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HIGHLIGHT

Nanocoated bacterial carriers enhance oral bacteriophage delivery efficiency

KEY WORDS

Polymer coating;
Bacterial carrier;
Phage therapy;
Bacterially induced enteritis and associated arthritis

Bacteriophages (phages) exhibit remarkable specificity and efficacy in eliminating pathogenic bacteria, marking a paradigm shift in the battle against drug-resistant pathogens and presenting themselves as a promising alternative to traditional antibiotics[1,2]. A growing body of clinical research supports this trend, with phage-based therapies being assessed across a range of infectious conditions, including gastrointestinal infections, pulmonary bacterial disorders, and genitourinary system infections[3-5]. As a non-invasive therapeutic strategy, oral administration enables anatomically targeted delivery of antimicrobial agents to pathogen-colonized regions within the gastrointestinal lumen, a critical advantage over systemic injectable approaches in managing enteric infections. Preserving phage activity during amplification, purification, and storage is essential to achieving optimal antibacterial efficacy. Nevertheless, phages are highly vulnerable to environmental factors, and their structural integrity is easily compromised upon release from host bacterial cells[6]. To overcome these limitations, innovative oral delivery systems with stabilization matrices have been engineered to preserve bacteriophage viability during gastrointestinal transit while ensuring the targeted release of sufficient viable therapeutic phages.

In a study published in *Nature Biomedical Engineering*, Lin et al.[7] demonstrated that encapsulation of bacteriophage-infected bacteria using the pH-responsive polymer (Eudragit L100-55) enabled selective release of latent phages through site-specific polymer dissolution. Orally administered phage-infected bacteria, coated with an enteric, stimulus-responsive nanocoating, were protected from gastric acid degradation. As the pH transitioned from acidic to neutral in the intestine, the nanocoating dissolved, enabling the selective release of latent phages from lysed host bacteria *in vivo*. This resulted in higher bioavailability and significantly improved therapeutic outcomes compared to uncoated phages. Researchers have developed a single-bacterium-isolating nanotechnology to artificially manipulate the lysis process of phage-invaded bacteria (Fig. 1A). This nanotechnology, in which a complete polymer nanocoating is formed around individual phage-infected bacteria, not only prevents phage exposure to the unfavorable extracellular conditions after bacterial lysis but also preserves the intrabacterial microenvironment to maintain the integrity of the isolated phages.

Recognizing that the vitality of phages is closely linked to their structural integrity[2-4], cryogenic transmission electron microscopy (cryo-TEM) was used to detect that both Ex-phages (phages generated from *Salmonella Typhimurium* (S. T) without the nanocoating) and In-phages (latent phages obtained from coated S. T) possessed long tails composed of an adsorption device (tip) and a tube (Fig. 1B). Both Ex-phages and In-phages featured a fork-shaped structure at the end of their tails (Fig. 1C, left). The three-dimensional (3D) classification analysis showed that the tip structures of both types exhibited C3 symmetry (Fig. 1C, right). Interestingly, a comparison of phage tips revealed that Ex-phages lacked the terminal density present in In-phages. Further measurements indicated that Ex-phage tips were approximately 3.6 nm shorter than those of In-phages, suggesting the loss of tail-associated functional proteins (Fig. 1D). Moreover, the decline in phage vitality after release from host bacterial cells and exposure to environmental stress was due to the hydrolysis of phage tail proteins, which were crucial for host cell infection. As anticipated, the nanocoating preserved the original morphology of S. T following infection (Fig. 1E). Employing a sophisticated nanostructured coating, bacteriophage viability was effectively safeguarded against harsh environmental stressors, thereby markedly improving its clinical applicability and therapeutic efficacy.

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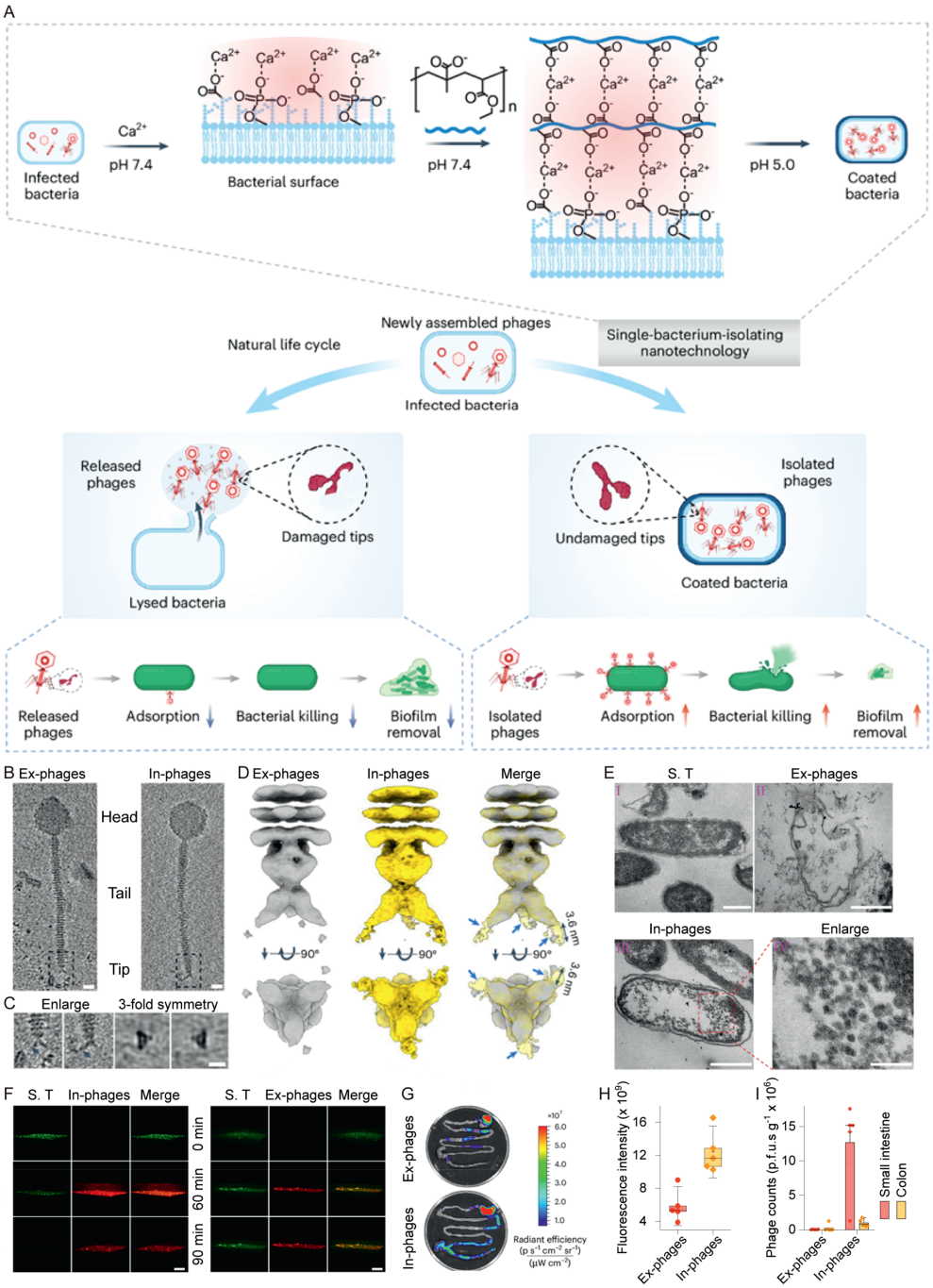

Figure 1 (A) The application of single-bacterium-isolating nanotechnology prevents phage release from infected host bacteria and protects phages from external stressors. It also highlights distinct differences in the antibacterial activity of released and isolated phages against pathogenic bacteria and associated biofilms. (B) Tomographic slices of Ex-phages and In-phages, along with Cryo-TEM analysis of their structures. Scale bars: 20 nm. (C) A magnified view of the black dashed boxes in B for Ex-phages and In-phages, showcasing the C3 symmetry of their tip structures. Scale bar: 10 nm. (D) Side and top views of the 3D-rendered tip structures illustrating the structural overlap between Ex-phages and In-phages, with blue arrows indicating regions of missing density. (E) TEM images of S. T, Ex-phages, and In-phages, with an enlarged view highlighting phages within the host bacterium. Scale bars: 400 nm (I, II, III) and 100 nm (IV). S. T. (F) Confocal images of biofilms formed by GFP-expressing S. T (green) after infection with Cy5.5-labeled In-phages or Ex-phages (red) at designated time points. Scale bars, 100 μm. (G) Representative *in vivo* imaging system images and (H) fluorescence intensity of gastrointestinal tracts collected from mice at 1 h after oral administration of Cy5.5-labeled Ex-phages and In-phages. (I) Quantification of viable bacteriophage populations in the small intestinal and colonic lumens at 1 h after oral administration ($n = 5$). For the boxplot, error bars represent the 95% confidence intervals, and '—' represents the means. All the data used in this study originates from the work of Lin et al.[7].

After confirming that In-phages exhibited greater vitality than Ex-phages, their effectiveness in inhibiting bacterial growth was investigated. It is well known that bacteria develop tolerance and resistance to both antibiotics and immune responses when protected by biofilms, posing a significant clinical challenge[8,9]. As indicated by the red fluorescence signal (Cy5.5-labelled phages), In-phages effectively infiltrated and killed GFP-expressing S. T embedded in biofilms after 60 min of co-culture. After extending the incubation to 90 min, the bacteria were nearly eliminated, as shown by the disappearance of green fluorescence (Fig. 1F, left). In contrast, biofilms co-cultured with Ex-phages still displayed significant green fluorescence even after 90 min, suggesting a reduced efficacy in biofilm removal (Fig. 1F, right). The fluorescence intensity in the intestines of the In-phage group was 2.12-fold higher than that of the Ex-phage group at 1 h after oral administration (Fig. 1G and H). Further results revealed that approximately 1.0×10^7 p.f.u./g of surviving phages were retained in the gut of the In-phage group (Fig. 1I). This result confirmed the significantly enhanced bioavailability of orally administered In-phages.

Compared to traditional phage therapy, studies have demonstrated that an enteric nanocoating enables pH-triggered in situ release of latent phages in the intestine, significantly improving oral phage bioavailability. This innovative approach significantly enhances therapeutic efficacy in treating intestinal infections and associated rheumatoid arthritis in mice[7]. More importantly, as the post-antibiotic era approaches, this study provides groundbreaking insights into phage vitality and its underlying mechanisms, introducing an innovative nanoplatform for advancing phage therapy. In future advancements, artificial intelligence (AI) is revolutionizing the development and optimization of oral delivery systems, paving the way for more effective and precise phage therapy. By leveraging machine learning and computational modeling, researchers can predict nanocarrier behaviors, enhance phage stability, and improve targeting precision within the biological environment, ultimately boosting therapeutic outcomes. Additionally, AI-driven drug discovery is accelerating the identification of novel antimicrobial agents, addressing the escalating challenge of antibiotic resistance. The convergence of AI and nanotechnology is poised to revolutionize phage therapy and antibiotic-resistant infection treatments. As these technologies continue to evolve and translate into clinical applications, they hold immense potential for reshaping modern therapeutic strategies against complex bacterial infections.

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Author contributions

Yingui Cao, Aodi Jiang, and Qiang Gao wrote the manuscript. Bo Xiao edited and revised the manuscript. All authors have given approval to the final version of the manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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