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BACTERIAL GHOSTS: NEW CHALLENGES IN MEDICAL AND PHARMACEUTICAL APPLICATIONS

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ABSTRACT: Bacterial Ghosts (BGs) represent bacterial envelopes which are produced by the controlled expression of the phage derived lysis gene E. Because the cytoplasmatic content is expelled through the generated lysis hole, the intracellular space can be filled with any biologically active substances. Like their viable counterpart, BGs provide fully intact surface structures including macromolecular assemblies such as flagella, pili or LPS and are therefore able to stimulate the innate immune system. Thus, the BG technology provides an innovative system for vaccine, drug or active substance delivery for various cells and tissues of animal, human or plant origin. However, the feasibility of BGs production method, trivial cost, and sustainable immunity can introduce this technique as a suitable method for medical and therapeutic objectives.

Keywords: Bacterial Ghost, Medicine, Pharmaceutics.

INTRODUCTION

Recently, BGs received an increasing interest for their promising medicinal and pharmaceutical applications. The BGs are the bacterial envelope without its internal content that retains all morphological, structural, and antigenic features of the cell wall [1, 2]. The production of BGs begins by creating a small hole in the external surface of bacteria. Due to the high internal osmotic pressure; the cytoplasm content is expelled through the tunnel. The main advantage of this method is production of the non-living of the bacterial ghosts, while all components antigenic structure of the initial bacterial, with its natural function [1, 3, 4].

Bacterial ghost production process

E gene expression in gram negative bacteria as a conventional method is used to create bacterial ghosts. In this process, E gene cloned into a bacteriophage ΦX174 under controlled conditions. E gene encoding the polypeptide of 91 amino acids[5, 6]. Unlike other lytic proteins, these proteins have no intrinsic enzymatic activity. Analysis of the primary structure of protein E revealed a hydrophobic region at its N terminal end suggesting it is membrane-associated [2].

Protein encoded by the gene E creates a tunnel in the bacterial cell wall and cytoplasmic contents through which it flows out [7, 8]. On average, the diameter of the tunnel varies from 40 to 200 nm [9, 10]. The variation in size and irregular tunnel structures indicated that the E-specific transmembrane tunnel structure is dynamically formed by the strong force ejecting the cytoplasmic content through the E-lysis hole which is most probably formed by E-oligomers [11].

Studies done on BGs by electron microscopy exhibited that the tunnel structure created by protein E, is not randomly distributed across the cell membrane, as it is limited to areas of potential sites that often in the middle of the cell or in the polar site[10, 12] There are additional requirements such as active growth and functional control elements of cell division and of autolytic activity of the host bacteria. Membrane adhesion sites, FtsZ protein in the septosome, cis-trans proline isomerases for conformational change of protein E, chaperones, the strength of the membrane potential, the activity of the autolytic system , the pH and osmotic strength of the medium, and other factors also influence the E-lysis process [13, 14]. Expression of gene E can be placed under transcriptional control of either the v thermosensitive EpL/pR-cI857 promoter, or under chemical inducible promoter repressor systems, like lacPO or the tol expression system.Mutations to the EpR promoter/operator regions have resulted in new expression systems, which stably repress gene E expression at temperatures of up to 37º C, but still allowed induction of cell lysis at a temperature range of 39–42 º C [15].

Another way for creation of BG is use from active chemical matherials such as sodium dodecyle sulfate (SDS), NaOH, $CaCO_3$ and H2O. Hence, minimum inhibition concentration (MIC) and minimum growth concentration (MGC) of the substances must be determined. The MIC is defined as the lowest concentration of each antimicrobial agent that inhibited visible growth of the tested isolate and the MGC is defined as the lowest concentration that permits survival of bacterial cells. In conventional methods, electron microscope to evaluate the quality of BGS and spectrophotometer to measure the amount of protein and manufactured DNA should be used. To determine the presence of DNA remaining after each load of BGs, agarose gel electrophoresis must be used. Living cells that existed after the implementation of the protocol were destroyed by induction lysosomic gene [14, 16]. The ghosts of bacteria, are including laboratory strains: *E.coli K12*، *E. coli* C, *Pectobacterium cypripedii*, enterotoxigenic *E.coli*, *Salmonella enterica* serovar *Typhimurium*, *Salmonella enteritidis*, *Shigella flexneri*, *Vibrio cholerae* serovar O1 and O139, *Helicobacter pylori*, *Neisseria meningitidis*, *Pseudomonas putida*, *Klebsiella pneumonia*, *Bordetella bronchiseptic*a , *actinobacillus. pleuropneumoniae*,. *Pasteurella. multocida*, *Mannheiiahaemolytica*, *Francisella tularensi*. Investigations with *E.coli* C and K12 strains were mostly done in genetic engineering to establish the BG system, and with the plant-specific *P. cypripedii* for plant pesticide targeting [17].

Applications

BGs have multiple applications in biomedicine but the most applications are including BGs as DNA transfection vehicle for DNA vaccines, as drug carrier for tumour therapy. Other applications are including miniature bioreactors, artificial bacterial life forms, and as construction modules for molecular machines in micro- and nanotechnology [10, 18, 19].

BGs as a Drug delivery system

The BG system provides a new promising platform for the delivery of drugs and other biologically active substances [20]. Studies exhibited that bacterial ghosts can use for drug delivery resveratrol. The drug inhibits the production of nitric oxide (NO) in the macrophage. Use the BG will be adjusted in the production of nitric oxide while it does not the cytotoxic effect on macrophage cells [21, 22]. BGs can also increase the viability of the cells after short and long term chemical treatment. As an example, BGs don't have toxic effect on human cell lines while can inactivate the affects toxic benzalkonium chloride (BAC) [22].

BG loaded by doxorubicin (DOX) is a therapeutic option in the cellular line of colon cancer in human. In compare with free drug, titer of intracellular DOX when it is loaded by BG is increased about 42 times [23]. DOX has side effects on heart. Tumor treatment by BGs must be finished immediately after reaching maximum critical dose. Accordingly, the reduction of consumed dose is the advantages of BG [23]. BG has a high capacity to enter into the cellular line of human conjunctiva without cytotoxinic effect on this line. Several studies introduce BGs as a suitable carrier for drug delivery in ocular diseases [3].

The role of bacterial ghosts in gene transfer

DNA or drugs can be used as active substances for treating tumors. Studies show that human melanoma and colon carcinoma cells can be targeted respectively for DNA or drugs transfer [24-26]. In a study, eight cellular lines of melanoma which have many functions in common with cells providing antigens have been examined the ability to connect and carry out the phagocytosis of BG. Bowes cells expressed about %80 of the marked gene delivered by BG. This study showed that BGs system is applied as a tool for transferring functional RNAs (e.g. siRNA) which are enzymes for medicinal conversion [26].

Bacterial ghosts as candidate vaccines

Bacterial ghosts like probiotics can be used as ideal vaccine candidates not only due to their ability in carriage of immunogenic antigens but also because of the adjuvant properties of bacterial membrane components such as lipopolysaccharides, peptidoglycans and lipid A [19, 27, 28]. High stimulation of natural immune system by pathogenic bacteria vs. non-pathogenic bacteria is one of the most benefits in use of bacterial wild type as a BG in vaccine. In a study the effects of BGs obtained from *E.coli* on stimulating immune system and producing proinflammatory cytokines and antimicrobial peptides was examined. It exhibited that BGs derived from the wild type of *E.coli* could further stimulate natural immune system responses and pro inflammatory cytokines as compared to other *E.coli [29, 30]*. In this study, it was shown that BGs with flagellin has further ability to induce the expression of natural immune mediators as compared to BGs without flagella [18].

Helicobacter pylori ghosts containing recombinant Omp18 is used as an efficient vaccine in immunizing rats infected by *H. pylori*. This vaccine considerably increased the specific antibody of H. pylori and reduced the colonization of this bacterium in stomach. Omp18 induced the production of IL-10, IL-12, and IFN-γ. It protected BALB/C rat against H. pylori infection [31].

Mannheimia hemolytica ghosts are applied to prepare vaccine as a transfer system. These bacterial ghosts adjusted Th1 and Th2 responses. Researchers showed that dendritic cells play a key role in stimulating immune system responses when bacterial ghosts are used as a DNA transfer system. BGs as an adjuvant reinforce the maturity and activation of dendritic cells [32, 33]. In several studies, the ability of BGs to stimulate the production of cytokines in dendritic cells has been assessed. Totally, these investigations exhibited that production of IL-1α and IL-6 in dendritic cells affected with BGs is very high in compare with non-stimulated dendritic cells, respectively [1, 34-36].

Studies done on BG of *Vibrio cholerae* in rabbits showed that the oral immunization of rabbit by BG increases immunity response against serovar O1 and serovar O139 [10, 37] Vibrio cholerae BGs could be used for treatment of Chlamydia trachomatis infections without any toxic effect. Vibrio cholerae BGs are useful means for transfer and have strong ability to stimulate immunity [38].

The role of aerosol BG in increasing of immunity is not clear but in several studies have been mentioned the beneficial effect of aerosol BGs. As an example, *Actinobacillus pleuropneumonia* aerosol BG with diameter of 1-3μm in pigs would cause significant increase in immunity. *Actinobacillus* pathogen and the experimental model of pig could be considered as a good model for immunizing human's lung or delivering aerosolic drugs deep to alveolar space [10, 34, 39, 40].

Conclusion

Bacterial ghosts (BGs) are considered as an appropriate candidate for stimulating immunity system and vaccination and as bacterial carriers, in compared to viral carrier have a bigger space for loading antigen, drug, and plasmid. Nevertheless, it must be noted that the presence of a huge number of lipopolysaccharide can induce fever and shock. Although, have been reported no side effects so far. The presence of numerous antigens existing in BG can excessively stimulate immunity system. Consequently, it can result in tissue damages. It is required to deactivate a part of these antigens before being delivered to body. Difficulties in BGs production in positive

gram bacterium is one of important limitations of BGs. Therefore further studies should be done to find more suitable methods for creating BGs in positive gram. The feasibility of BGs production method, trivial cost, and sustainable immunity can introduce this technique as a suitable method for therapeutic options. Studies have showed that using genetic techniques for producing vaccine enhances the stimulation of immunity system as compared to chemical methods.

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