura, H. Arachchi, S. Volant, M.A. Dillies, et al., Bacteriophages to reduce gut carriage of antibiotic resistant uropathogens with low impact on microbiota composition, Environ. Microbiol. 18 (2016) 2237–2245, https://doi.org/10.1111/1462-2920.13284.

[104] S. Zelasko, A. Gorski, K. Dabrowska, Delivering phage therapy per os: benefits and barriers, Exp. Rev. Anti Infect. Ther. 15 (2017) 167–179, https://doi.org/10.1080/14787210.2017.1265447 Taylor & Francis.

[105] S. McCallin, S. Alam Sarker, C. Barretto, S. Sultana, B. Berger, S. Huq, et al., Safety analysis of a Russian phage cocktail: from MetaGenomic analysis to oral application in healthy human subjects, Virology 443 (2013) 187–196, https://doi.org/10.1016/j.virol.2013.05.022.

[106] R. Nannapaneni, K.A. Soni, Use of bacteriophages to remove biofilms of *Listeria monocytogenes* and other foodborne bacterial pathogens in the food environment, Biofilms in the Food Environment, John Wiley & Sons, Ltd, Chichester, UK 2015, pp. 131–144, https://doi.org/10.1002/9781118864036.ch5.

[107] L. Endersen, J. O'Mahony, C. Hill, R.P. Ross, O. McAuliffe, A. Coffey, Phage therapy in the food industry, Annu. Rev. Food Sci. Technol. 5 (2014) 327–349, https://doi.org/10.1146/annurev-food-030713-092415.

[108] F.L. Nobrega, A.R. Costa, J.F. Santos, M.F. Siliakus, J.W.M. Van Lent, S.W.M. Kengen, et al., Genetically manipulated phages with improved pH resistance for oral administration in veterinary medicine, Sci. Rep. 6 (2016)https://doi.org/10.1038/srep39235.

[109] E. Jończyk, M. Kłak, R. Międzybrodzki, A. Górski, The influence of external factors on bacteriophages–review, Folia Microbiol. (Praha) 56 (2011) 191–200, https://doi.org/10.1007/s12223-011-0039-8.

[110] Z. Tang, X. Huang, S. Baxi, J.R. Chambers, P.M. Sabour, Q. Wang, Whey protein improves survival and release characteristics of bacteriophage Felix O1 encapsulated in alginate microspheres, Food Res. Int. 52 (2013) 460–466, https://doi.org/10.1016/j.foodres.2012.12.037.

[111] J. Colom, M. Cano-Sarabia, J. Otero, P. Cortés, D. Maspoch, M. Llagostera, Liposome-encapsulated bacteriophages for enhanced oral phage therapy against Salmonella spp, Appl. Environ. Microbiol. 81 (2015) 4841–4849, https://doi.org/10.1128/AEM.00812-15.

[112] C. Dini, G.A. Islan, G.R. Castro, Characterization and stability analysis of biopolymeric matrices designed for phage-controlled release, Appl. Biochem. Biotechnol. 174 (2014) 2031–2047, https://doi.org/10.1007/s12010-014-1152-3.

[113] J.R. Turner, Intestinal mucosal barrier function in health and disease, Nat. Rev. Immunol. 9 (2009) 799–809, https://doi.org/10.1038/nri2653 Nature Publishing Group.

[114] A. Ermund, A. Schütte, M.E.V. Johansson, J.K. Gustafsson, G.C. Hansson, Studies of mucus in mouse stomach, small intestine, and colon. I. Gastrointestinal mucus layers have different properties depending on location as well as over the Peyer's patches, Am. J. Physiol. Liver Physiol. 305 (2013) G341–G347, https://doi.org/10.1152/ajpgi.00046.2013.

[115] Barbara P. de Santa, G.R. van den Brink, D.J. Roberts, Development and differentiation of the intestinal epithelium, Cell. Mol. Life Sci. Inserm 60 (2003) 1322–1332, https://doi.org/10.1007/s00018-003-2289-3.

[116] J.K. Sheehan, M. Kesimer, R. Pickles, Innate immunity and mucus structure and function, Innate Immun. Pulm. Infect. (2008) 155–169, https://doi.org/10.1002/9780470035399.ch13.

[117] M. Weiss, E. Denou, A. Bruttin, R. Serra-Moreno, M.-L. Dillmann, H. Brüssow, In vivo replication of T4 and T7 bacteriophages in germ-free mice colonized with Escherichia coli, Virology 393 (2009) 16–23, https://doi.org/10.1016/j.virol.2009.07.020.

[118] J.J. Barr, R. Auro, M. Furlan, K.L. Whiteson, M.L. Erb, J. Pogliano, et al., Bacteriophage adhering to mucus provide a non-host-derived immunity, Proc. Natl. Acad. Sci. 110 (2013) 10771–10776, https://doi.org/10.1073/pnas.1305923110.

[119] E.M. Ryan, S.P. Gorman, R.F. Donnelly, B.F. Gilmore, Recent advances in bacteriophage therapy: how delivery routes, formulation, concentration and timing influence the success of phage therapy, J. Pharm. Pharmacol. (2011) 1253–1264, https://doi.org/10.1111/j.2042-7158.2011.01324.x.

[120] L.V. Hooper, Molecular analysis of commensal host-microbial relationships in the intestine, Science (80-) 291 (2001) 881–884, https://doi.org/10.1126/science.291.5505.881.

[121] K. Ishikawa, Y. Satoh, Y. Oomori, M. Yamano, M. Matsuda, K. Ono, Influence of conventionalization on cecal wall structure of germ-free Wistar rats: quantitative light and qualitative electron microscopic observations, Anat. Embryol. (Berl) 180 (1989) 191–198 , Available http://www.ncbi.nlm.nih.gov/pubmed/2802183.

[122] A. Oliveira, R. Sereno, A. Nicolau, J. Azeredo, The influence of the mode of administration in the dissemination of three coliphages in chickens, Poult. Sci. 88 (2009) 728–733, https://doi.org/10.3382/ps.2008-00378 Oxford University Press.

[123] M. Łobocka, M.S. Hejnowicz, K. Dąbrowski, A. Gozdek, J. Kosakowski, M. Witkowska, et al., Genomics of staphylococcal twort-like phages – potential therapeutics of the post-antibiotic era, Adv. Virus Res. (2012) 143–216, https://doi.org/10.1016/B978-0-12-394438-2.00005-0.

[124] B.M. Fihn, A. Sjöqvist, M. Jodal, Permeability of the rat small intestinal epithelium along the villus-crypt axis: effects of glucose transport, Gastroenterology 119 (2000) 1029–1036, https://doi.org/10.1053/gast.2000.18148.

[125] R.D. Berg, Bacterial translocation from the intestines, Jikken Dobutsu 34 (1985) 1–16 , Available http://www.ncbi.nlm.nih.gov/pubmed/3987819.

[126] J. König, J. Wells, P.D. Cani, C.L. García-Ródenas, T. MacDonald, A. Mercenier, et al., Human intestinal barrier function in health and disease, Clin. Transl. Gastroenterol. 7 (2016) e196, https://doi.org/10.1038/ctg.2016.54 Nat. Publ. Group.

[127] M.R. Geier, M.E. Trigg, C.R. Merril, M.R. Grier, M.E. Trigg, C.R. Merril, Fate of bacteriophage lambda in non-immune germ-free mice, Nature 246 (1973) 221–223, https://doi.org/10.1038/246221a0.

[128] A. Vallon-Eberhard, L. Landsman, N. Yogev, B. Verrier, S. Jung, Transepithelial pathogen uptake into the small intestinal lamina propria, J. Immunol. 176 (2006) 2465–2469, https://doi.org/10.4049/JIMMUNOL.176.4.2465 American Association of Immunologists.

[129] M. Rescigno, M. Urbano, B. Valzasina, M. Francolini, G. Rotta, R. Bonasio, et al., Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria, Nat. Immunol. 2 (2001) 361–367, https://doi.org/10.1038/86373 Nature Publishing Group.

[130] J.H. Niess, S. Brand, X. Gu, L. Landsman, S. Jung, B.A. McCormick, et al., CX3CR1-Mediated Dendritic Cell Access to the Intestinal Lumen and Bacterial Clearance, Science (80-) 307 (2005) 254–258, https://doi.org/10.1126/science.1102901.

[131] M. Chieppa, M. Rescigno, A.Y.C. Huang, R.N. Germain, Dynamic imaging of dendritic cell extension into the small bowel lumen in response to epithelial cell TLR engagement, J. Exp. Med. 203 (2006) 2841–2852, https://doi.org/10.1084/jem.20061884.

[132] T. Kucharzik, N. Lügering, K. Rautenberg, A. Lügering, M.A. Schmidt, R. Stoll, et al., Role of M cells in intestinal barrier function, Ann. N. Y. Acad. Sci. 915 (2000) 171–183 , Available http://www.ncbi.nlm.nih.gov/pubmed/11193574.

[133] V. Fievez, L. Plapied, C. Plaideau, D. Legendre, A. des Rieux, V. Pourcelle, et al., In vitro identification of targeting ligands of human M cells by phage display, Int. J. Pharm. 394 (2010) 35–42, https://doi.org/10.1016/j.ijpharm.2010.04.023.

[134] Helen M. Amerongen, Richard Weltzin, Chris M. Farnet, Pierre Michetti, William A. Haseltine, M.R. Neutra, Transepithelial transport of HIV-1 by intestinal m cells: a mechanism for transmission of AIDS, J. Acquir. Immune Defic. Syndr. 4 (1991) 760–765.

[135] Y. Fujimura, M. Takeda, H. Ikai, K. Haruma, T. Akisada, T. Harada, et al., The role of M cells of human nasopharyngeal lymphoid tissue in influenza virus sampling, Virchows Arch. 444 (2004) 36–42, https://doi.org/10.1007/s00428-003-0898-8.

[136] Y. Takahashi, S. Misumi, A. Muneoka, M. Masuyama, H. Tokado, K. Fukuzaki, et al., Nonhuman primate intestinal villous M-like cells: an effective poliovirus entry site, Biochem. Biophys. Res. Commun. 368 (2008) 501–507, https://doi.org/10.1016/j.bbrc.2008.01.120.

[137] J.L. Wolf, D.H. Rubin, R. Finberg, R.S. Kauffman, A.H. Sharpe, J.S. Trier, et al., Intestinal M cells: a pathway for entry of reovirus into the host, Science 212 (1981) 471–472, https://doi.org/10.1126/science.6259737.

[138] S. Chibani-Chennoufi, J. Sidoti, A. Bruttin, E. Kutter, S. Sarker, H. Brüssow, In vitro and in vivo bacteriolytic activities of Escherichia coli phages: implications for phage therapy, Antimicrob. Agents Chemother. 48 (2004) 2558–2569, https://doi.org/10.1128/AAC.48.7.2558-2569.2004.

[139] A. Bruttin, H. Brüssow, H. Bru, Human volunteers receiving Escherichia coli Phage T4 Orally: a safety test of phage therapy these include: Human volunteers receiving escherichia coli phage T4 orally: a safety test of phage therapy, Antimicrob. Agents Chemother. 49 (2005) 2874–2878, https://doi.org/10.1128/AAC.49.7.2874.

[140] R. Payne, V.A.A. Jansen, Phage therapy: the peculiar kinetics of self-replicating pharmaceuticals, Clin. Pharmacol. Ther. 68 (2000) 225–230, https://doi.org/10.1067/mcp.2000.109520.

[141] K.E. Cerveny, A. DePaola, D.H. Duckworth, P.A. Gulig, Phage therapy of local and systemic disease caused by Vibrio vulnificus in iron-dextran-treated mice, Infect. Immun. 70 (2002) 6251–6262, https://doi.org/10.1128/IAI.70.11.6251-6262.2002.

[142] B.A. Wiggins, M. Alexander, Minimum bacterial density for bacteriophage replication: implications for significance of bacteriophages in natural ecosystems, Appl. Environ. Microbiol. 49 (1985) 19–23 , Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC238337/pdf/aem00147-0035.pdf.

[143] M.E. Goodrich, D.W. McGee, Preferential enhancement of B cell IgA secretion by intestinal epithelial cell-derived cytokines and interleukin-2, Immunol. Investig. 28 (1999) 67–75.

[144] M. Żaczek, M. Łusiak-Szelachowska, E. Jończyk-Matysiak, B. Weber-Dąbrowska, R. Międzybrodzki, B. Owczarek, et al., Antibody production in response to staphylococcal MS-1 phage cocktail in patients undergoing phage therapy, Front. Microbiol. 7 (2016) 1681, https://doi.org/10.3389/fmicb.2016.01681.

[145] M. Łusiak-Szelachowska, M. Żaczek, B. Weber-Dąbrowska, R. Międzybrodzki, M. Kłak, W. Fortuna, et al., Phage neutralization by sera of patients receiving phage therapy, Viral Immunol. 27 (2014) 295–304, https://doi.org/10.1089/vim.2013.0128.

[146] J.J. Barr, R. Auro, N. Sam-Soon, S. Kassegne, G. Peters, N. Bonilla, et al., Subdiffusive motion of bacteriophage in mucosal surfaces increases the frequency of bacterial encounters, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 13675–13680, https://doi.org/10.1073/pnas.1508355112 National Academy of Sciences.

[147] A. Górski, R. Międzybrodzki, J. Borysowski, K. Dąbrowska, P. Wierzbicki, M. Ohams, et al., Phage as a modulator of immune responses, Adv. Virus Res. (2012) 41–71, https://doi.org/10.1016/B978-0-12-394438-2.00002-5.

[148] J.S.K. BOYD, Immunity of lysogenic bacteria, Nature 178 (1956) 141, https://doi.org/10.1038/178141a0 Nature Publishing Group.

[149] H.T.P. Williams, Phage-induced diversification improves host evolvability, BMC Evol. Biol. (2013)https://doi.org/10.1186/1471-2148-13-17.

[150] J.M. Norman, S.A. Handley, M.T. Baldridge, L. Droit, C.Y. Liu, B.C. Keller, et al., Disease-specific alterations in the enteric virome in inflammatory bowel disease, Cell 160 (2015) 447–460, https://doi.org/10.1016/j.cell.2015.01.002.

[151] P. Lepage, J. Colombet, P. Marteau, T. Sime-Ngando, J. Doré, M. Leclerc, Dysbiosis in inflammatory bowel disease: a role for bacteriophages? Gut (2008)https://doi.org/10.1136/gut.2007.134668.

[152] W.M. Pardridge, Blood-brain barrier drug targeting: the future of brain drug development, Mol. Interv. 3 (2003) 90–105, https://doi.org/10.1124/mi.3.2.90.

[153] N.J. Abbott, L. Rönnbäck, E. Hansson, Astrocyte-endothelial interactions at the blood-brain barrier, Nat. Rev. Neurosci. (2006) 41–53, https://doi.org/10.1038/nrn1824.

[154] B. Solomon, Filamentous bacteriophage as a novel therapeutic tool for Alzheimer's disease treatment, J. Alzheimers Dis. 15 (2008) 193–198 , Available http://www.ncbi.nlm.nih.gov/pubmed/18953108.

[155] A. Ksendzovsky, S. Walbridge, R.C. Saunders, A.R. Asthagiri, J.D. Heiss, R.R. Lonser, Convection-enhanced delivery of M13 bacteriophage to the brain, J. Neurosurg. 117 (2012) 197–203, https://doi.org/10.3171/2012.4.JNS111528 NIH Public Access.

[156] J.B. Fishman, J.B. Rubin, J.V. Handrahan, J.R. Connor, R.E. Fine, Receptor-mediated transcytosis of transferrin across the blood-brain barrier, J. Neurosci. Res. 18 (1987) 299–304, https://doi.org/10.1002/jnr.490180206.

[157] F.I. Staquicini, M.G. Ozawa, C.A. Moya, W.H.P. Driessen, E.M. Barbu, H. Nishimori, et al., Systemic combinatorial peptide selection yields a non-canonical iron-mimicry mechanism for targeting tumors in a mouse model of human glioblastoma, J. Clin. Invest. 121 (2011) 161–173, https://doi.org/10.1172/JCI44798.

[158] A. Sergeeva, M.G. Kolonin, J.J. Molldrem, R. Pasqualini, W. Arap, Display technologies: application for the discovery of drug and gene delivery agents, Adv. Drug Deliv. Rev. 58 (2006) 1622–1654, https://doi.org/10.1016/j.addr.2006.09.018.

[159] E. Urich, R. Schmucki, N. Ruderisch, E. Kitas, U. Certa, H. Jacobsen, et al., Cargo delivery into the brain by in vivo identified transport peptides, Sci. Rep. 5 (2015) 14104, https://doi.org/10.1038/srep14104 Nat. Publ. Group.

[160] H. Dimant, N. Sharon, B. Solomon, Modulation effect of filamentous phage on α-synuclein aggregation, Biochem. Biophys. Res. Commun. 383 (2009) 491–496, https://doi.org/10.1016/j.bbrc.2009.04.048.

[161] H. Dimant, B. Solomon, Filamentous phages reduce α-synuclein oligomerization in the membrane fraction of SH-SY5Y cells, Neurodegener. Dis. 7 (2010) 203–205, https://doi.org/10.1159/000295664.
[162] R. Krishnan, H. Tsubery, M.Y. Proschitsky, E. Asp, M. Lulu, S. Gilead, et al., A bacteriophage capsid protein provides a general amyloid interaction motif (GAIM) that binds and remodels misfolded protein assemblies, J. Mol. Biol. 426 (2014) 2500–2519, https://doi.org/10.1016/j.jmb.2014.04.015.
[163] J. Messing, Phage M13 for the treatment of Alzheimer and Parkinson disease, Gene (2016) 85–89, https://doi.org/10.1016/j.gene.2016.02.005.
[164] G. Thwaites, T. Chau, N. Mai, F. Drobniewski, K. McAdam, J. Farrar, Tuberculous meningitis, J. Neurol. Neurosurg. Psychiatry 68 (2000) 289–299, https://doi.org/10.1016/j.rmed.2006.08.006.
[165] K.V. Sergueev, Y. He, R.H. Borschel, M.P. Nikolich, A.A. Filippov, Rapid and sensitive detection of Yersinia pestis using amplification of plague diagnostic bacteriophages monitored by real-time PCR, PLoS One 5 (2010)https://doi.org/10.1371/journal.pone.0011337.
[166] P. Barrow, M. Lovell, Berchieri a. Use of lytic bacteriophage for control of experimental Escherichia coli septicemia and meningitis in chickens and calves, Clin. Diagn. Lab. Immunol. 5 (1998) 294–298, https://doi.org/10.1128/IAI.70.11.6251.
[167] C. Carrière, P.F. Riska, O. Zimhony, J. Kriakov, S. Bardarov, J. Burns, et al., Conditionally replicating luciferase reporter phages: improved sensitivity for rapid detection and assessment of drug susceptibility of Mycobacterium tuberculosis, J. Clin. Microbiol. 35 (12) (1997) 3232–3239.
[168] I.J. Eltringham, S.M. Wilson, F.A. Drobniewski, Evaluation of a bacteriophage-based assay (phage amplified biologically assay) as a rapid screen for resistance to isoniazid, ethambutol, streptomycin, pyrazinamide, and ciprofloxacin among clinical isolates of Mycobacterium tuberculosis, J. Clin. Microbiol. 37 (11) (1999) 3528–3532.
[169] I.J. Eltringham, F.A. Drobniewski, J.A. Mangan, P.D. Butcher, S.M. Wilson, Evaluation of reverse transcription-PCR and a bacteriophage-based assay for rapid phenotypic detection of rifampin resistance in clinical isolates of Mycobacterium tuberculosis, J. Clin. Microbiol. 37 (11) (1999) 3524–3527.
[170] F.A. Drobniewski, S.M. Wilson, The rapid diagnosis of isoniazid and rifampicin resistance in Mycobacterium tuberculosis – a molecular story, J. Med. Microbiol. (1998)https://doi.org/10.1099/00222615-47-3-189.
[171] S.M. Wilson, Z. Al-Suwaidi, R. McNerney, J. Porter, F. Drobniewski, Evaluation of a new rapid bacteriophage-based method for the drug susceptibility testing of Mycobacterium tuberculosis, Nat. Med. (1997)https://doi.org/10.1038/nm0497-465.
[172] D.A. Schofield, C. Westwater, Phage-mediated bioluminescent detection of Bacillus anthracis, J. Appl. Microbiol. (2009)https://doi.org/10.1111/j.1365-2672.2009.04332.x.
[173] M.J. Loessner, C.E. Rees, G.S. Stewart, S. Scherer, Construction of luciferase reporter bacteriophage A511::luxAB for rapid and sensitive detection of viable Listeria cells, Appl. Environ. Microbiol. 62 (4) (1996) 1133–1140, https://doi.org/10.1128/JCM.39.11.3883-3888.2001.
[174] D.A. Schofield, I.J. Molineux, C. Westwater, Diagnostic bioluminescent phage for detection of Yersinia pestis, J. Clin. Microbiol. (2009)https://doi.org/10.1128/JCM.01533-09.
[175] C.E. O'Connell-Rodwell, S.M. Burns, M.H. Bachmann, C.H. Contag, Bioluminescent indicators for in vivo measurements of gene expression, Trends Biotechnol. (2002)https://doi.org/10.1016/S0167-7799(02)02001-2.
[176] M.R.A. Carrera, G.F. Kaufmann, J.M. Mee, M.M. Meijler, G.F. Koob, K.D. Janda, Treating cocaine addiction with viruses, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 10416–10421, https://doi.org/10.1073/pnas.0403795101.
[177] W. Hauser, S. Schug, A. Furlan, The opioid epidemic and national guidelines for opioid therapy for chronic noncancer pain: a perspective from different continents, Pain Rep. 2 (2017), e599. https://doi.org/10.1097/PR9.0000000000000599.
[178] Z. Steel, C. Marnane, C. Iranpour, T. Chey, J.W. Jackson, V. Patel, et al., The global prevalence of common mental disorders: a systematic review and meta-analysis 1980–2013, Int. J. Epidemiol. 43 (2014) 476–493, https://doi.org/10.1093/ije/dyu038.
[179] A.C.H. Chen, Y. Shirayama, K.H. Shin, R.L. Neve, R.S. Duman, Expression of the cAMP response element binding protein (CREB) in hippocampus produces an antidepressant effect, Biol. Psychiatry 49 (2001) 753–762, https://doi.org/10.1016/S0006-3223(00)01114-8.
[180] H.A. Lankes, C.N. Zanghi, K. Santos, C. Capella, C.M.P. Duke, S. Dewhurst, In vivo gene delivery and expression by bacteriophage lambda vectors, J. Appl. Microbiol. 102 (2007) 1337–1349, https://doi.org/10.1111/j.1365-2672.2006.03182.x.
[181] A. Muruganandam, J. Tanha, S. Narang, D. Stanimirovic, Selection of phage-displayed llama single-domain antibodies that transmigrate across human blood-brain barrier endothelium, FASEB J. 16 (2002) 240–242, https://doi.org/10.1096/fj.01-0343fje.
[182] M. Kutateladze, R. Adamia, Phage therapy experience at the Eliava Institute, Médecine Mal Infect. 38 (2008) 426–430, https://doi.org/10.1016/J.MEDMAL.2008.06.023 Elsevier Masson.
[183] V. Ki, C. Rotstein, Bacterial skin and soft tissue infections in adults: a review of their epidemiology, pathogenesis, diagnosis, treatment and site of care, Can. J. Infect. Dis. Med. Microbiol. 19 (2008) 173–184 , J Can des Mal Infect la Microbiol medicale. Hindawi Limited. Available http://www.ncbi.nlm.nih.gov/pubmed/19352449.
[184] B.A. Pruitt, A.T. McManus, S.H. Kim, C.W. Goodwin, Burn wound infections: current status, World J. Surg. (1998) 135–145, https://doi.org/10.1007/s002689900361.
[185] C.S. McVay, M. Velásquez, J.A. Fralick, Phage therapy of Pseudomonas aeruginosa infection in a mouse burn wound model, Antimicrob. Agents Chemother. 51 (2007) 1934–1938, https://doi.org/10.1128/AAC.01028-06.
[186] B. Weber-Dabrowska, M. Mulczyk, A. Górski, Bacteriophage therapy of bacterial infections: an update of our institute's experience, Arch. Immunol. Ther. Exp. (Warsz) 48 (2000) 547–551.
[187] D.D. Rhoads, R.D. Wolcott, M.A. Kuskowski, B.M. Wolcott, L.S. Ward, A. Sulakvelidze, Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial, J. Wound Care. 18 (2009)https://doi.org/10.12968/jowc.2009.18.6.42801 237–8, 240–3.
[188] J. Debattista, Phage therapy: where East meets West, Expert Rev. Anti-Infect. Ther. 2 (2004) 815–819.
[189] K. Markoishvili, G. Tsitlanadze, R. Katsarava, J.G. Morris, A. Sulakvelidze, A novel sustained-release matrix based on biodegradable poly(ester amide)s and impregnated with bacteriophages and an antibiotic shows promise in management of infected venous stasis ulcers and other poorly healing wounds, Int. J. Dermatol. 41 (2002) 453–458, https://doi.org/10.1046/j.1365-4362.2002.01451.x.
[190] S.T. Abedon, S.J. Kuhl, B.G. Blasdel, E.M. Kutter, Phage treatment of human infections, Bacteriophage 1 (2011) 66–85, https://doi.org/10.4161/bact.1.2.15845.
[191] S.T. Abedon, Phage therapy of pulmonary infections, Bacteriophage 5 (2015), e1020260. https://doi.org/10.1080/21597081.2015.1020260.
[192] S. Hoe, D.D. Semler, A.D. Goudie, K.H. Lynch, S. Matinkhoo, W.H. Finlay, et al., Respirable bacteriophages for the treatment of bacterial lung infections, J. Aerosol. Med. Pulm. Drug. Deliv. 26 (2013) 317–335, https://doi.org/10.1089/jamp.2012.1001.
[193] H.L. Sadoff, J.W. Almlof, Testing of filters for phage removal, Ind. Eng. Chem. 48 (1956) 2199–2203, https://doi.org/10.1021/ie50564a044.
[194] E. Morello, E. Saussereau, D. Maura, M. Huerre, L. Touqui, L. Debarbieux, Pulmonary bacteriophage therapy on pseudomonas aeruginosa cystic fibrosis strains: first steps towards treatment and prevention, Aziz R, editor PLoS One 6 (2011) e16963, https://doi.org/10.1371/journal.pone.0016963 Public Libr. Sci.
[195] L. Golshahi, K.D. Seed, J.J. Dennis, W.H. Finlay, Toward modern inhalational bacteriophage therapy: nebulization of bacteriophages of burkholderia cepacia complex, J. Aerosol. Med. Pulm. Drug. Deliv. 21 (2008) 351–360, https://doi.org/10.1089/jamp.2008.0701 Mary Ann Liebert, Inc. 2 Madison Avenue Larchmont, NY 10538 USA.
[196] S.S.Y. Leung, T. Parumasivam, F.G. Gao, N.B. Carrigy, R. Vehring, W.H. Finlay, et al., Production of inhalation phage powders using spray freeze drying and spray drying techniques for treatment of respiratory infections, Pharm, Res. 33 (2016) 1486–1496, https://doi.org/10.1007/s11095-016-1892-6 Springer US.
[197] C.J. Cooper, S.P. Denyer, J.Y. Maillard, Stability and purity of a bacteriophage cocktail preparation for nebulizer delivery, Lett. Appl. Microbiol. 58 (2014) 118–122, https://doi.org/10.1111/lam.12161.
[198] S.S.Y. Leung, T. Parumasivam, F.G. Gao, E.A. Carter, N.B. Carrigy, R. Vehring, et al., Effects of storage conditions on the stability of spray dried, inhalable bacteriophage powders, Int. J. Pharm. 521 (2017) 141–149, https://doi.org/10.1016/j.ijpharm.2017.01.060 Elsevier.
[199] L. Golshahi, K.H. Lynch, J.J. Dennis, W.H. Finlay, In vitro lung delivery of bacteriophages KS4-M and ΦKZ using dry powder inhalers for treatment of Burkholderia cepacia complex and Pseudomonas aeruginosa infections in cystic fibrosis, J. Appl. Microbiol. 110 (2011) 106–117, https://doi.org/10.1111/j.1365-2672.2010.04863.x.
[200] S. Matinkhoo, K.H. Lynch, J.J. Dennis, W.H. Finlay, R. Vehring, Spray-dried respirable powders containing bacteriophages for the treatment of pulmonary infections, J. Pharm. Sci. 100 (2011) 5197–5205, https://doi.org/10.1002/jps.22715 Wiley Subscription Services, Inc., A Wiley Company.
[201] D. Vandenheuvel, A. Singh, K. Vandersteegen, J. Klumpp, R. Lavigne, G. Van Den Mooter, Feasibility of spray drying bacteriophages into respirable powders to combat pulmonary bacterial infections, Eur. J. Pharm. Biopharm. 84 (2013) 578–582, https://doi.org/10.1016/j.ejpb.2012.12.022 Elsevier.
[202] R.Y. Chang, J. Wong, A. Mathai, S. Morales, E. Kutter, W. Britton, et al., Production of highly stable spray dried phage formulations for treatment of Pseudomonas aeruginosa lung infection, Eur. J. Pharm. Biopharm. 121 (2017) 1–13, https://doi.org/10.1016/j.ejpb.2017.09.002 Elsevier.
[203] D. Vandenheuvel, J. Meeus, R. Lavigne, G. Van Den Mooter, Instability of bacteriophages in spray-dried trehalose powders is caused by crystallization of the matrix, Int J Pharm. 472 (2014) 202–205, https://doi.org/10.1016/j.ijpharm.2014.06.026 Elsevier.
[204] S. Morrison, R.E. Gardner, The treatment of a lung abscess due to bacillus coli with a lytic filtrate, J. Am. Med. Assoc. 107 (1936) 33, https://doi.org/10.1001/jama.1936.92770270005010c American Medical Association;.
[205] B. Burrowes, D.R. Harper, J. Anderson, M. McConville, M.C. Enright, Bacteriophage therapy: potential uses in the control of antibiotic-resistant pathogens, Expert Rev. Anti-Infect. Ther. 9 (2011) 775–785, https://doi.org/10.1586/eri.11.90.
[206] D. Harper, H. Parracho, J. Walker, R. Sharp, G. Hughes, M. Werthén, et al., Bacteriophages and biofilms, Antibiotics 3 (2014) 270–284, https://doi.org/10.3390/antibiotics3030270.
[207] I. Takemura-Uchiyama, J. Uchiyama, M. Osanai, N. Morimoto, T. Asagiri, T. Ujihara, et al., Experimental phage therapy against lethal lung-derived septicemia caused by Staphylococcus aureus in mice, Microbes Infect. 16 (2014) 512–517, https://doi.org/10.1016/j.micinf.2014.02.011.
[208] Y. Hua, T. Luo, Y. Yang, D. Dong, R. Wang, Y. Wang, et al., Phage therapy as a promising new treatment for lung infection caused by carbapenem-resistant acinetobacter baumannii in mice, Front. Microbiol. 8 (2018) 2659, https://doi.org/10.3389/fmicb.2017.02659 Frontiers.
[209] M.G. Montague, C. Lartigue, S. Vashee, Synthetic genomics: potential and limitations, Curr. Opin. Biotechnol. (2012) 659–665, https://doi.org/10.1016/j.copbio.2012.01.014.
[210] M. Di Giovine, B. Salone, Y. Martina, V. Amati, G. Zambruno, E. Cundari, et al., Binding properties, cell delivery, and gene transfer of adenoviral penton base displaying

bacteriophage, Virology 282 (2001) 102–112, https://doi.org/10.1006/viro.2000.0809 Academic Press.
[211] L.K. Medina-Kauwe, Endocytosis of adenovirus and adenovirus capsid proteins, Adv. Drug Deliv. Rev. 55 (2003) 1485–1496, https://doi.org/10.1016/j.addr.2003.07.010.
[212] A. Hajitou, M. Trepel, C.E. Lilley, S. Soghomonyan, M.M. Alauddin, F.C. Marini, et al., A hybrid vector for ligand-directed tumor targeting and molecular imaging, Cell 125 (2006) 385–398, https://doi.org/10.1016/j.cell.2006.02.042 Elsevier.
[213] A. Hajitou, R. Rangel, M. Trepel, S. Soghomonyan, J.G. Gelovani, M.M. Alauddin, et al., Design and construction of targeted AAVP vectors for mammalian cell transduction, Nat. Protoc. 2 (2007) 523–531, https://doi.org/10.1038/nprot.2007.51.
[214] C.A. Stoneham, M. Hollinshead, A. Hajitou, Clathrin-mediated endocytosis and subsequent endo-lysosomal trafficking of adeno-associated virus/phage, J. Biol. Chem. 287 (2012) 35849–35859, https://doi.org/10.1074/jbc.M112.369389.
[215] P. Tao, M. Mahalingam, B.S. Marasa, Z. Zhang, A.K. Chopra, V.B. Rao, In vitro and in vivo delivery of genes and proteins using the bacteriophage T4 DNA packaging machine, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 4–9, https://doi.org/10.1073/pnas.1300867110/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1300867110.
[216] K. Dabrowska, A. Opolski, J. Wietrzyk, K. Switala-Jelen, J. Godlewska, J. Boratynski, et al., Anticancer activity of bacteriophage T4 and its mutant HAP1 in mouse experimental tumour models, Anticancer Res. 24 (2004) 3991–3995.
[217] K. Dąbrowska, A. Opolski, J. Wietrzyk, D. Nevozhay, K. Szczaurska, K. Switała-Jeleń, et al., Activity of bacteriophages in murine tumor models depends on the route of phage administration, Oncol. Res. 15 (2005) 183–187.
[218] F. Eriksson, P. Tsagozis, K. Lundberg, R. Parsa, S.M. Mangsbo, M.A.A. Persson, et al., Tumor-specific bacteriophages induce tumor destruction through activation of tumor-associated macrophages, J. Immunol. 182 (2009) 3105–3111, https://doi.org/10.4049/jimmunol.0800224.
[219] N. Cerf-Bensussan, V. Gaboriau-Routhiau, The immune system and the gut microbiota: friends or foes? Nat. Rev. Immunol. (2010) 735–744, https://doi.org/10.1038/nri2850.