Integrating nanomedicine and immunotherapy: Bacterial membrane–derived vesicle-encapsulated prodrug assemblies for chronic infections

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Chronic bacterial infections present a significant clinical challenge due to microbial persistence within host cells and the formation of biofilm structures.[1] Intracellular pathogens, including *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*), have developed immune escape mechanisms that hinder phagolysosomal killing. By shielding themselves from humoral immune factors and limiting exposure to antibiotics, these pathogens can establish long-term reservoirs and sustain recurrent infections.[2] In parallel, many chronic infections are also driven by polymicrobial biofilm formation. The adherence of structured microbial communities on surfaces with the help of extracellular polymeric substances and glycocalyx makes them resistant to antimicrobial agents.[3] Compounding this issue, biofilm formation acts as a physical and chemical barrier contributing to host immunity evasion.[4] Therefore, successful therapy depends on overcoming significant delivery barriers to access bacterial niches and reprogramming the highly immunosuppressive microenvironment. Intracellular drug delivery strategies, including the use of nanocarriers, liposomes, and nanoparticles, present distinct advantages in the targeted treatment of bacterial infections.[5] While immunomodulatory therapies have shown significant promise, their effectiveness remains constrained due to inadequate antigen exposure in biofilms, adaptive immune imbalance, and abnormal activation of suppressive immune cells.[6-8] A pressing imperative exists for dual strategies aimed at optimizing drug delivery systems while simultaneously enhancing immune responses. Bacterial membrane–derived vesicles (BMVs) serve as a highly versatile platform by facilitating the transport of pathogen-associated molecular patterns (PAMPs) from the parent bacterium directly to the host cell.[9,10] However, their robust structure is not conducive to the ideal absorption and distribution of pharmaceutical compounds. To circumvent these limitations, combining chemical and immunological therapies has recently attracted attention as a cooperative strategy.

In a recent study, Li *et al.*[11] from the First Affiliated Hospital of Wenzhou Medical University created a prodrug conjugation method for antibiotics that self-assembles when encountering bacterial membranes to address chronic bacterial infections.[11] The prodrug conjugate (CpE) includes two phenylboronic acid (pba)-modified ciprofloxacin (Cip) molecules and ellagic acid (Ea).[11] This innovative approach is shown in **Figure 1**. It employs a designed multifunctional nanomedicine, CpE@BMV. The surface PAMPs of CpE@BMV bind to toll-like receptors. This binding triggers immune cells, enhancing their ability to engulf pathogens. The study highlights how CpE@BMV achieves precise delivery to intracellular bacteria and immunomodulatory effects, presenting a promising alternative to conventional antibiotic regimens.

The CpE, created by linking two Cip molecules modified with pba to one Ea, is able to assemble itself because of its water-repelling properties. While uncoated CpE assemblies are prone to uncontrolled aggregation in liquid, BMV of *E. coli* significantly stabilizes the resultant structures, making uniform nanoparticles that preserve native PAMPs. Functionally, the authors conducted a comprehensive evaluation, focusing on extracellular infections, intracellular infections in macrophages, and established biofilms, also assessing immunomodulatory effects in RAW264.7 cells. The results showed that CpE@BMV significantly enhanced antibacterial effectiveness against *S. aureus*, *E. coli*, and *Salmonella* Typhimurium compared to free Cip and the CpE. In macrophages, CpE@BMV resulted in markedly improved intracellular clearance of three pathogens, as evidenced by the rapid loss of *S. aureus* fluorescence and lower colony-forming unit (CFU) counts. Regarding

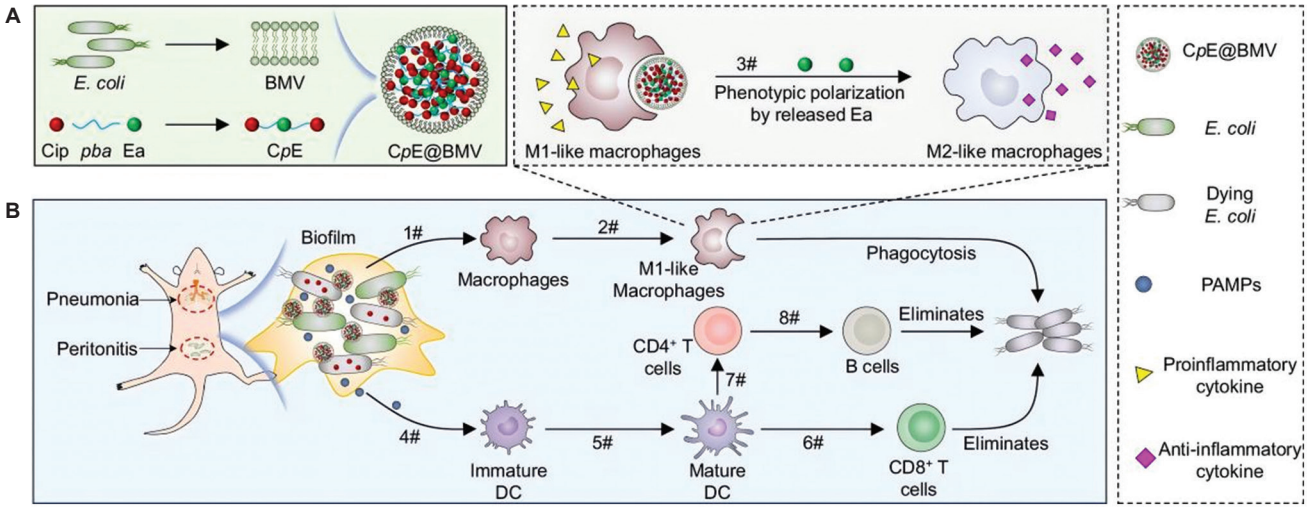

Figure 1. The preparation of CpE@BMV and the proposed mechanism behind the enhanced antimicrobial immune response. (A) Preparation of CpE@BMV via BMV cloaked prodrug CpE. BMV was derived from *Escherichia coli*, and the prodrug CpE was created by connecting Cip and Ea using a *pba* linker. (B) CpE@BMV enhanced bacterial eradication and immune modulation. CpE@BMV induced *E. coli* killing, releasing PAMPs that activated macrophage polarization from M0 to M1-like macrophages, enhancing bacterial phagocytosis. The nanoparticles further triggered Ea release, promoting the reprogramming of M1-like to M2-like macrophages. PAMPs were delivered to immature DCs, which activated naïve T cells. Reprinted with permission from Li *et al.*[11] Copyright © 2025 The Author(s).
Abbreviations: BMV: Bacterial membrane–derived vesicle; Cip: Ciprofloxacin; CpE: Prodrug conjugate; Ea: Ellagic acid; PAMPs: Pathogen-associated molecular patterns.

biofilms, mature *E. coli* biofilms were effectively penetrated and eradicated, as shown by confocal imaging and viable CFUs. Immunologically, PAMPs on BMV promoted macrophage activation. CpE@BMV enhanced M1-like polarization and significantly improved phagocytosis. BMV coating facilitated the uptake of the CpE core, ensuring targeted delivery to the intracellular environment. Moreover, CpE@BMV adjusted the redox balance by reducing intracellular reactive oxygen species (ROS) levels in macrophages activated by lipopolysaccharide, by inhibiting interleukin (IL)-6, tumor necrosis factor alpha (TNF-α), and IL-1β while enhancing IL-10. Earlier investigations frequently suffered from limited colloidal stability, whereas chemical carriers typically lacked patterns to activate host immunity.[12,13] This innovative platform exemplifies a skillful integration of nanocarrier engineering and immunotherapy design.

In another study involving mouse pneumonia and peritonitis models established using *E. coli*,[14] CpE@BMV treatment significantly reduced bacterial burden across infected tissues, while mitigating tissue damage and inflammation. In the pneumonia model, CpE@BMV achieved over 4 log unit bacteria reductions and preserved alveolar structure. Immunofluorescence staining showed a transition from M1-like macrophages, which are pro-inflammatory, to M2-like phenotypes, which are anti-inflammatory.[14] The analysis of immune profiles indicated improved dendritic cell maturation and the activation of $CD4^+$ and $CD8^+$ T cells, along with increasing plasma cells. In addition, the analysis also showed a marked reduction in pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) and an increase in IL-10, indicating effective resolution of inflammation. In the peritonitis model, CpE@BMV rapidly eliminated both extracellular and intracellular pathogens and reduced systemic infection. The authors also assessed the capacity for sustained immune memory within a living organism. Analysis showed a significant increase in memory B cells in the spleen after reinfection compared to untreated mice. Moreover, flow cytometry data indicated increased levels of mature dendritic cells, $CD8^+$ T cells, and $CD4^+$ T cells in the spleen of CpE@BMV-treated mice, further suggesting the activation of adaptive immune responses. These outcomes imply that CpE@BMV not only eradicates pathogens but also provides enduring protective immunity against reinfection, which is critical for treating chronic infections.

RNA sequencing analysis of infected lung tissues showed that CpE@BMV upregulated anti-inflammatory and regenerative genes (*Dusp1*, *Fasl*), whereas it downregulated inflammation genes (*Wnt7b*, *Tnf*, *Saa3*), indicating the mechanism to reduce inflammation. Gene ontology analysis revealed enhanced adaptive immunity, T cell proliferation, and angiogenesis, alongside peroxisome proliferator activated receptor (PPAR) signaling activation. In addition, 2bRAD-M sequencing demonstrated that CpE@BMV restored microbial diversity, reducing pathogenic *Enterobacteriaceae* and *Neisseriaceae* while preserving beneficial *Burkholderiaceae* and *Rikenellaceae*. Functional predictions indicated a recovery of metabolic activities about protein synthesis and glycolysis. These bioinformatic insights support the mechanism by which CpE@BMV not only eradicates pathogens but also promotes immune resolution.

The innovative design and compelling efficacy of CpE@BMV represent an important step forward in combating chronic bacterial infections. Many nanodrugs, such as polymeric nanoparticles and liposomes, primarily deliver a chemotherapeutic or antibiotic payload and often rely on passive targeting.[15] However, the BMV shell not only enhances targeting and uptake through innate immune receptor

engagement but also supplies immunogenic surface antigens that promote dendritic cell activation and adaptive immune responses. Moreover, compared to other exosomes, such as red blood cell membranes, BMV offers the advantage of its natural PAMPs, which better activate immune responses.[16] Compared with previously reported BMV-based delivery systems, which often focus simply on immunomodulation or cargo transport, CpE@BMV advances the field by addressing biofilm penetration, intracellular infection, and immune re-education in tandem. Despite these strengths, several challenges remain for further refinement. First, BMV structural integrity is sensitive to environmental factors such as temperature, pH, and ionic strength, limiting deployment in diverse scenarios.[17] Second, BMV can indeed interact with pattern recognition receptors due to the presence of PAMPs, offering distinct advantages over other biomimetic carriers.[18,19] However, there is still uncertainty about balancing robust immune activation with systemic safety in translational contexts. Detoxified or genetically modified BMVs with tailored PAMP profiles may be constructed to achieve safer immune activation.[20] Third, while microbiome alterations are reported, the deep mechanism between community shifts and immune restoration remains to be clarified. Furthermore, expanding *in vivo* evaluations to encompass chronic, recurrent infection models, as well as larger animal studies, is necessary for studying long-term bacterial clearance and off-target immune effects. CpE@BMV exemplifies how bacterial outer membrane vesicles can be utilized as precise, bio-inspired nanoplatforms that integrate antimicrobial delivery with immune modulation to overcome chronic infections. Beyond the present application, BMVs show great promise as programmable and immuno-active carriers. While BMV shows great potential for chronic infection treatment, several challenges remain for clinical translation, including large-scale production, immune safety, and regulatory approval. Overcoming these obstacles will require advancements in production technologies,[19] strategies to further reduce immunogenicity[21], and compliance with regulatory standards.

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Conflicts of interest statement

The authors declare no conflicts of interest.

Author contributions

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