Use of Bacterial Ghosts as Novel Drug Delivery Systems to Improve Cancer Treatment

Amin Afkhami-Poustchi¹, Maryam M. Matin¹,²,³*

ABSTRACT

Despite the large number of various anti-cancer drugs on the market, proper delivery systems are needed to decrease serious toxic and non-curative side effects. In order to enhance compliance, several delivery systems such as polymeric micro- and nanoparticles, liposomal systems and erythrocyte ghosts have been developed. Bacterial ghosts (BGs) represent novel advanced delivery and targeting vehicles suitable for delivery of hydrophobic or water-soluble drugs. BGs are empty bacterial envelopes of Gram-negative bacteria produced by controlled expression of cloned gene E, forming a lysis tunnel structure within the envelope of the living bacteria. BGs are devoid of cytoplasmic content and possess all bacterial bio-adhesive surface properties in their original state while not posing any infectious threat. BGs are ideally suited as an advanced drug delivery system for toxic substances in tumor therapy. The inner space of BGs can be loaded with either single components or combinations of peptides, drugs or DNA which provides an opportunity to design new types of (polyvalent) drug delivery vehicles. In particular, Doxorubicin-loaded bacterial ghosts have been used to target colon carcinoma cells. DOX, a cytotoxic drug commonly used in cancer therapy, was used as a model substance to demonstrate the delivery of moderate water-soluble drugs by BGs. The application of DOX with BGs increased the efficacy of treatment by two folds. The same effect was observed after incubation of leukemia cells and melanoma cells with DOX loaded BGs.

Keywords: Bacterial ghost (BG), Drug delivery, Tumor therapy, Doxorubicin loaded BG

The bacterial ghost (BG) system represents a novel and progressive approach in the development of bacterial mediated cancer immunotherapy. The empty inner space of BGs can be filled with drugs, proteins, DNA, enzymes and other compounds. The induced lysis process does not harm the essential structural components of the bacteria, giving rise to immunologically active particles capable of stimulating the host immune system and delivering specific antigen (Ag) to professional antigen-presenting cells (APCs) or active substances to the target cells (1,2).

References


Author Information

1. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
2. Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
3. Stem Cell and Regenerative Medicine Research Group, Iranian Academic Center for Education.

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Corresponding Author E-Mail: matin@um.ac.ir
Production of bacterial ghosts

BGs are produced by expression of cloned gene E from bacteriophage φX174 resulting to cell lysis in Gram-negative bacteria, such as, Escherichia coli K12 strains, Klebsiella pne-moniae, Mannheimia (Pasteurella) haemolytica, Neisseria menin-gitidis, Salmonella typhimurium, Vibrio cholera, Helicobacter pylori, and others (3,4). Expression of gene E can be placed under transcriptional control of either the thermo sensitive EpL/pR-cl857 promoter, or under chemical inducible promoter repressor systems, like lacPO or the tol expression system. Gene E codes for 91 amino acids and exerts its lytic function by fusion of the inner and outer cell membranes, forming a specific transmembrane tunnel structure through which all the cytoplasmic content is expelled, thus leaving a bacterial envelope called a BG devoid of nucleic acids, ribosomes and other intracellular constituents. The inner membrane (IM) and outer membrane (OM) structures of BGs remain intact during the lysis process (5,6). Electron microscopy studies and enzymatic studies clearly showed a sealed periplasmic space at the border of the lysis tunnel. The efficiency of the E-mediated lysis process, and quantification of generated BGs and non lysed viable bacteria are determined by flow cytometry assays using a specific dye that is sensitive to the changes of discriminatory power of membrane potential and stains only cells that have lost membrane potential (BGs or dead bacteria).

Bacterial ghosts as advanced drug delivery systems

Many diseases including cancers require the systemic administration of highly aggressive drugs to already immunocompromised patients. Deleterious and often severe side effects result from a lack of cellular and tissue selectivity. Another major issue is the poor solubility of some drugs used in cancer treatment. Considering these limitations, the development of a safer and more efficient drug delivery system (DDS) is the priority for future advanced cancer treatments (7).

Recently, bacterial ghosts made from the colonic commensal Mannheimia haemolytica were used for in vitro delivery of doxorubicin (DOX) to human colorectal adenocarcinoma (Caco-2) cells. Adherence studies showed that the M. haemolytica ghosts targeted the Caco-2 cells and released the loaded DOX within the cells. Cytotoxicity assays showed a two folds enhancement in cytotoxic and anti proliferative activity in cells incubated with DOX-loaded ghosts as compared with cells for which DOX was directly added to the culture media (8). This phenomenon might be caused by degradation of DOX-loaded BGs within the endolysosome of target cells allowing DOX to bypass the multi-drug resistance (MDR) efflux pumps and resulting in enhanced accumulation of DOX in the cytoplasm and then in the nuclear area of target cells.

References

Current work with bacterial ghosts lies in the investigation of the carrier capacity of the cytoplasmic lumen. This intracellular space of BGs can be filled either with water-soluble substances or emulsions such that the drug(s) of interest can be coupled to streptavidin anchored on the inside of the cytoplasmic membrane. Moreover, bacterial ghosts can be filled and sealed for the delivery of fluid, non-anchored substances. In a recent study, E. coli ghosts were filled with the reporter substance calcein and were sealed by fusion with membrane vesicles to maintain inner membrane integrity. Adherence and uptake studies showed that murine macrophages and human Caco-2 cells took up the bacterial ghosts, and calcein was released within the cells (1,7,9).

**Bacterial ghosts as immunologically active particles**

Because of the unique structure of the BG’s envelope with preserved pathogen-associated molecular patterns (PAMPs), BGs can be used in biomedicine alone as an adjuvant or as a delivery vehicle for drugs or genes. The inner space of BG’s empty envelope can be loaded with a combination of peptides, drugs or foreign DNA which gives us an opportunity to design new types of polyvalent vaccines (9). BGs have excellent DNA loading capacity varying from 4000 to 6000 plasmid copies per BG depending on the concentrations of DNA solution used (10-13). BGs loaded with plasmid DNA are efficiently internalized and phagocytosed by both professional APCs and tumor cells. Cross-presentation of Ag delivered to dendritic cells (DCs) by BGs can activate both CD4+ and CD8+ T cells and stimulate the immune system to enhance the immune response against Ag expressed by target cells. Inner and outer membrane structures of BGs including lipopolysaccharide (LPS) and other PAMPs remain intact after protein E-mediated lysis of Gram-negative bacteria. Thus, beside possessing a high loading capacity; BGs carry highly effective molecules for the stimulation of cross presentation by DCs on their surface, specially, tumor-associated antigens (TAAs). BGs with their intact envelope structures are not only immune stimulatory to professional phagocytes but are also capable of providing stimulatory signals to tumor cells. It is known that melanoma cells have the capacity to behave as non-professional APCs and can phagocyte both apoptotic and live cells, and it was recently shown that melanoma cells actively respond to exposure to BGs by increasing their rate of phagocytosis. Using BGs for gene delivery to the immunocompetent cells, in particular DCs as well as tumor cells, could initiate or restore the immune response against the delivered TAAs as well as induce and increase the expression of target genes by APCs and tumor cells (14).

**References**


Conclusions

These observations indicate high capacity of BGs to target various histological types of cancers. Bacterial ghosts are very useful non-living carriers, as they can carry foreign antigens, nucleic acids and drugs in one or more cellular locations simultaneously. Optimization and improvement of the selected prospective model type of BGs would help to progress the development of microbial-mediated disease treatment and drug delivery systems and their application in future clinical trials.