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Review Article

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

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ABSTRACT

Virus-like particles (VLPs) are spontaneously generated from viral capsid proteins. VLPs imitate genuine viruses visually and physiologically, but lack viral DNA. Various VLP designs provide structural and functional appeal. Spontaneous polymerization of viral capsid proteins may result in the formation of VLPs with geometrical symmetry, which are often icosahedral, spherical, or rod-like. Moreover, functionalized VLPs may precisely target cancer cells and recruit macrophages to destroy them. The ability to target tumors for therapeutic drug delivery through VLP-based delivery platforms in novel and intriguing aspects related to cancer treatment is the primary goal of VLP design. Cancer therapies require precise targeting of diagnostic or therapeutic elements to tumor cells while avoiding healthy cells and tissues. VLPs offer an innovative approach as site-specific drug delivery systems, reducing systemic toxicity and minimizing injury to healthy cells. Immunotherapy, which boosts the host's immune system, has fewer side effects. Cancer vaccines aim to induce an immune response that provides protection against tumor cells. Due to their naturally fitted particle size and repetitive structural order, VLPs may be employed as a vaccine without any adjuvant. Recombinant VLP structures can be enhanced by including antigenic epitopes of viruses or different disease-related antigens, and targeting peptides to the interior and exterior surfaces, making them potential tools for future immunizations with preventive and regenerative qualities. Additionally, VLP-based delivery strategies may enhance immunogenicity and provide a more effective and safer approach to managing solid cancers with fewer side effects compared to 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. This review aims to provide basic information on VLPs and outline current studies on their use as drug and vaccine delivery systems in different cancers, highlighting their potential as a promising cancer treatment strategy. The key terms in the literature search-including drug delivery, gene therapy, multi-capsid VLPs, and virus-like particles (VLPs)-were searched in international databases, namely Web of Science, PubMed, and Scopus from 2003 to 2022.

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1. Context

Virus-like particles (VLPs) are self-assembling viral proteins that are generated through recombinant technology. They make subviral or viral particles with diameters ranging 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 (such as 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). Nontheless, 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 enhance the effectiveness of VLPs as a delivery strategy, allowing for the addition of affinity tags or targeting peptides through genetic engineering (6). (Figure 2).

2. Data Acquisition

2.1. Structural Classification of VLPs

VLPs are formed spontaneously by interacting with one or more viral structural capsid proteins. VLPs mimic natural viruses in both physical structure and appearance, but they do not contain a complete viral genome or any part of the viral genome. The variety of designs adopted by distinct VLPs endows them 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).

2.1.1. 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.

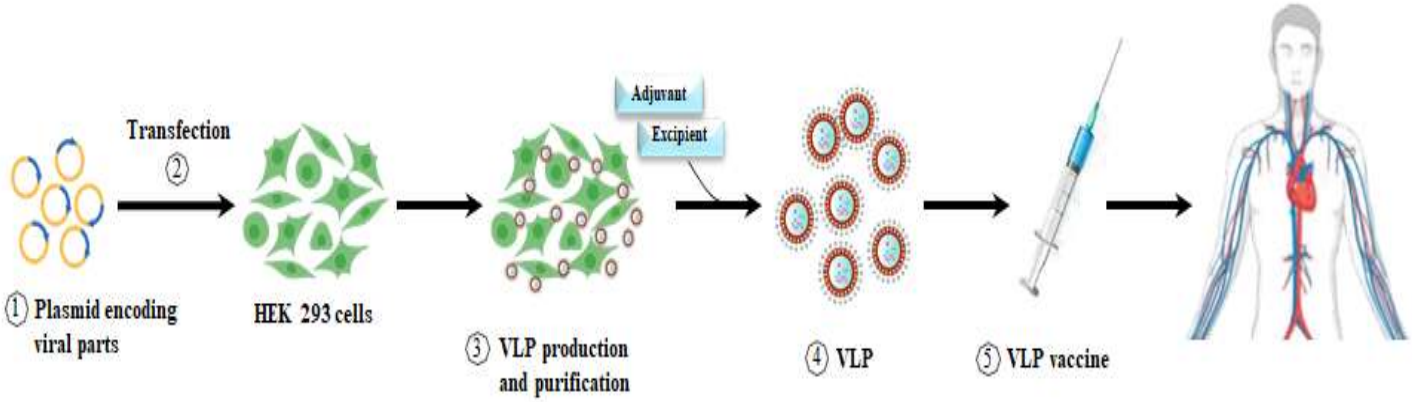

Figure 1. The process of producing a VLP-based vaccine involves three steps: 1) The production stage involves cloning the viral genes and producing self-assembling viral proteins using a suitable expression platform such as HEK 293T cells. The result is the collection of VLPs in particle form without 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.

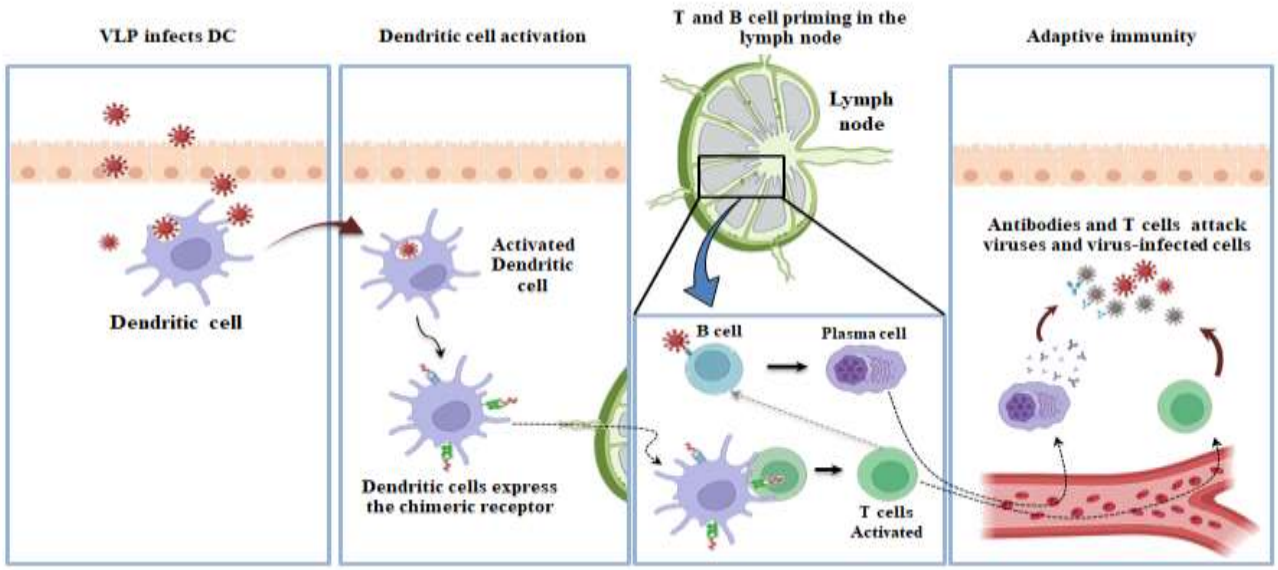

Figure 2. The induction of both innate and adaptive immunological responses, which includes humoral immunity and cell-mediated immunity, by using VLPs (virus-like particles), results in improved absorption and presentation of antigens by APCs (antigen-presenting cells) such as dendritic cells, which 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.

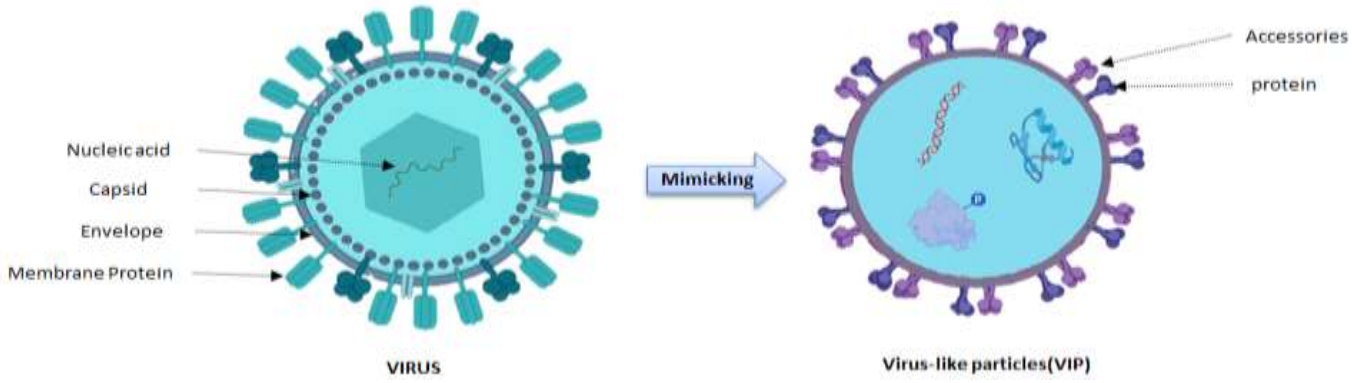

Figure 3. Non-enveloped and enveloped virus VLP as a platform for the delivery of foreign small molecules.

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 can be synthesized into VLPs through the co-expression of these two proteins (11).

2.1.2. 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 envelope's structure, origin, and composition differ among 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).

2.1.3. Various VLPs Development Methods

Virus-like nanoparticles might be employed as a new delivery strategy, due 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 and are commonly formed in living organisms. Internal, external, and inter-subunit functional elements of the nanoparticles may be employed to fine-tune their stability, optimize drug encapsulation, adjust surface charge, and enhance ligand presentation (14).

Some virus-like particles (VLPs) are more effective as nanocarriers. In particular, Bacteriophage MS2 VLPs excel as delivery vehicles for transporting therapeutic agents. Recombinant-protein technology makes it easy to generate these VLPs (15, 16). The MS2 capsid interacts with 'PAC' DNA or RNA, encapsulating it at the five prime termini to protect against nuclease damage. It also effectively delivers antigenic components and epitopes to patients (17). Moreover, the epitope peptide may be presented 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, they can be used as selective and passive Nano transporters. Drugs may be packaged to MS2CP, MS2 (19). The target environment, the type of drug being loaded, and the structure all influence the current particulate techniques used (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, while murine polyomavirus has been investigated for delivering methotrexate (19, 22). Researchers have 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 destroy DNA and may help treat lymphoma, leukemia, and breast cancer (25). Furthermore, the cucumber mosaic virus was utilized to encapsulate doxorubicin specifically to target folate-expressing cancer cells, resulting in anti-tumor responses that were more effective than those achieved with free doxorubicin (26).

RNA interference (RNAi) is another cancer treatment option that is challenging in vivo. However, the JC virus VLPs are 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 targeted 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 recently gained popularity in vaccine research and has been applied to various therapeutic uses such as antigen processing and immunostimulant adjuvants.

Nanovaccinology is largely used to treat cancer, Alzheimer's, hypertension, and nicotine addiction as well (29). Self-assembly of viral capsids requires just one viral capsid protein. Most virus-like particles (VLPs) are produced using insect or yeast cells, as these systems are straightforward and capable of generating complex viral protein targets for vaccines (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 1980s using 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), produced using recombinant DNA technology (32). Engerix-B and Recombivax-HB are two vaccines for hepatitis B, while GSK's Gardasil and Cervarix are vaccines produced using yeast and insect cells, respectively. All four vaccines were developed 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 vaccins because they include Pre-S1, Pre-S2, and hepatitis B surface antigens. The third-generation Bio-Hep B vaccine includes three types of surface proteins from the hepatitis B virus (HBV): Pre-S1 (large), Pre-S2 (middle), and small (s) proteins. These proteins are produced using mammalian cells, which are utilized to create approved virus-like particle (VLP)-based vaccines E. coli has been used to produce many recombinant protein products, including the first human insulin. Between 1986 and 2015, about 50 VLP vaccines were developed. E. coli is used to produce the VLP vaccines such as Hecolin and Heber Nasvac. 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 stimulate protective immune responses when combined with an adjuvant. The adsorbed VLPs of each HPV type are combined to form GARDASIL (aluminum-containing adjuvant) (33).

The impact of aluminum adjuvant surface exposure on VLP morphology is investigated. The results indicated that the adjuvant's adsorption had minimal influence on the morphology, and MelQbG10 virus-like nanoparticles containing CpG-oligonucleotides and a Melan-A peptide were developed as an anticancer vaccine (37). Researchers used Gag and M1, two influenza virus capsid proteins, to create chimeric viruses featuring a variable fragment region that targets colon cancer cells. These chimeric virus-like particles (VLPs) were packaged in huge, single-walled unilamellar containers to protect them from cancer-killing agents. VLPs are highly selective for cancer cells and effective in transporting dyes and drugs (38). Lentiviral vectors were long the preferred method of gene silencing in mice. These vectors may cause mutagenesis and cancer through DNA incorporation into the host's genome, which limits their use in medicine (39).

2.1.4. Vaccination and Gene Therapy

Delivering DNA, mRNA, and tiny RNA molecules to target cells to replace faulty genes or control protein production is one of the most complex components of creating gene therapy. Contrary to common opinion, gene therapy has had minor success using anything other than adenoviral vectors, adeno-associated viruses, and lentiviral vectors in vitro and in vivo (40). The lentiviral system, in particular, has the potential to generate mutagenesis and carcinogenesis; consequently, it must be extensively examined for safety before it can be employed. Using their reversible construction, VLPs may be created in vitro with nucleic acids, peptides (like p19), or stem-loop RNAs; a favorable influence of this on the field of nano vehicles for nucleic acid delivery has been documented in the literature (41).

VLPs protect therapeutic cargos before they reach the target cell. They are non-toxic and inert. In contrast to lipid agents, cationic polymers and polypeptides are typical penetrating agents (42). This approach of targeted gene delivery may lower the immune response, boost therapeutic molecule efficiency, reduce therapeutic cargo dosage, promote bioavailability, decrease cytotoxicity, and limit undesirable effects (43). Capsid proteins applied for gene therapy are most commonly derived from plant and animal viruses (such as TMV, CPMV, CCMV and BMV) and from cytomegalovirus (like JCV and HBV). Plant virus-derived VLPs may often be assembled in vitro or in vivo, depending on the conditions. The process of in

vitro purification involves isolating the virus from an infected plant by dissecting the capsid protein and reassembling it in various solutions (44). Viral particles produced using this method may have morphologies that differ from the original virus. A host produces viral proteins to generate VLPs without encapsulating infectious DNA in vivo. Proteins that form the capsids of plant viruses, yeast, or E. coli can self-assemble into more complex structures (icosahedral and helical symmetry) (45).

It is critical, given the lower cost of adenovirus, adeno-associated virus, and lentiviral expression one systems compared to mammalian or insect-based expression systems. This can be achieved either before or after capsid protein assembly, using ultracentrifugation, 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 require the JCV VP1 epitopes to target cancer cells. Recently, bacteriophage-based VLPs have been utelized for medicinal purposes, including the delivery of antisense oligonucleotides and siRNA both in vitro and in vivo using MS2 bacteriophage-based VLPs. Additionally the delivery of vaccine adjuvants containing unmethylated CG motifs has been achieved using Q VLPs. Some of these applications overlap with the mRNA-based vaccines previously mentioned. However, improving the efficiency of VLP production and packaging remains a challenge in the vaccine development industry (15). An essential initial step in the development of effective drug delivery systems is determining the packaging signal required to effectively and selectively encapsulate the payload within the host cell. The viral genome contains sequences or structural elements that ensure proper packaging of genetic material. Each batch of VLPs will contain the same RNA content if it includes the correct capsid protein packaging signal. However, further study is required to understand the selective packaging of ssRNA viruses (50).

2.1.5. The Challenges and Potential Development of VLP

Designing, purifying and storing vaccines based on eVLPs presents 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 compared to protein-only VLPs. Temperature, shear stress, and chemical treatment can affect particle integrity and stability. Structural degradation induced by environmental factors reduces the immunogenicity of eVLPs. Low temperatures are used to modify virus-like particles (VLPs) in order to enhance their thermostability and improve their stability during storage and transportation.

Stabilizing mutations are common. Stabilizing mutations are common in virus-like particles (VLPs). A study on poliovirus type 3 VLPs showed that their structure is preserved despite changes in coat proteins. Enhancing transmembrane glycoprotein expression can be achieved by replacing the transmembrane region, facilitating the release of enveloped virus-like particles (eVLPs) (JEV) (1, 8, 52, 53). This could be better when membrane-integration-dependent oligomerization is necessary for a protein's function or immunogenicity. Adding a signal peptide can enhance eVLP secretion. Cells that co-express prM and E cannot generate eVLPs, which is an essential stage DENV VLP formation. Cells can create eVLPs when a JEV signal sequence is introduced at the N terminus of prME. Signal peptides affect the translocation and topology of downstream proteins (54). Purified eVLPs must be inactivated to reduce baculovirus infectivity to meet security criteria; however, this process may affect antigenicity (52). To improve product purity without compromising eVLP immunogenicity, employ successive purification approaches such as clarification, intermediate purification, and polishing. The purification procedures may include centrifugation, precipitation, ultrafiltration, and chromatography (55).

3. Results

Virus-like particles (VLPs) present a groundbreaking method for targeted drug delivery systems, minimizing systemic toxicity and reducing harm to healthy cells. Immunotherapy, which enhances the host's immune response, typically has fewer adverse effects. Cancer vaccines are designed to trigger an immune reaction that protects against tumor cells. Thanks to their naturally optimized particle size and repetitive structural organization, VLPs can function as vaccines without the need for adjuvants. Recombinant VLP architectures can be improved by incorporating antigenic epitopes from viruses or various disease-related antigens, as well as targeting peptides on both their internal and external surfaces, positioning them as promising candidates for future immunizations with both preventive and regenerative properties. Furthermore, VLP-based delivery methods may boost immunogenicity, offering a more effective and safer strategy for managing solid tumors with fewer side effects than traditional chemotherapy or radiation therapies. Nonetheless, the production of chimeric VLPs continues to encounter challenges, including the necessity for more dependable preclinical animal models and associated costs. Despite these hurdles, ongoing research is expected to enhance VLP-based technologies and amplify their potential benefits.

4. Conclusion

Unique properties of VLPs, such as multimer antigens, particle shape, and non-infectivity have recently emerged as promising candidates for vaccination and targeted drug delivery. The assembly, disassembly/reassembly, and self-assembly properties of VLPs have enabled various drug encapsulation and targeting approaches. The ability to safely stimulate humoral and cellular immune responses is a crucial advantage of VLPs, making them suitable for in vivo therapy without concern about toxicity or inflammation. VLPs may be chemically or genetically modified for specific purposes. Recombinant VLP structures can be enhanced by including antigenic epitopes from viruses or different disease-related antigens, along with targeting peptides on the interior and exterior surfaces, making them potential tools for future immunizations with both preventive and regenerative qualities. Additionally, VLP-based delivery strategies may enhance immunogenicity and provide a more effective and safer approach to managing solid cancers, with fewer side effects compared to chemotherapy or radiation therapy. However, the production of chimeric VLPs still face 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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Authors' Contribution

Study concept and design: M.F
Acquisition of data: M.F, HEGG
Analysis and interpretation of data: M.M, A.Z
Drafting of the manuscript: M.F, HEGG, M.M, A.Z, M.M.N
Critical revision of the manuscript for important intellectual content: M.F, HEGG, M.M, A.Z, M.M.N
Statistical analysis: M.M, A.Z, M.F
Administrative, technical, and material support: M.F, HEGG

Ethics

The study protocol was reviewed and approved by the ethics committee of the Baqiyatallah University of Medical Sciences (IR.BMSU.REC.1401.060).

Conflict of Interest

The authors report no conflicts of interest.

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Data Availability

Not applicable

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