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REVIEW

Chemically induced bacterial ghosts: a novel approach for advancing biomedical applications

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Abstract

Background Bacterial ghosts (BGs) are empty cell envelopes derived from bacteria, making them safe and non-replicative, and BGs have shown great potential as a vaccine platform. Specifically, chemically induced BGs are generated by selectively removing the cytoplasmic content of bacterial cells while preserving the structural integrity of the cell envelope.

Objective Generally, BGs are genetically engineered, but this is limited to Gram-negative bacteria. However, the utilization of chemically induced BGs can be extended to Gram-positive bacteria, resulting in empty bacterial envelopes that hold potential as a platform for drug delivery.

Results Chemically induced BGs offer several advantages, including improved safety profile and immunogenicity, and efficient antigen presentation. Preclinical studies have yielded promising results, exhibiting enhanced immune responses and protection against diverse pathogens.

Conclusion Chemically induced BGs represent a novel and promising approach for vaccine development, holding the potential for advancing disease prevention and public health.

Purpose of review In this review, we discuss key aspects of chemically induced BGs, including their production principles, mechanisms of formation, characterization techniques, immunogenicity, and medical applications. We also discuss the challenges and direction of future research for optimizing production methods for chemically induced BGs, evaluating long-term safety, and undertaking clinical trials to assess their efficacy.

Keywords Chemically induced bacterial ghosts · Empty cell envelopes · Immunogenicity · Drug delivery platform

Introduction

Bacteria are prokaryotes, lacking a nucleus and membrane-bound organelles (Gitai 2005). They have a simple cellular structure consisting of a cell membrane, cytoplasm, genetic material (DNA or RNA), and often a cell wall (Lewis 2004). Bacteria have wide applications in various aspects of human life, including in medicine (Sender et al. 2016; Eckhardt et al. 2013). While some bacteria cause infections and diseases (van Elsland and Neefjes 2018; Fernebro 2011; Bunchorntavakul et al. 2016), many beneficial bacteria have important medical applications (Taylor et al. 2002; Hinchliffe et al. 2021). Beneficial bacteria have several noteworthy medical applications (Yan et al. 2023). First, live microorganisms provide health benefits when consumed in adequate amounts as probiotics (Garbacz 2022; Mu and Cong 2019). Probiotics, such as certain strains of *Lactobacillus* and *Bifidobacterium*, are used to prevent and treat various gastrointestinal conditions, including diarrhea, irritable bowel syndrome, and inflammatory bowel disease (Ringo et al. 2020; Fioramonti et al. 2003). They help maintain a healthy balance of microorganisms in the gut microbiota, which is crucial for digestion, nutrient absorption, immune system modulation, and overall gut health (de Moreno de LeBlanc and LeBlanc 2014; Wallace et al. 2011; Abenavoli et al. 2019). In addition, numerous antibiotics are derived from bacteria or their metabolic products (Anke and Zahner 1978; Gunnarsson et al. 2004; Korp et al. 2016). Antibiotics are used to treat bacterial infections by inhibiting the growth or killing bacteria causing infections (Hutchings et al. 2019; Kummerer 2003). Certain bacteria can break down or degrade environmental pollutants (Segura and Ramos 2013; Le Borgne et al. 2008). Moreover, bacteria are employed in

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various industrial processes. For instance, bacteria are used in the production of enzymes, such as amylase or cellulase, which find applications in the food, textile, and detergent industries (Awasthi et al. 2018; Mohammad et al. 2017; Dai et al. 2020). Additionally, bacteria like *Escherichia coli* (*E. coli*) have been genetically engineered to produce therapeutic proteins like insulin, growth factors, and vaccines (Waegeman and Soetaert 2011; Goldstein and Thomas 2004). Bacteria are also important in medical diagnostics. They can be isolated from patient samples and cultured to identify the causative agents of bacterial infections. Although bacteria have numerous medical applications, certain infectious strains cause deadly diseases. It is crucial to maintain a balance between the beneficial and harmful aspects of bacteria and undertake appropriate measures, such as hygiene practices and responsible antibiotic use, to effectively manage health issues related to bacteria.

Bacterial ghosts (BGs) are empty cell envelopes derived from bacteria. BGs generated through controlled biological or chemical treatment offer unique advantages such as retained antigenicity, structural integrity, and versatile cargo loading capacity (Abenavoli et al. 2019; Li et al. 2021; Kang et al. 2022). Herein, we discuss the mechanisms, characterization, and diverse applications of chemically induced BGs in vaccine development, drug delivery, and diagnostics. Additionally, we highlight the potential of chemically induced BGs in advancing biomedical strategies and contributing to innovative biomedical approaches.

Principles of BG production

BGs, which are primarily hollow cell envelopes, are produced from Gram-negative bacteria by controlled expression of the PhiX174 lysis gene E (Park et al. 2016; Lee et al. 2008; Witte et al. 1990). This gene encodes a small protein with hydrophobic regions at its N-terminal (Hajam et al. 2017). Upon binding to the inner membrane of the bacterial cell wall, the hydrophobic N-terminal of lysis protein E enhances internal osmotic pressure, leading to the formation of transmembrane structures (Park et al. 2016). These structures facilitate the release of cellular contents, including ribosomes and nucleic acids, generating BGs (Hajam et al. 2017). The resulting transmembrane tunnels have a diameter of approximately 40–200 nm and allow the expulsion of the cytoplasm while preserving the integrity and morphology of the cellular envelope (Lubitz et al. 2009; Witte et al. 1992). Electron microscopy revealed that these empty bacterial envelopes retain the surface structures of native bacteria, such as outer membrane proteins, adhesions, lipopolysaccharides (LPS), and the peptidoglycan layer (Li et al. 2021; Chen et al. 2021). Therefore, BGs are promising candidates for vaccine development and as delivery systems for antigens in humans and other animal.

Mechanisms of chemically induced BG formation

While gene E-induced BGs have proven effective in conferring protective immunity against specific infections, their formation has been limited to Gram-negative bacteria due to the lack of suitable inner and outer membranes in Gram-positive bacteria (Park et al. 2016). However, a novel protocol has been developed to overcome this constraint and expand the scope of BGs to use Gram-positive bacteria (Amara et al. 2013). This innovative approach involves the application of various chemical agents, including sodium hydroxide (NaOH), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), sodium dodecyl sulfate (SDS), nitric acid (HNO_3), hydrogen peroxide (H_2O_2), and Tween-80, at their minimum inhibitory concentrations (MIC) or minimum growth concentrations (MGC) (Li et al. 2021; Park et al. 2016; Ji et al. 2022; Rabea et al. 2018, 2022; Sheweita et al. 2022). These chemically induced BGs by specific chemical agents disrupt the cell membrane and release the cytoplasmic contents, selectively removing the cellular contents of bacterial cells while preserving the cell envelope. This multi-step process begins with the growth of bacterial cells under suitable conditions to promote cell envelope production. Subsequently, the cells are treated with a chemical agent that selectively disrupts the cytoplasmic membrane while leaving the cell envelope intact (Fig. 1). The resulting BGs retain the structural and functional properties of the original cell, including the cell wall and outer membrane, while maintaining its antigenicity. These BGs interact with immune cells, eliciting both innate and adaptive immune responses (Lubitz et al. 2009; Vinod et al. 2015, 2017). Additionally, their well-preserved morphology and high loading capacity make BGs excellent vehicles for delivering subunit vaccines, DNA vaccines, as well as chemotherapeutics and antibiotics (Szostak et al. 1996; Liu et al. 2006; Hensel et al. 2000; Mayr et al. 2005a). In conclusion, BGs represent a versatile platform for the development of vaccines and therapeutic carriers for a wide range of infectious and non-infectious diseases.

Characterization of chemically induced BGs

Chemically derived BGs undergo morphological and structural changes compared to their original bacterial cells. Morphological analysis of chemically derived BGs is commonly conducted using electron microscopy such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (Chaudhari et al. 2012; Wu et al. 2017;

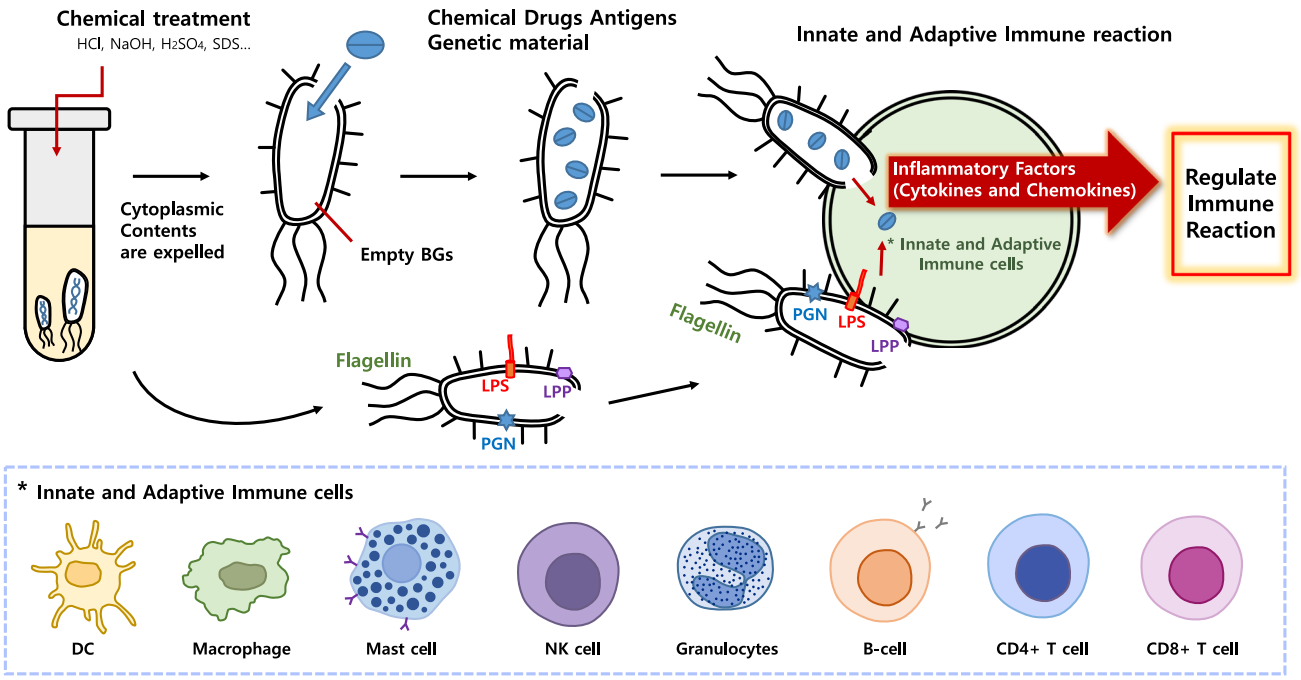

Fig. 1 Schematic illustration of preparation of BGs using the chemical treatment and effect of BGs on immune reaction

Ma et al. 2021). SEM reveals that chemically induced BGs retain the surface features and structures of the parent bacterial cells, including outer membrane proteins, adhesions, and other surface components, maintaining morphological similarity (Park et al. 2016; Jawale et al. 2014) (Fig. 2). TEM allows for the examination of the internal structures of chemically derived BGs, displaying empty cell envelopes with intact cell walls and outer membranes devoid of internal components (Wu et al. 2017). Apart from morphological analysis, various techniques help assess the structural characteristics of chemically derived BGs. These include analysis of their biochemical composition through techniques like Fourier-transform infrared spectroscopy (FTIR) (Huang et al. 2017; Parvulescu et al. 2021). Furthermore, the integrity and stability of BGs can be assessed through techniques like dynamic light scattering (DLS) (Go et al. 2019; Myers et al. 2022) and atomic force microscopy (AFM) (Eslaminejad et al. 2022; Anderson et al. 2004) by determining their size distribution, surface charge, and stability. As the antigenic properties of chemically derived BGs are preserved, immunological techniques like enzyme-linked immunosorbent assay (ELISA) and immunofluorescence can confirm the presence and accessibility of specific surface antigens, providing insight into their immunogenicity and antigen presentation capabilities. These comprehensive analyses help characterize BGs by providing details about

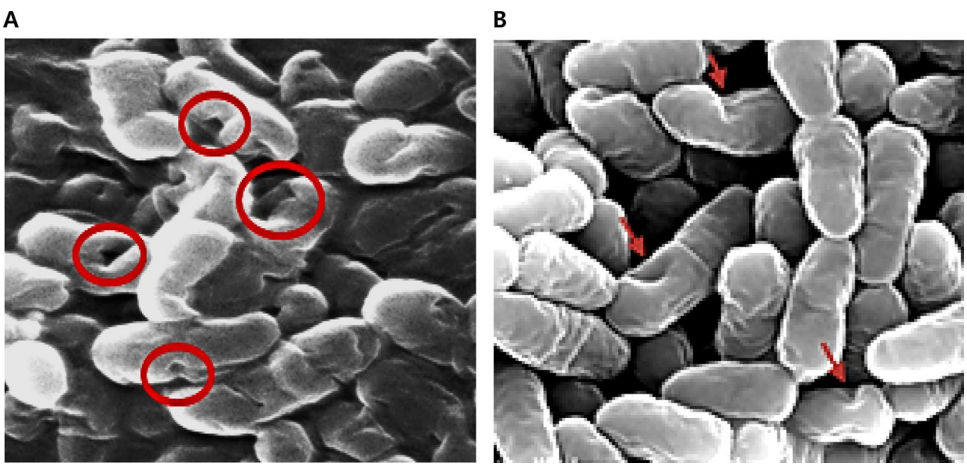

Fig. 2 Analysis of SEM for transmembrane lysis tunnel of BGs. The red circles (**A**) and arrows (**B**) indicate the areas where the chemicals are dissolved by NaOH-treated *Weissella kimchii* cells (**A**) and HNO_3-treated *Weissella koreensis* cells (**B**). The ratio of scanning electron microscopy was 65,000 times

their morphology, surface features, biochemical composition, integrity, stability, and antigenicity, as well as, highlight the potential of chemically derived BGs for various biomedical applications.

Roles of chemically induced BGs in immunogenicity and immune response modulation

Chemically induced BGs play a significant role in immunogenicity and immune response modulation. They retain the original bacterial cells' structural components and surface antigens, such as LPS, outer membrane proteins, and other surface molecules (Li et al. 2021). These components are recognized by the immune system as foreign antigens, triggering immune responses (Kang et al. 2022). The presence of intact surface structures in BGs enhances their immunogenicity, eliciting robust immune responses. BGs can activate innate immune responses through the recognition of pathogen-associated molecular patterns (PAMPs) on their surface (Akira et al. 2006; Schnare et al. 2001). Components such as LPS and other bacterial surface molecules are recognized by pattern recognition receptors (PRRs) on immune cells, such as macrophages and dendritic cells (Medzhitov and Janeway 2000; Kawai and Akira 2011; Akira and Hemmi 2003). This recognition leads to the secretion of pro-inflammatory cytokines and further recruitment of immune cells, promoting the immune response against the target antigen (Quevedo-Diaz et al. 2010; Steinhagen et al. 2011).

BGs also stimulate adaptive immune responses through the activation of B and T cells. The presence of antigens on the surface or within the BGs facilitates antigen presentation to immune cells, leading to the generation of antigen-specific antibodies by B cells (Hajam et al. 2017; Szostak et al. 1996) and the activation of T cells (Felnerova, et al. 2004; Kudela et al. 2010; Langemann et al. 2010; Mayr et al. 2005b; Dobrovolskiene et al. 2018). This ultimately results in the production of specific antibodies, memory B cells, and cytotoxic T lymphocytes, contributing to long-term immunity. BGs possess intrinsic adjuvant properties, meaning that they can enhance the immune response to co-administered antigens (Abenavoli et al. 2019; Langemann et al. 2010; Riedmann et al. 2007; Szostak et al. 1997; Jalava et al. 2002). The presence of bacterial surface components, such as LPS, can activate immune cells and promote the secretion of pro-inflammatory cytokines (Ciesielska et al. 2021; Yang et al. 2015). This adjuvant effect helps to amplify the immune response and improve the effectiveness of the vaccine (Zariri and van der Ley 2015; Kumar et al. 2019; Melssen et al. 2019). Depending on the properties of the bacterial components retained in the BGs and the type of antigen presented, they can skew the immune response in a particular direction. For example, BGs containing specific bacterial components can promote a Th1 response characterized by cellular immunity (Hensel et al. 2000; Cao et al. 2018; Won et al. 2017), or a Th2 response characterized by antibody production (Ebensen et al. 2004; Cai et al. 2010; Senevirathne et al. 2021). Therefore, chemically induced BGs can enhance immunogenicity, activate innate and adaptive immune responses, exhibit adjuvant properties, and modulate the immune response. These features make them valuable tools in vaccine development and immunotherapy to improve the effectiveness of vaccines and tailor immune responses to specific pathogens or diseases.

Discussion

Chemically induced BGs have shown great potential in vaccine development due to their unique properties and immunogenicity (Chen et al. 2021; Ebensen et al. 2004; Chiang et al. 2005). BGs serve as efficient carriers for delivering antigens to the immune system (Ebensen et al. 2004). They can be loaded with specific antigens by incorporating them into the inner spaces of the ghost or by presenting them on the surface of the cell envelope (Li et al. 2021; Lubitz et al. 2009). This allows for the targeted delivery of antigens to antigen-presenting cells, leading to effective immune responses. In terms of adjuvant properties, BGs possess intrinsic adjuvant properties, meaning that they enhance the immune response to co-administered antigens (Szostak et al. 1997). Chemically induced BGs are produced from various bacterial species of both Gram-negative and Gram-positive bacteria and have wide applications. This expands the scope of potential antigens and their applications in vaccine development, targeting a wide range of infectious diseases (Batah and Ahmad 2020). Furthermore, BGs have improved stability compared to live bacterial vaccines (Szostak et al. 1996). Chemically induced BGs offer numerous advantages in vaccine development, including efficient antigen delivery, enhanced immunogenicity, intrinsic adjuvant properties, safety, and scalability. Moreover, the production of BGs can be easily scaled up, making them highly suitable for large-scale vaccine manufacturing (Amara et al. 2013). The flexibility of BGs has been exploited to generate foreign antigens in a biologically active form that are firmly attached to the envelope. It has been effective as a vaccine delivery or immune modulator in animal studies (Chen et al. 2021; Riedmann et al. 2007; Jalava et al. 2002). In in vivo studies, they have successfully used BGs to deliver antigens form various pathogens, such as *Salmonella*, *Escherichia coli*, *Klebsiella pneumonia*, and *Shigella* (Mayr et al. 2005a; Kim et al. 2016; Hur et al. 2015; Osorio et al. 2007; Hoseini Shahidi et al. 2019). The administration of these ghost-based vaccines induced specific immune responses and provided

protection against subsequent infections in the animal. These desirable features make chemically induced BGs compelling candidates for the development of vaccines against a wide range of infectious diseases.

Chemically induced BGs have promising applications in the field of diagnostics. They can be engineered to present specific surface antigens, derived from various pathogens such as bacteria, viruses, or parasites. BGs carrying these antigens can be used for developing diagnostic assays to detect specific antibodies or immune responses in patient samples, enabling the sensitive and specific detection of infectious agents or disease biomarkers. BGs can also be employed in serological assays, including ELISA or immunoblotting, where they serve as capture agents to detect corresponding antibodies in patient sera. This allows for the diagnosis of infectious diseases and the identification of specific antibody responses. Furthermore, BGs can be incorporated into point-of-care diagnostic devices, facilitating rapid and on-site testing. These devices use BGs as capture agents for target analytes, enabling the direct detection of pathogens or disease markers at the point of patient care. This approach offers the advantage of timely and convenient diagnostics, particularly in resource-limited settings. The integration of bacterial BGs into biosensor platforms enables the detection of a wide range of analytes. The integration of BGs with specific receptors or ligands can serve as highly sensitive and selective sensing elements. This allows for the detection of target molecules, including nucleic acids, proteins, or small molecules, in patient samples. Owing to the unique characteristics of BGs, such as their antigenicity, stability, and cargo-carrying capacity, they can be used in diverse diagnostic strategies for the accurate detection of infectious agents, disease biomarkers, and other analytes of interest. These applications can significantly enhance diagnostic accuracy through the development of efficient and reliable diagnostic tools and improve patient management.

Despite the wide applicability of chemically induced BGs, several challenges impede their use and some aspects require further improvement. One challenge is optimizing the production process to enhance efficiency, yield, and reproducibility. This involves identifying safe and effective chemical agents, determining optimal concentrations and exposure times, and refining purification and characterization methods. Another challenge is improving the stability and storage of BGs. Developing suitable preservation techniques, such as lyophilization or freeze-drying, and identifying stabilizing agents can enhance their long-term stability, facilitating storage, transportation, and distribution. Strategies to enhance the immunogenicity of BGs can be explored, including surface modification techniques to increase antigen loading or incorporation of immune-enhancing molecules like adjuvants or immunomodulatory agents. Additionally, optimizing formulation and delivery methods can improve the immune response elicited by BG-based vaccines. The efficiency of chemically induced BG production may vary depending on bacterial species and the chemical agents used. The stability of BG envelopes is also a concern, as they may have less practical application due to reduced stability and shelf life. Furthermore, using BGs as immune enhancers has challenges, such as achieving a comparable immune response to that of live bacterial infection and the risk of undesired immune responses to immunogenic peptides or proteins resulting from the chemically induced process. Moreover, BGs may not be as efficient in triggering the adaptive immune response as a live bacterial infection, which could lead to inadequate protection against future infections. BGs might still retain some residual components that could trigger an immune response in the host. This could potentially impact their use in vaccine production, as the immune response could be undesirable or interfere with the desired immune response generated by the vaccine. Taken together, addressing these challenges and focusing on areas such as production efficiency, stability, immunogenicity, expansion to Gram-positive bacteria, targeted delivery, improved cargo loading strategies, and conducting clinical studies will promote the development of safe, effective, and versatile BG-based technologies for various biomedical applications.

Conclusion

Chemically induced BGs have emerged as safe and non-replicative vaccine platform. These BGs are created by selectively removing the cytoplasmic content of bacterial cells while preserving the structural integrity of the cell envelope through chemical treatments. This process results in empty bacterial envelopes that possess key advantages for various biomedical applications, including drug delivery and vaccine development. Chemically induced BGs offer several desirable properties, such as safety, effective antigen presentation, immunogenicity, and structural integrity. The key features of chemically derived BGs (Fig. 3) make them highly promising tools for vaccine development, drug delivery, and other biomedical applications. Considering their unique attributes, chemically induced BGs provide a novel and safe avenue for improving disease diagnosis and treatment.

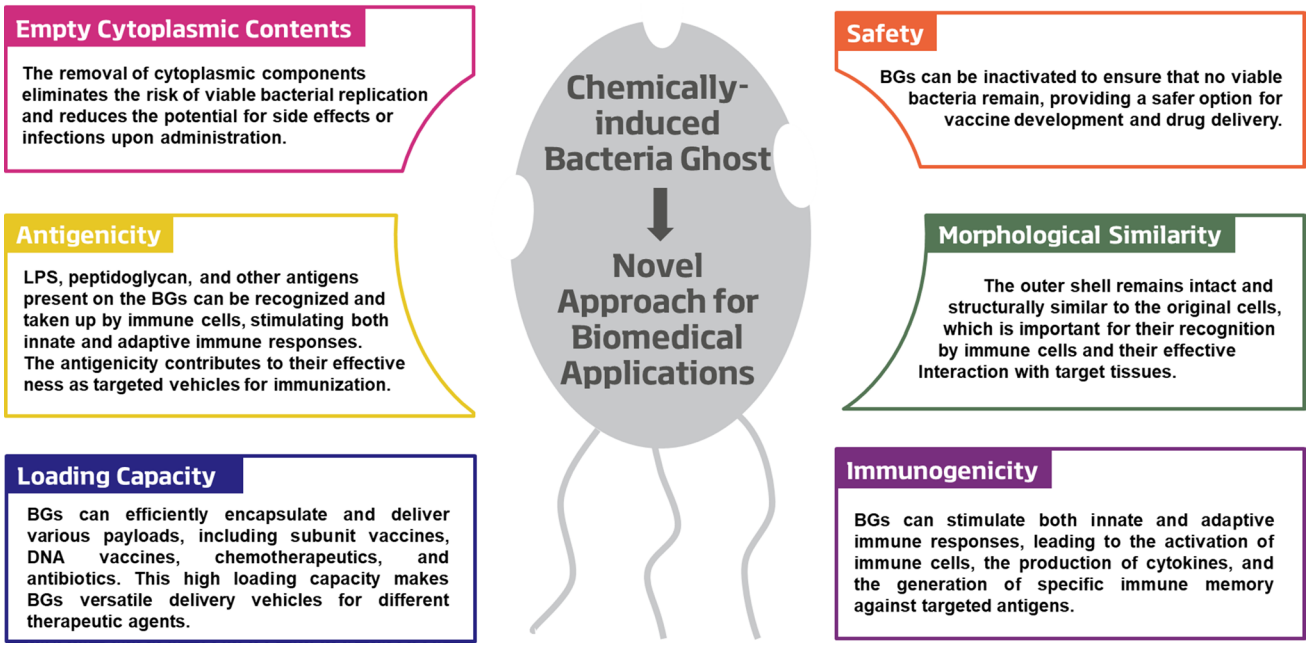

Fig. 3 Chemically induced BGs provide empty cytoplasmic contents, safety, antigenicity, morphological similarity, loading capacity, and immunogenicity in biomedical applications

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Author contributions Shin-Young Park supervised the manuscript and project and acquired research funding.

Data availability The datasets used during the present study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest Author Shin-Young Park declares that she has no conflict of interest. All the authors approved the manuscript.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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