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Bacteriophages and phage-inspired nanocarriers for targeted delivery of therapeutic cargos☆

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ABSTRACT

The main goal of drug delivery systems is to target therapeutic cargoes to desired cells and to ensure their efficient uptake. Recently a number of studies have focused on designing bio-inspired nanocarriers, such as bacteriophages, and synthetic carriers based on the bacteriophage structure. Bacteriophages are viruses that specifically recognize their bacterial hosts. They can replicate only inside their host cell and can act as natural gene carriers. Each type of phage has a particular shape, a different capacity for loading cargo, a specific production time, and their own mechanisms of supramolecular assembly, that have enabled them to act as tunable carriers. New phage-based technologies have led to the construction of different peptide libraries, and recognition abilities provided by novel targeting ligands. Phage hybridization with non-organic compounds introduces new properties to phages and could be a suitable strategy for construction of bio-inorganic carriers. In this review we try to cover the major phage species that have been used in drug and gene delivery systems, and the biological application of phages as novel targeting ligands and targeted therapeutics.

Contents

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1. Introduction

In nature, viruses are natural gene carriers that transfer biological information between different species, which can range from prokaryotic cells such as bacteria to more complicated biological systems like mammalian cells. A wide variety of viruses have been used as drug delivery systems (DDSs) with varying properties. The most commonly-employed eukaryotic viruses such as adenoviruses, retroviruses and lentiviruses, although efficient in infecting target cells, might stimulate immune responses and could cause cancer by disrupting tumor suppressor genes. These possible drawbacks have encouraged researchers to consider bacteriophages and other virus-like particles that are considered safer. The basic structure of viruses has inspired the construction of newly engineered viral particles by a process called pseudotyping. This involves changing their surface proteins and glycoproteins that can lead to generation of de novo particles such as virosomes, that consist of a virion-like phospholipid bilayer that can be coated with specifically chosen surface glycoproteins.

Among all viruses, bacteriophages have a wide range of potential applications in both clinical and non-clinical fields. Phages have been used for food preservation in the food industry [1], as an eradicator of bacterial biofilms [2], and in bio-sensing in wastewater treatment [3]. The fundamental benefits of bacteriophage systems include their ease of use and development, a great capacity for packaging cargos, an ability to be loaded with non-DNA cargos, and their relative safety in humans. These favorable properties suggest that phages can be considered as nanocarriers [1,3,4]. Moreover bacteriophages do not undergo alterations in their natural tropism or mutation which is an advantage compared to eukaryotic viruses. This property makes them safer to be used for treatment and prevention of infections in human and animal populations. Bacteriophages have two main stages in their life cycle, which are the lytic cycle and the lysogenic cycle, although few phages are capable of carrying out both [4]. Since DNA and RNA molecules are unstable in their native form in body fluids [5–60 min for RNA and 10 min for DNA] because they are destroyed by nucleases, phages are ideal vehicles for transferring nucleic acids. Their simple production and purification methods as well as their large capacity for containing genetic material are additional advantages.

The therapeutic application of phages began in the former Soviet Union and Eastern Europe and their unique properties suggested they could be promising agents in clinical applications as anti-infectives. Phages are highly specific for their target and their self-regulating systems, such as a limitation of propagation to specific cells, make them versatile and modifiable carriers in targeted delivery systems [5]. Rather than using intact phages, some phage-derived molecular products can also be used as therapeutic agents. For instance phage endolysins specifically hydrolyze the peptidoglycan component of different bacterial species and are therefore bactericidal [6]. In recent years, more researchers have studied new application of phages in the pharmaceutical and biotechnology industries. In this review we will cover recent studies that have focused on phages as delivery vehicles.

2. Basic properties of bacteriophages

Bacteriophages are viruses with a highly efficient ability for compressing and wrapping DNA to form a compact particle. The phage genome encodes only a few to hundreds of genes and is considered a useful model to study DNA replication [7]. The discovery of bacteriophages first took place in 1915 by Fredrick Twort [8]. In 1917 D'Herelle discovered the potential ability of phages to kill the bacteria they infected via the lytic phase. He suggested that phages could be used as antimicrobial agents in a therapeutic application if they were targeted to bacteria that caused human diseases [8]. After the discovery of penicillin in the 1940s, the application of phages in therapy for human infectious disease was abandoned. Concurrent studies categorized phages as obligate intracellular parasites, and research on phages provided fundamental discoveries of biological systems such as the identification of nucleic acids as the genetic material. During these studies the nature, chemical composition and life cycles of phages were identified. It became clear that the phage was composed of a protein envelope encapsulating nucleic acids. Thanks to the efforts of Martha Chase and Al Hershey who discovered that the nucleic acids entered the bacterial cells during phage infection while the proteins did not, this led to the realization that hereditary information was solely transmitted by DNA molecules [9]. The properties of phages vary depending on the regulatory mechanism of host cells, their genetic content and physiological variants [10]. Based on their properties, phages have been classified into different groups of which the double stranded (ds) DNA tailed phages are the most common type. It has been calculated that there are no less than 10^{31} different phages that exist on the planet Earth, making them the most diverse grouping of biological entities in existence. The new classification system called the “Phage Proteomic Tree”, has classified phages in a sequence-based taxonomic system according to their genome in which average distance between pairs of phages is determined and phages are grouped in the context of all other phages [11].

Improvements in electron microscopy have provided more information about the phage morphology, and their interactions with bacterial cells [12]. Studies in this area have found different morphologies for the phage structure. Each type of phage has a particular shape and shows different mechanisms of supramolecular assembly. Some basic morphological and other properties of selected phages are shown in Table 1.

A variety of phages exist with a different range of hosts and mechanisms of production. Phages are divided into three main classes based on their production and generation: lytic phages such as T4; temperate phages like lambda; and lysogenic phages such as M13. M13 is a filamentous phage that converts the host cell into a generation factory without lytic disruption. This phage can cross the bacterial membrane to escape by leaking out without disruption of the cell wall and membrane. By contrast in lytic phage infections, bacterial cells are lysed and disrupted by replication of the virions inside the bacterial cell and the infection is rapidly transferred to new hosts [13]. The third type, temperate phages can choose between a lytic and lysogenic pathway of development. In temperate phages, the phage genes become integrated into the bacterial genome. The phages replicate through the natural bacterial cell cycles and are transmitted into the next generation of bacteria, although in stressful conditions such as UV irradiation, the phages become activated and the lytic phase begins.

The genome sequence of phages that have been integrated into bacteria is called the “prophage” and 2/3rd of the genomes of “low CG” bacteria contain these sequences. This phenomenon can alter the properties of the host bacterial cells and can alter their pathogenicity and virulence by changing their physiology and pathology [14]. The actual mechanism of lysogeny is due to the presence of repressor genes that are encoded in temperate phages. These genes generate repressor proteins that repress various bacterial operons. They bind to the phage genomes and interrupt the formation of intact phage particles, but the prophage sequence is translated in bacteria and leads to expression of new properties in the host cells. This phenomenon, which is called “lysogenic conversion”, is important in medicine. Some bacteria such as *Corynebacterium diphtheriae*, streptococci and *Clostridium botulinum* are not able to produce their toxins without prophage sequences.

Cell penetration of the phage into the bacterial cell occurs through the recognition and attachment of phages to specific receptors that are expressed on the surface of the host cell, and the surface properties of the host membrane also affect this phenomenon [15]. The tolerance of the phage surface to be chemically altered provides opportunity for insertion of foreign peptides such as RGD (Arg-Gly-Asp) or DGEA (Asp-Gly-Glu-Ala). These moieties can have therapeutic effects in a targeted delivery approach. Studies of Young's group showed that not only could the surface of phages be modified, but also their genetic