

REVIEW ARTICLE

Research progress and medical applications of material-functionalized phages

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Abstract

With the escalating crisis of antimicrobial resistance, the slow pace of traditional antibiotic development presents a critical and growing threat to global public health. Phage therapy, which offers benefits such as high specificity, self-replication, and minimal disruption to the microbiota, has emerged as a promising treatment option. However, its therapeutic potential faces several hurdles, including a limited antibacterial spectrum, inadequate *in vivo* efficacy, susceptibility to immune clearance, and inability to target intracellular bacteria and biofilm-associated infections. To overcome these issues, new delivery systems have been developed by combining engineered phages with functional materials, such as nanomaterials, hydrogels, and liposomes. This approach significantly improves phage targeting, stability, drug delivery capacity, and controlled release. Nevertheless, engineered phages face challenges such as complex host interactions and intestinal delivery. Future research should incorporate artificial intelligence-assisted design, cross-disciplinary teamwork, and innovative materials to develop phage delivery systems as groundbreaking tools against drug-resistant infections and complex diseases. This review discusses the potential uses of engineered phages in combination with antibiotic therapy, clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein gene editing, innovative smart drug delivery, and tumor immune environment control. It also examines the key challenges in translating laboratory studies to clinical use, including technical issues, immune response regulation, and safety concerns.

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1. Introduction

In recent years, the escalating problem of bacterial drug resistance has emerged as a formidable challenge to global public health, with an increase in “superbugs,” such as carbapenem-resistant *Klebsiella pneumoniae* (CRKP) and methicillin-resistant *Staphylococcus aureus* (MRSA).¹ The development of traditional antibiotics has

significantly stagnated. Consequently, nanotechnology has played a vital role in numerous biology-related fields, such as diagnostics, drug delivery, and molecular imaging, paving the way for novel approaches to combat drug-resistant infections.² In this context, phage therapy has emerged as a promising remedy for the “post-antibiotic era” owing to its inherent advantages, such as high host specificity, preservation of normal microbiota, specific lysis of drug-resistant bacteria, high transduction efficiency, and capacity to carry large host DNA fragments.^{3,4} However, phage therapy has its own limitations, such as a narrow antimicrobial spectrum, physical barriers to phage entry into intracellular pathogens, and inconsistent efficacy due to the lack of *in vivo* delivery.^{5,6} To overcome these limitations and offer efficient targeted phage treatment, pioneering research has focused on developing advanced phage delivery systems.

Innovative drug delivery systems employed in phage therapy are essential for enhancing phage stability, bioavailability, and targeted delivery to infection sites.⁷ Nanoconfinement using nanoparticles or hydrogels protects phages from gastrointestinal or bloodstream degradation and can specifically target bacterial pathogens.⁸ Recent advancements in phage delivery systems have achieved significant success in three domains: synergistic enhancement, controlled release, and targeted delivery. This synergistic enhancement, exemplified by the cooperative behavior of engineered phages with antibiotics, has been shown to be effective *in vivo*. Phages, for instance, destabilize bacterial biofilms or compromise cell wall integrity, making bacteria susceptible to antibiotics. This combined approach can significantly reduce the minimum inhibitory concentration of antibiotics and minimize the chance of resistance mutations.⁹ Liu *et al.*¹⁰ utilized a temperate phage to deliver clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein (Cas) 9 gene editing tools, precisely cleaving bacterial resistance genes to restore the sensitivity of susceptible bacteria to β -lactam antibiotics. Single-bacterium isolation nanotechnology has been used to encapsulate a fully polymeric nanocoating on phage-infected bacteria.¹¹ This strategy not only prevents phage exposure to harsh extracellular conditions following lysis of the bacteria, but it also preserves the intracellular microenvironment to maintain phage integrity.¹² In controlled-release drug delivery, phages are encapsulated within nanomaterial carriers, such as liposomes or biomimetic hydroxyapatite nanocrystals, to protect them from enzymatic degradation or gastric acid, while targeted modifications achieve site-specific accumulation at infection sites.¹³ For instance, hydrogel-based nanotechnology platforms allow phage particles to self-assemble into hydrogels for drug delivery

targeted at tumors.¹⁴ Targeted modification aims to genetically modify receptor-binding proteins (RBPs), which determine the phage host range. By modulating RBPs, phages with decreased toxicity and narrow host ranges can be engineered into more therapeutic phages with highly specific, well-defined targets.¹⁵ Targeted peptides/proteins displayed on the phage surface can be conjugated to nanoparticles, conferring them with targeted functionality and a high drug-loading capacity. Moreover, phage peptides or proteins can self-assemble into phage-mimetic nanoparticles as self-guided drug-delivery nanocarriers.¹⁶ This review explores the integration of phage delivery systems with functional materials (e.g., nanomaterials, hydrogels, polymers), with a focus on elucidating how their combination compensates for mutual shortcomings and thereby synergistically enhances targeting, stability, payload capacity, and therapeutic efficacy. Furthermore, we examine recent advances in this multidisciplinary field and discuss their translational potential in clinical practice.

2. Biological foundations and engineering design of phage delivery systems

2.1. Biological properties and engineering potential of phages

Phages exhibit distinctive biological attributes that empower them to selectively infect and lyse bacteria, showcasing their immense promise as antimicrobial therapies. The strategy of injecting toxic substances into a target through wounds is far more common in the living world than is generally recognized. Plants, fungi, protists, bacteria, and viruses have evolved functionally similar “venom” delivery systems, with phages serving as prime examples among viruses.^{17,18} Their inherent biological characteristics and engineering potential are revolutionizing research within the field, transcending the limitations of conventional antibiotic treatments. The pivotal components of phages include the genome, capsid, and tail filament, which synergistically interact to facilitate efficient host recognition and infection. Certain phages also possess specialized structural components, such as the spike tip protein gp5.4 of T4 phage. Gp5.4 is a proline-alanine-alanine-arginine repeat protein exhibiting unique structural features, including three β -hairpin structures surrounding a central metal-binding site. Its high efficiency and programmability make gp5.4 a promising tool for developing therapeutic protein delivery systems.¹⁹ Phages demonstrate remarkable host specificity, precisely targeting and infecting particular bacterial types without affecting other microbes or host cells. This specificity is analogous to that observed in other prokaryotic delivery systems, such as contractile injection systems (CISs), which also exhibit

precise targeting functions.²⁰ This attribute renders phage therapy advantageous in combating drug-resistant bacterial infections, as it mitigates the collateral damage to the normal microbiota often caused by traditional antibiotics.²¹ Similar targeting precision is also seen in extracellular CISs, which mediate interbacterial interactions without broad-spectrum toxicity.²² This specific infection relies on a conformational rearrangement occurring at the tail tip during host binding. For instance, the cage-like structure at the tail tip of the Bxb1 phage rotates and flattens to penetrate the complex cell wall of *Mycobacterium smegmatis*, creating a channel for DNA injection.²³ Investigations into multidrug-resistant *K. pneumoniae* have illustrated that phage KP002 effectively lyses resistant strains while sparing the normal human flora.²⁴ Notably, bacteria have not only evolved defenses against phage attacks but also developed phage-like offensive systems known as bacterial CISs.^{25,26} For instance, the intracellular CISs-CIS^{sc} discovered in *Streptomyces* can trigger programmed cell death under stress conditions, thereby affecting the developmental cycle and secondary metabolite synthesis

of the bacterial cell.²⁷ Lytic phages execute their replicative life cycle through adsorption, penetration, biosynthesis, assembly, and release, culminating in the lysis of the host bacterium (Figure 1). This process is highly efficient and specific, governed by genome-encoded phage proteins, forming the basis for phage engineering. Phages possess the capability of self-replication, amplifying through the lytic cycle. This inherent ability enables phage therapy to sustain long-term antimicrobial efficacy while mitigating the emergence of resistant bacteria.²⁸ Furthermore, phages can harbor exogenous genes that encode bacterial toxins, which contribute to their ongoing inhibitory effects against resistant bacteria.²⁹ In nature, bacteria have evolved into a protein delivery system structurally similar to the tail of a phage but functionally highly specialized, extracellular CISs. These extracellular CISs are produced and released by bacteria into the extracellular space. Their core function is not replication and infection, as with phages, but rather to act as nanoscale “injectors” that precisely deliver effector proteins into target eukaryotic or prokaryotic cells, thereby mediating complex biological interactions.³⁰

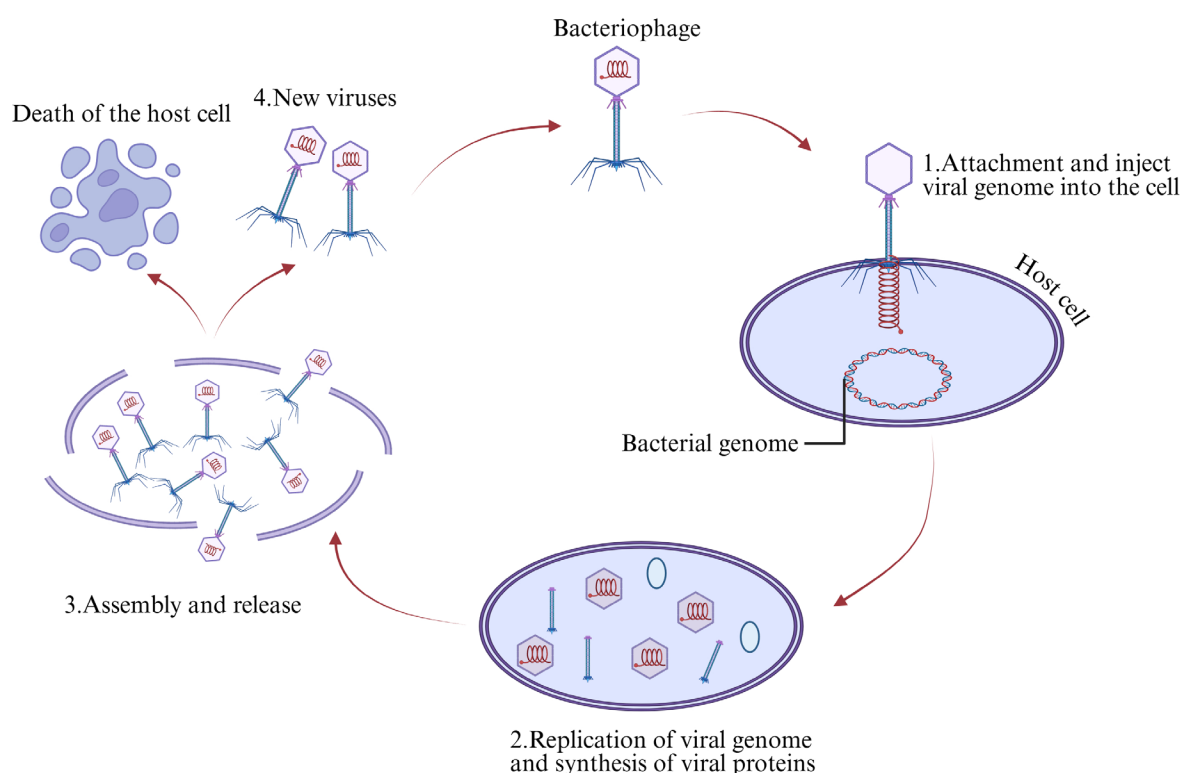


Figure 1. Mechanism diagram of the lytic cycle. Phages recognize and attach to specific receptors on the surface of host bacteria, injecting their genetic material into the bacterial cell. They utilize the host cell's biosynthetic machinery to replicate their own nucleic acids and synthesize components such as the protein coat. These newly synthesized components are assembled into complete daughter phage particles, which are ultimately released by lysing the host cell. Created with BioRender.com. Li, B. X. (2026) <https://BioRender.com/vn6z7r9>.

The modular architectures of phages offer exceptional programmability, enabling precise modifications that enhance their therapeutic efficacy. These modifications can improve delivery efficiency, stability, and retention time within the host, while also altering interactions between host cells and the immune system, ultimately leading to better therapeutic outcomes.³¹ Furthermore, certain bacterial-derived CISs, such as the *Photorhabdus* virulence cassette (PVC), exhibit distinctive structural characteristics, including a cage-like enclosure around the central spike, an enlarged sheath adaptor, and a plug protein exposed within the tube lumen.³² These attributes present novel avenues for bioengineering. Targeted modifications of the tail filament receptor-binding domain have facilitated the expansion of the host range.³³ For instance, experiments have demonstrated that a chimeric construct of the λ phage tail achieves bipartite targeting of *Escherichia coli* and *K. pneumoniae*, significantly improving DNA delivery efficiency through the phage-induced chromosomal island mechanism.³⁴ Structural insights into phage tail conformations, such as the ultra-long, extendable tails of Myoviridae phages, further inform engineering strategies to enhance delivery.³⁵ Furthermore, genome-wide streamlined designs, such as CRISPR/Cas9-based iterative phage genome reduction strategy³⁶ and the integration of exogenous elements, contribute to increased functional specificity. Notably, the recently discovered CRISPR-Cas π system (a novel Cas12f derivative) by Sun *et al.*³⁷ exhibits a reduced protospacer adjacent motif restriction range (requiring only 5'-NTTN-3') and enhanced editing efficiency. This system holds great promise as a powerful tool for future gene-editing endeavors, enabling the miniaturization of phage delivery systems and improving efficiency.

Phage delivery systems have made substantial progress in treating drug-resistant bacterial infections, primarily through genetic modification, targeted delivery, and vector optimization. Genetic engineering techniques enable scientists to combine phages with antibiotics or utilize phage RBPs and cell wall-binding domains (CBDs) as targeting modules to ensure precise delivery.¹³ Recent structural studies of contractile nanomachines like myophage Mu provide a blueprint for engineering stable and efficient delivery vectors.³⁸ Additionally, phages can be integrated with CRISPR systems for targeted delivery. By leveraging the specific adsorption and invasion characteristics of phages, they can deliver CRISPR-Cas systems into target bacteria, enabling precise gene editing and antimicrobial therapy.^{39,40} This technology holds immense potential for addressing antimicrobial resistance, diseases, and microbiome control. Evidence suggests that introducing the CRISPR-

Cas9 system into the genome of a temperate phage enables overcoming the limitations of conventional phage delivery systems, thereby enhancing delivery efficiency and host specificity.⁴¹ Research has shown that using a modified λ phage vector to package a plasmid carrying the CRISPR-Cas9 system enables targeted cleavage of the *bla*CTX-M gene in *E. coli*, which mediates resistance to β -lactam antibiotics. This approach achieves precise attenuation by specifically eliminating the resistance gene, thus restoring bacterial sensitivity to antibiotics without directly lysing the bacteria.⁴² Additionally, phages and antibiotics can synergistically form a delivery system. First, phages infect and lyse bacteria, significantly increasing bacterial permeability by degrading cell walls and disrupting membrane integrity, thereby creating entry pathways for co-delivered antibiotics. Antibiotics then efficiently target intracellular sites, achieving potent bactericidal effects by capitalizing on the metabolic disruption caused by the phage. Ultimately, this spatiotemporally coordinated “dual strike” overcomes bacterial resistance. Although the above-mentioned strategies have shown immense potential in functionality, stability during delivery remains a critical bottleneck limiting their application. To enhance delivery stability, nanomaterial carriers offer unique advantages: liposomes and hydrogels can physically encapsulate phages, protecting them from gastric acid³⁴; pH-responsive polymers, such as methacrylate copolymers, can trigger phage release under colon-targeting pH conditions (Figure 2).⁴³

Phages, owing to their precise host specificity and highly efficient lytic capabilities, facilitate the targeted eradication of drug-resistant bacteria. Their targeting properties, environmental tolerance, and therapeutic potential can be substantially augmented through genetic engineering modifications specifically directed at their capsid proteins, tail fiber receptors, and genomes. High-resolution structural studies of simple contractile DNA injection machines, such as *Vibrio* phage XM1, offer valuable insights for optimizing these engineering approaches.⁴⁴ Furthermore, the development of novel delivery systems optimizes their stability and controlled-release characteristics within complex physiological environments. Despite limitations such as host range, the modular design and programmability of phages have unveiled new avenues for precision antimicrobial therapy. Furthermore, phage tail-like nanostructures may also perform roles within host cells that extend beyond their traditional lytic functions. For instance, intracellularly localized *Streptomyces* phage tail-like particles discovered in *Streptomyces lividans* participate in responses to osmotic stress by influencing cell wall synthesis and ribosomal function.⁴⁵

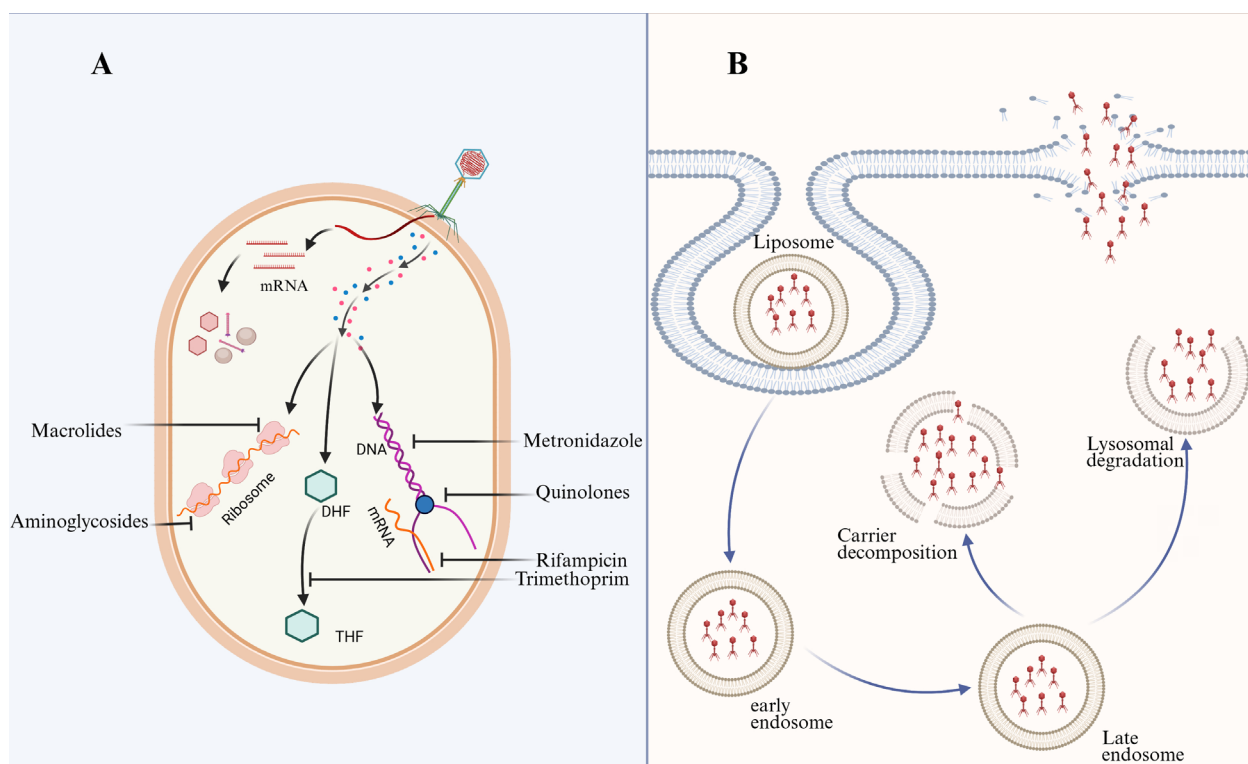


Figure 2. Mechanism of phage-mediated co-delivery systems. (A) Phage-antibiotic synergy. This system can specifically target and effectively deliver antibiotics to the site of infection, increasing local antibiotic concentrations and enhancing therapeutic efficacy. Phages induce stress responses in bacteria, activating biosynthetic processes such as DNA replication, transcription, and translation. Antibiotics targeting these processes—such as macrolides that inhibit translation, quinolones that disrupt DNA, rifampicin that blocks transcription, and trimethoprim that inhibits the conversion of dihydrofolate (DHF) to tetrahydrofolate (THF)—can thus act more effectively. (B) Nanomaterial phage delivery system. Liposomes are nanoscale carriers composed of phospholipid bilayers that enhance therapeutic efficacy by protecting the phage, regulating its release, and facilitating penetration of biological barriers. This system ensures phage delivery to target cells, enhancing phage killing efficiency and enabling highly effective cell lysis. Since liposomes readily diffuse through epithelial cells without obstruction, encapsulating phages within them may yield excellent results in eliminating intracellular bacteria. Created with BioRender.com. Li, B. X. (2025) <https://BioRender.com/4vza5t4>.

2.2. Key technologies for phage engineering

The fundamental technologies driving phage engineering encompass three critical dimensions: vector design, precise control, and functional enhancement (Table 1). At the heart of these advancements lies the synergistic optimization of targeting, efficiency, and safety, achieved through the integration of synthetic biology tools and structural biology methodologies. High-resolution structural elucidation of delivery complexes, such as the tail-baseplate of extracellular CIs, is crucial for understanding and engineering their infectivity.⁴⁶ In the realm of vector design, the heterogeneous compatibility of CRISPR-Cas systems stands as a cornerstone consideration. The Type II CRISPR-Cas9 system, widely favored for its user-friendly nature, is extensively employed for site-directed insertion and knockout in phage genomes.⁴⁷ Additionally, the Type V CRISPR-Cas12a system demonstrates the capability to cleave glucose-hydroxymethylcytosine-modified genomes,

positioning it as a valuable tool for the engineering of T4 phage.⁴⁷ Understanding the molecular mechanisms of phage contraction and host penetration, as revealed in studies of S-layer-penetrating phages, can inspire new engineering strategies to overcome delivery barriers.⁴⁸ The advent of base editing tools has further expanded the horizons of precision editing; for instance, the cytosine base editor derived from λ phage can mediate single-base editing in *E. coli*, thereby circumventing the risks of genomic instability associated with double-strand breaks.⁴⁹ RNA editing tools, such as LbuCas13a, employ a two-step strategy of “phage editing + sequence enrichment” to significantly elevate editing efficiency, showcasing notable advantages in suppressing host escape mutations.⁴⁷

A pivotal aspect of precise control technology lies in empowering phages to counteract host defense mechanisms.⁵⁵ Phages can acquire escape mutations, undergo DNA methylation modifications, or synthesize

Table 1. Key technologies for phage engineering

Topic	Technical name	Specifics
Carrier design	CRISPR-Cas9 genome editing	Utilize its ease of operation for point-specific insertion and knockout in the phage genome ⁴⁷
	CRISPR-Cas12a genome editing	It can cut the genome modified by glucose hydroxymethyl cytosine, which is a powerful tool for modifying T4 phages ^{47,50}
	Cytosine base editor (CBE)	Delivered using λ phages, it can achieve single-base modification in <i>Escherichia coli</i> , bypassing the risk of genomic instability caused by double-strand breaks ⁴⁹
	RNA editing and enrichment strategies	Through the two-step strategy of “phage editing + sequence enrichment,” the editing efficiency is improved, and the host escape mutation is effectively curbed ⁴⁷
Precise regulation	Escape CRISPR-Cas immunity	Escape host immune responses by evolution to produce escape mutations, perform DNA methylation modifications, or express anti-CRISPR proteins ⁵¹
	Protects against restriction enzymes	Defense breakthroughs are achieved by modifying their own DNA (e.g., introducing methylation homing enzyme genes) or directly inhibiting the activity of host-restriction enzymes ⁵²
Feature enhancements	Host range expansion	The receptor binding site of tail fiber is rationally designed, or the gp37/gp38 domain of T4 phage is modularly replaced, so that it can recognize unnatural hosts ⁵³
	Capsid modification	Through chemical modification or genetic engineering, the capsid is effectively reduced by the recognition of the immune system, and its persistence and targeting ability in the host body is enhanced ^{23,54}

Abbreviations: Cas: CRISPR-associated protein; CRISPR: Clustered regularly interspaced short palindromic repeats.

anti-CRISPR proteins, such as AcrVIA1, to neutralize the VI-A type Cas13 system to evade the host's CRISPR-Cas-based immune system.⁵¹ In response to restriction enzyme assaults, designer phages achieve defensive triumphs by rearranging their own DNA (e.g., incorporating methyltransferase genes) or directly inhibiting host restriction enzyme activity.⁵²

From a functional enhancement perspective, expanding the host range depends on the structural redesign of receptor-binding sites. This includes the rational design of receptor-binding sites on tail fibers or the modular replacement of the gp37/gp38 domains of the T4 phage to target non-native host outer membrane proteins.⁵³ Evolutionary analysis of tail sheath proteins offers new insights for engineering. The number of domains correlates positively with genome size, and large phages commonly possess multi-domain tail sheath proteins. These additional domains (often featuring immunoglobulin-like folds) may confer competitive advantages by enhancing adhesion or stability, making them potential targets for modification.⁵⁶ Capsid modification approaches, whether through chemical alterations or genetic engineering, dramatically reduce immune recognition, thereby enhancing persistence

and targetability within host cells.^{23,54}

The multidimensional innovation driving phage engineering is propelling their evolution from simple antimicrobial agents to designable biotherapeutics, establishing a novel paradigm for the clinical treatment of drug-resistant bacterial infections. This transformative approach promises to revolutionize therapeutic strategies and address the urgent challenges posed by antimicrobial resistance.

2.3. Optimization strategies for delivery efficiency and stability

In addition to genetic and structural enhancements, the route of administration and formulation design play a critical role in determining the *in vivo* efficacy of bacteriophage therapies. From the perspective of delivery route and formulation design, oral delivery systems have garnered significant attention due to their non-invasive advantages. However, challenges such as gastric acid degradation and poor retention in the intestinal lumen persist. To address these limitations, innovative strategies have been explored. Yoo *et al.*⁵⁷ utilized an M13 phage display library to screen peptides targeting M cells,

such as CKS9. After displaying these peptides on gp3, they observed a substantial enhancement in the phage's penetration efficiency into intestinal Peyer's patches. Nanoparticle encapsulation technology has emerged as a promising solution to maximize phage drug-loading capacity, shield phages from inactivation by low pH, and protect them from digestive enzymes or bile. This approach further enables controlled phage release.⁵⁸ Nanoparticle delivery systems, including liposomes and hydrogels, enhance the stability and penetration of phage therapy into biofilms. The structural dynamics and mechanical properties of CISs also inform the design of nanoparticles for efficient payload delivery and environmental adaptation.⁵⁹ By delivering polysaccharide-degrading enzymes and endolysins, these systems facilitate the lysis of biofilm matrices. Moreover, improvements in nanoparticle targeting and permeability increase delivery efficiency.⁶⁰ The integration of phages with nanoparticle carriers also introduces new functionalities, such as increased payload capacity and biocompatibility. Stimuli-responsive release systems provide programmed delivery of drugs or genes, effectively counteracting the issues of poor bioavailability and instability inherent in traditional systems.⁶¹

To improve immune evasion and long-term stability, strategies such as capsid-engineering mutations, suicide-switch architecture, and phage encapsulation have been employed. Genetic engineering of the phage capsid protein gene can eliminate or conceal immunogenic epitopes. For instance, glycosylation acts as an immunoglobulin barrier to phage-specific antibodies by preventing their binding.⁶² Furthermore, evidence suggests that glycosylation interferes with CD4⁺ and CD8⁺ T-cell epitope recognition.⁶³ Research has demonstrated that combining polyethylene glycol with *Salmonella* phage increases circulation time in non-immunized individuals.⁶⁴ Enhancing suicide-switch design is a pivotal strategy to improve the safety and control of phage delivery systems. The primary objectives are to achieve clear spatiotemporal activation and rapid off-switch mechanisms. Synthetic biology approaches incorporate inducible promoters, such as light- or chemical-inducible systems, into phage genomes to manage crucial gene expression. This allows for spatiotemporal regulation of phage activity. For example, replacing the early gene promoter in T7 phage with a TetR response element enables dynamic regulation of phage replication and lysis via tetracycline induction, preventing uncontrolled release.⁶⁵ Experiments have confirmed that phage encapsulation facilitates the gradual release of phages, leading to low phage-specific antibody production and prolonged phage circulation in the blood.⁶⁶ Encapsulation strategies can be informed by natural examples of compartmentalized delivery, such as the

thylakoid-anchored CISs in cyanobacteria, which ensure targeted payload release.⁶⁷ Kim *et al.*⁶⁸ encapsulated phages in a polylactic copolymer/alginate composite, enabling slow release from the capsules and inducing low phage-specific antibody responses. These innovative strategies collectively advance phage delivery systems, enhancing their therapeutic potential and safety profiles.

Future advancements in phage delivery systems in the medical field will primarily focus on technological innovation, interdisciplinary convergence across sciences, and pathways to clinical translation. In the era of artificial intelligence (AI), phage design has emerged as a critical breakthrough. Structural prediction of host-interaction proteins is being leveraged to enhance phage targeting properties, while genome engineering is applied to create artificially engineered phages with high infectivity.^{69,70} Bojar *et al.*⁷¹ have pioneered a groundbreaking study in this domain. By utilizing natural language processing, they constructed a comprehensive database of glycan structures. This database not only includes directly applicable phage-binding feature data but also facilitates the comparison of variation patterns across different bacterial species. This innovative approach establishes a novel paradigm for AI-driven research in phage therapy, particularly valuable for monitoring microbial evolution and optimizing targeted therapeutic strategies. An interdisciplinary integration methodology that combines systems biology, nanotechnology, and synthetic biology is also being explored. This approach utilizes multi-omics sequencing, organ-on-a-chip platforms, and AI-driven complex network analysis tools to comprehensively dissect the mechanisms of host-microbiome interactions at the molecular level, thereby enabling targeted interventions. Furthermore, integrating single-cell sequencing with high-throughput droplet microfluidics enables quantitative exploration of dynamic phage-host interactions. This capability significantly enhances the efficiency of phage library screening and design. These advancements collectively pave the way for more precise, effective, and clinically translatable phage-based therapies.⁷²

Collectively, these advancements herald a new era in phage-based therapies, promising more precise, effective, and clinically viable solutions to combat drug-resistant bacterial infections and other microbial challenges. Continued research and development in these areas are crucial for realizing the full potential of phages as designable biotherapeutics.

3. Medical applications of combining phage delivery systems with functional materials

In contrast to classical antibiotics, phage delivery systems

offer several advantages, including specificity, self-replication, and low host toxicity. These aspects make them excellent tools against drug-resistant bacterial infections.⁷³ In clinical medicine, phage delivery systems and functional materials have great potential, particularly in the treatment of resistant bacterial infections, targeted drug and gene delivery, and in precision cancer treatment (Figure 3).⁷⁴

In treating drug-resistant bacterial infections, engineered phages modified with functional materials can enhance phage targeting and bactericidal efficiency. The principles of cell-specific targeting, as demonstrated in engineered protein delivery vehicles for erythroid cells, can be translated to enhance the precision of phage-based antimicrobials.⁷⁵ Peng *et al.*⁴⁷ developed chimeric phages

targeting five distinct bacterial species by modifying the RBPs of M13 phages. The developed chimeric phage-gold nanorod composite platform utilizes near-infrared photothermal effects to achieve localized hyperthermic killing upon specific recognition of multidrug-resistant *E. coli*, thereby effectively preventing endotoxin release from bacterial lysis.⁷⁶ The integration of nanodelivery systems with phages significantly enhances targeted antibiotic delivery efficacy. For instance, Zhao *et al.*¹³ demonstrated that combining nanodelivery systems (liposome-encapsulated antibiotics) with phage protein-RBPs or CBDs can specifically eliminate CRKP and MRSA, demonstrating promising efficacy in a mouse pneumonia infection model. Tao *et al.*⁷⁷ prepared a gelatin-sensitive bioresponsive hydrogel, ΦxGel, employed for phage-

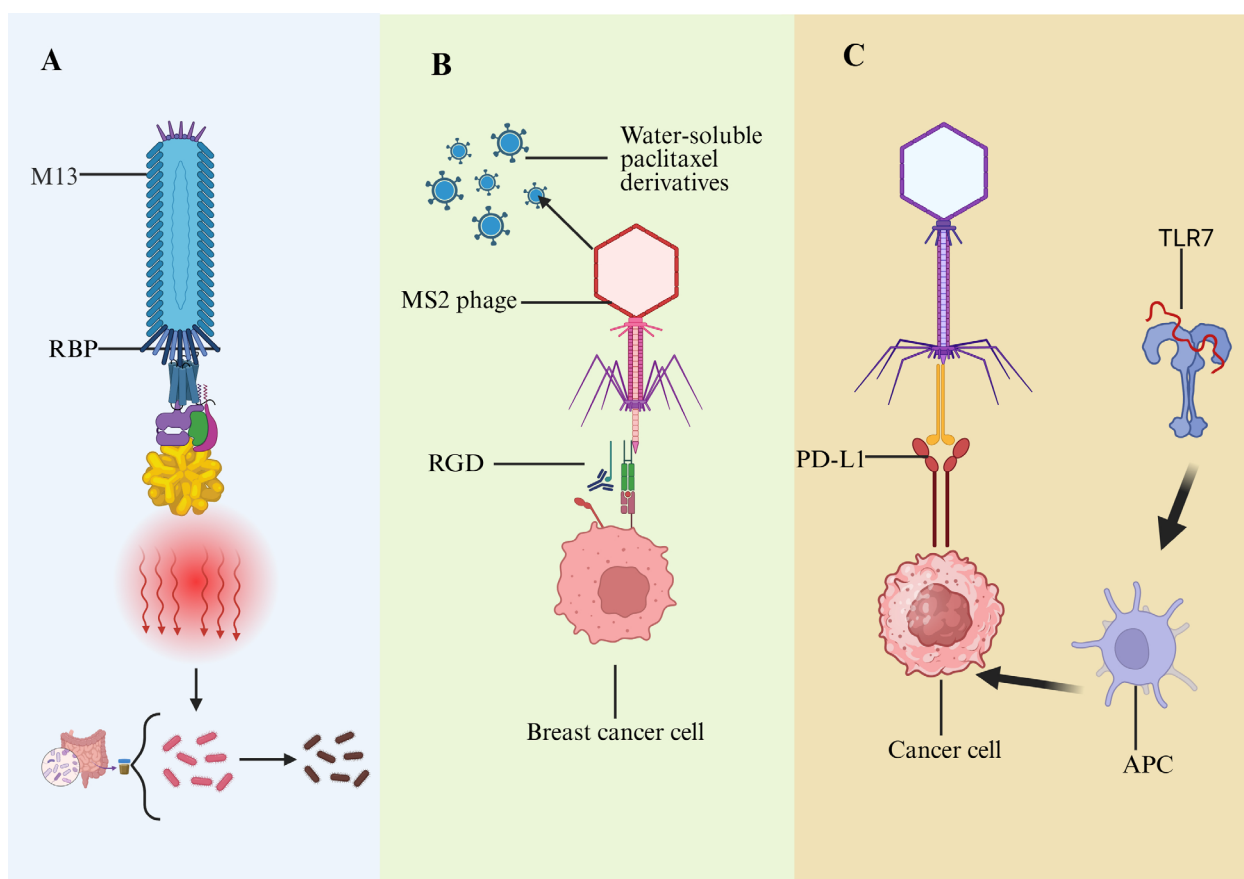


Figure 3. Medical applications of phage delivery systems. (A) Drug-resistant bacteria infections: Phages functionalized with nanomaterials (e.g., gold nanorods) or encapsulated in hydrogels enable targeted killing of multidrug-resistant bacteria. Near-infrared irradiation triggers photothermal effects for localized bacterial eradication, while enzyme-responsive hydrogels allow on-demand phage release at infection sites. (B) Targeted drug delivery: Phage display technology presents tumor-targeting peptides (e.g., Arg-Gly-Asp [RGD]) on phage surfaces, enabling precise delivery of chemotherapeutic agents (e.g., paclitaxel-loaded MS2 phage capsids) to cancer cells. Liposomal encapsulation further protects phages and prolongs circulation. (C) Tumor treatment: Phage-displayed nanobodies (e.g., anti-programmed death-ligand 1 [PD-L1]) combined with immune agonists (e.g., toll-like receptor 7 [TLR7] agonist) remodel the tumor immune microenvironment by blocking immune checkpoints and activating innate immunity, thereby enhancing antitumor responses. Abbreviations: APC: Antigen-presenting cell; RBP: Receptor-binding protein. Created with BioRender.com. Li, B. X. (2026) <https://BioRender.com/tlhp6f2>.

targeted delivery in the treatment of multidrug-resistant *Pseudomonas aeruginosa* infection. Schiff base crosslinking technology is applied to this hydrogel, which preserves phage activity while enabling controlled degradation by bacterial gelatinase, facilitating on-demand drug release at the infected site.⁷⁸

For drug- and gene-targeted delivery, phage display is combined with functional materials to achieve controlled delivery. Alternative protein delivery platforms, such as the PVC, have been characterized and repurposed for heterologous cargo translocation, offering complementary strategies to phage-based systems.⁷⁹ This technique is capable of screening tumor-targeting peptides (such as RGD peptides) to be subsequently conjugated with chemotherapy agents. MS2 phage capsids loaded with water-soluble paclitaxel derivatives can specifically target breast cancer cells, enhancing tumor suppression and reducing systemic toxicity.⁸⁰ Meanwhile, the bacteria's extracellular shrink-injection system offers novel insights for protein drug delivery. For instance, the PVC system has been demonstrated to load and deliver various bacterial effectors. Upon entering host cells, these effectors exert their inherent biological functions—such as cleaving host proteins or activating specific signaling pathways—fully validating PVC's immense potential as a universal protein delivery platform for precisely regulating cellular activities.⁸¹ In parallel, phage delivery systems based on liposomes are multifunctional drug delivery systems.⁸² Liposomes possess various benefits: not only do they improve the delivery efficiency of phages to target areas, but also encapsulate phages from environmental influences—e.g., low gastric pH during transit through the intestine and neutralizing antibodies—thus extending *in vivo* drug action duration.⁸³ Smart responsive system development has enhanced controlled-release efficiency. Such systems regulate drug release in response to external stimuli, such as specific pH levels, temperatures, and bacteria-derived proteins, cytokines, and signaling molecules.⁸² By releasing the drugs at the targeted time, they can achieve the high local concentrations while precluding phages from external stress.⁸⁴

Phage-functional material fusion systems also hold immense potential in cancer therapy. These systems rely on multistage targeting, synergistic enhancement, and integrated diagnosis and treatment, with engineered adjustments and material composites, offering a new paradigm to overcome traditional treatment obstacles. Research indicates that bacteria not only induce severe resistance to cancer chemotherapy but also promote the formation of immunosuppressive tumor microenvironments, ultimately weakening the antitumor

response of immune cells.^{85,86} Conversely, phages can precisely regulate the gut microbiota and activate immune cells, aiding in the restoration of equilibrium within the tumor immune microenvironment.⁸⁷ The fusion system of phages and functional materials achieves multilevel targeting, synergistic enhancement, and integrated diagnosis and treatment through engineered modifications and material composites. The study of other contact-dependent delivery systems, such as the archaeal type VI secretion system mediating antagonism, provides insights into intercellular targeting mechanisms that can inspire phage engineering.⁸⁸ Regarding the targeting mechanism, phage display technology can be used to screen for tumor-specific peptides. Meanwhile, filamentous phage pIII/pVIII coat protein display systems are used to generate high-affinity binding to cancer cell-surface receptors, thereby significantly enhancing targeting accuracy.⁸⁹ Representative examples are monoclonal antibodies IgG-69⁹⁰, IgG-H1⁹¹, and L6-E5-IgG.⁹² Functional material integration enhances tumor treatment strategy. Yu *et al.*⁹³ designed a PD-L1/TLR7 dual-targeted nanobody-drug conjugate. Using phage display technology, they obtained the blocking anti-PD-L1 nanobody Nb16, which demonstrated significant tumor growth inhibition and superior tumor penetration compared to monoclonal antibodies. TLR7 agonist SZU-101 activates innate immune cells, thereby enhancing PD-L1 expression on tumor cells and antigen-presenting cells. The medication shows enhanced antitumor activity and can reconstruct the tumor immune microenvironment.

Phage delivery systems, possessing precise targeting ability and self-replication features, have been incorporated into functional materials. Such integration holds promises to break through the war against drug-resistant bacterial infections, site-specific drug/gene delivery, and immune regulation of tumor growth (Table 2). Engineered improvements and smart response designs build on their targeted-killing and on-demand-release properties, significantly improving efficacy while minimizing side effects. Future research must overcome clinical translation hurdles to develop phage-functional material hybrid systems as a new generation of therapeutic tools against drug-resistant infections and cancer.

4. Conclusion

Engineering and functionalization of phages with advanced materials have demonstrated remarkable potential to overcome conventional barriers in drug delivery. By integrating the strengths of synthetic biology and materials science, this approach has evolved phage-based systems from naturally occurring antimicrobial agents into highly

Table 2. Comparative analysis of phage delivery systems

Delivery system type	Core material/technology	Advantages	Disadvantages/limitations	Primary application scenarios
Nanomaterial encapsulation system	Liposomes, polymeric nanoparticles	(i) Protect phages from degradation by gastric acid and digestive enzymes, enhancing stability. (ii) Increase drug loading capacity. (iii) Liposomes readily penetrate epithelial cells, aiding in the elimination of intracellular bacteria. (iv) Enable passive or active targeting (e.g., through binding to RBP or CBDs).	(i) Synthesis may be relatively complex. (ii) The biocompatibility and long-term toxicity of nanomaterials require thorough evaluation. (iii) They may face clearance by the immune system.	(i) Oral delivery requires crossing the gastrointestinal barrier. (ii) For biofilm infections, enhanced penetration is necessary. (iii) Targeted delivery of antibiotics to the site of infection.
Smart responsive hydrogel systems	Bioresponsive hydrogels (e.g., Φ xGel)	(i) Excellent biocompatibility. (ii) Enables controlled, sustained release of phages. (iii) Capable of responding to specific environmental stimuli (e.g., bacterial enzymes, pH) to achieve on-demand release.	(i) The drug release rate is influenced by material degradation kinetics and may not be suitable for acute infections requiring a rapid onset of action. (ii) Typically, suitable for local administration.	(i) Treatment of localized chronic infections (e.g., wound infections) (ii) Implant-associated infections. (iii) Scenarios requiring sustained antimicrobial activity at the infection site.
Phage display targeting systems	Engineered phages (displaying targeting peptides, e.g., CKS9, RGD)	(i) Significantly enhances penetration and targeting capabilities for specific cells (e.g., intestinal M cells, tumor cells). (ii) Uses the phage's inherent structure to achieve efficient delivery. (iii) Can be used for screening high-affinity targeting molecules.	(i) The stability and half-life of displayed peptides or proteins within the body may be limited. (ii) Large-scale production of engineered phage with specific targeting functions presents challenges. (iii) Immunogenicity may be present.	(i) Mucosal immune delivery (e.g., oral vaccines targeting intestinal Peyer's patches). (ii) Tumor-targeted therapy (e.g., delivery of chemotherapeutic agents). (iii) Drug/gene delivery requires cell-level specific targeting.
Photothermal synergistic therapy systems	Nanomaterial composites (e.g., gold nanorods)	(i) Combining the specific targeting of phages with the photothermal effects of nanomaterials to achieve synergistic physical sterilization. (ii) Effectively prevents the release of endotoxins caused by bacterial lysis.	(i) Requires an external light source excitation, limiting its application in deep tissue infections. (ii) The potential phototoxicity and long-term <i>in vivo</i> fate of nanomaterials must be considered.	(i) Superficial or endoscopically accessible localized infections caused by drug-resistant bacteria. (ii) Situations require rapid, potent bactericidal action with the goal of controlling endotoxemia.

(Cont'd...)

Table 2. (Continued)

Delivery system type	Core material/technology	Advantages	Disadvantages/limitations	Primary application scenarios
CRISPR-Cas gene editing delivery systems	Engineered phages carrying CRISPR-Cas systems	(i) Enables precise editing of bacterial resistance genes to restore antibiotic sensitivity without direct lysis, potentially reducing endotoxin release. (ii) Provides a new paradigm for precise intervention at the genetic level.	(i) High technical complexity, with off-target effects posing a key challenge. (ii) Delivery efficiency (particularly <i>in vivo</i>) requires optimization. (iii) Currently predominantly in the laboratory research phase, with a lengthy path to clinical translation.	(i) Precisely reversing bacterial drug resistance. (ii) Research on bacterial gene function. (iii) Regulating specific microbial communities.
Biomimetic/bacterial-derived systems	Bacterial contractile injection systems (e.g., PVC)	(i) As a natural protein delivery nanomachine, it has high-efficiency delivery potential. (ii) It can be engineered for the delivery of exogenous substances.	(i) Limited understanding exists of its interactions with human cells and immunogenicity. (ii) Engineering modifications pose significant challenges, and technology is still immature.	(i) Exploratory research on novel protein drug delivery platforms. (ii) Basic research applications for understanding bacterial-bacterial or bacterial-host interactions.

Abbreviations: Cas: CRISPR-associated protein; CBD: Cell wall-binding domains; CRISPR: Clustered regularly interspaced short palindromic repeats; PVC: *Photorhabdus* virulence cassette; RBP: Receptor-binding proteins; RGD: Arg-Gly-Asp.

programmable, intelligent biotherapeutics. As a result, they have shown exceptional performance in targeted antimicrobial therapy, gene delivery, and precision tumor treatment. Nevertheless, several fundamental challenges must be addressed to enable broader clinical translation. Foremost among them are three interrelated hurdles that impede clinical translation. First, immunogenicity remains a critical safety concern; phage-specific antibodies and complement activation can rapidly clear administered phages, reducing efficacy and potentially triggering adverse inflammatory responses. While strategies such as capsid engineering (e.g., glycosylation) and polyethylene glycol conjugation show promise in preclinical models to evade immune detection, their long-term impact on human immunity and phage pharmacodynamics requires rigorous clinical evaluation. Second, scalable and standardized manufacturing presents a formidable engineering and logistical barrier. Unlike small-molecule drugs, producing clinical-grade, well-characterized phage preparations with consistent potency, purity, and genomic stability under Good Manufacturing Practice conditions is complex and costly. Developing robust, high-yield fermentation, purification, and formulation pipelines is essential for a cost-effective and reliable supply. Third, the absence of clear, harmonized regulatory frameworks globally creates significant uncertainty for clinical development and market approval.

To overcome these technical and scientific hurdles, advances in basic research must be accompanied by globally harmonized regulatory guidelines and ethical consensus. Such integration will facilitate the transition of novel therapeutic strategies from bench to bedside, offering innovative solutions to combat antibiotic resistance and improve cancer chemotherapy. The ultimate success of this technology will largely depend on elucidating the co-evolutionary dynamics between phages and their hosts. To overcome these challenges, future research should focus on several key research areas. To address issues of immunogenicity and targeting, AI-assisted design should be leveraged not only to predict phage-host compatibility but also to *de novo* design phage capsids with minimized immunogenic epitopes and enhanced tissue-specific targeting tendencies. For instance, AI can analyze large datasets of phage and host genomes to predict optimal modifications to RBPs, thereby expanding host range or enhancing specificity. Furthermore, AI-driven complex network analysis tools can dissect mechanisms of host-microbiome interactions, guiding the design of more effective and safer delivery systems. Concurrently, developing advanced stimulus-responsive delivery systems—such as those using biomimetic materials responsive to pH, enzymes, or redox gradients—is crucial for achieving

spatiotemporally controlled release, thereby minimizing off-target effects and immune clearance. To address production and standardization challenges, advancing more efficient and versatile phage genome-editing tools (e.g., novel CRISPR-Cas systems such as Cas π) is essential to creating standardized, well-defined therapeutic phages. Furthermore, fostering interdisciplinary integration of synthetic biology, nanotechnology, and systems biology is vital for optimizing vector design and enhancing functionality. Ultimately, collaborative efforts among academia, industry, and regulatory bodies are required to establish clear regulatory pathways and accumulate robust clinical data on pharmacokinetics, safety, and efficacy, paving the way for these innovative therapies to transition from the laboratory to clinical practice.

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Conflict of interest

The authors declare that they have no competing interests.

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