Synthetic Biology in the Design and Development of Virus-Like Particle Vaccines

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Abstract. With the frequent emergence of new infectious diseases and the growing demand for precision medicine, the limitations of traditional vaccines are becoming increasingly apparent. Virus-like particles (VLPs) have become a key platform for the development of next-generation vaccines due to their combination of structural characteristics typical of natural viruses and a favorable safety profile. From the perspective of synthetic biology, this article reviews the working principles, production processes, and key construction technologies of VLP vaccines, with a focus on research progress regarding chassis protein design, engineering modification of antigen modules, optimization of linkage systems, and enhancement of structural stability. Meanwhile, the development status of marketed VLP vaccines, such as those for hepatitis B virus (HBV), hepatitis E virus (HEV), and human papillomavirus (HPV) is summarized, and the application of VLP vaccines currently in clinical research, including those for influenza, COVID-19, and HIV is analyzed. The results demonstrate that synthetic biology has significantly enhanced the programmability, immunogenicity, and development efficiency of VLP vaccines, driving their evolution toward multivalent, intelligent, and personalized forms. However, issues such as complex antigen display, large-scale production, and the evaluation of long-term protective efficacy remain to be addressed. Future research could further integrate AI-driven protein design, novel expression systems, and precision delivery technologies to advance the clinical translation and industrial application of VLP vaccines.

Keywords: Virus-like particle, COVID-19, biosynthesis.

1. Introduction

Synthetic biology is a field of research that adopts an engineering-based design approach to design and construct standardized biological components and modules, with the aim of modifying naturally occurring biological systems or synthesizing artificial life systems *de novo*. It has a history spanning a century [1]. At the same time, the journal *Science* identified "how to develop next-generation vaccines" as one of the 125 frontier scientific questions [2]. Traditional vaccines include inactivated vaccines, attenuated vaccines, and protein subunit vaccines; however, due to technical limitations in their production, they suffer from various drawbacks, such as insufficient immunogenicity, the risk of viral virulence reversion, and even rare instances of disease exacerbation [3]. Among them, virus-like particles (VLPs)—protein particles assembled from viral structural proteins—have emerged as an ideal platform for the development of novel vaccines; they exhibit exceptional biological activity and immunogenicity, as they closely mimic the morphological structure of natural viruses while lacking genetic material [4]. Synthetic biology has provided new ideas for the development of the next generation of vaccines. At present, vaccine research and development has adopted diversified technical approaches, mainly including: genome codon-optimized vaccines, fully synthetic nucleic acid vaccines, recombinant virus vector vaccines, and virus-like particle (VLP) vaccines [1]. In addition, VLP vaccines can be mainly classified into two types: enveloped type and non-enveloped type. Under these two types, the vaccines can be further classified into single-layer, double-layer and multi-layer VLP vaccines based on their protein structure. At present, the VLP vaccines that have been approved for market release include human papillomavirus (HPV) vaccines and hepatitis B virus (HBV) vaccines, among others [5]. This article mainly focuses on the current development and application of synthetic biology in the manufacturing and production of VLP vaccines. The development of VLP vaccines in conjunction with synthetic

biology holds significant implications. From a scientific perspective, the integration of these two directions can effectively promote the advancement of interdisciplinary research methods, which include molecular biology, virology, systems immunology, synthetic biology, nanotechnology, and more. The collaboration of these interdisciplinary approaches can facilitate researchers in developing VLP vaccines that are cost-effective, simple in process, and have better immunogenicity. Furthermore, in the field of medicine, the interdisciplinary combination of synthetic biology and vaccine synthesis also provides new ideas for precision medicine. For instance, customized vaccines have been developed for patients with melanoma and pancreatic cancer [6]. In conclusion, synthetic biology can create a more intelligent, controllable and personalized antigen presentation platform for VLP vaccines.

Therefore, from the perspective of synthetic biology, this article reviews the construction methods, key technologies, and clinical application progress of virus-like particle vaccines, with the aim of providing a reference basis for the development of a new generation of efficient, safe, and programmable vaccine platforms, and promoting the further application of synthetic biology in the field of vaccines.

2. The Principle of VLP Vaccine

2.1. Highly Repetitive Surface Structures

The surface antigens of the VLP vaccine are presented in a granular form. When antigens are repeatedly arranged on the VLP platform at intervals of 5-10 nanometers per epitope, it will prompt the immune system to respond to the VLP particles. These highly ordered and repetitive structures, which are recognized by the immune system and resemble the surface features of many pathogens, are called "pathogen-associated molecular patterns" (PAMPs). This feature can efficiently cross-link the B-cell receptors (BCR) on the surface of B cells, thereby directly and strongly activating B cells, inducing the production of high-titer antibody responses, and the response lasts for a long time [7].

2.2. The size of VLP Particles

The diameter of VLP particles is usually around 20 to 200 nanometers, which is exactly similar to the size of many lymphocytes. This size enables them to pass through the lymphatic vessels preferentially and effectively drain to the lymph nodes. After entering the lymph nodes, VLPs can be captured by follicular dendritic cells and remain there for a long time. They can also be efficiently taken up by antigen-presenting cells (APCs) such as macrophages and dendritic cells. Subsequently, APCs will continue to react with B cells, thereby mediating effective interactions between B cells and T cells [7].

2.3. Flexible Loading of Exogenous Antigens

At the same time, VLP particles can display exogenous antigens through various methods such as gene fusion and chemical coupling [8,9]. Currently, the antigens that can be carried include viral antigens, as well as some cytokines that are used to treat human or animal diseases (such as chronic inflammation, Alzheimer's disease, cancer, etc.). Finally, these antigens, delivered by the VLPs, can be recognized by the immune system and efficiently induce specific antibodies against these exogenous molecules [7].

3. General Preparation Method of VLP Vaccine

3.1. Carrier Protein Design

The common cytoskeletal proteins can be classified into two types: naturally self-assembled proteins and artificially synthesized proteins. In the field of naturally self-assembling proteins, the p239 particles used by Li et al. are self-assembled from the truncated capsid protein p239 of hepatitis E virus (HEV) [8]. This naturally self-assembling protein serves as both an antigen and a scaffold in VLP vaccines. In the field of synthetic proteins, Kou et al. expressed the Mi3 scaffold protein with the LPETGG sorting signal peptide in Escherichia coli through genetic engineering and allowed it to self-assemble into nanocages [9]. This artificially synthesized scaffold protein can stably form particles, thus serving as an antigen display platform.

3.2. Antigen Module Design

Currently, synthetic biology's antigen module design mainly focuses on multi-epitope antigen design, which involves combining multiple antigens together. For example, Li et al. used the p239 particles of the hepatitis E vaccine that were already on the market to non-covalently bind multiple respiratory virus antigens through the nanobody P1-5B, while also retaining the immunogenicity of the original particles, and finally achieved the rapid construction of a multi-antigen combination vaccine [8]. Belghith et al. used the SpyTag/SpyCatcher covalent system to display the M1, A35 and B6 proteins of monkeypox virus on the Mi3 nanoscaffold, ultimately forming a 60-mer VLP [10]. At the same time, the chirality of the antigen is also used to display the vaccine, thereby significantly enhancing its ability to resist protease degradation. For example, Kou et al. used mirror Spy chemistry to connect D peptides to L protein scaffolds through L sorting enzyme, and then covalently coupled by D SpyStapler catalysis to finally achieve chiral anti-degradation [9].

3.3. Selecting a Modular Connection System

Currently reported common ligation methods include gene fusion, sorting enzyme ligation, and ligation using the SpyTag system. First, regarding gene fusion: Li et al. screened and obtained nanobody P1 5B from alpacas, which can specifically bind to the depression site on the surface of hepatitis E vaccine p239 particles. Then the antigen was fused with P1 5B and expressed, and then simply mixed with p239 particles to form a stable complex through non-covalent binding [8]. It is evident that the advantage of gene fusion method lies in its simple process and preservation of the original particle's immunogenicity. Secondly, sorting enzymes are used for ligation. For example, Kou et al. used the transpeptidation reaction of L sorting enzyme A to link D SpyTag/D BDTag to the L protein scaffold; then, D SpyStapler catalyzes the formation of heteropeptide bonds, and finally the antigen is covalently coupled to VLP. Finally, the SpyTag system was used for connection[9]. After Belghith et al. fused monkeypox virus antigen with SpyTag for expression, the SpyCatcher003 mi3 scaffold self-assembled into a 60-mer. After the two are mixed, SpyTag will spontaneously form heteropeptide bonds with SpyCatcher, covalently coupling the antigen to the surface of Mi3 to achieve multivalent display [10].

3.4. Optimization of VLP Structure and Stability

Common methods include using TEM technology to observe whether particles form regular nanostructures, while also detecting their particle size uniformity, antigen density, stability, and integrity. Meanwhile, flexible linkers are screened to reduce steric hindrance and improve antigen conjugation efficiency. Mirror biology is also introduced to utilize chirality to improve the stability of VLP vaccines [9].

4. Current Status of VLP Vaccine Research

4.1. VLP Vaccines Already on the Market

Currently approved VLP vaccines mainly target HBV, hepatitis E virus (HEV), and HPV.

4.1.1. HBV

HBV is a hepatotropic DNA virus. Its infection process begins with the binding of viral surface proteins to heparan sulfate proteoglycans (HSPGs) and sodium taurocholic acid cotransporter (NTCP) on the surface of hepatocytes. After HBV enters hepatocytes through the NTCP receptor, it forms a stable cccDNA reservoir in the cell nucleus and uses it as a template to complete viral replication and protein expression. HBV-induced liver damage is mainly mediated by the host immune response rather than by the direct cytotoxic effects of the virus. The long-term presence of cccDNA is the main reason for persistent viral infection and the difficulty in eradicating chronic hepatitis B, and it is also an important target for new HBV vaccines and treatment strategies[11]. Recombivax HB® is a VLP vaccine manufactured by Merck based on a Saccharomyces cerevisiae expression system. It was approved for marketing in 1986. A subsequent 30-year follow-up study of 539 healthcare workers who received the hepatitis B vaccine showed that, after dividing the subjects into three groups based on their antibody titers after initial vaccination, the incidence of anti-HBs loss was 52.1/1000 person-years in low-responders (10–99 mIU/mL, 8 individuals), 11.3/1000 person-years in moderate-responders (100–999 mIU/mL, 37 individuals), and only 1.4/1000 person-years in high-responders (≥1000 mIU/mL, 494 individuals). The mean duration of antibody levels in the three groups were 19.2 years (95% CI: 15.6–22.8), 25.4 years (95% CI: 23.0–27.9), and 31.0 years (95% CI: 31.0), respectively. (30.5–31.5), indicating that when the titer reaches 100 mIU/mL or higher after the initial immunization, the protective antibody can persist for up to 30 years without the need for booster vaccination [12]. Secondly, Engerix-B® is a VLP particle vaccine based on the Saccharomyces cerevisiae system, developed by GlaxoSmithKline. It was approved for marketing in 1989. The seroprotection rate of test subjects 1–2 months after the third dose was 98% in children aged 6 months to 10 years and 96% in the test group aged 16 to 65 years [13]. The HBV vaccines approved for production and marketing in recent years are Heplisav-B® and PreHevbrio®, which were approved for marketing by Dynavax and VBI Vaccines in 2017 and 2020, respectively, and are based on the expression of pleomorphic Hansenula yeast and Chinese hamster ovary cells, respectively [14].

4.1.2. Hepatitis E virus

Hepatic veno-viral (HEV) is a single-stranded positive-sense RNA virus transmitted via the fecal-oral route. After infection, the virus replicates in the intestines and liver, and can further spread to extrahepatic organs such as the kidneys, brain tissue, and placenta. Most infected individuals can clear the virus spontaneously, but immunocompromised individuals are more susceptible to chronic infection, which can lead to liver fibrosis and cirrhosis. HEV also has the ability to cross the blood-brain barrier and placental barrier, which is an important reason for its extrahepatic injury [15]. Among vaccines designed for HEV, the most well-known is Hecolin®, which was approved for marketing in 2012 by Xiamen Innovax Biotech. In a phase III clinical trial, researchers administered three doses of the recombinant hepatitis E vaccine HEV239 or a placebo at 0, 1, and 6 months to evaluate its efficacy and safety. The results showed that no cases of hepatitis E occurred in the vaccine group within 12 months after the full course of vaccination, while 15 cases occurred in the placebo group. The protective efficacy reached 100.0% (95% CI 72.1–100.0), and the antibody levels of 98.7% of the recipients increased more than 4 times. The vaccine was well tolerated, and the adverse reactions were mainly local reactions at the injection site [16].

4.1.3. HPV

HPV is a double-stranded DNA virus that primarily infects epithelial cells. During persistent infection, its genome can integrate into the host chromosome, leading to the inactivation of the E2 gene and the persistent expression of the E6 and E7 oncoproteins. E6 and E7 promote abnormal cell

proliferation and genomic instability by inhibiting tumor suppressor pathways such as p53 and pRb, respectively, ultimately inducing the development of cervical cancer and other related tumors. Therefore, E6, E7 and viral capsid protein L1 have become important targets for HPV vaccines and treatment strategies [17]. Cervarix®, manufactured by GlaxoSmithKline, was approved for marketing in 2007. In its Phase III double-blind randomized controlled trial, researchers enrolled 18,644 women aged 15–25 years who received either the AS04 adjuvanted bivalent HPV-16/18 L1 VLP vaccine or the hepatitis A control vaccine at 0, 1, and 6 months. According to the population analysis of the protocol, after a median follow-up of 14.8 months, the efficacy of the vaccine in preventing HPV-16/18-related CIN2+ was 90.4% (97.9% CI 53.4–98.8), the efficacy in preventing persistent infection for 6 months was 97.5% (80.7–99.9), and the protective efficacy in those under 18 years of age with negative baseline serology was 100% [18]. At the same time, Merck also launched Gardasil® and Gardasil 9® in 2006 and 2014, respectively [14].

4.2. VLP Vaccines in Clinical Trials

As shown in Table 1, the virus-like particle (VLP) vaccines currently in clinical trials are mainly being studied for important pathogens such as influenza virus, COVID-19, HIV, and HPV. Among these, research on influenza VLP vaccines is the most extensive, with 18 clinical trials covering various candidate products including H5, H7, and quadrivalent influenza vaccines. COVID-19-related VLP vaccines have 7 clinical trials, including candidate vaccines such as VBI-2901, VBI-2902, and ABNCoV2. In addition, VLP vaccines for diseases such as HIV and HPV have entered the clinical evaluation stage. Overall, the application of VLP vaccines has gradually expanded from traditional preventative vaccines to emerging infectious diseases and complex pathogens, demonstrating broad development prospects.

Table 1. Statistics on VLP Vaccines in Clinical Trial Phase

Disease type	NCT number	Clinical stage	Vaccine
Influenza	NCT04622592	2	Instramuscular Vaccine
	NCT03739112	3	Quadrivalent VLP Vaccine
	NCT03321968	3	Quadrivalent VLP Vaccine
	NCT03301051	3	Quadrivalent VLP Vaccine
	NCT02768805	2	Quadrivalent VLP Vaccine
	NCT02307851	2	Quadrivalent VLP Vaccine
	NCT02236052	2	Quadrivalent VLP Vaccine
	NCT02233816	2	Quadrivalent VLP Vaccine
	NCT02022163	1	H7 VLP vaccine + Alhydrogel
	NCT01991561	2	H5 VLP vaccine + Alhydrogel
	NCT01991587	1/2	Quadrivalent VLP Vaccine
	NCT01561768	2	Novavax Quadri valentvaccine
	NCT01072799	2	A/H1N1 2009 Influenza VLP Vaccine
	NCT01014806	2	Influenza VLP Vaccine
	NCT00984945	1	H5 VLP pandemic influenza vaccine
	NCT00903552	2	Influenza VLP Vaccine
	NCT00754455	2	Influenza VLP Vaccine (recombinant)
	NCT00519389	1/2	H5N1 VLP Vaccine
HIV	NCT00339040	2	Quadrivalent HPV vaccine
COVID-19	NCT05614245	1	VBI-2901e
	NCT05548439	1	VBI-2901a
	NCT04962893	2	SARS-CoV-2 VLP Vaccine-Wuhan
	NCT04839146	1	ABNCoV2 Vaccine
	NCT04818281	1	SARS-CoV-2 VLP Vaccine
	NCT04773665	1	VBI-2902a
	NCT04636697	2/3	Intramuscular vaccine

All registered clinical trials are listed on www.clinicaltrial.gov.

As shown in Table 1, the development of VLP vaccines is primarily focused on viral diseases with a high public health burden, especially influenza and COVID-19. This indicates that the VLP platform possesses advantages such as rapid response to outbreaks, flexible antigen replacement, and efficient induction of immune responses. Meanwhile, most candidate vaccines are still in Phase I or Phase II clinical trials, indicating that although the technology has shown good safety and immunogenicity, there is still room for improvement in large-scale clinical validation and industrial application. Furthermore, with the development of synthetic biology, modular antigen design, artificial protein scaffold construction, and the application of novel expression systems are continuously driving the optimization of VLP vaccine performance, providing crucial technical support for the future development of broad-spectrum vaccines, personalized vaccines, and therapeutic vaccines. Therefore, VLP vaccines are expected to become one of the important directions for next-generation vaccine development.

5. Conclusion

This article provides a systematic review of the basic principles, construction strategies, preparation methods, and research progress of virus-like particles (VLPs) vaccines from the perspective of synthetic biology. VLP vaccines, with their highly repetitive surface structure, suitable particle size, and flexible antigen display capabilities, can effectively activate the body's immune system. In recent years, with the development of synthetic biology, new technologies such as modular scaffold design, multi-epitope antigen engineering, SpyTag/SpyCatcher connection systems, and mirror biology have been continuously applied to VLP vaccine development, significantly improving the immunogenicity, stability, and programmability of vaccines. Currently, HBV, HEV, and HPV vaccines based on VLP technology have been successfully industrialized and applied. Meanwhile, VLP vaccines for diseases such as influenza, COVID-19, and HIV have also entered the clinical research stage, showing promising application prospects.

This review indicates that the deep integration of synthetic biology and VLP vaccines is driving a shift in vaccine development from traditional experience-driven to rational design-driven approaches. As mentioned in the introduction, traditional vaccines have limitations such as insufficient immunogenicity, safety risks, and long development cycles, while synthetic biology gives the VLP platform more intelligent, controllable, and modular design capabilities. By engineering chassis proteins, antigen modules, and connection systems, researchers can rapidly construct candidate vaccines against emerging and re-emerging infectious diseases, and further expand into the fields of tumor immunotherapy and personalized medicine. Therefore, VLP vaccines not only provide a new technological route for the development of next-generation vaccines, but also provide an important example of interdisciplinary integration and innovation.

Despite their promising immunogenicity and safety, VLP vaccines still face challenges in complex antigen display, large-scale manufacturing, and long-term efficacy validation. Future research will focus on AI-assisted VLP design, cost-effective production platforms, and novel delivery strategies to expand their applications in infectious diseases, cancer immunotherapy, and animal health.

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