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Short Communication

Bacteriophage-based Vaccine: A New Dawn for Vaccine Design and Development

Fazal Mehmood Khan*

Key Laboratory of Marine Microbiome Engineering, Institute for Advanced Study, Shenzhen University, Shenzhen 518060, China

Abstract: The COVID-19 epidemic has strained healthcare systems, causing stress among personnel and facing significant economic and social issues. COVID-19 patients have significant symptoms, necessitating prompt treatment. It is a global urgency to develop effective vaccinations against COVID-19. Quick immunization of the whole world population against an ever-changing, extremely deadly virus is alarming, and various vaccine techniques are being researched. Bacteriophages are helpful in the treatment of multidrug-resistant bacterial infections. But, their clinical efficacy may go far beyond. One of the most significant bioproducts in medicine is thought to be vaccines. Vaccines for a variety of diseases have been made. However, certain vaccinations have disadvantages, such as high prices and immunological responses. In this regard, the use of bacteriophages has been suggested as an exciting alternative for making more inexpensive vaccines. Bacteriophage-displayed vaccines are based on the antigens being expressed on the phage surface. This tactic uses the inherent advantages of these particles, including their high stability, inexpensive production, and adjuvant capacity. Phage-displayed, phages DNA and hybrid phage-DNA vaccines are the three phage-based vaccines that are currently offered. The traditional method for finding novel barrier protection epitopes, antigens, and mimotopes is phage display. In this frame of reference, phage particles serve as a versatile, effective, and promising strategy for making vaccine delivery systems that are more effective and should be widely applied in the future. The phage-vaccine technique can potentially address the growing demand for innovative vaccinations against emerging diseases. This short communication addresses bacteriophage uses in vaccine development and discusses recent developments in bacteriophage-based vaccinations. It also focuses on and describes bacteriophages as a novel vaccine candidate for COVID-19.

Keywords: Antigen delivery, Bacteriophage, COVID-19, Vaccine.

1. INTRODUCTION

Bacteriophages (phages) are natural bacteria predators that identify, target, and kill a bacterial host while causing no harm to normal flora and human cells. Bacteriophages, bacteria's natural predators, are valuable in modern biotechnology. They've been proposed as antibiotic substitutes for various antibiotic-resistant bacterial species. Additionally, phages have applications such as DNA and proteins vaccine delivery systems, harmful bacterial strain identification agents, and protein and antibody display systems [1]. Phages are broadly used in biotechnology and have helped to illuminate essential molecular biology concepts. Phage biology offers many new Genetic technologies, medical diagnostics, and synthetic biology methods [2].

Bacteriophages are used as vaccine delivery systems [1]. Because of their excellent stability under harsh environmental circumstances, simplicity, low-cost, large-scale production, and robust adjuvant powers, phages are well-suited for vaccine formulation. Phage vaccines have a high safety level and good immunostimulatory effects because bacteriophages have a rich legacy of association with the mammalian body. The advent of bacteriophage display technology is a watershed moment in the evolution of phage-based

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*Corresponding Author: Fazal Mehmood Khan <fazalmehmoodhu@hotmail.com>

vaccinations [3].

Vaccine development is one of the tremendous benefits of phage display, which has recently received considerable attention [3]. Vaccine development represents one of the essential impacts in the medical field, as it has saved countless animal and human lives [4]. Numerous research groups worldwide are currently focusing on developing medical vaccines that are more effective, safe, and less expensive. They possess a prolonged immune response [5]. Multiple vaccination programs, such as live attenuated, inactivated, and synthetic, have been developed and successfully tested for preventive purposes. Traditional vaccinations include destabilized or inactivated microorganisms [6]. Despite significant improvements in conventional vaccinations, there have been reports of challenges in microbes, low efficacy, and possible risks from virulent conversion or transmission to immunocompromised people [5].

Bacteriophage-based vaccines are thought to be a viable alternative to traditional vaccines. The intrinsic properties of bacteriophages increase the lifetime and immunogenicity of the expressed antigen [7]. Modern molecular tools, which allow for bacteriophage genome manipulation via phage display technology, facilitate the synthesis of phage-based vaccines. The current use of bacteriophages in vaccine direction has created an entirely new market opportunity [8]. Recombinant bacteriophage technology is one proposed method for overcoming the drawbacks faced by present vaccinations. Moreover, the phage-vaccine technique has the potential to meet the growing demand for novel vaccines against emerging diseases [9].

This brief communication article explains recent advances in synthesizing bacteriophage-based vaccines, particularly emphasizing the current phage COVID-19 vaccine strategy. In addition, the advantages of phage-based vaccines. Finally, critical issues and prospects for phage-based vaccinations are addressed.

2. BACTERIOPHAGE-BASED VACCINE DEVELOPMENT

The development of vaccines represents an array of significant medical and scientific breakthroughs, protecting numerous animals and humans [4]. Many research organizations worldwide are now concentrating on making a vaccine that is more efficient, cheaper, and safe for clinical use and has a more extended immune response [5].

To overcome the limitations of traditional vaccines, phage-based immunization is a promising alternative. This method uses bacteriophage properties to extend the shelf life and high stability of expressed antigens [10]. This method uses phage ability to promote both humoral and cell-mediated immunity [8, 11].

Bacteriophages are helpful in the treatment of multidrug-resistant bacterial illnesses. But, their clinical utility may go far beyond. Some researchers are enthusiastic about putting phages to work since the COVID-19 global epidemic presented a new and demanding challenge [12]. Bacteriophages have several characteristics that make them appealing for vaccine development. Phages are extremely simple and inexpensive to make vaccines on a large scale. They are also exceptionally stable in challenging, hostile environments, necessitating no sophisticated refrigeration for shipping and storage. Bacteriophages have also been found to stimulate both the innate and adaptive body's immune system. Bacteriophages are safe and have no severe side effects [13]. A turning point in applying bacteriophages in vaccine production occurred in 1985 with the invention of phage display technology. During phage display, a nucleotide sequence encoding a necessary amino acid or protein is inserted into the phage's coat protein gene. The protein or amino acid is then shown on the phage's surface. The surface of phages can be coated with foreign antigens to stimulate an immune response in vaccination applications. Using phage display technology, phages may be detached and put back together like jigsaw puzzle pieces. Whatever we layer on their surface will cause our immune systems to respond [14].

The most important technical advancement in molecular biology in recent years may be bacteriophage displays, which are effective research tools. This strategy is based on the bacteriophage surface's fusion amino acid proteins depiction. The virus genome is simultaneously encoded in DNA and wrapped around the fusion-displaying

mechanism. Phage display offers, at the very least, different approaches and strategies for developing vaccines [15].

Escherichia coli, a typical and unharmful bacterium in the human digestive system, is infected by phage T4. The finding made by Rao of Catholic University has opened the door for the phage to be used as a vehicle for the delivery of novel vaccines and treatments to patients. To accomplish this, he and his team wrap therapeutic Genetic code in proteins and attach them to the capsid's top shell. In Rao's work, chimera phages for vaccine and gene-targeted treatments are produced using the genome-editing tool CRISPR and bacteriophage display technology [16]. Professor Rao's T4 bacteriophage research has allowed his team to study the bacteriophages' therapeutic potential, including the invention of a dual anthrax-plague vaccination that has proven efficient in protecting mice models from pathogenic bacteria [17]. Rao's team was also involved with another T4 bacteriophage application. They give excellent proof studies for developing next-generation influenza vaccines employing bacteriophage T4 virus-like particle (VLP) platforms [18].

By including protective antigen expression mimicking epitopes or cassettes in the phage genomes, phage DNA vaccines are produced. Due to the protection provided by the coat protein, bacteriophage DNA vaccines are safer for injection, preservation, and transportation than conventional DNA vaccinations. This allows for the oral delivery of phage DNA vaccines. Nonetheless, phage-displayed vaccines are more widespread and popular than phage DNA vaccines. Phage-displayed vaccines are recombinant phages expressing immunostimulatory peptides or proteins on their surface through transcriptional fusing or by entrapping antigens with previously expressed antigen-binding peptides [19].

3. BACTERIOPHAGE-BASED VACCINE AND COVID-19

Rao and his colleagues took on a new challenge with the T4 phage platform and the novel idea of incorporating any microbe into a vaccine. Their efforts resulted in the development of a phage vaccine candidate that inhibited SARS-CoV-2 infections in cell cultures and led to elevated antibody levels in inoculated animals [16]. The work caught the attention of Adaptive Phage Therapeutics, a biotech start-up investigating the clinical uses of phages against multi-drug resistant pathogens. The focus shifted from using bacteriophages to treat multiple drug-resistant infections to making a COVID-19 vaccination using bacteriophages. The US defense department financed Adaptive Phage Therapeutics and the Catholic University Rao group to make the vaccine. If the Phase-one trials are effective, the T4 vaccine will enter humans Phase 3 clinical trials [20].

4. CLINICAL TRIALS OF A BACTERIOPHAGE-BASED VACCINE

In a clinical phase I/II trial (NCT04839146), healthy volunteers were tested with ABNCoV2, a vaccine based on virus-like particles (VLPs) of Phage AP205 designed with recombinant receptor domain (RBD) of SARS-CoV-2 produced in S2 Drosophila cells [21]. This study was completed on February 25, 2022, but no research findings have been published on clinicaltrials.gov. In addition, an open-label phase two trial with ABNCoV2 (NCT 05077267;EUCTR2021-001393-31) is underway in Germany. A phase three trial (NCT05329220) assessed the safety, tolerability, and Immunogenicity of ABNCoV2 in adults who had previously received SARS-CoV-2 vaccination [22].

Human aspartate-hydroxylase (ASPH) has been linked to several types of cancer and has been found to have good vaccine tolerance, provoking an immune response. SNS-301 is a bacteriophage lambda with an ASPH fragment coated inframe with the coat protein gpD. The safety, immunogenicity, and preliminary clinical efficacy of the phage-based vaccine SNS-301 were evaluated in a clinical trial (clinicalTrials.gov, identifiers NCT04217720, and NCT04034225). Individuals with persistent myelomonocytic leukemia and high-risk myelodysplastic syndromes were given SNS-301 in conjunction with pembrolizumab. According to the findings, the combination of SNS-301 and pembrolizumab was well tolerated by sick people, resulting in disease normalization and tumor response. Because these are preliminary findings, more research into the effectiveness of the combination treatment is required [23].

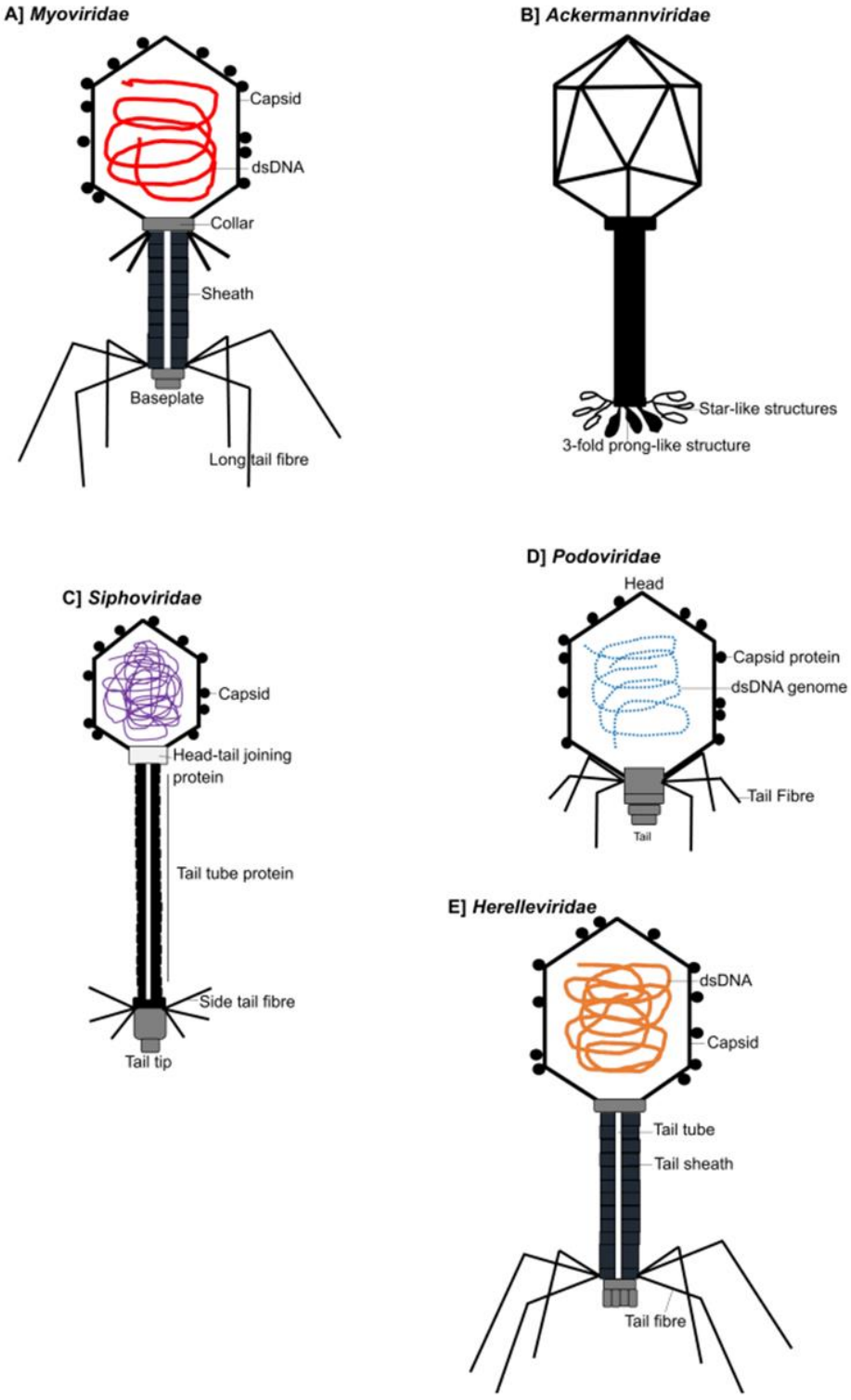

Fig. 1. A schematic representation of various morphologies of dsDNA bacteriophages. These are tailed phages belonging to the Caudovirales order.

5. PHAGE DISPLAYED AND COVID-19 PROTEIN TARGETS

A bacteriophage library can contain millions of diverse and distinct displayed peptide ligands. Using the ligand-receptor interactions that underpin phage display and an affinity selection-based method called "biopanning," epitope mapping and antigen display on the surface of bacteriophages has been effectively accomplished. This versatile approach has been considerably refined over the last three decades, resulting in various multimodal peptide display platforms. These recently developed diagnostic methods have been used in hazardous bacteria and viruses. Researchers are looking for neutralizing antibodies in the current COVID-19 pandemic to find possible treatment targets for the new SARS-CoV-2. This strategy contributes to studies on host-pathogen interactions and novel methods to discover coronavirus medication [24].

Knowing how coronaviruses interface with human receptor molecules has been made possible by extensively using bacteriophage display technology to aid in investigating targeted therapies via epitope mapping. While symptomatic treatment is provided to SARS-CoV-2 pandemic victims, there are currently no established methods for avoiding COVID-19 infection. By providing a library of inhibiting peptides and neutralizing antibodies, phage display could aid in our understanding of the infectious biology, pathogenesis, and blocking pathways of the very pathogenic, presently circulating SARS-CoV-2 [24].

Shortly, Phage display advancement will drive innovations to build practical techniques to uncover the processes associated with infections, cellular interaction, and the development of SARS-CoV-2 and other virus post-exposure therapies [24].

6. APPLICATIONS OF BACTERIOPHAGE-BASED VACCINES

Phages have a variety of unique characteristics that can be used to produce a potent inflammatory immune response against cancer or viral infection. Phage vaccines activate stimulatory pathways and present antigens to the immune system, like other nanotechnologies. Beyond vaccination, the medical industry is researching the use of nanotechnology for things like disease characterization, targeted drug delivery, and tissue regeneration. Mainly phages have been used in various applications, from cancer immunotherapies to biosensors [14]. Phage-based vaccines can be used therapeutically to treat non-infectious diseases as well as preventatively to fight microbial and parasitic infections. Phage-based vaccines are therapeutically applied using immunotherapy, which relies on the body's natural ability to fight disease. This way, phage-inspired vaccines can treat diseases like drug addiction, cancer, and neurodegenerative disorders. Phages may be a novel means of delivering genes or drugs because the mounting evidence suggests they can interact with and release their cargo inside mammalian cells. Phages are prokaryotic antagonists, so their capacity to deliver eukaryotic cargo naturally is severely constrained. The effectiveness of these viral particles at delivering preventative, diagnostic, and therapeutic cargoes into eukaryotic hosts can be significantly enhanced by phage surface engineering [3].

7. LIMITATIONS AND FUTURE PROSPECTS

Although bacteriophages have been used as vaccines, much research still needs to be done before they can be used clinically. The US FDA authorized the first phage therapy human clinical trial. A phages combination is used in this phage therapy study to cure a resilient *Staphylococcus aureus* infection. By using phages, this procedure enables the design of more fruitful clinical studies for vaccine development [13].

Although bacteriophage-based vaccines have been the subject of many preclinical studies, both *in vivo* and *in vitro,* demonstrating their potential for preventing infectious diseases, none of these vaccines have yet been approved for use in clinical settings. Numerous potentially fruitful preclinical studies aren't documented and never brought to market [13]. Regarding the activation of adaptive immune responses by phage-based vaccines, there is still considerable measure that we do not fully understand. It also shows how aspects of phage preparation, such as the amount of endotoxin bound to phages, can affect both quantitative and qualitative elements of immunogenicity [25].

It is essential to point out that the rising cost of

such clinical evaluations limits the use of phage-based products. In addition, the wide range of phage-based vaccines has the potential to assist in developing combinatorial vaccination strategies, which may prove to be more successful than those currently in use. Priority should be given to the design of clinical trials to expedite the approval process for these bioproducts. This is necessary because of the numerous applications for phage-based bioproducts and the effectiveness of phage-based vaccines that are currently under development [8]. Because there is no possibility of genetic transfer, vaccines based on phages established through *in vitro* display rather than the insertion of outside DNA into the phage genome can be categorized as natural bioproducts. These aspects are necessary to speed up commercializing vaccines based on phages [13]. Phage-based vaccines are an intriguing possibility for vaccine development because they have several benefits that cannot be found in traditional vaccine delivery systems. More study is needed to comprehend the immunological mechanism underlying phage vaccines to develop more highly specialized antigen delivery systems [13].

8. CONCLUSION

The SARS-CoV-2-related COVID-19 pandemic is wreaking havoc on public health, education, travel, and economic situations worldwide. How should we prepare for future outbreaks? Phages can contribute to the pandemic. The current situation highlights the critical significance of a novel therapeutic vaccination and diagnostic measures to combat COVID-19. A universal vaccine design platform capable of rapidly creating multiplex vaccine candidates is required to control future pandemics. As a result, a global effort is needed to make an effective vaccination widely available. This demands the participation of skilled scientists and financing to produce a vaccine that will help reduce future pandemics.

The benefits of bacteriophage-based vaccines overhead other vaccine technical advancements. Phage-based vaccinations get the potential to give considerable benefits by launching a novel strategy that allows the vaccine to change in response to coronavirus alterations quickly. Furthermore, phage-based vaccines are self-adjuvanted, automatically stimulating and boosting immune response while displaying multiple antigens. Phage therapy in humans is well recognized and has a satisfactory safety profile. The key benefits of phage-based vaccine:

1) Phage has an excellent safety profile.
2) Rapid adaptation of new vaccines to potential coronavirus mutations.
3) Manufacturing costs less than alternative vaccine approaches.
4) Immune response enhancement through self-adjuvant. https://aphage.com/science/vaccines/.
5) Phages stimulate innate and adaptive immune systems, making them particularly interesting for vaccines and immunotherapies.

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11. CONFLICT OF INTEREST

No conflict of interest.

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