scientific reports

Check for updates

OPEN

EDITORIAL

Current and future directions in bacteriophage research for developing therapeutic innovations

Longzhu Cui[1]✉, Kotaro Kiga[2], Kiran Kondabagil[3] & Alicja Węgrzyn[4]

Phages are gaining attention for their ability to target drug-resistant bacteria, disrupt biofilms, and reach intracellular pathogens, offering promising alternatives to traditional antibiotics. The Collection discusses advances in phage therapy, including their application in vaccine development, cancer immunotherapy, and gene delivery systems. Key research gaps are identified, such as challenges related to phage stability, immune response, and regulatory hurdles. Despite the progress, phage therapy faces obstacles in maintaining phage viability, evading immune detection, and navigating complex regulatory frameworks. The articles collectively address these challenges and propose potential solutions to enhance the effectiveness and acceptance of phage-based treatments. By overcoming these barriers, bacteriophage research has the potential to revolutionize medical therapies, providing innovative approaches to some of the most pressing healthcare challenges today.

The alarming rise in antibiotic-resistant bacterial infections has necessitated the exploration of alternative therapeutic strategies. Bacteriophages, or phages, naturally occurring viruses that infect and lyse bacteria, have emerged as promising solutions[1]. Phage therapy, harnessing these viruses, holds promise in addressing some of the most challenging medical issues, including drug-resistant, biofilm-generating, and intracellular bacterial infections.

Phages offer direct applications in treating bacterial infections that no longer respond to conventional antibiotics. They have demonstrated efficacy against a range of drug-resistant pathogens, including those responsible for chronic infections such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*[2]. An article featured in this Collection, for instance, has shown that combining phage endolysins with antibiotics like colistin enhanced antibacterial activity against resistant strains such as *Acinetobacter baumannii*[3]. Unlike antibiotics, phages can co-evolve with their bacterial hosts, potentially outpacing bacterial resistance mechanisms[4]. This adaptability is crucial for developing long-term solutions to bacterial infections that continuously evolve resistance to available treatments.

Biofilms, complex communities of bacteria that adhere to surfaces and are encased in a protective matrix, pose a significant challenge to treatment due to their heightened resistance to antibiotics. Some phages have shown a unique ability to penetrate and disrupt biofilms, offering a viable solution for infections associated with medical devices and chronic wounds[5]. Phage-derived enzymes, such as depolymerases, are being studied for their role in degrading biofilm matrices, further enhancing the efficacy of phage therapy[6]. These enzymes can specifically target the extracellular polymeric substances that protect bacteria within biofilms, making the bacteria more susceptible to both phage attack and conventional antibiotics. Recent studies have also demonstrated the potential of phage-antibiotic combinations in treating biofilms, particularly those involving multi-species communities like *Pseudomonas aeruginosa* and *Candida* spp.[7].

Intracellular bacteria, which reside within host cells, evade many traditional antibiotics. However, recent advances in phage research have identified phages capable of targeting and lysing intracellular pathogens. By modifying phage delivery mechanisms, researchers aim to enhance the ability of phages to reach and act within intracellular environments[8]. This involves innovative approaches such as engineering phages to bind to receptors that facilitate their entry into host cells, thus ensuring they reach intracellular bacteria effectively. Techniques

[1]Division of Bacteriology, Department of Infection and Immunity, Jichi Medical University, Shimotsuke, Tochigi 329-0498, Japan. [2]Research Center for Drug and Vaccine Development, National Institute of Infectious Diseases, Shinjuku-ku, Tokyo, Japan. [3]Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay Powai, 400076 Mumbai, India. [4]University of Gdansk, University Center for Applied and Interdisciplinary Research, Kładki 24, 80-822 Gdansk, Poland. ✉email: longzhu@jichi.ac.jp

like phage-assisted evolution are being employed to create phages with improved capabilities, such as enhanced replication and specificity[9].

Beyond infection control, phages are being explored as platforms for vaccine development[1]. Phage display technology holds promise for the rapid development of vaccines against emerging pathogens by efficiently identifying and displaying target antigens. This approach has shown promise in generating robust immune responses against various pathogens[10]. Phage-based vaccines are particularly advantageous because of their ability to display a wide variety of antigens, making them versatile tools in the fight against infectious diseases.

Moreover, phage-based vaccines are also being developed to target cancer cells, leveraging the specificity and versatility of phages to induce anti-tumor immunity[11]. The ability of phages to present tumor-specific antigens can stimulate the immune system to recognize and attack cancer cells. This form of immunotherapy holds promise for developing highly personalized cancer treatments, potentially leading to better patient outcomes and fewer side effects compared to conventional therapies.

In cancer therapy, phages are being engineered to deliver therapeutic agents directly to tumor cells. This targeted delivery system aims to minimize damage to healthy tissues and enhance the efficacy of anti-cancer treatments[12]. For instance, phages can be modified to carry genes encoding pro-apoptotic proteins, which can induce cell death specifically in cancer cells while sparing normal cells. The use of phages as vectors for gene therapy is also under investigation, with the goal of correcting genetic defects or delivering genes that can induce cancer cell death[13,14]. This approach not only provides a targeted treatment option but also reduces the likelihood of off-target effects that are common with traditional chemotherapy[1]. Additionally, phage therapy's potential for scalable production has been explored, with novel methods being developed to enhance the efficiency of phage generation and purification[15].

Phages are also being integrated into advanced drug delivery systems (DDS) to enhance the precision and effectiveness of therapeutic agents. By coupling phages with nanoparticles or other delivery vehicles, researchers are developing sophisticated systems capable of delivering drugs, genes, or other therapeutic molecules directly to target sites within the body. This approach not only improves the targeting of specific cells or tissues but also reduces systemic side effects, thereby improving patient outcomes[13,14]. For example, phage-nanoparticle conjugates can be designed to release their therapeutic payload in response to specific environmental triggers such as pH changes or the presence of certain enzymes, ensuring that the treatment is delivered precisely where it is needed. Additionally, the discovery and characterization of novel phage genomes from diverse environments, such as urban microbiomes, expand the potential applications of phages in both therapeutic and diagnostic contexts[16].

Despite the promising advances, several challenges must be addressed to fully realize the potential of phage therapy. Phage stability is a critical concern, as phages can be sensitive to environmental conditions such as temperature and pH, which can affect their viability and efficacy[17]. Ensuring the stability of phage preparations during storage and administration is essential for their practical use in clinical settings. Techniques such as lyophilization (freeze-drying) and encapsulation in protective coatings are being explored to enhance the shelf-life and stability of phage products. Furthermore, the development of phage formulations that maintain activity across diverse physiological conditions is crucial for clinical success[18].

The human immune response to phages presents another significant challenge, given its ability to recognize and neutralize phages, potentially reducing their therapeutic effectiveness. Strategies to evade immune detection, such as the use of encapsulation techniques or immune-suppressive agents, are being explored to prolong the activity of phages within the body[19]. On the other hand, results of recent investigations indicated that the innate immune response to bacteriophages is interrupted in birds and mammals, providing evidence that, unlike other viruses, phages can be safely used in therapeutic approaches, at least not inducing a strong anaphylactic response[20]. Nevertheless, another problem is that repeated administration of phages may lead to the development of anti-phage antibodies, which can neutralize the therapeutic phages. Research is ongoing to develop phage cocktails (combinations of different phages) and phages that can evade immune detection to mitigate this issue. Phage-layer interferometry is also being explored as a companion diagnostic tool for phage therapy, which could help monitor and mitigate immune responses during treatment[21].

Regulatory approval poses additional hurdles for phage therapy. The unique nature of phages, their specificity, and their ability to evolve necessitate a regulatory framework that differs from traditional drugs. Establishing standardized protocols for phage characterization, production, and clinical testing is crucial for gaining regulatory acceptance and ensuring the safety and efficacy of phage-based treatments[22]. Regulatory agencies must develop guidelines that address the specific challenges associated with phage therapy, including the potential for horizontal gene transfer, the variability of phage populations, and the need for personalized phage preparations[1]. In fact, such guidelines have already been prepared and published recently by the European Medicines Agency regarding the quality, safety, and efficacy of veterinary medicinal products specifically designed for phage therapy[23]. Optimized preparation pipelines, such as those developed for emergency phage therapy, are crucial steps toward meeting these regulatory standards and ensuring that phage therapy can be rapidly deployed in clinical settings[24].

In conclusion, bacteriophage research holds immense potential for revolutionizing medical therapies. As we continue to advance our understanding of phages and their applications, the development of phage-based treatments for bacterial infections, vaccines, anti-cancer therapies, and gene delivery systems will likely expand. Addressing the challenges related to phage stability, immune response, and regulatory approval is essential for translating this potential into clinical reality. By overcoming these obstacles, phage therapy can become a cornerstone of modern medicine, offering innovative solutions to some of the most pressing healthcare challenges of our time.

Published online: 17 October 2024

References

1. Cui, L. et al. A comprehensive review on phage therapy and phage-based drug development. *Antibiotics* **13**, 870. https://doi.org/10.3390/antibiotics13090870 (2024).
2. Salmond, G. P. C. & Fineran, P. C. A century of the phage: Past, present and future. *Nat. Rev. Microbiol.* **13**, 777–786. https://doi.org/10.1038/nrmicro3564 (2015).
3. Sitthisak, S. et al. Antibacterial activity of vB_AbaM_PhT2 phage hydrophobic amino acid fusion endolysin, combined with colistin against Acinetobacter baumannii. *Sci. Rep.* **13**, 7470–7470. https://doi.org/10.1038/s41598-023-33822-8 (2023).
4. Chan, B., Stanley, G., Modak, M., Koff, J. & Turner, P. (Authorea, Inc., 2020).
5. The promise of phages. *Nat. Biotechnol.* **41**, 583-583. https://doi.org/10.1038/s41587-023-01807-7 (2023).
6. Chang, C. et al. Bacteriophage-mediated control of biofilm: A promising new dawn for the future. *Front. Microbiol.* **13**, 825828–825828. https://doi.org/10.3389/fmicb.2022.825828 (2022).
7. Manohar, P., Loh, B., Nachimuthu, R. & Leptihn, S. Phage-antibiotic combinations to control Pseudomonas aeruginosa-Candida two-species biofilms. *Sci. Rep.* **14**, 9354–9354. https://doi.org/10.1038/s41598-024-59444-2 (2024).
8. Lu, T. K. & Collins, J. J. Dispersing biofilms with engineered enzymatic bacteriophage. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 11197–11202. https://doi.org/10.1073/pnas.0704624104 (2007).
9. Goicoechea Serrano, E., Blázquez-Bondia, C. & Jaramillo, A. T7 phage-assisted evolution of riboswitches using error-prone replication and dual selection. *Sci. Rep.* **14**, 2377–2377. https://doi.org/10.1038/s41598-024-52049-9 (2024).
10. Abedon, S. T., Kuhl, S. J., Blasdel, B. G. & Kutter, E. M. Phage treatment of human infections. *Bacteriophage* **1**, 66–85. https://doi.org/10.4161/bact.1.2.15845 (2011).
11. Bao, Q. et al. Phage-based vaccines. *Adv. Drug Deliv. Rev.* **145**, 40–56. https://doi.org/10.1016/j.addr.2018.12.013 (2019).
12. Smith, G. P. & Petrenko, V. A. Phage display. *Chem. Rev.* **97**, 391–410. https://doi.org/10.1021/cr960065d (1997).
13. Veeranarayanan, S., Azam, A. H., Kiga, K., Watanabe, S. & Cui, L. Bacteriophages as solid tumor theragnostic agents. *Int. J. Mol. Sci.* **23**, 402. https://doi.org/10.3390/ijms23010402 (2021).
14. Azam, A. H., Tan, X.-E., Veeranarayanan, S., Kiga, K. & Cui, L. Bacteriophage technology and modern medicine. *Antibiotics (Basel)* **10**, 999. https://doi.org/10.3390/antibiotics10080999 (2021).
15. Wiebe, K. G., Cook, B. W. M., Lightly, T. J., Court, D. A. & Theriault, S. S. Investigation into scalable and efficient enterotoxigenic Escherichia coli bacteriophage production. *Sci. Rep.* **14**, 3618–3618. https://doi.org/10.1038/s41598-024-53276-w (2024).
16. Flores, V. S. et al. Discovery and description of novel phage genomes from urban microbiomes sampled by the MetaSUB consortium. *Sci. Rep.* **14**, 7913–7913. https://doi.org/10.1038/s41598-024-58226-0 (2024).
17. Sauvageau, D. & Cooper, D. G. Two-stage, self-cycling process for the production of bacteriophages. *Microb. Cell Fact.* **9**, 81–81. https://doi.org/10.1186/1475-2859-9-81 (2010).
18. Bogun, K. et al. Investigating bacteriophages as a novel multiple-hurdle measure against Campylobacter: Field trials in commercial broiler plants. *Sci. Rep.* **14**, 3182–3182. https://doi.org/10.1038/s41598-024-53365-w (2024).
19. Krut, O. & Bekeredjian-Ding, I. Contribution of the immune response to phage therapy. *J. Immunol.* **200**, 3037–3044. https://doi.org/10.4049/jimmunol.1701745 (2018).
20. Podlacha, M. et al. Bacteriophage DNA induces an interrupted immune response during phage therapy in a chicken model. *Nat. Commun.* **15**, 2274–2274. https://doi.org/10.1038/s41467-024-46555-7 (2024).
21. Needham, P., Page, R. C. & Yehl, K. Phage-layer interferometry: a companion diagnostic for phage therapy and a bacterial testing platform. *Sci. Rep.* **14**, 6026–6026. https://doi.org/10.1038/s41598-024-55776-1 (2024).
22. Faltus, T. The medicinal phage-regulatory roadmap for phage therapy under EU pharmaceutical legislation. *Viruses* **16**, 443. https://doi.org/10.3390/v16030443 (2024).
23. Encyclopedia of Toxicology. 557 (Elsevier, 2014).
24. Würstle, S. et al. Optimized preparation pipeline for emergency phage therapy against Pseudomonas aeruginosa at Yale University. *Sci. Rep.* **14**, 2657–2657. https://doi.org/10.1038/s41598-024-52192-3 (2024).

Author contributions

Conceptualization: L.C.; Writing—original draft preparation and editing: L.C.(entire text, especially the part of phage-based innovations in vaccine and cancer therapy), K.Kiga. (the part of advanced applications of phages in medicine), K.Kiran (the part of the role of bacteriophages in addressing antibiotic resistance), and A.W. (the part of challenges and future directions for phage-based therapies) All authors have read and agreed to the published version of the manuscript.

Funding

LC was supported by the Japan Agency for Medical Research and Development (no. JP24ae0121045 and JP-24gm1610002), the Japan Society for the Promotion of Science KAKENHI grants (no. JP24H00662), and partially by Moonshot R&D Program for Agriculture, Forestry, Fisheries (grant no. JPJ009237). KK was supported by the Department of Science and Technology, DST (Indo-RSF, DST/IC/RSF/2024/460) and Council of Scientific and Industrial Research (CSIR, 37/1752/23/EMR-II).

Declarations

Competing interests

The authors declare no competing interests.

Correspondence and requests for materials should be addressed to L.C.