Nasal Nanovaccines for SARS-CoV-2

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A vaccine-based nanoparticle (NP) delivery vehicle is the inoculum to deliver an antigen in vivo. The nanovaccine has been a novel vaccine delivery platform in recent years. NPs function as an adjuvant to enhance the immune response and the effect of cross-reactivity. Functional NPs in SARS-CoV-2 vaccines mainly include promoting cell uptake of antigens, protecting antigens, and fully mimicking pathogens (like nano-virus). NPs are mainly divided into four categories: polysaccharide NPs; lipid NPs and protein NPs; Nano-biomimetic delivery vehicles; polymer NPs

COVID-19 nasal vaccination nanovaccine

1. Polysaccharide Nanoparticles

Polysaccharide nanoparticles belong to a class of natural polymers composed of carbohydrate monomers connected by glycosidic bonds [1]. With inherent immunomodulatory, biocompatibility, biodegradability, low toxicity, and safety characteristics, polysaccharides have attracted much attention in the preparation of nanovaccines and nanomedicine. Polysaccharide adjuvants mainly include chitosan and its derivatives, in addition to glucan, mannan, inulin, and Chinese medicinal herbs.

Chitosan is a cationic polysaccharide biopolymer that exists in the exoskeleton of crustaceans and is produced by acetylation [2]. Chitosan NPs have a large surface area, are capable of the controlled release of drugs, have excellent antibacterial and other biological properties, are non-toxic to humans, and are environmentally friendly and used as a drug delivery vehicle [3][4][5]. Chitosan nanovaccines have proven that the vaccines with chitosan as a carrier can stimulate immune responses in animals [6][7]. In particular, chitosan is soluble in acidic environments and has adhesive properties. The excellent adhesion of chitosan reduces the nasal clearance of the vaccine [8][9][10]. Chitosan can prolong the retention time of drugs or vaccines and improve their efficacy. It has significant advantages as an adjuvant for oral or nasal nanovaccines. Priscila Diniz Lopes et al. [11] confirmed that a chitosan-based IBV-cs vaccine, alone or in combination with a heterologous live attenuated vaccine, can cause humoral and cell-mediated immune responses at the primary site of virus replication and can be localized (the trachea) or in the whole body (kidney) and provide effective protection against IBV infection. Santosh Dhakal et al. [12] confirmed that chitosan NPs improve mucosal immunity and influenza vaccine protection in pigs. Mucosal immune response and systemic immunity are generated after nasal vaccination with chitosan-based nanovaccines. Chitosan NPs are theoretically feasible as the delivery system and adjuvant of SARS-CoV-2 nanovaccines. Adel M. Talaat et al. [13] developed a quil-A-loaded chitosan (QAC) nanovaccine for COVID-19. Neutralizing antibodies and IgA were tested in vaccinated mice. The effect of cationic chitosan-based nanovaccines in improving animal humoral immunity is

more significant than other chitosan-based nanovaccines [14]. The feasibility of chitosan and its derivatives as SARS-CoV-2 nanovaccine carriers is emphasized in some reviews [15][16]. Chitosan can also be associated with other poly nanoparticles, such as association chitosan-polymers. The associated nanoparticles may be an option in nanovaccine development [17].

2. Lipid Nanoparticles

2.1. Liposomes

Driven by hydrophobicity in water, self-assembled liposomes are spherical vesicles encased by at least a double layer of phospholipids. They are highly fat-soluble and can fuse with cell membranes. Liposome-based vaccines enter the cell by endocytosis. Liposomes were first discovered by Bangham et al. using electron microscopy in the early 1960s [18] and later named “Liposome” by Sessa and Weissmann in 1968 [19]. Generally, liposomes are composed of different types of amphiphilic phospholipids. Combined with other lipids, liposomes can modify the surface characteristics and electrical charge. Liposomes include multilamellar vesicles (MLV), large unilamellar vesicles (LUV), and small unilamellar vesicles (SUV). Gregoriadis et al. [20] have confirmed that liposomes have inherent adjuvant properties. Vaccinated mice produced strong antibody immune responses to the Ags (such as diphtheria toxoid) carried. Moreover, it was found that mice vaccinated with liposome-based vaccines did not have the side effects brought about by conventional vaccine adjuvants, such as granulomas. Most liposomes are negatively charged, and positively charged liposomes composed of positively charged lipids can better adsorb to the nasal mucosa [21]. Ellen K. Wasan et al. [22] intranasally inoculated mice with the L-TriADJ complex coated with cationic liposomes and produced a stronger immune response in mice. Rui Tada et al. [23] found that adhesion of class B CpG ODN to DOTAP/DC-Chol liposomes in nasal vaccine preparation enhances antigen-specific immune responses in mice. Liposomes, especially cationic liposomes, have great potential in the development of SARS-CoV-2 nasal nanovaccines.

2.2. Other Lipid Nanoparticles

Liposomes are only an early version of the nanomedicine delivery platform. Many different lipid nanoparticles have been developed, such as solid lipid nanoparticles, lipid nanocapsules and virosomes. These lipid nanoparticles are used in vaccine delivery [24][25][26]. They may provide a direction in the development of nasal nanovaccines for SARS-CoV-2.

3. Protein Nanoparticles

3.1. Self-Assembled Proteins

Self-assembled proteins are a higher-level structure made by self-assembly of oligopeptides, nucleotides, and non-biological amphipathic building blocks. To achieve different purposes, researchers have designed different self-assembled proteins. Self-assembled proteins have been widely used in biomolecular engineering and biomedical

platforms [27]. In the field of vaccine development, self-assembling proteins can be fused with inactivated pathogens or parts of antigens to produce safe molecular entities that can be effectively delivered to cells to induce immune responses [28]. The development of candidate vaccines based on protein assemblies is a powerful strategy. Ferritin self-assembled NPs are already in clinical trials as nasal nanovaccines [29].

4. Nano-Biomimetic Delivery Vehicles

Nano-biomimetic delivery vehicles are generally assembled from nanomaterials with a variety of different functions. It is more capable of delivery with nanocarriers synthesized with polymers and lipids [30]. Nano-biomimetic delivery vehicles are made with pathogen antigens into nanovaccines, such as virus-like particles (VLPs), a virus-derived structure composed of one or more different molecules with the ability to self-assemble [31][32]. VLPs mimic the form and size of viruses, however, they lack genetic material, so they have high biological safety due to low infectious doses [33][34]. So far, a series of VLPs candidate vaccines against COVID-19 have been developed, and the effect is being evaluated. Cyrielle Fougerou et al. [35] developed two vaccines based on capsid-like particles (CLP), showing RBD of the SARS-CoV-2 spike protein. Furthermore, the vaccines stimulated strong virus-neutralizing activity in mice. Jing et al. [36] designed a genetic vaccine encoding SARS-CoV-2 virus-like particles. This vaccine induces a strong antiviral-like immune response in mice. Typically, VLPs require nano-biomimetic delivery vehicles in nanovaccines [37]. By improving the charge, size, and other characteristics of VLPs, NPs can better deliver VLPs to the host. Zheng bin et al. [38] designed a nasal nanovaccine, which can induce mucosal immunity by nasal delivery to prevent virus infection. The nanovaccine was composed of poly(I:C) mimicking viral genetic material as adjuvant, biomimetic pulmonary surfactant liposomes as capsid structure of virus and RBDs of SARS-CoV-2 as “spike” to completely simulate the structure of the SARS-CoV-2. NPs may be assembled with antigens to form a SARS-CoV-2-like molecule that mimics the process of viral infection for effective vaccination.

5. Polymer Nanoparticles

Polymer NPs are nanoparticles formed by the polymerization of one or more organic substances. Poly(D,L-lactic-co-glycolic acid), or PLGA, is the most commonly used synthetic polymer in developing nanoparticle delivery vaccines due to its biodegradability and biocompatibility [39][40]. It was originally used as a suture material for surgery as PLGA is non-toxic and can be degraded into two safe and non-toxic monomers, lactide and glycolide [41][42]. Later, it was found that PLGA functions as an adjuvant and an antigen delivery vehicle. As an antigen delivery vector, PLGA can either encapsulate antigens to form nanocapsules or make antigens adhere to the surface to form nanospheres. The nanocapsules formed by PLGA are similar to liposomal nanovesicles. The pharmacokinetics is regulated by encapsulating the antigen in PLGA particles, and continuous and controlled protein release is allowed to improve the immune response. The sustained release characteristics of PLGA can be used in a single-dose vaccine, which is important for the development of the SARS-CoV-2 vaccine. Some researchers tend to develop single-dose vaccines to achieve rapid vaccination [43][44][45][46][47].

PLGA can also prevent the degradation of antigens. The preservation of antigens is considered by many developers. PLGA-encapsulated vaccines have advantages in antigen protection and can delay the release of antigens. Patki M. et al. [48] found that PLGA loaded with the anti-SARR-CoV-2 drug Remdesivir can continuously and stably release antigen. Qingqin Tan et al. [49] determined that drugs with PLGA as a vector can neutralize a variety of pro-inflammatory cytokines and effectively inhibit the activation of macrophages and neutrophils. Inhibiting inflammation is conducive to reducing the side effects caused by the SARS-CoV-2 vaccine, which means that a nanovaccine with PLGA as a vector is safe. As a nanoparticle, PLGA can provide a characteristic delivery system for antigens and be used as an adjuvant [50][51]. It has great prospects in the development of the SARS-CoV-2 vaccine [52]. In addition to PLGA, other polymer nanoparticles, such as Poly (I:C) as an agonist, also play a similar role [53].

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