Immobilization and Stabilization of Bacteriophages

Subjects: Others

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Phages are effective at targeting and killing bacterial strains of interest and have yielded encouraging results when administered as part of a tailored treatment to severely ill patients as a last resort. The incorporation of bacteriophages into therapeutic formulations typically involves encapsulating them within a stabilizing substance. Immobilization refers to the chemical, physio-chemical or electrostatic binding of bacteriophages to a surface.

phage therapy phage formulations immobilization encapsulation therapeutic delivery

1. Introduction

The emergence of antibiotic-resistant pathogens is becoming increasingly problematic in the treatment of bacterial diseases. The search for alternative strategies to solve these problems has rekindled interest in bacteriophages. These viruses can kill specific bacterial targets, leaving other cells unharmed. Additionally, their ability to propagate to high concentrations at the site of infection reduces the need for continuous application [1][2]. A considerable proportion of bacteriophage-related research now focuses on their practical application for the treatment of diseases including respiratory, gastro-intestinal, wound and skin infections [3][4][5][6][7][8]. While phages can be applied to areas of infection in liquid form, this does not necessarily represent the most effective means of treatment, with the few controlled clinical trials that have been carried out yielding mixed results thus far [9][10][11]. Apart from the difficulty in applying a liquid preparation to a site of infection, adverse conditions brought about by the body's natural physio-chemical environment as well as its immune response could present a considerable challenge to bacteriophage stability [12]. There has therefore been increased attention towards the development of alternative phage formulations, with a view to improving both their efficiency of application as well as their long-term stability [13][14].

2. Stabilization of Bacteriophage Therapeutics

The incorporation of bacteriophages into therapeutic formulations typically involves encapsulating them within a stabilizing substance [15][16]. Through such an approach, various antimicrobial materials such as powders, semisolids and nanofibers can be produced, providing more options for effective delivery at the site of infection and, consequently, improved patient outcomes.

2.1. Emulsification

Emulsions are mixtures of immiscible liquids in which one liquid acts as the continuous phase with the other(s) dispersed within it with the help of a surfactant. The dispersal and/or encapsulation of bacteriophages in emulsions to improve their stability whilst facilitating their bioactivity [17][18]. And emulsions can facilitate bacteriophage infectivity [19]. Despite the improved bioavailability and delivery of emulsified bacteriophages, there are challenges to the use of such a technique in an upscaled scenario.

2.2. Freeze-Drying

In freeze-drying (lyophilization) the phase change process of sublimation is used to remove all traces of water from a sample [20]. The final product is typically a powder of varying grain sizes. This type of formulation can therefore be applied to a range of delivery scenarios, such as incorporation into oral capsules or topical creams. The powder-form product of the freeze-drying process makes it an interesting option with respect to large scale production. Powders are generally light and stable, making them easy to pack and transport. Additionally, the potential for freeze dried phage formulations to retain viability over periods of months would allow for a non-disruptive incorporation into a production chain.

2.3. Spray-Drying

Spray-drying allows for the transformation of a liquid substance to a dried particulate form through evaporation [21]. This is achieved by spraying concentrated feed droplets (typically between 10–100 µm in diameter) into a hot drying chamber containing hot air. An attractive aspect of spray-drying is the relative simplicity of the process, and for this reason, the technique has received increased attention with respect to bacteriophage formulations. The resulting product of the spray-drying process is a dry powder which much like the product of the freeze-drying process, can be applied to creams, tablets and inhalable formulations [22]. The advantages with respect to storage, transport and stability associated with freeze-dried powder also hold true for spray dried powder. Additionally, spray drying is considerably less expensive to run than the former.

2.4. Liposomes

Liposomes have been used to encapsulate a wide variety of substances, including hydrophilic and hydrophobic drugs, proteins, living cells, nanoparticles, quantum dots and plasmid DNAs [23]. Like cell membranes, liposomes vesicles are composed of phospholipid bilayers [24]. Liposome encapsulation has been demonstrated to be a viable option for both bacteriophage stabilization as well as the delivery of phage therapeutics [25][26][27][28]. The fact that liposomes can fuse readily with cells they come into contact with broadens their scope of applications by allowing for the potential to target intracellular pathogens. The increased protection afforded to bacteriophages by liposomes also increases their retention time in vivo.

2.5. Electrospinning

The production of nanofibers through electrospinning can be used to stabilize bacteriophages and produce antibacterial fibers [29]. For this technique, a charged, molten polymer solution is drawn onto an electrode of

opposite charge. The final, dried nanofibers typically measure 100 nm or less in diameter [29]. The addition of bacteriophages to the liquid polymer prior to carrying out the process results in encapsulated bacteriophages within the nanofibers, which can be applied for both water-soluble and insoluble polymers.

3. Bacteriophage Immobilization

The advantages and drawbacks of the various immobilization techniques that have been described would need to be considered for this application (Table 3 and Table 4). As with encapsulation, immobilized phage can potentially be integrated into various formulations including powders, patches, wound dressings and creams, however the fact that it can technically be carried out on most surfaces increases the potential range of applications.

Table 3. Examples of studies involving immobilization of bacteriophages onto surfaces.

Immobilization Approach	Bacteriophage (Host Genus)	Surface	Observations	Reference
Physical Adsorption	T4 (*Escherichia*)	Gold surface modified with cysteine and glutaraldehyde	Phage surface concentration of 18 ± 0.15 phages per um^2	[30]
Protein-Ligand	T4 (*Escherichia)*	Magnetic beads, microcrystalline cellulose beads	Up to 81% improved binding efficiency compared to physical adsorption	[31]
Electrostatic	T7 (*Escherichia*)	Cellulose microfibers	15–25% phage loading efficiency on surface	[32]
Covalent Linkage	AG10 (*Escherichia*) CG4 (*Salmonella*)	Magnetic-fluorescent beads	Phage activity equivalent to 10^8 PFU/mL observed in material	[33]

Table 4. Summary of benefits and limitations associated with various bacteriophage immobilization techniques for the production of therapeutic phage formulations.

Immobilization Approach	Benefits	Limitations
Physical Adsorption	Simple process Inexpensive	Undirected, inconsistent Phage not strongly bound to substrate
Protein-Ligand	Strongly bound phage High binding efficiency	Complicated process Expensive

Immobilization Approach	Benefits	Limitations
	Tail-up orientation	
Electrostatic	High binding efficiency Applicable to most tailed phages Tail-up Orientation	Electrostatically charged surface may not be desirable
Covalent Linkage	Strongly bound phage Potentially longer shelf life	Can be a costly and complex process (in the case of linker-based immobilization)

3.1. Physical Adsorption

Physical adsorption, or physisorption, refers to the adhesion of particles onto a surface brought about by van der Waal's forces, dipole-dipole moments, electrostatic forces and steric and hydrophobic interactions [34]. Van der Waals forces, although weak, occur in all molecular species. This makes physical adsorption a ubiquitous occurrence. It represents a quick and relatively simple ways to immobilize a species onto a given surface and, because it occurs through non-chemical interactions, it typically does not result in any chemical alteration of the absorbate. However, because of these physical stresses as well as extremes of acidity, temperature and ionic strength can act to reduce attachment or reverse it post immobilization [15].

3.2. Charge-Directed Immobilization

In charge-directed immobilization, electrostatic attraction between permanent opposing charges on the surface and adsorbent is used to bring about immobilization. These are considerably stronger than the interactions discussed thus far. Bacteriophages often consist of charged regions; phage heads usually possess a net negative charge with the opposite being true for their tails [35][36]. For this reason, phages have been shown to bind tail-down or tail-up, depending on the net charge present on the surface [37].

3.3. Protein Ligand

The natural tendencies of proteins to adsorb to certain ligands can be exploited for the purpose of bacteriophage immobilization. The surface and absorbate are coupled with a binding protein and its corresponding ligand, respectively, with the interaction and subsequent immobilization occurring once they encounter one another. Streptavidin is a protein that occurs in the bacterium *Streptomyces avidinii* [38].

3.4. Covalent

All of the approaches discussed so far have not involved the alteration of substances through the formation of new chemical bonds. Covalent immobilization represents the most permanent and irreversible form of attachment,

demonstrated by the ability of covalently immobilized phages to remain bound to substrate even after prolonged exposure to sonication forces [39].

4. Future Prospects

Bacteriophages represent a viable treatment alternative for bacterial-borne diseases. The application of specialized formulations will be key to any future clinical trial successes in bacteriophage therapy.

In addition to the other considerations, formulations will need to be considered in terms of the ease at which their production can be scaled up. In cases such as linker-based immobilization, multiple processing steps would be involved, while higher running cost and increased lead times would be associated with other processes such as freeze-drying. The most attractive phage products from a production point of view will therefore be those that are relatively straightforward to produce consistently. Formulations which can be stored for extended periods of time at ambient temperatures are also more likely to be favored.

It is likely that different formulation methods will be required for different applications and that further research is needed in this area to facilitate the widespread use of phages as genuine viable alternatives to other antibiotics in human therapy.

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