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Chapter 5

Bacteriophages for treatment of biofilm infections

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History of bacteriophage use in human infections

Bacteriophages – or literally “bacteria-eaters” from the Greek *phagein* meaning "to devour” – were first mentioned in 1915 by Frederick Twort. They owe their therapeutic applications to Félix d’Hérelle, who isolated them in 1917 in stool samples of patients suffering from shigellosis [1]. Shortly thereafter, d'Herelle used bacteriophages to treat bacillary dysentery (shigellosis). This was probably the first attempt of phage application to treat pathogenic bacterial infections. The bacteriophage preparation was first ingested by d'Herelle and some colleagues in order to evaluate its safety before being administered to a 12-year-old boy with severe dysentery. After a single application of the anti-dysentery bacteriophage, the patient's symptoms terminated, and the boy fully recovered within days [2]. Inspired by these results, d'Herelle continued studies on the therapeutic use of bacteriophages, carried many non-randomized trials in humans [3], and co-founded with George Eliava an institute, known today as the “Eliava Institute of Bacteriophages, Microbiology and Virology” in Tbilisi, Georgia, to carry out basic bacteriophage research and provide bacteriophages to treat human bacterial infections.

The development of bacteriophages as antimicrobials continued for about three decades, i.e. from about 1915–42 [4]. During this period, bacteriophages were used, among other indications, in France against avian typhoid caused by *Salmonella gallinarum,* and in the United States against chronic furunculosis. Phage therapy was also used during the Winter War between the former Soviet Union and Finland (1939–40), with 6,000 Soviet soldiers treated against streptococcal or staphylococcal wound infections, which prevented limb amputations and reduced mortality due to gangrene. Companies such as Behring in Germany and Eli Lilly in the United States produced phage preparations against *streptococci*, *staphylococci* and *Escherichia coli*. During World War II in Africa, the German army and the allied forces applied bacteriophages against dysentery [5].

The progress made on the synthesis of penicillin during the 1940’s ushered in the era of antibiotic use, a golden age of medicine that largely continues to this day, which lead to an almost complete abandonment of interest in the development of phage as clinically-used antibacterial agents, especially in the Western countries [4]. In the Eastern countries, however, phage therapy was never abandoned, persisting to this day in countries such as Poland, Georgia and Russia. Much knowledge of phage therapy in human patients comes from numerous publications in either Russian or Polish journals from that period, for example, involving the use of oral phages to treat gastrointestinal infections, including shigellosis and salmonellosis [4].

Phage therapy was rediscovered by the English-language literature starting with the work of Smith and Huggins in the 1980s, and progressively gained attention during the 1990s followed by the start of human experiments in the 2000s [6]. The first placebo-controlled phase I trial in the United States was published in 2009, and showed no safety concerns [7].

The emergence of multidrug-resistant bacterial infections has led to recent efforts investigating and promoting phage therapy to treat multitude of infections. Despite the costly and time-consuming requirements for the production of bacteriophages under current guidelines in the United States and the European Union, some countries are trying to accelerate the implementation of phage therapy through the so-called "Magistral Approach". Belgium, for instance, is currently implementing a pragmatic framework on phage therapy that centers on magistral preparation of individual therapeutic bacteriophages by pharmacies, and although the final products will not fully comply with the European requirements for medicinal products for human use (Directive 2001/83/EC), such magistral phage preparations can be used to treat patients in Belgium [8].

Principles of bacteriophage therapy

Molecular background

Bacteriophages (also referred as phages) are viruses that specifically infect bacteria. Phages are the most abundant organisms on Earth, with an estimation of about 10^{31} particles distributed over all ecosystems on our planet. A phage is usually conformed by its genome (single or double-stranded DNA or RNA) encapsulated in a protein capsid, which is sometimes completed with a tail and more or less complex appendages (e.g. spikes, tail fibers, etc.) (Figure 5.1) [9, 10]. As nonliving microorganisms, they rely on the bacterial cellular machinery to reproduce. The viral infection begins by attachment of the phage to its bacterial host through specific recognition of one or more receptors on the bacterial cell. These receptors can be found in the cell wall, bacterial capsules, slime layers, pili or flagella, often consisting of proteins, lipopolysaccharides, teichoic acids and other cell surface structures serving as irreversible phage-binding receptors [11]. Upon recognition of the cell receptors, the phage injects its genetic material into the cytoplasm of the infected cell, and depending on its nature (virulent or temperate), it follows the lytic or lysogenic cycle.

Virulent phages follow the lytic cycle, where the host's genome is first degraded and the bacterial metabolic machinery is employed to copy the viral genome and produce viral proteins. After that, the viral particles are self-assembled, and the bacterial cell is lysed by phage enzymes, releasing the progeny phages and killing the bacterial host. Tempered phages, on the other hand, can follow the lysogenic cycle. They become latent by inserting their genome either as a free plasmid inside the host cell or integrated into the bacterial chromosome. By this mean, they propagate to the next generations of bacterial cells. Under specific stressful environmental conditions, temperate phages can eventually shift towards the lytic cycle. As a result, the phage genome will be excised from the host chromosome, replicated, encapsulated and then the phage particles will be released from the host bacterium by cell lysis, causing the death of the bacterial host cell [12]. Temperate phages have

been described to transfer new genes to their hosts, including antibiotic resistance genes. In addition, they can alter the expression of host genes or provide protection to the host against infection by other phages [13]. Thus, strictly virulent phages, with immediate bactericidal effect, are favored for use in clinical practice.

Resistance Mechanisms

Bacteria can develop resistance against phages at any stage of the phage infection cycle, classified as adaptive or non-adaptive mechanism [11]. Common non-specific anti-phage resistance mechanisms that have been described until now include (a) preclusion of phage adsorption and prevention of nucleic acid entry by surface modifications and receptor mutations; (b) superinfection exclusion systems preventing a secondary viral infection with the same or a closely related virus, (c) restriction-modification systems responsible for the cleavage of exogenous dsDNA and protection of bacterial genetic material and (d) abortive infection leading to cell death or stasis when phage replication takes place. A second line of defense (adaptive defense) is associated with restriction enzymes and the CRISPR-Cas system (Clustered Regularly Interspaced Short Palindromic Repeats and associated proteins), which can identify and cleave phage nucleic acids in a highly specific and effective manner [14].

Phages can evolve and develop counterstrategies to circumvent bacterial anti-phage mechanisms. Based on their genomic plasticity and rapid replication rates, phages can overcome adsorption inhibition by point mutations in specific genes or escape from restriction-modification mechanisms by genome rearrangements. Furthermore, phages can use anti-CRISPR proteins to evade the CRISPR/Cas system, or avoid the abortive infection by hijacking bacterial antitoxins [11].

Contrary to antibiotics, phages have minimal influence to normal microbiome due to their high bacterial species or strain specificity, and have the ability to increase in number at the site of infection due to their “multiplicity” [15], what theoretically would imply that a little phage dose is sufficient for effective treatment.

Antibiotic-bacteriophage interactions

Although the use of phages alone would potentially demonstrate clinical success, their combination with antibiotics have shown to be more effective than phage monotherapy in numerous *in vitro* and animal studies. These studies have proved statistically or clinically significant phage-antibiotic synergism, biofilm minimization or reductions in resistance emergence [12]. Regardless of the antibiotic resistance state of the bacteria, the combinatorial approach using phages and antibiotics have demonstrated a range of benefits [16]. For instance, it has been shown for some phage/antibiotic combinations that sub-inhibitory concentration of antibiotics can foster phage

productivity and consequently decrease bacterial counts. A restoration of antibiotic sensitivity, by loss of bacterial fitness or by phage interaction with the bacterial drug efflux systems, has also been described. Phages can additionally work as adjuvants of antibiotics against biofilms by enabling antibiotics to reach bacterial cells deeper within biofilms through degradation of the exopolysaccharide matrix by depolymerases and by infecting antibiotic-tolerant persisters (further information in section "*Activity of phages against bacterial biofilms and persisters*"). However, antagonistic effects can also occur with phage/antibiotic combinations depending on the treatment conditions (e.g., dosage, order of administration, timing, etc.). Thus, combinatorial therapies require a careful choice of dosing and time points at which either antimicrobial substance is administered. *In vitro* studies determined better outcomes if phage was administered before antibiotics, than if antibiotics were introduced before or simultaneously with phage. This is possibly due to the killing of host bacteria, which are essential for phage production, by antibiotics [12]. Other competing dynamics between phage and antibiotics may also play a role. Antibiotics are expected to interfere with aspects of bacterial physiology that can be crucial to phage activities, as for instance by interfering with bacterial ribosome functioning, necessary for phage protein production [17].
The pharmacokinetic/pharmacodynamic (PK/PD) modeling techniques traditionally used for antibiotics differ for phages. The phage concentration is expected to increase at the site of infection through their replication in living bacteria. PK/PD models for phage therapy should integrate the classical antimicrobial pharmacological view (drug impact on the body, drug interactions, absorption, distribution, metabolism, secretion, etc.) with the self-replication characteristic of phages [18].
The immune system plays also a key role in phage inactivation and/or clearance from the body, which may pose a problem maintaining sufficient phage titers for therapeutic activity. Based on limited reports on immune response in clinical studies using virulent phages, their immunogenicity does not seem to represent a safety risk. Major concerns encompass an increase in pro-inflammatory cytokines as response to the potentially massive liberation of bacterial endotoxins after bacterial lysis, as it has been observed with the use of certain antibiotics [19]. So far, there is not enough evidence-based data for a better understanding of the phage pharmacokinetics and the phage immune interaction as well as the clinical relevance of all these parameters.

Activity of bacteriophages against bacterial biofilms and persisters

Biofilms are complex clusters of bacteria, formed by single or multiple species, merged by extracellular polymeric substances (EPS) and adhered to surfaces, including living tissue or medical devices, among others. Biofilm microorganisms are metabolically less active and have a minimal growth rate. Therefore, they are tolerant to many antibiotics [20][21]. Bacteriophages showed

promising results for biofilm eradication due to their multiplicity at the infection site, but also by producing specific enzymes that allow them to actively penetrate and disrupt biofilms and for their ability to infect persisters which are less metabolically active bacteria [20].

Phages encoding EPS-degrading enzymes are particularly useful against biofilms. A diverse group of phage-encoded enzymes, called depolymerases, capable of degrading polymers – either associated with the cell surface (e.g. capsule polysaccharides) to facilitate phage adsorption, or EPS involved in biofilm matrix in order to promote phage diffusion through the biofilm – have been described [22]. Depolymerases can be associated with virions, forming part of the phage particle (e.g. in their tail spikes), or be in soluble form.

Depolymerases derived from phages have been tested against biofilms of different bacterial species, exhibiting dose-dependent activity and reducing significantly the biofilm biomass [20, 23, 24]. Similar to the host specificity of bacteriophages, phage-associated depolymerases can be highly specific for host-derived EPS. Since different species of bacteria produce different EPS components, depolymerase active against the polysaccharides produced by one species may not act on that produced by other bacteria [24]. However, some depolymerases are capable of degrading EPS of several genera [23]. Moreover, some bacteriophages can induce their host bacteria to produce and release depolymerases, which could be a phage mechanism to make the biofilm matrix more porous, facilitating infection by progeny bacteriophage or, alternatively, a fight response by infected bacteria, seeking to facilitate movement away from the focus of infection [24], leading in any case to a disaggregation of the biofilm.

Unlike other antimicrobials, bacteriophages replicate within their host cells, which can result in self-sustaining infections with ongoing amplification leading to an increasing number of bacteriophages. The localized spread of phage progeny continues infecting and killing more bacteria, which is called multiplicity at the infection site. These mechanisms require a critical mass of host bacteria at the same location, which is typically the case in biofilm infections [24]. Hence, by spreading through the biofilm, bacteriophages can progressively remove the biofilm and reduce the potential for regrowth. The regrowth of bacteria within the biofilm it is thought to arise from the presence of persisters. Unlike resistant bacterial cells, where resistance mechanisms are based on genetic changes that block antimicrobial activity, persisters present a transient non-heritable phenotype that is thought to be less sensitive to antibiotics because the cells are not undergoing cellular activities that antibiotics can corrupt, which results in tolerance [25]. Thus, persisters can remain viable over the course of antibiotic exposure and repopulate the biofilm when the levels of antibiotic drop, causing the relapse of the infection. Some studies have reported on the ability of bacteriophages to infect persisters, and initiate a productive lytic infection when persisters switch to normal growth, ultimately causing their lysis [24].

New concepts are emerging nowadays in the design of phage-based treatments to maximize phage therapy efficacy minimizing the likelihood of resistance emergence. A schematic illustration of phage-based treatments for biofilm removal is shown in figure 5.2 [20].

Designing phage cocktails that include phages against multiple species have been shown to be especially effective against multi-species biofilms [23, 26]. Phage cocktails, besides conferring activity against a broader host range, can help also preventing the emergence of phage resistant bacteria if multiple phages active against a given target are included in the cocktail [20]. However, in order to avoid possible undesired effects when using phage cocktails, a rational approach to designing cocktails is crucial. In a phage cocktail, the various phages should not compete with each other, in order to minimize the risk for reduction of efficacy. In addition, the mechanisms of phage resistance by bacteria should be different in order to minimize the risk of cross-resistance. [26].

Bacteriophages can be genetically modified to improve their bacterial killing properties. Existing examples of genetically engineered phages include a phage with altered tail fiber proteins to extend its host range [23], a phage designed to produce a soluble hydrolase that enhances biofilm degradation, a temperate phage turned into a lytic phage by removal of all genes related to lysogeny or a chimeric phage encoding a short peptide with broad-spectrum anti-biofilm effect [20].

Bacteriophage susceptibility testing: the phagogram

Phage therapy is still not approved in most parts of the world, and extensive research on its efficacy and safety is still to be conducted. However, if the particular conditions according to the Declaration of Helsinki (article 37) are met and bacteriophages are to be applied, the magistral approach ("compounded" drug product in United States) is to be followed. In practice, it means that, only when no other option of treatment is available, a phage preparation is prescribed by a physician for an individual patient and prepared by the hospital pharmacist following strict safety regulations [8].

Due to the high host-specificity of bacteriophages (mostly infecting single species or even single strains of bacteria), when preparing the phage solution for an individual patient, it is important to select bacteriophages active against the patient's isolated strain. To this end, similarly to an antibiogram (antibiotic susceptibility testing), a so-called "phagogram" needs to be performed [8].

Various methods for testing bacteria susceptibility to bacteriophages have been described until now, such as the spot test, efficacy of plating (EOP) or killing assays. The simplest method among them is the spot test, where small droplets of a bacteriophage lysate are applied on a plate prepared with the bacterial strain to be tested and the appearance of a clear zone (lysis) determines for bacterial susceptibility to the bacteriophage. However, lysis observed by this method may be the result not only from the bacteriophage infection that give rise to lysis and production of new phage, but also due to residues of bacteriocins on the phage lysate that kills bacteria, or to the phages themselves

causing abortive infections or lysis from without, leading to false positive results [27]. Performing an EOP assay, by plating different titers of bacteriophages, quantifying plaque-forming-units (PFU) and comparing it to the PFU count of a reference bacterial strain can provide more information on the efficacy of a particular bacteriophage [27]. Nevertheless, the absence of plaque formation does not necessarily correlate with a lack of bacteriophage ability for a productive infection. Plaque formation might depend on several factors, including phage diffusion in agar, adsorption rate, electrolyte requirements, growth phase of the host, etc. [28].

Killing assays, in which bacteria and bacteriophages are incubated together in liquid medium and the optical density or heat flow production are measured as indicators of bacterial presence, represent useful methods for determination of the minimum phage titer needed for successful bacteria killing or to better monitor phage virulence [29, 30]. On the other hand, these assays are usually less cost-efficient as clinical laboratory tests due to a higher instrumental cost, minimum automation or limitations in their throughput.

A standardized method, easy to perform, fast and available to everyone is still to be developed. Currently, several projects for the development of an automated, reliable and reproducible phagogram technique are ongoing. Some examples include the PHAGOGramme project under development by Pherecydes Pharma (https://www.pherecydes-pharma.com/phagogramme.html), the combined PhageBank™and HRQT™approaches of the clinical-stage company Adaptive Phage Therapeutics (http://www.aphage.com/the-science/#phagebank) or the PhagoFlow project as a joint effort of different institutions including the Charité-Universitätsmedizin, the Bundeswehr Hospital Berlin, the Leibniz Institute DSMZ and the Fraunhofer ITEM (https://www.phagoflow.de/en/phagogram/).

Experimental and clinical evidence with bacteriophage treatment

Despite long history of use of phages for antibacterial therapy since their discovery in the early 1900s, and even with the availability of phage products for the treatment of bacterial infections in some countries (e.g. Georgia, Poland, Russia), extensive *in vitro* and experimental studies as well as clinical trials to fulfill the requirements for phage therapy according to good manufacturing practice guidelines are lacking [31].

The efficacy of phage therapy has been investigated for bloodstream, gastrointestinal, urinary tract, burn wounds and respiratory infections [19, 26]. We limit our focus on experimental and clinical evidence of phage therapy in bone and joint infections.

Most preclinical studies investigated the efficacy of bacteriophages on monomicrobial *S. aureus* or *P. aeruginosa* infections demonstrating large reduction of planktonic bacteria, successful prevention of

bacterial adherence to foreign material and synergism between antibiotics and phages to eradicate biofilms [32]. However, numerous experimental limitations need to be addressed. For instance, limited data exist about phages active against *S. epidermidis* despite its high prevalence in implant-associated infections, its strong biofilm forming capability, and its extensive resistance to antibiotics [33]. Moreover, *in vivo* models that replicate the joint and peri-implant microenvironment are lacking, which makes the translation of preclinical findings into clinical settings difficult [34]. One promising *in vivo* model published by Carli *et al.* in 2017 [35] replicates accurately the clinical setting of total joint replacement, and could therefore be adopted in the future for phage therapy testing. Other studies reported a concentration dependency of phage therapy, suggesting that low-titer phage administration or single instead of multiple doses are unlikely to be successful. In addition, considering for instance possible vascular impairments in open fractures or the wish for reduction of systemic effects, local treatment is often preferred in bone and joint infections [36]. Hence, in aiming for phage stability and appropriate release kinetics during treatment, an important part of research in phage therapy is focused on the encapsulation of phages into sustained release systems. Numerous strategies regarding bacteriophage formulation and encapsulation are being implemented, showing promising outcomes under experimental settings [36]. Still, great challenges involve a rational design of carriers loaded with precise doses of encapsulated phage able to support controlled releases in patients.

Osteomyelitis is another clinical field where phage therapy has been applied, often using anti-staphylococcal bacteriophages. A summary of clinical studies on phage therapy for musculoskeletal infections is presented in Table 5.1 (reprinted with permission from [32]). The largest clinical study with 120 participants was conducted in Tbilisi, Georgia, assessing the therapeutic efficacy of a custom-made staphylococcal cocktail against arthritis and osteomyelitis. The summarized results do not allow to evaluate phage efficacy. All 120 patients had complete recovery of osteomyelitis and/or arthritis, namely 9 patients with phage therapy alone, 51 patients with phage plus antibiotics and 60 patients with antibiotics alone. These results are much better in each treatment group, than could be expected.

In Western countries, due to strict regulations in application of phage therapy, clinical experience with bacteriophages is limited to individual cases with a total of 5 case reports and 1 case series published between 2017 and 2019. As shown in Table 5.1, two case reports investigated the use of bacteriophages in osteomyelitis, another two in prosthetic joint infection, and one in a fracture related infection. The applied bacteriophages were used to target *P. aeruginosa*, *S. aureus*, *Acinetobacter baumannii, Klebsiella pneumoniae, S. epidermidis and E. faecalis, respectively*. Bacteriophages were administered either intravenously or locally, and in combination with

intravenous antibiotics. Eradication of infection was seen in all case reports except for one, where the outcome of infection was unclear due to death caused by the primary disease of the patient. Although bacteriophages have so far demonstrated good efficacy and safety, experience is still lacking and comprehensive and well-organized studies on the production and processing of bacteriophages, their administration and dosage, as well as exhaustive clinical monitoring of results, are still needed.

Local delivery and systemic bacteriophage application

A major hurdle of phage therapy is the achievement of a sufficient number of phages at the site of infection to accomplish therapeutic activity. Phage ability to disseminate throughout the body strongly depends on the route of administration and the initial phage dose [37]. Administration of high or repeated phage doses might increase the chances for successful distribution. Furthermore, encapsulation of phages might allow a controlled phage release and act as shield against chemical degradation or immunological neutralization, prolonging its systemic circulation period [18]. Routes of phage application include the use of parenteral administration, being oral dosing, topical and aerosolization also commonly applied. A summary of some advantages and disadvantages of the administration routes can be seen in Table 5.2 (reprinted with permission from [19]).

Systemic delivery

Systemic phage delivery by intravenous, intraperitoneal or intramuscular injection allows rapid phage dissemination in different organs and tissues such as the liver, spleen, kidneys and lungs [38]. A nearly complete recovery of administered phages was shown several minutes after intravenous application [37]. Phages distribution was documented in the heart, skeletal muscles, bladder, thymus, bone narrow, lungs or brain but not yet in joints, bone or eyes [18].
The use of highly purified phage preparations without bacterial components or endotoxins is essential to minimize the risk of side-effects due to impurities.

Oral or inhaled delivery

Oral route of administration has been successfully used in gastrointestinal infections. However, phage stability in acidic environment in the stomach and duodenum may reduce the phage concentration or activity [38]. Thus, protection of phages from the gastric acidity could be achieved by phage encapsulation, as shown in a study against *Salmonella* spp. [39].

In respiratory infections, liquid and dry powder phage formulations were investigated for nebulization and inhalation for topical delivery in acute and chronic lung infections [40].

Local delivery

Effective local delivery of antibacterial substances is essential in patients with biofilm infections associated either to implanted medical devices or chronic wounds, since antibacterial drugs have limited activity in such infections. Bacteriophages may have better efficacy in such infections, provided that they reach the infectious site. Therefore, a major focus is being set in the implementation of drug carriers to allow local and prolonged release of phages. Unfortunately, there is insufficient data available on processing phages into well-defined pharmaceutical formulations, their long-term stability and impact on phage efficacy *in vivo* [40]. Wound healing is one of the therapeutic areas where local application of phages has received a lot of attention. Advances in the development of phage formulations including hydrogels, liposome entrapment or phage-immobilized wound dressings have led to increasing successful rates in the topical application of phage therapy [41]. Numerous reviews report on the widespread clinical use of phage preparations for the treatment of skin infections, purulent and surgical wounds, mostly by the former Soviet Union countries. In Europe, the project *Phagoburn*, launched in June 2013, was the first prospective multicentric, randomized, single blind and controlled clinical trial on phage therapy to treat *Escherichia coli* and *Pseudomonas aeruginosa* skin infections in burn patients [42]. It allowed significant advances regarding the regulatory framework of phage therapy as well.
Some clinical cases and pre-clinical studies also support the effective local delivery of bacteriophages to treat local bone infections (see section "*Experimental and clinical evidence with bacteriophage treatment*"). Currently, the application of phages in patients with severe musculoskeletal infections has generally consisted in local administration through a draining system (Figure 5.3) [43]. Although this approach has shown successful outcomes, it has the drawback of the usage of the drainage tube as delivery route, which could favor the emergence of superinfections, besides being a cumbersome method. Thus, the optimization of local phage delivery strategies might help overcoming these issues. Some examples are an engineered hydrogel for controlled delivery of phage targeting *Pseudomonas aeruginosa* to the site of orthopedic infections [44] or the use of fibrin glue for sustained delivery of viable phages [45]. Phages have also been immobilized on surfaces for the prevention of biofilm formation with examples on urinary catheters or on nylon sutures for wound healing applications [36].

Outlook and future perspectives

The rising threat of multi-resistant bacterial infections has brought together many research institutions, hospitals and the industry in a joint effort to seek alternative treatments to the conventional use of antibiotics.

Phages have unique features that make them convincing antibacterial agents, alone or in combination with other antimicrobials, while the constraints associated with the implementation of phage therapy could be overcome through a combination of proper phage selection, effective formulation and greater clinician understanding of and familiarity with product application.

Phages have been used to treat bacterial infections since their discovery, being for decades and also today the standard of care in several countries of Eastern Europe and having demonstrated clinical success in recent compassionate care cases in Western Europe and the United States, with no serious adverse events been reported to date.

The increasing number of publications that have appeared during the last decade and the growing interest of the industry in phage therapy represent very encouraging progress in addressing the knowledge gap required for phage therapeutic applications.

Key Points

- With increasing antimicrobial resistance of bacteria, there is a rising interest in the therapeutic potency of bacteriophages.
- Biofilm infections are tolerant to most antibiotics. Phage therapy of such infections could be an attractive new option.
- There are a few clinical studies showing that bacteriophages are able to eradicate musculoskeletal infections without serious adverse events. However, controlled trials of high quality are still lacking.
- Despite current restrictions in the application of phage therapy, commercialization of phage-based technologies in Western countries is garnering a surge of interest.

Legends to Figures

Figure 5.1. Representative structures of tailed phages. All tailed phages have a capsid that encloses and protects the genome and connects to the tail. **(a)** Phages in the *Myoviridae* family are the only tailed phages with a contractile tail sheath. **(b)** Both phages belonging to the *Myoviridae* and *Siphoviridae* families have a baseplate at the distal end of the tail to which receptor-binding proteins (RBPs), such as tail fibres and tail spikes, are attached. **(c)** | Because members of the *Podoviridae* have no baseplate, the RBPs directly attach to the tail. *Siphoviridae* and *Podoviridae* additionally have a central tail fibre or spike that protrudes from the distal end of the tail or baseplate. Reprinted with permission from Nobrega et al. [10]

Figure 5.2. Main phage-based treatments for biofilm removal. Reprinted with permission from Ferriol-González&Domingo-Calap [20]

Figure 5.3. Phage therapy of pelvic osteomyelitis (pathogen: pan-resistant *Pseudomonas aeruginosa*). **(a)** Removal of foreign bodies and surgical debridement of necrotic tissue. **(b)** Preparation of a wound filler impregnated with the phage solution. **(c)** Insertion of the instillation tubes before wound closure. **(d)** Daily administration of 50 ml of the bacteriophage suspension after pre-treatment of the wound with bicarbonate buffer (over one week). Reprinted with permission from Vogt et al. [43]

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Tables

Table 5.1. Human clinical studies on phage therapy for musculoskeletal infections. Reprinted with permission from Onsea et al. [32].

Reference	Sample size	Patient characteristics	Intervention	Outcome
Lang *et al.*, 1979	7	PJI (*n* = 2) OM (*n* = 1) Septic arthritis (*n* = 1) Spinal infection (*n* = 1) FRI (*n* = 2)	Phages adapted to isolated strains Administration either topical or by injection through a draining system. Some cases received combination treatment with antibiotics	5/7 treated Recurrence of spinal infection and one FRI
Kutateladze and Adamia, 2010	120	Patients with staphylococcal OM or arthritis	Three groups: - antibiotics (*n* = 60) - phage monotherapy (*n* = 9) - phage + antibiotics (*n* = 51) Administration of Eliava staphylococcal phage preparation topically or intravenously	100 % success rate in all groups
Slopek *et al.*, 1987	100	Purulent arthritis and myositis (*n* = 19) OM of the long bones (*n* = 40) FRI (*n* = 41)	Administration locally and/or orally Some cases received combination treatment with antibiotics	Success rates: - purulent arthritis and myositis: 89.5 % - OM of the long bones: 95 % - FRI: 90.2 %
Weber-Dabrowska *et al.*, 2000	81	OM of the long bones (*n* = 40) FRI (*n* = 41)	Administration locally and/or orally Unclear if some patients received combination treatment with antibiotics	Success rates: OM of the long bones: 95 % FRI 60 %
Vogt *et al.*, 2017	1	OM	Repeated dosing of phage cocktail Pyo bacteriophage through draining system, in combination with antibiotic therapy	Eradication of the infection
Ferry *et al.*, 2018a	1	OM (post-radiation)	Application of customised phage cocktail every 3 d, in combination with intravenous antibiotic therapy	Patient died 45 d after treatment due to cancer progression
Ferry *et al.*, 2018b	1	PJI	Single intraoperative injection of a customised phage cocktail in combination with intravenous antibiotic therapy	Eradication of the infection
Nir-Paz *et al.*, 2019	1	FRI	Intravenous repeated administration of customised phage cocktail, in combination with intravenous antibiotic therapy	Eradication of the infection (after two phage therapy regimens)
Tkhilaishvili *et al.*, 2019	1	PJI	Repeated dosing of customised phage cocktail, in combination with intravenous antibiotic therapy	Eradication of the infection
Onsea *et al.*, 2019	4	OM	Repeated dosing of BFC1 cocktail or Pyo bacteriophage cocktail in combination with intravenous antibiotic therapy	Eradication of the infection in all cases

Abbreviations: PJI, prosthetic joint infection; OM, osteomyelitis; FRI, fracture-related infection.

Table 5.2. Routes of administration for phage therapy. Reprinted with permission from Romero-Calle et al. [19]

Delivery Route	Advantages	Disadvantages	Mitigations to Hurdles
Intraperitoneal	Higher dosage volumes possible. Diffusion to other sites.	Extent of diffusion to other sites may be overestimated in humans (most data from small animals)	Multiple delivery sites.
Intramuscular	Phages delivered at infection site.	Slower diffusion of phages (possibly). Lower dosage volumes.	Multi-dose courses.
Subcutaneous	Localized and systemic diffusion.	Lower dosage volumes.	Multi-dose courses.
Intravenous	Rapid systemic diffusion.	Rapid clearing of phages by the immune system.	*In vivo* selection of low-immunogenic phages may be possible.
Topical	High dose of phages delivered at infection site.	Run-off from target site if phages suspended in liquid.	Incorporate phages into gels and dressings.
Suppository	Slow, stable release of phages over long time.	Limited applications/sites. Risk of insufficient dosing. Technically challenging to manufacture.	Careful consideration of phage kinetics required.
Oral	Ease of delivery. Higher dosage volumes possible.	Stomach acid reduces phage titer. Non-specific adherence of phages to stomach contents and other microflora.	Add calcium carbonate to buffer pH. Microencapsulation to deliver phages to target area.
Aerosol	Relative ease of delivery. Can reach poorly perfused regions of infected lungs.	High proportion of phages lost. Delivery can be impaired by mucus and biofilms.	Use of depolymerases to reduce mucus.