Virus-like Particles in Vaccine Development for Infectious Diseases

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Abstract. Throughout the last two decades, virus-like particles (VLP), a nano scale multi-protein structure, have been vigorously studied and became a crucial and unique tool for clinical use. Due to VLPs' structural resemblance of viable virus particles, highly modifiable nature, and lack of viral genome, they are excellent candidates for vaccine development for infectious diseases, offering many advantages over traditional vaccine development methods. Capable of eliciting both potent humoral and cell-mediated immunity, VLPs become one of the best nano-vectors for vaccines for infectious diseases. In addition, VLPs' flexibility in composition and expression systems also contribute to their versatility as a vaccine platform. Various VLP-based vaccines are commercially available, including Cervarix®, Gardasil®, and Gardasil9® for Human Papillomavirus (HPV), Heptavax-B and Sci-B-Vac™ for Hepatitis B Virus, and COVIFENZ® for SARS-CoV-2. In this review, classification of VLPs, different expression systems, as well their application in vaccine development for several infectious diseases will be discussed.

Keywords: Virus-like particles, Vaccines, Nanoparticles, Infectious diseases.

1. Introduction

Virus-like particles are nanoscale structures that are made by self-assembly viral structural proteins, resembling the structural features of natural viruses while lacking the viral genetic materials. VLPs can be self-assembled from virus structural, envelope, and, sometimes, core proteins from either a single type of virus or multiple different types of viruses (called Chimeric VLPs). Structural proteins from human immunodeficiency virus (HIV), Hepatitis C virus (HCV), Adenoviruses, bacteriophages, and many plant viruses are rigorously used as VLPs' development platforms. Due to their highly organized geometry, VLPs resemble pathogen-associated structural patterns (PASP), which is a potent immunogen the immune system can effectively recognize and react to.

Like virus capsids, VLPs typically consist of a surface structure that displays repetitive protein epitopes. One of the most important features that make VLPs one of the best vaccine platforms is their ability to stimulate both cellular and humoral immunity. The inflexible and repetitive surface structure serves as a strong PASP. Despite stimulating innate immune responses, such a feature induces B cells activation directly by cross-linking B cell receptors (BCRs), producing neutralizing antibodies. As one of the most essential components of the immune system, dendritic cells (DCs), through micropinocytosis and phagocytosis, can normally take up small particles that are about 100-500 nm. VLPs, normally around 10-200 nm, can interact with DCs after administration through DCs'pattern recognition receptors (PRRs) including Toll-like receptors (TLRs) and C-type lectin receptors (CLRs). These same receptors are also used to detect natural viruses [1]. After the interaction, VLPs will be transferred to a secondary lymphoid organ such as spleen, where the uptake of the VLPs will initiate DC maturation and stimulate production of TNF-α, IL-1β, and other proinflammatory cytokines and factors. These factors will encourage DCs to lyse the VLP vaccine molecules into smaller peptides and present them onto DCs' MHC class II molecules. Together with many costimulatory molecules on the DC surface, MHC-peptide complex activates naïve T-cells into CD4+ T-helper cells, further proliferating and differentiating both B and T cells [2]. Besides activating CD4+ T-helper cells, VLP can also activate CD8+ cytotoxic T cells by being processed in the cytosol of DCs as endogenous antigens and, consequently, presented by MHC class I [3]. This cross-presentation ensures a potent and comprehensive immunological response, hence making VLPs

a strong candidate for use as carriers to deliver and present epitopes for vaccine design, especially for infectious diseases.

2. Structural Classification

Virus Like Particle (VLP)'s formation requires one or more viral structural capsid proteins. The virus of which the VLPs are derived from heavily determines their morphology.

Spontaneous self-assembly of the viral structural proteins produces VLPs with geometrically symmetrical shapes, including icosahedral, rod-like, or spherical conformation. VLPs can be identified into two categories: Enveloped VLPs (eVLPs) and Non-enveloped VLPs, depending on the presence or absence of a lipid envelope. Non-enveloped VLPs are often composed of one or more viral structural proteins while enveloped VLPs display their viral proteins on the outer surface of a host-cell-derived membrane, of which properties depend on the expression platform [4]. Although non-enveloped VLPs have certain advantages in production and purification, enveloped VLPs demonstrate a higher flexibility as antigens of distinct pathogens can be integrated. However, enveloped VLPs' application may be affected by containing the host's proteins [5].

3. Expression platform

Different expression platforms can be used to produce VLPs for vaccines. These platforms contain both prokaryotic and eukaryotic systems, which includes mammalian/avian cell culture, plants and Baculovirus/Insect cells (B/IC). Among prokaryotic systems, bacteria are most commonly used. Besides producing VLPs in a cell, a cell-free system can also be implemented. An appropriate VLP expression platform is critical not only for its production efficiency but also its immunogenicity. These varying platforms offer different levels of protein folding and post-translational modifications (PTM), which include steps such as glycosylation and phosphorylation, affecting the immunogenicity of the VLP as stimulation of a sufficient immune response is often affected by the PTMs.

3.1. Bacteria

Bacteria cell culture is one of the most rigorously used expression platforms for the production of recombinant proteins and, consequently, various VLPs.

Escherichia coli (E. coli) is a robust protein expression host cell for VLP production. It has various benefits, including potent protein production levels, cost-efficient culturing, simplicity in scale-up, and short turnaround time [6]. E. coli is a preferred expression platform for protein production if correct protein folding can be achieved. In vaccine development, utilization of the E. coli expression system benefits especially in developing countries, where expensive production costs restrict vaccine utilization. With frequent emergence of infectious diseases that threatens both developed and the developing countries, E. coli and other microbe-based platforms have the advantages to offer efficient, economically friendly, secure, and efficacious vaccines as E. coli allow high yield of structural and capsid proteins in a cost-efficient manner [6].

Various VLP vaccines have been generated from E. coli. For instance, Hecolin® is the world's first commercially synthesized VLP vaccine using the E. coli-based expression system. It is in the form of p239 VLPs with epitopes, which can elicit robust immunogenicity against HEV genotype 1, decorated on the particle's exterior [7]. Also, MalariVax, a chimeric VLP-based malaria vaccine, has also been produced in E. coli successfully [8]. Furthermore, various VLP-based vaccines that prevent non-infectious illness, such as multiple forms of cancers, and allergies have been produced using the E. coli expression platform [6].

However, there are some major drawbacks from using E. coli as the main expression platform. These disadvantages include: (1) production of incorrect disulfide bonds; (2) not capable to produce a potent level of post-translational modifications compared to utilization of the mammalian cell expression system; (3) issues with protein solubility; (4) the presence of endotoxins such as

lipopolysaccharide (LPS) [9]. Fortunately, researchers are working on reducing or eliminating these drawbacks. For instance, through multi-step mutagenesis and demonstrating versatility in protein production, researchers are able to produce proteins using E. coli cell culture without worrying about the side-production of endotoxins. [10].

3.2. Yeast

Yeast cells have long been acknowledged for their ability to produce recombinant proteins and thus have also been implemented to produce VLPs. Among the yeast strains, Saccharomyces cerevisiae and Pichia pastoris are commonly used commercially. They have several advantages including efficient cell cultivation, high yield of expressed proteins, scalability, advantageous production, and certain level of post-translational modification processes [11]. Despite these advantages, yeast expression systems lack complex PTM pathways, having high levels of mannose glycosylation, loss of plasmid and lower yield of protein production than some other expression systems such as the bacterial platform.

3.3. Baculovirus/Insect cells (B/IC)

The Baculovirus/Insect cells system favors the production of VLPs, including both enveloped and non-enveloped ones, the most. It can yield a higher quantity of molecular products compared to bacterial or yeast systems, form multi-protein VLPs, and possess mammalian-cell-like-PTM pathways, which include fatty acid acetylation and glycosylation [12]. Consisting of two Human Papillomavirus (HPV) serotypes' L1 protein, Cervarix®, an FDA approved VLP-based vaccine for HPV, is produced using the B/IC expression platform. In addition, B/IC system can also produce vaccines against various infectious diseases including Rift Valley Fever viru(RVFV), Dengue virus, Ebola virus, Severe acute respiratory syndrome(SARS), Chikungunya virus(CHIKV), Influenza virus, and HIV [13]. One main drawback of the B/IC cell platform, when compared to mammalian cells, is that it expresses a less complex form of N-glycosylation for the yielded glycoproteins, causing troubles for some VLP applications [14].

3.4. Plant cells

Compared to conventional expression platforms, including Mammalian and Yeast cell culture, Plants expression systems have various advantages, such as low costs of refining, efficient expression process, and an 80% yield of total soluble protein [15]. Through molecular farming, a production system that uses transgenic plants, modified Agrobacterium tumefaciens, or recombinant plant virus vectors, designed proteins can be produced in high yield transiently. With the help of this technique, several experimental VLP vaccines are developed. About 60 different types of plant viruses, some of which are detected from the human GI tract and human feces, can be used as an antigen expressing platform.

In addition, many animal and human viruses and viral proteins can assemble in plants. These included HPV L1 proteins, norovirus capsid protein, foot and mouth disease virus, and the capsid protein of bluetongue virus [16]. In the recent COVID-19 pandemic, the only authorized plant-based SARS-CoV-2 vaccine, COVIFENZ, is produced by Medicago, which is able to produce vaccines against various infectious diseases, including coronavirus, rotavirus, and norovirus, using a plant-based system that is termed molecular farming [17].

3.5. Mammalian and Avian cells

The best advantage of using mammalian and avian cells as VLP manufacturing platform is that they allow processing of complex and comprehensive post-translational modifications, which are necessary for correct protein folding. With the help of plasmid transfection or viral transduction, genes that encode the viral proteins of interest are inserted into the host mammalian cells [3]. With the help of mammalian cell culture systems such as Vero or MDCK cells, the manufacturing process

of the vaccine viruses will have increasing flexibility and consistency. Also, such processes, unlike in egg- or baculovirus-dependent platforms, recover the host-dependent specific glycosylation [14].

Despite the benefits they provide, mammalian and avian cell expression platforms have high production costs, possible safety concerns such as contamination of opportunistic pathogens, and difficult scaling up production.

3.6. Cell Free System

Cell-free expression platform is a relatively recent development. The system uses E. coli or yeast cell extracts to enable primary production of viral capsid proteins that contain toxic intermediate protein forms in vitro. Additionaly, with the help of the system, VLPs containing unnatural amino acids (UAAs) can be produced. However, the system has some limiting features for commercial application such as limiting scalability and high production cost due to the system's non-replenishable nature. Despite the limitations, several vaccines have been successfully developed, including Inflexal®, a VLP vaccine against influenza, and Epaxal®, a VLP hepatitis A vaccine [3].

4. VLP-based vaccines for infectious disease

More than 110 viral proteins from about 40 coral families have the capability to assemble into VLPs. A variety of vaccines have been licensed for human use, including Gardasil, Gardasil-9, and Cervaris for Human papillomavirus (HPV), and Engerix-b for HBV. Several others are also in different stages of development and clinical evaluation, including Medicago's COVIFENZ® and Novavax NVAX-CoV2373 for the recent SARS-CoV-2 outbreak.

4.1. VLP-based vaccine for Human Papillomavirus (HPV)

Human Papillomavirus (HPV) is a group of viruses that are spread through sexual intercourse. Among the high-risk HPV types that cause several types of cancer, HPV16 and 18 are responsible for most HPV-related cancers, including cervical, oropharyngeal, anal, penile, vaginal, vulvar cancers. Every year, in the United States, around 3% and 2 % of all cancers in women and men are caused by HPVs. Annually, in the 45,000 cases of cancer where HPV is found in the cancerous body parts, about 36,000 of these are caused by HPV [18]. In recent years, FDA approved several HPV vaccines that use VLP as carrier for the prevention of certain serotypes of this cervical-cancer-causing virus. Gardasil® is the first HPV VLP vaccine, consisting of recombinant VLPs that are spontaneously polymerized from four types of HPV's major capsid protein L1 with neutral salt aluminum hydroxy phosphate sulfate as adjuvant. Gardasil® effectively protects people from infection of HPV types 6,11,16, and 18. Covering additional HPV types 31, 33, 45, 52, and 58, Gardasil9® quickly replaced Gardasil® in 2014. Cervarix® is another VLP-based HPV vaccine. It uses self-assembly of L1 protein from the HPV types 16 and 18 to form the VLP nanostructure and is formulated in AS04 adjuvant. Although covering less serotypes than Gardasil9®, it might have an enhanced immunogenicity due to its carriage of MPL, a TLR4 agonist, in AS04 adjuvant system [19].

4.2. VLP-based vaccine for Hepatitis B

Hepatitis B is a disease that is caused by infection from Hepatitis B virus (HBV), which contributes to both acute and chronic symptoms by attacking the liver. HBV is usually transmitted perinatally during childbirth and delivery, contact of blood or body fluid during sex, and exposures to sharp instruments. According to WHO, 296 million people will suffer from chronic HBV infection in 2019, with annually new infectant's number rising to 1.5 million. From 2019 alone, hepatitis B contributed over 820,000 mortalities, most commonly from cirrhosis and hepatocellular carcinoma [20]. The most effective way to control the infection is vaccination. VLP-based vaccines for HBV in development mostly utilize VLP particles that are self-assembled from Hepatitis B surface antigen (HBsAg). There are three generations of HBV vaccines. The first generation of HBV vaccine made from VLPs is Heptavax-B, consisting of the HBsAg directly separated from infected patients' body fluid and blood

serum. Having an improved safety and immunogenicity, the second-generation HBV VLP-based vaccines were produced by expressing small HBV protein by incorporating a gene called SH2 Domain Containing Adaptor Protein B(SHB) in the genome of Saccharomyces cerevisiae yeast. These second-generation vaccines demonstrated efficacious results, significantly reducing HBV prevalence globally. The third generation VLP-based HBV vaccine is developed to mitigate some of the disadvantages previous generation vaccines had. Sci-B-Vac™ is such a third generation HBsAg VLP-based vaccine, consisting of S, Pre-S1, and Pre-S2 antigenic domain. These three antigens from the HBV surface are all produced from mammalian Chinese hamster ovary cells. In addition to protecting the people from HBV infection, Sci-B-Vac™ might also serve potential therapeutic effects in patients with chronic HBV infections [19].

4.3. VLP-based vaccine for SARS-CoV-2

The recent COVID-19 pandemic has already reported more than 640 million cases with about 7 million related deaths as of December 2022 [21]. The causative agent of this pandemic is the SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), a single-stranded RNA coronavirus. Many VLP-based vaccines have emerged recently. The NDVLP-S2P is an enveloped VLP vaccine self-assembled from proteins of different viral origins, utilizing the structure of Newcastle disease virus (NDV). Developed by researchers in National Institute of Allergy and Infectious Diseases (NIAID), NDVLP-S2P is made by integrating the NDV fusion protein's transmembrane domain into S2P of SARS-CoV-2, exposing the S2P, a vigor immunogen, on the chimeric VLP's surface. Such a method shows more immunogenicity than the approach that uses a trimeric S protein alone [22]. COVIFENZ®, developed by Medicago/GSK, is another VLP-based vaccine for prevention of SARS-CoV-2 infection. COVIFENZ®, formulated with AS03, displays a mutated S2P protein, some substitutions in the S1/S2 site, and a certain antigenic sequence of the H5 from influenza virus. Due to its formulation in plant cells, COVIFENZ® possesses many production advantages over other VLP vaccine candidates. COVIFENZ® just went through its phase 3 clinical trials, demonstrating a high serum virus neutralizing antibodies titers and an efficacy ranging from 69.5% against symptomatic infection to 78.8% against moderate-to-severe disease [23]. Produced from the baculovirus/insect cell system, the Novavax NVAX-CoV2373 is another VLP-based vaccine that protects against SARS-CoV-2 infection. It also consists of S2P protein from the virus, but the protein is locked in a prefusion conformation and formulated with Matrix-M® adjuvant. The modified Spike protein is later integrated into polysorbate 80 detergent. NVAX-CoV2373 demonstrated high immunogenicity and safety while conferring almost a 90% efficacy [24].

5. Conclusion

With fast advancing in the field of nanotechnology and molecular engineering, unique and novel findings have been explored for vaccine platforms' improvement and development. As a result, viruses are also utilized as malleable tools for vaccine design as functional nanoparticles, rather than being regarded solely as the cause of diseases. VLPs is one of these nanoparticles that offer newer and safer alternatives to vaccine production, where conventional vaccines approaches predominate the industry.

A large exploited application of them is in vaccinology of infectious diseases, where VLPs provide many advantages over conventional vaccine approaches. VLPs are capable of eliciting potent immune responses, including both humoral and cellular responses, while offering safety especially for immunocompromised or elderly populations. More effectively, VLPs can be associated with immune-modulators to provoke even stronger immune responses.

Not only being structurally attractive, VLPs also demonstrate functional diversity. Despite their application in vaccinology, VLPs are amazing drug delivery vectors thanks to their possession of an internal cavity. Consequently, they show potent capabilities in delivering a variety of biological material, which includes genes, proteins, drugs, and peptides. Due to their highly modifiable character,

VLPs are further vigorously utilized for precise delivery of drugs, making them an excellent approach for cancer treatment and tumor imaging.

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