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Virus-Like Particles (Vlps) from Synthesis to Targeted Drug Delivery, Vaccine Approaches, and Gene Therapy

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Abstract

٤ Virus-like particles (VLPs) are spontaneously generated from viral capsid proteins. VLPs imitate
٥ genuine viruses visually and physiologicallybut lack viral DNA. Various VLP designs provide
٦ structural and functional appeal. Spontaneous polymerization of viral capsid proteins may result
٧ in VLPs with geometrical symmetry, which are often icosahedral, spherical, or rod-like.Moreover,
٨ functionalized VLPs may precisely target cancer cells and recruit macrophages to destroy them.
٩ The ability to target tumors for therapeutic drug delivery through using VLP-based delivery
١٠ platforms in novel and intriguing aspects related to cancer treatment is the primary goal of VLP
١١ design. Cancer therapies require precise targeting of diagnostic or therapeutic elements to tumor
١٢ cells while avoiding healthy cells and tissues. VLPs offer an innovative approachas site-specific
١٣ drug delivery systems reducing systemic toxicity and injury to healthy cells. Immunotherapy,
١٤ which boosts the host's immune system, has fewer side effects. Cancer vaccines aim to induce an
١٥ immune response that provides protection against tumor cells. Due to their naturally fitted particle
١٦ size and repetitive structural order, VLPs may be employed as a vaccine without any adjuvant.
١٧ Recombinant VLP structures can be enhanced by including antigenic epitopes of viruses or
١٨ different disease-related antigens and targeting peptides to the interior and exterior
١٩ surfaces,making them potential tools for future immunizations with preventive and regenerative
٢٠ qualities. Additionally, VLP-based delivery strategies may enhance immunogenicity and provide
٢١ a more effective and safer approach to managing solid cancers with fewer side effects compare to
٢٢ chemotherapy or radiation. However, the production of chimeric VLPs still faces challenges, such
٢٣ as the need for more reliable preclinical animal models and associated costs Despite these
٢٤ obstacles, ongoing research will improve VLP-based technologies and increase their potential
٢٥ advantages. This review aims to provide basic information on VLPs and outline current studies on
٢٦ their use as drug and vaccine delivery systems in different cancers, highlighting their potential as
٢٧ a promising cancer treatment strategy. The key terms in the literature search included drug
٢٨ delivery, gene therapy, multi-capsid VLPs, virus-like particles (VLPs) were searched in
٢٩ international databases, namely, Web of Science, PubMed, and Scopus from 2003 to 2022.

٣٠ **Keywords**: Drug delivery, Gene therapy, Multi-capsid VLPs, Virus-like particles (VLPs)

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1. Background

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Virus-like particles (VLPs) are self-assembling viral proteins that are recombinantly generated. They make subviral or viral particles with diameters that range from 20 to 100 nm due to the proteins' ability to self-assemble. Due to their high stability, symmetry, and safety, VLPs are ideal carriers for pharmaceuticals, physiologically active peptides, and complete proteins (antigens, receptors, enzymes, etc.) (1). Previously, VLPs have been created for approximately thirty human and animal viruses. VLPs are composed of structural proteins and can self-assemble. The morphology of VLPs resembles that of natural viruses. Unlike real viruses, VLPs lack infectious and genetic material and cannot replicate or infect (2). Virus-like particles are a non-adjuvant-based vaccine candidate **(Figure 1).** Viruses create virus-like particles using a variety of different topologies. Parvoviruses have simple capsids composed of one or two main proteins; in contrast, picornaviruses have complex capsids with several protein layers expressed by many unique mRNAs or a single polyprotein (3). In contrast, certain viruses, such as influenza, HIV, and hepatitis C, acquire capsids, glycoprotein spikes, and their membranes and bilayers of lipids from the host cell. In addition to their diverse forms, VLPs display unusual three-dimensional nanoscale. VLP synthesis may be significantly increased by biological amplification and expansion. They may exhibit exogenous protein insertions due to genetic or chemical modifications. By selecting organic or inorganic materials to deposit at specific locations on the VLP, exact control of nanomaterial assembly, size, and spacing is possible, resulting in a uniform and reproducible Nano architectures (4). Certain VLPs are more immunogenic than recombinant protein immunogens and elicit an immune response at both humoral and cellular levels. Vaccinations against viruses may be substituted with VLPs that structurally mimic the original virus. Direct immune responses can also be induced by binding to VLP pattern recognition and B-cell receptors.VLPs may be used to create vaccines due to their intrinsically adjusted particle size and repetitive structural order (5). Apart from vaccinations, VLPs are useful for immunological treatment, targeted medicine administration, and gene therapy due to their immunogenicity. VLPs are biocompatible, uniform in size, scalable, and amenable to various functionalization procedures. These features make VLPs an effective delivery strategy. Through genetic engineering, affinity tags or targeting peptides may be added (6) **(Figure 2).**

2. Structural Classification of VLPs

VLPs are formed spontaneously by interacting with one or more viral structural capsid proteins. VLPs mimic natural viruses both physically and aesthetically but lack either a whole viral genome or the entire virus genome. The variety of designs adopted by distinct VLPs endows with both

structural and functional appeal. Spontaneous polymerization of various viral capsid proteins can produce geometrically symmetrical VLPs, often icosahedral, spherical, or rod-like, depending on the virus from which they are generated. VLPs may be categorized into various groups based on their structural complexity. Capsid proteins may be arranged in layers of one, two, or three (1, 7). Additionally, certain single-layer VLPs may include multiple structural proteins. Multi-protein VLPs, in contrast to single-protein VLPs, have a more complicated structure that contains numerous capsid layers. Other VLPs, such as those generated from HIV-1 and influenza virus, include a lipid coating bearing viral surface antigens encircling the capsid structure, mimicking the lipid envelope observed on infectious virus particles in nature. Generally, enclosed VLPs comprise matrix proteins directly incorporated into host-derived lipid membranes containing viral glycoproteins. There are limits to the manufacturing technique that can be employed for VLPs because of the necessity for a lipid envelope and the requirement to target viral proteins to the lipid bilayer (1,4, 8). VLPs are categorized into enveloped and non-enveloped based on the presence or absence of lipid envelopes **(Figure 3).**

2.1.Non-enveloped VLPs

Non-enveloped VLPs may also be classified as single- or multi-capsid protein VLPs, which can be single-, double-, or triple-layered. Single capsid VLP structures, such as those used in Human papillomavirus (HPV) VLP vaccines, are currently the most well-known basic form of non-enveloped VLP structures. These basic VLPs comprise a single capsid protein that can be synthesized in eukaryotic or prokaryotic cells. For some basic VLPs, capsid proteins may be produced in a cell-free environment. To generate a homogenous VLP capsomer soluble in these situations, the proteins can first be generated in a cell-based expression system and then assembled in a cell-free environment to fold appropriately (1, 9). Furthermore, non-enveloped multi-capsid VLPs are more challenging to synthesize and typically require eukaryotic expression systems such as yeast, insect cells, and plants (8, 10). Multiple capsid proteins may be expressed and assembled in a single cell. For instance, Bluetongue, Enterovirus, infectious bursal disease virus, poliovirus, and rotavirus, among other viruses, have successfully created multi-capsid proteins. Additional non-enveloped multicapsid VLPs include the HPV L1 and L2 proteins, which may be synthesized into VLPs from two proteins (11).

2.2.Enveloped VLPs (eVLPs)

There are three lipid-enveloped VLPs: single-layer, double-layer, and multi-layer, each with its innate structure. During the assembly and budding of VLPs from the cell, the lipid membrane of encapsulated VLPs is obtained. When glycoprotein anchors are introduced into the lipid membrane, antibodies can be produced to neutralize them since the immune system recognizes these glycoproteins as major antigens. The envelop's structure, origin, and composition differ between enveloped VLPs, and their precise nature is governed by the virus from which the VLP is formed, which in turn determines the process of assembly and budding of the VLP from the host cell line employed to make them (1, 12).

2.3. Various VLPs Development Methods

Virus-like nanoparticles might be employed as a new delivery strategydue to their diverse characteristics that allow them to transport peptide fragments, nucleic acids, and medicinal drugs into protein structures (13). Protein cages are hollow structures made of self-assembling protein components, are commonly formed in living organisms. Internal, exterior, and inter-subunit functional elements may be employed to fine-tune particle stability, drug encapsulation, surface charge, and ligand display (14). Some VLPs work better as nanocarriers. As a delivery vehicle, Bacteriophage MS2 VLPs excel. The recombinant-protein technology makes generating these VLPs easy (15, 16). The MS2 capsid sympathizes with "PAC" DNA or RNA and may enclose it by placing itself at the five termini; this minimizes the chance of nuclease-induced RNA or DNA damage. MS2 VLPs may transfer antigenic components and epitopes to patients (17). Moreover, the epitope peptide may be given to the immune system once the MS2CP gene is expressed. VLP capsids may contain viral antigens that trigger a robust immune response (15, 18). MS2 VLPs are highly stable and large enough to expose viral antigenic epitopes. In addition, MS2 VLPs can be used as selective and passive Nano transporter. Drugs may be packaged to MS2CP, MS2 (19). The target environment, the kind of drug being loaded, and the structure all determine current particulate techniques (20), tumor-targeted drug delivery shows promise in cancer treatment (21). The MS2 bacteriophage has been studied for its potential to deliver 5-fluorouracil and doxorubicin, as well as murine polyomavirus for methotrexate (19, 22). Researchers observed that modified adenoviruses destroy tumor cells more effectively while causing less harm to healthy organs (23, 24). The cancer-killing chemical doxorubicin was encapsulated in VLPs, which destroys DNA and may help treat lymphoma, leukemia, and breast cancer (25). Furthermore, the cucumber mosaic virus was used to encapsulates doxorubicin to target folate-expressing cancer cells. The anti-tumor responses outperformed free medicines (26). RNA interference (RNAi) is another cancer treatment option that is challenging in vivo. However, the JC virus VLPs is shown to be an effective vector

for delivering anti-IL-10 silencing drugs, reducing gene expression by up to 88-90% compared to VLPs alone (27). Galway and Stockley could target HeLa cells using reassembled VLPs in vitro, utilizing the RNA bacteriophage's MS2 coat protein and an RNA conjugate comprising a siRNA and the known capsid assembly signal (28).

Using Nanovaccinology's VLPs could potentially lead to the elimination of cancer cells. The term "nanovaccine" has acquired popularity in vaccine research recently in various therapeutic applications such as antigen processing and immunostimulant adjuvants. Nanovaccinology is largely used to treat cancer, Alzheimer's, hypertension, and nicotine addiction as well (29). A single viral capsid protein is needed for self-assembly. Most VLPs are made from insect or yeast cells because they are simple and can create complex viral protein targets for vaccinations (30). Viruses have been linked to human cancer development; as1.9 million cancer cases (17.8% of all cancer cases) in 2002 were due to viral infections. Viruses may play a role in cancer development at different stages, ranging from 15% to 100%. Between 15 and 20% of cancers have been observed to be of viral origin (31). Vaccination for cancer prevention gained popularity in the late 1980susing recombinant virus-like particles as antigens in hepatitis B and E vaccines. Vector-based vaccinations against HBV have been approved, including Recombivax and Engerix-B vaccines.Both vaccines use non-infectious HBV surface antigen (HBsAg) through using recombinant DNA technology (32). Engerix-B from Recombivax-HB, and GSK's Gardasil (yeast) and Cervarix (insect cells) were two vaccines produced in the late 2000s. The HBV and HPV vaccines include recombinant virus-like particles. Clinical trials have demonstrated that vaccines elicit protective and neutralizing antibodies that may prevent illness. Cloning a portion of the HBV genome into Saccharomyces cerevisiae produces recombinant HBsAg. Recombivax and Engerix-B vaccines are immunogenic and induce HBsAg antibodies (33, 34). They are more immunogenic than earlier VLP vaccinations because they include Pre-S1, Pre-S2, and hepatitis B surface antigens. The third-generation Bio-Hep Bvaccine contains Pre-S1 (large), Pre-S2 (middle), and tiny (s) surface proteins of HBV and mammalian cells that were used to make approved VLP-based vaccines. E. coli created many recombinant protein products, including the first human insulin. Between 1986 and 2015, about 50 VLP vaccines were developed. E. coli produces the VLP Hecolin and Heber Nasvac vaccines. Hecolin is the first commercially available E. coli hepatitis E vaccine (35). Heber Nasvac (ABX203) is a VLP vaccine for hepatitis B treatment licensed by Cuban regulators in 2015. (36) GARDASIL's HPV VLPs are a great example of a cancer vaccine. Cervical cancer and warts may be prevented with this medication. Baculovirus or yeast systems can generate HPV L1 VLPs. It resembles viroid epitopes, which can

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١٦٠ stimulateprotective immune responses when adjuvanted. The adsorbed VLPs of each HPV type
١٦١ are combined to form GARDASIL (aluminum-containing adjuvant) (33). The impact of aluminum
١٦٢ adjuvant surface exposure on VLP morphology is investigated. The esults indicate the adjuvant's
١٦٣ adsorption had minimal influence on the morphology and developed MelQbG10 virus-like
١٦٤ nanoparticles containing CpG-oligonucleotides and a Melan-A peptide as an anticancer vaccine
١٦٥ (37). Deo et al., 2015, used Gag and M1, two influenza virus capsid proteins, to create chimeric
١٦٦ viruses with a variable fragment region that target colon cancer cells. These chimeric virus-like
١٦٧ particles (VLPs) were packaged in huge, single-walled unilamellar containers to protect them from
١٦٨ cancer-killing agents. VLPs are highly selective for cancer cells and effective in transporting dyes
١٦٩ and drugs (38). Lentiviral vectors were long the preferred method of gene silencing in mice. These
١٧٠ vectors may cause mutagenesis and cancer through DNA incorporation into the host's genome.
١٧١ limits their use in medicine (39).

١٧٢ **2.4.Vaccination and Gene Therapy**

١٧٣ Delivering DNA, mRNA, and tiny RNA molecules to target cells to replace faulty genes or control
١٧٤ protein production is one of the most complex components of creating gene therapy. Contrary to
١٧٥ common opinion, gene therapy has had minor success using anything other than adenoviral
١٧٦ vectors, adeno-associated viruses, and lentiviral vectors in vitro and in vivo (40). The lentiviral
١٧٧ system, in particular, has the potential to generate mutagenesis and carcinogenesis; consequently,
١٧٨ it must be extensively examined for safety before it can be employed. Using their reversible
١٧٩ construction, VLPs may be created in vitro with nucleic acids, peptides (like p19), or stem-loop
١٨٠ RNAs; a favorable influence of this on the field of nano vehicles for nucleic acid delivery has been
١٨١ documented in the literature (41). VLPs protect therapeutic cargos before they reach the target cell.
١٨٢ However, they are non-toxic and inert. In contrast to lipid agents, cationic polymers and
١٨٣ polypeptides are typical penetrating agents (42) This approach of targeted gene delivery may lower
١٨٤ the immune response, boost therapeutic molecule efficiency, reduce therapeutic cargo dosage,
١٨٥ promote bioavailability, decrease cytotoxicity, and limit undesirable effects (43). Capsid proteins
١٨٦ for gene therapy are most typically taken from plant and animal viruses (TMV, CPMV, CCMV,
١٨٧ BMV) and cytomegalovirus (JCV, HBV) (JCV, HBV). Plant virus-derived VLPs may often be
١٨٨ assembled in vitro or in vivo, depending on the conditions. In vitro purification of an infected
١٨٩ plant, the virus is achieved by dissecting the capsid protein and reassembling it in different
١٩٠ solutions (44). Viral particles made using this method may have morphologies that vary from the
١٩١ original virus. A host produces viral proteins to generate VLPs without encasing the infectious

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DNA in vivo. Those proteins that make up the capsids of plants, yeast, or E. coli self-assemble into more complex structures (icosahedral and helical symmetry) (45). This third consideration is critical, given the lower cost of adenovirus, adeno-associated virus, and lentiviral expression 1systems than mammalian or insect expression systems. This can be achieved before or after the capsid proteins have been assembled through ultracentrifuge, immobilized metal affinity, and size exclusion chromatography (46). Plant viruses and bacteriophages can be produced in less biosafety-concerned facilities as an alternative to animal virus-based systems. Animal virus-based VLPs are effective gene carriers (47). CpG oligodeoxynucleotides and siRNAs, for example, are readily transmitted by HBV CP particles (48). Additionaly, RANKL, a type of tumor necrosis factor, was turned off in rat osteoblasts using JCV virus-infected neurotropic polyomavirus (JCV) VLPs to convey siRNA to the cells. These VLPs do not need a unique cancer epitope to target glioblastoma cells since JCV may infect human GBM cells (49). These VLPs presumably just need the JCV VP1 epitopes that already exist to target cancer cells. Lastly, bacteriophage-based VLPs have been used for medicinal purposes,including the delivery of antisense oligonucleotides, and siRNA in vitro and in vivo using MS2 bacteriophage-based VLPs,and the delivery of vaccine adjuvants containing unmethylated CG motifs was also achieved using Q VLPs. Some of these uses overlap with the mRNA-based vaccines antecedently mentioned. However, improving the efficiency of VLP production and packaging remains a challenge in this industry (15). An essential initial step is determining which packaging signal is required to effectively and selectively package the payload inside the host cell. The viral genome has sequences or structural elements that ensure the proper packaging of genetic material,with Each batch of VLPs will have the same RNA content if it contains the correct capsid protein packaging signal. However, further study is required to understand the selective packaging of ssRNA viruses (50).

2.5.The Challenges and Potential Development of VLP

Designing, purifying, and storing vaccines based on eVLP offer several technological problems. The stability of the vaccination over time is one of the primary problems that should be addressed immediately. Multimeric VLP vaccines are more stable than subunit vaccinations; nonetheless, their lack of a viral genome makes them unstable when environmental circumstances change, especially following further therapy (DSP) (51). eVLPs with a host-derived envelope are more susceptible to environmental impacts than protein-only VLPs. Temperature, shear stress, and chemical treatment can affect particle integrity and stability. This structural degradation reduces eVLPs' immunogenicity. Shallow temperatures (VLPs) are changed to increase particle

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٢٢٤ thermostability. Stabilizing mutations are common. According to a study on poliovirus type 3
٢٢٥ VLPs, the antigenic conformation and repetitive structure of the original viral particle are
٢٢٦ conserved with stabilizing changes in the coat proteins. The wild-type VLPs are less stable and
٢٢٧ viral protein expression on different platforms can vary (8). Secretory glycoprotein expression is
٢٢٨ problematic. Because the envelope must emerge from the cell membrane, if eVLP is not adequately
٢٢٩ secreted, cell lysis or another extraction step may be needed. These processes complicate the
٢٣٠ subsequent purification. To boost transmembrane glycoprotein expression, delete or replace the
٢٣١ transmembrane region. It is a standard way to increase expression (1, 52). Exchanging the stem-
٢٣٢ transmembrane domain of DENV2 E, which contains a strong ER retention signal, with the
٢٣٣ homologous component of the Japanese encephalitis virus can release enveloped virus-like
٢٣٤ particles (eVLPs) (JEV) (53). This could be better when membrane-integration-dependent
٢٣٥ oligomerization is necessary for a protein's function or immunogenicity. Adding a signal peptide
٢٣٦ can enhance eVLP secretion. Cells that co-express prM and E cannot generate eVLPs, an essential
٢٣٧ DENV VLP stage. Cells can create eVLPs when a JEV signal sequence is introduced at the N
٢٣٨ terminus of prME. Signal peptides affect the translocation and topology of downstream proteins
٢٣٩ (54). Purified eVLPs must be inactivated to reduce baculovirus infectivity to meet security criteria;
٢٤٠ however, this process may affect antigenicity (52). Improve product purity by employing
٢٤١ successive purification approaches, such as clarifying, intermediate purification, and polishing,
٢٤٢ without reducing eVLP immunogenicity (1, 8). Purification procedures include centrifugation,
٢٤٣ precipitation, ultrafiltration, and chromatography (55).

٢٤٤ **3. Conclusion**

٢٤٥ Unique properties of VLPs, such as multimer antigens, particle shape, and non-infectivity, have
٢٤٦ recently emerged as promising candidates for vaccination and targeted drug delivery. The
٢٤٧ assembly, disassembly/reassembly, and self-assembly properties of VLPs have enabled various
٢٤٨ drug encapsulation and targeting approaches.The ability to safely stimulate humoral and cellular
٢٤٩ immune responses is a crucial advantage of VLPs, making them suitable in vivo therapy without
٢٥٠ concern about toxicity or inflammation. VLPs may be chemically or genetically modified for
٢٥١ specific purposes. Recombinant VLP structures can be enhanced by including antigenic epitopes
٢٥٢ of viruses or different disease-related antigens and targeting peptides to the interior and exterior
٢٥٣ surfaces,making them potential tools for future immunizations with preventive and regenerative
٢٥٤ qualities. Additionally, VLP-based delivery strategies may enhance immunogenicity and provide
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chemotherapy or radiation. However, the production of chimeric VLPs still faces challenges, such as the need for more reliable preclinical animal models and associated costs Despite these obstacles, ongoing research will improve VLP-based technologies and increase their potential advantages.

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Author contributions

HEG, MM, MF, AZ, MMN: developed the theoretical formalism,and contributed to the final version of the manuscript. MF: supervised the project.

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Availability of data and material

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References

1. Nooraei S, Bahrulolum H, Hoseini ZS, Katalani C, Hajizade A, Easton AJ, et al. Virus-like particles: preparation, immunogenicity and their roles as nanovaccines and drug nanocarriers. J Nanobiotechnology. 2021; 19:1-27. doi.org/10.1186/s12951-021-00806-7.
2. Taghizadeh MS, Niazi A, Afsharifar A. Virus-like particles (VLPs): A promising platform for combating against Newcastle disease virus. Vaccine: X. 2024:100440. doi.org/10.1016/j.jvacx.2024.100440.

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3. Tariq H, Batool S, Asif S, Ali M, Abbasi BH. Virus-like particles: Revolutionary platforms for developing vaccines against emerging infectious diseases. Front microbiol. 2022; 12:790121. doi.org/10.3389/fmicb.2021.790121.
4. Martins SA, Santos J, Silva RDM, Rosa C, Cabo Verde S, Correia JDG, et al. How promising are HIV-1-based virus-like particles for medical applications. Front Cell Infect Microbiol. 2022; 12:997875. doi: 10.3389/fcimb.2022.997875.
5. Mohsen MO, Bachmann MF. Virus-like particle vaccinology, from bench to bedside. Cell Mol Immunol. 2022;19(9):993-1011. doi.org/10.1038/s41423-022-00897-8.
6. Travassos R, Martins SA, Fernandes A, Correia JD, Melo R. Tailored Viral-Like Particles as Drivers of Medical Breakthroughs. Int J Mol Sci. 2024;25(12):6699. doi.org/10.3390/ijms25126699.
7. Mohsen MO, Gomes AC, Vogel M, Bachmann MF. Interaction of viral capsid-derived virus-like particles (VLPs) with the innate immune system. Vaccines. 2018;6(3):37. doi.org/10.3390/vaccines6030037.
8. Gupta R, Arora K, Roy SS, Joseph A, Rastogi R, Arora NM, Kundu PK. Platforms, advances, and technical challenges in virus-like particles-based vaccines. Front Immunol. 2023;14:1123805. doi: 10.3389/fimmu.2023.1123805.
9. Yan D, Wei YQ, Guo HC, Sun SQ. The application of virus-like particles as vaccines and biological vehicles. Appl Microbiol Biotechnol. 2015;99:10415-32. doi.org/10.1007/s00253-015-7000-8.
10. Fuenmayor J, Gòdia F, Cervera L. Production of virus-like particles for vaccines. New biotechnol. 2017;39:174-80. doi.org/10.1016/j.nbt.2017.07.010.
11. Roldão A, Silva AC, Mellado MCM, Alves PM, Carrondo MJT. Viruses and Virus-Like Particles in Biotechnology: Fundamentals and Applications. Comprehensive Biotechnology. 2017:633–56. doi: 10.1016/B978-0-12-809633-8.09046-4.
12. Brémaud E, Favard C, Muriaux D. Deciphering the assembly of enveloped viruses using model lipid membranes. Membranes. 2022;12(5):441. doi.org/10.3390/membranes12050441.
13. Rohovie MJ, Nagasawa M, Swartz JR. Virus-like particles: Next-generation nanoparticles for targeted therapeutic delivery. Bioeng Transl Med. 2017;2(1):43-57. doi: 10.1002/btm2.10049.

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۳۱۹ 14. Wang Y, Douglas T. Protein nanocage architectures for the delivery of therapeutic proteins.
۳۲۰ Current Opinion in Colloid & Interface Science. 2021;51:101395.
۳۲۱ doi.org/10.1016/j.cocis.2020.101395.
۳۲۲ 15. Fu Y, Li J. A novel delivery platform based on Bacteriophage MS2 virus-like particles.
۳۲۳ Virus Res. 2016;211:9-16. doi: 10.1016/j.virusres.2015.08.022.
۳۲٤ 16. He J, Yu L, Lin X, Liu X, Zhang Y, Yang F, et al. Virus-like particles as nanocarriers for
۳۲٥ intracellular delivery of biomolecules and compounds. Viruses. 2022;14(9):1905. doi:
۳۲٦ 10.3390/v14091905.
۳۲۷ 17. Naskalska A, Heddle JG. Virus-like particles derived from bacteriophage MS2 as antigen
۳۲۸ scaffolds and RNA protective shells. Nanomedicine. 2024; 1103-1115.
۳۲۹ doi.org/10.2217/nnm-2023-0362.
۳۳۰ 18. Chehelgerdi M, Chehelgerdi M. The use of RNA-based treatments in the field of cancer
۳۳۱ immunotherapy. Mol Cancer. 2023;22(1):106. doi.org/10.1186/s12943-023-01807-w.
۳۳۲ 19. Ashley CE, Carnes EC, Phillips GK, Durfee PN, Buley MD, Lino CA, et al. Cell-specific
۳۳۳ delivery of diverse cargos by bacteriophage MS2 virus-like particles. ACS nano. 2011
۳۳٤ 26;5(7):5729-45. doi: 10.1021/nn201397z.
۳۳٥ 20. Lino CA, Caldeira JC, Peabody DS. Display of single-chain variable fragments on
۳۳٦ bacteriophage MS2 virus-like particles. J nanobiotechnology. 2015;15:1-0.
۳۳۷ doi.org/10.1186/s12951-016-0240-7.
۳۳۸ 21. Kolesanova EF, Melnikova MV, Bolshakova TN, Rybalkina EY, Sivov IG. Bacteriophage
۳۳۹ MS2 As a Tool for Targeted Delivery in Solid Tumor Chemotherapy. Acta Naturae.
۳٤۰ 2019;11(2):98-101. doi: 10.32607/20758251-2019-11-2-98-101.
۳٤۱ 22. Chung YH, Cai H, Steinmetz NF. Viral nanoparticles for drug delivery, imaging,
۳٤۲ immunotherapy, and theranostic applications. Adv Drug Deliv Rev. 2020;156:214-235.
۳٤۳ doi: 10.1016/j.addr.2020.06.024.
۳٤٤ 23. Hajeri PB, Sharma NS, Yamamoto M. Oncolytic Adenoviruses: Strategies for Improved
۳٤٥ Targeting and Specificity. Cancers (Basel). 2020;12(6):1504. doi:
۳٤٦ 10.3390/cancers12061504.
۳٤۷ 24. Wold WS, Toth K. Adenovirus vectors for gene therapy, vaccination and cancer gene
۳٤۸ therapy. Curr Gene Ther. 2013;13(6):421-33. doi:
۳٤۹ 10.2174/1566523213666131125095046.

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٣٥٠ 25. Wang M, Bergès R, Malfanti A, Préat V, Bastiancich C. Local delivery of doxorubicin
٣٥١ prodrug via lipid nanocapsule–based hydrogel for the treatment of glioblastoma. Drug
٣٥٢ Deliv Transl Res. 2023:1-7. doi: 10.1007/s13346-023-01456-y.
٣٥٣ 26. Zeng Q, Wen H, Wen Q, Chen X, Wang Y, Xuan W, et al. Cucumber mosaic virus as drug
٣٥٤ delivery vehicle for doxorubicin. Biomaterials. 2013;34(19):4632-42. doi:
٣٥٥ 10.1016/j.biomaterials.2013.03.017.
٣٥٦ 27. Chou MI, Hsieh YF, Wang M, Chang JT, Chang D, Zouali M, et al. In vitro and in vivo
٣٥٧ targeted delivery of IL-10 interfering RNA by JC virus-like particles. J Biomed Sci. 2010
٣٥٨ ;17(1):51. doi: 10.1186/1423-0127-17-51.
٣٥٩ 28. Galaway FA, Stockley PG. MS2 viruslike particles: a robust, semisynthetic targeted drug
٣٦٠ delivery platform. Mol Pharm. 2013;10(1):59-68. doi: 10.1021/mp3003368.
٣٦١ 29. Facciolà A, Visalli G, Laganà P, La Fauci V, Squeri R, Pellicanò GF, et al. The new era of
٣٦٢ vaccines: the "nanovaccinology". Eur Rev Med Pharmacol Sci. 2019;23(16):7163-7182.
٣٦٣ doi: 10.26355/eurrev_201908_18763.
٣٦٤ 30. Hadj Hassine I, Ben M'hadheb M, Almalki MA, Gharbi J. Virus-like particles as powerful
٣٦٥ vaccination strategy against human viruses. Rev Med Virol. 2024;34(1):e2498. doi:
٣٦٦ 10.1002/rmv.2498.
٣٦٧ 31. Parkin DM. The global health burden of infection-associated cancers in the year 2002. Int
٣٦٨ J Cancer. 2006;118(12):3030-44. doi: 10.1002/ijc.21731.
٣٦٩ 32. Moradi Vahdat M, Hemmati F, Ghorbani A, Rutkowska D, Afsharifar A, Eskandari MH,
٣٧٠ et al. Hepatitis B core-based virus-like particles: A platform for vaccine development in
٣٧١ plants. Biotechnol Rep (Amst). 2021;29:e00605. doi: 10.1016/j.btre.2021.e00605.
٣٧٢ 33. Wang JW, Roden RB. Virus-like particles for the prevention of human papillomavirus-
٣٧٣ associated malignancies. Expert Rev Vaccines. 2013;12(2):129-41. doi:
٣٧٤ 10.1586/erv.12.151.
٣٧٥ 34. Zhao H, Zhou X, Zhou YH. Hepatitis B vaccine development and implementation. Hum
٣٧٦ Vaccin Immunother. 2020;16(7):1533-1544. doi: 10.1080/21645515.2020.1732166.
٣٧٧ 35. Shouval D, Ilan Y, Adler R, Deepen R, Panet A, Even-Chen Z, et al. Improved
٣٧٨ immunogenicity in mice of a mammalian cell-derived recombinant hepatitis B vaccine
٣٧٩ containing pre-S1 and pre-S2 antigens as compared with conventional yeast-derived
٣٨٠ vaccines. Vaccine. 1994;12(15):1453-9. doi: 10.1016/0264-410x(94)90155-4.
٣٨١ 36. Fleites YA, Aguiar J, Cinza Z, Bequet M, Marrero E, Vizcaíno M, et al. HeberNasvac, a
٣٨٢ Therapeutic Vaccine for Chronic Hepatitis B, Stimulates Local and Systemic Markers of
Preprint

٣٨٣ Innate Immunity: Potential Use in SARS-CoV-2 Postexposure Prophylaxis. Euroasian J
٣٨٤ Hepatogastroenterol. 2021;11(2):59-70. doi: 10.5005/jp-journals-10018-1344.
٣٨٥ 37. Braun M, Jandus C, Maurer P, Hammann-Haenni A, Schwarz K, Bachmann MF, et al.
٣٨٦ Virus-like particles induce robust human T-helper cell responses. Eur J Immunol.
٣٨٧ 2012;42(2):330-40. doi: 10.1002/eji.201142064.
٣٨٨ 38. Deo VK, Kato T, Park EY. Chimeric virus-like particles made using GAG and M1 capsid
٣٨٩ proteins providing dual drug delivery and vaccination platform. Mol Pharmaceutics.
٣٩٠ 2015;12(3):839-45. doi: 10.1021/mp500860x.
٣٩١ 39. Manjunath N, Wu H, Subramanya S, Shankar P. Lentiviral delivery of short hairpin RNAs.
٣٩٢ Adv Drug Deliv Rev. 2009;61(9):732-45. doi: 10.1016/j.addr.2009.03.004.
٣٩٣ 40. Uddin F, Rudin CM, Sen T. CRISPR Gene Therapy: Applications, Limitations, and
٣٩٤ Implications for the Future. Front Oncol. 2020;10:1387. doi: 10.3389/fonc.2020.01387.
٣٩٥ 41. Dong W, Kantor B. Lentiviral Vectors for Delivery of Gene-Editing Systems Based on
٣٩٦ CRISPR/Cas: Current State and Perspectives. Viruses. 2021;13(7):1288. doi:
٣٩٧ 10.3390/v13071288.
٣٩٨ 42. Torres-Vanegas JD, Cruz JC, Reyes LH. Delivery Systems for Nucleic Acids and Proteins:
٣٩٩ Barriers, Cell Capture Pathways and Nanocarriers. Pharmaceutics. 2021;13(3):428. doi:
٤٠٠ 10.3390/pharmaceutics13030428.
٤٠١ 43. Puhl DL, D'Amato AR, Gilbert RJ. Challenges of gene delivery to the central nervous
٤٠٢ system and the growing use of biomaterial vectors. Brain Res Bull. 2019;150:216-230. doi:
٤٠٣ 10.1016/j.brainresbull.2019.05.024.
٤٠٤ 44. Agranovsky A. Enhancing Capsid Proteins Capacity in Plant Virus-Vector Interactions and
٤٠٥ Virus Transmission. Cells. 2021;10(1):90. doi: 10.3390/cells10010090.
٤٠٦ 45. Peyret H, Steele JFC, Jung JW, Thuenemann EC, Meshcheriakova Y, Lomonossoff GP.
٤٠٧ Producing Vaccines against Enveloped Viruses in Plants: Making the Impossible, Difficult.
٤٠٨ Vaccines (Basel). 2021;9(7):780. doi: 10.3390/vaccines9070780.
٤٠٩ 46. Naso MF, Tomkowicz B, Perry WL 3rd, Strohl WR. Adeno-Associated Virus (AAV) as a
٤١٠ Vector for Gene Therapy. BioDrugs. 2017;31(4):317-334. doi: 10.1007/s40259-017-0234-
٤١١ 5.
٤١٢ 47. Czapar AE, Steinmetz NF. Plant viruses and bacteriophages for drug delivery in medicine
٤١٣ and biotechnology. Curr Opin Chem Biol. 2017;38:108-116. doi:
٤١٤ 10.1016/j.cbpa.2017.03.013.
preprint

48. Malanchère-Brès E, Payette PJ, Mancini M, Tiollais P, Davis HL, Michel ML. CpG oligodeoxynucleotides with hepatitis B surface antigen (HBsAg) for vaccination in HBsAg-transgenic mice. J Virol. 2001;75(14):6482-91. doi: 10.1128/JVI.75.14.6482-6491.2001.

49. Hoffmann DB, Gruber J, Böker KO, Deppe D, Sehmisch S, Schilling AF,et al. Effects of RANKL Knockdown by Virus-like Particle-Mediated RNAi in a Rat Model of Osteoporosis. Mol Ther Nucleic Acids. 2018;12:443-452. doi: 10.1016/j.omtn.2018.06.001.

50. An M, Raguram A, Du SW, Banskota S, Davis JR, Newby GA, Chen PZ, Palczewski K, Liu DR. Engineered virus-like particles for transient delivery of prime editor ribonucleoprotein complexes in vivo. Nat Biotechnol. 2024:1-2. doi: 10.1038/s41587-023-02078-y.

51. Liu S, Hu M, Liu X, Liu X, Chen T, Zhu Y, Liang T, Xiao S, Li P, Ma X. Nanoparticles and Antiviral Vaccines. Vaccines (Basel). 2023;12(1):30. doi: 10.3390/vaccines12010030.

52. Dai S, Wang H, Deng F. Advances and challenges in enveloped virus-like particle (VLP)-based vaccines. J Immunol Sci. 2018;2(2). doi:10.29245/2578-3009/2018/2.1118.

53. Hsieh SC, Liu IJ, King CC, Chang GJ, Wang WK. A strong endoplasmic reticulum retention signal in the stem-anchor region of envelope glycoprotein of dengue virus type 2 affects the production of virus-like particles. Virology. 2008;374(2):338-50. doi: 10.1016/j.virol.2007.12.041.

54. Ponndorf D, Meshcheriakova Y, Thuenemann EC, Dobon Alonso A, Overman R, Holton N, et al. Plant-made dengue virus-like particles produced by co-expression of structural and non-structural proteins induce a humoral immune response in mice. Plant Biotechnol J. 2021;19(4):745-756. doi: 10.1111/pbi.13501.

55. Joung JK, Cabeceiras P, inventors; Harvard College, General Hospital Corp, assignee. Enhanced virus-like particles and methods of use thereof for delivery to cells. United States patent application US 18/158,173. 2023.

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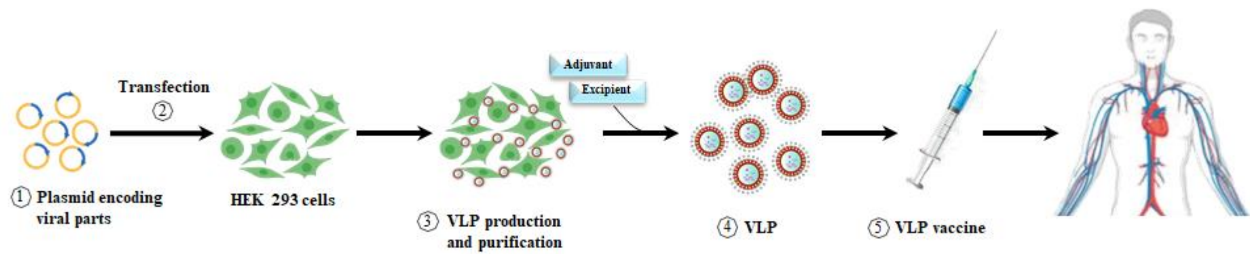

Figure 1. The process of producing a VLP-based vaccine involves three steps: 1) The production stage involves cloning the viral genes and producing self-assembling viral proteins using a suitable expression platform such as HEK 293T cells. The result is the collection of VLPs in particle form without infectious properties. 2) Tthe purification stageinvolves further processing to obtain purified VLPs without any residual debris. 3) The formulation stage, where adjuvants and other ingredients are added to create a safe and effective vaccine for administration.

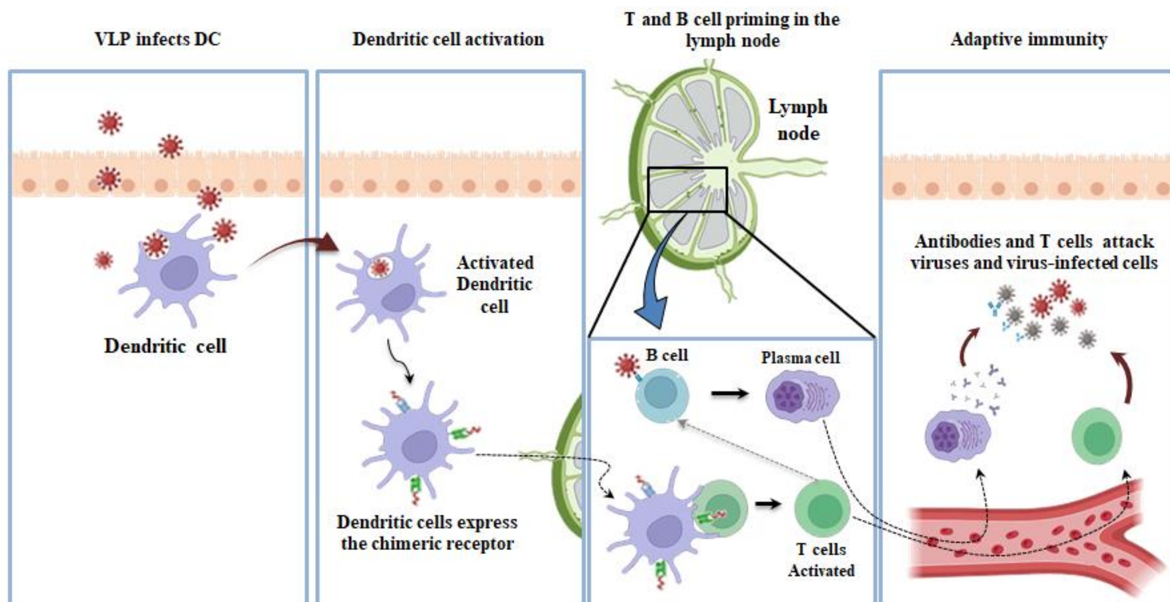

Figure 2. The induction of both innate and adaptive immunological responses, which includes humoral immunity and cell-mediated immunity, by using VLPs (virus-like particles), results in improved absorption and presentation of antigens by APCs (antigen-presenting cells) such as dendritic cells, which alert T cells to potential dangers, efficient transportation of the VLPs to lymph nodes, an important location for adaptive immunological responses, better communication between B cells, T cells, and APCs, and the ability of VLP-based antigens to effectively activate and link B cell receptors, leading to the formation of memory cells and both long-livedand short-lived plasma cells after exposure to the antigen.

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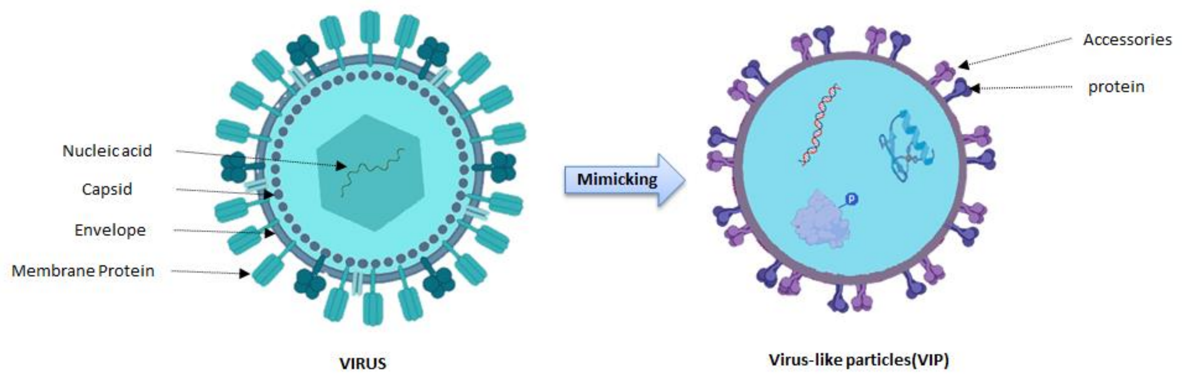

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٤٦١ Figure 3. Non-enveloped and enveloped virus VLP as a platform for the delivery of foreign small molecules

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