



Research Article

Virus Like Particles: Nanoparticles for Targeted Drug Delivery

Khaled A. El-DougDoug¹, Wael S. El-Araby^{2*} and Rehab, A. Dawoud³

¹Department of Microbiology, Faculty of Agriculture Ain Shams University, Cairo, Egypt; ²Department of Virus and Phytoplasma, Plant disease institute, Agri. Res. center. Giza, Egypt; ³Department of Biology, Faculty of Science, Jazan University, Jazan 45142, P.O.Box:114, Saudi Arabia.

Abstract | Virus-like particles (VLPs) are biological entities at the nanoscale level that are made of viral proteins organized in a morphology that resembles the original virion but lacks the viral genetic material. VLPs are appealing as a system because their proteins can be altered both chemically and genetically, opening up a wide range of potential uses. Virus-like particles (VLPs) are ideal for antigen and medication administration because viruses are strong immune activators and efficient vectors for transporting genetic elements into host cells. Recent developments in VLP platform engineering, activation, and release strategies, in addition to their application to drug administration, are discussed. The utilize of VLPs for drug delivery in model systems is discussed, and various examples of its effectiveness are highlighted.

Received | November 26, 2022; **Accepted** | March 13, 2023; **Published** | July 15, 2023

***Correspondence** | Wael S. El-Araby, Virus Research Dept, Plant Pathology Research Institute, ARC, Giza 12619, Egypt; **Email:** biowael@yahoo.com

Citation | El-DougDoug, K.A., W.S. El-Araby and R.A. Dawoud. 2023. Virus like particles: Nanoparticles for targeted drug delivery. Journal of Virological Sciences, 11(2): 25-44.

DOI | <https://dx.doi.org/10.17582/journal.jvs/2023/11.2.25.44>

Keywords | VLPs, Drug-delivery, Nanoparticles, Virus plants, Host



Copyright: 2023 by the authors. Licensee ResearchersLinks Ltd, England, UK.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Introduction

Viral-like particles (VLPs) are consisting of assembled viral proteins at the nanoscale. These structures are devoid of viral genetic material as well as are consequently non-pathogenic (Bai *et al.*, 2008). Dispersed nanoparticles called very long polymers (VLPs) may be manufactured in a wide range of organisms. Due to the presence of a cavity in their structure, VLPs have the potential to be utilized as vehicles for the delivery of biomaterials and nanomaterials, including pharmaceuticals, vaccines, quantum dots, and imaging compounds (Steinmetz, 2010; Chung *et al.*, 2020). Virus-like particles (VLPs)

are self-assembled entities of icosahedral or rod form that are composed of viral structural proteins (Pushko *et al.*, 2013). In 1968, researchers discovered these nanoparticle formations in the blood samples of people with Down syndrome, leukemia, and hepatitis. Although it was demonstrated that these particles have antigenic sites on their surface, their actual biological origin remained a mystery (Bayer *et al.*, 2018). It was subsequently established that VLP structures may be formed by the capsid, envelope, and even core viral proteins of some viruses. Recombinant viral proteins may be created in a number of different expression systems, involving prokaryotic cells, plant cell lines, yeast, insect cell lines, as well as mammalian

cell lines, can be used to make VLPs in the lab for use in end experiments.

Chimeric VLPs can be prepared through assembling structural proteins from various viruses; although most VLPs are made utilizing proteins from a single viral species.

Viral structural proteins have been used in the production of VLPs, and these viruses include HIV, AAV, HBV and HCV, as well as bacteriophages.

These particles come in a variety of sizes, with the majority falling between twenty to two hundreds nm. VLPs are very ordered structures that, according to the geometry on which they are based, resemble pathogen-associated structural patterns (PASP) that are readily identified by the immune system's cells and molecules. VLPs may be broken down into two broad categories, enveloped and non-enveloped, and further subdivided into those with proteins arranged in a single layer, two layers, or more. VLPs are being utilized for a variety of uses. Due to the presence of an internal cavity, these entities possess the capability to function as highly effective carriers for various biological substances, such as proteins, genes, peptides, and minor medications. One notable characteristic of these carriers is their capability to facilitate delivery of targeted drugs. Furthermore, their improved attributes of permeability and retention render them an appealing option for drug delivery to tumor tissues, enabling both therapy administration as well as tumor imaging.

VLPs have been put to good use in the field of vaccinology due to the benefits they provide over more traditional vaccination methods. This is because VLPs are similar in both form and size to native viruses, which allows them to effectively elicit immune responses; additionally, because VLPs lack viral genomes, Replication is not conceivable within the target cells, providing increased safety, especially for immunocompromised or elderly vaccines. Because of their versatility, VLPs may elicit humoral and cellular immune responses, and they can be enhanced by the addition of immunomodulators such stimulants for the innate immune system. Several vaccines depend on VLPs have been licensed to be utilized in the clinic, thus these vaccines are now commercially accessible.

A largely exploited application of VLPs is their

potential in vaccinology where they can offer several advantages over conventional vaccine approaches (Garg and Dewangan, 2020; Donaldson *et al.*, 2018). Because of their size and shape, which resembles the actual size and shape of native viruses, these structures can efficiently elicit the immune responses and in VLPs lacking viral genomes there is no potential for replication within the target cells, which offers improved safety especially for immunocompromised or elderly vaccines (Balke and Zeltins, 2019). While VLPs can stimulate both humoral and cellular immune responses (Wang *et al.*, 2017) they can also be loaded with immune-modulators, such as innate immune system stimuli to provoke more effective immune responses. Several VLP-based vaccines have been approved for use in the clinic and are now commercially available with others in various phases of clinical trials (Table 1). This review article describes the classification of VLPs and considers the immunogenicity of VLP-based vaccines. Different expression systems for recombinant protein production and production of VLP proteins are discussed. Applications of VLPs as vaccines in the prevention of infectious diseases and cancers as well as their future prospects, are discuss.

Structural and classification of VLPs

The ultimate structure of a VLP is generated from the unplanned combination of single or more viral structural capsid proteins. VLPs look and behave like real viruses but shortage either the viral genome or the viral genome in its entirety. VLPs are visually and functionally appealing due to the wide range of structures they adopt. VLPs can take the shape of a sphere, a rod, or an icosahedron according to the viral capsid protein that was used to create them during spontaneous polymerization. The structural complexity of VLPs allows for convenient categorization. Proteins in capsids can be stacked in a single, double, or triple layer structure. Multiple structural proteins may be present in other types of single-layer VLPs. Multi-protein VLPs, in contrast to single-protein VLPs, exhibit complex structures, including the existence of several capsid layers (Figure 1a). Other VLPs, such as those derived from HIV-1 and influenza virus, have a layer of lipid that contains viral surface antigens surrounding the capsid structure, reflecting the lipid envelope found in the natural infectious virus particle. The presence or absence of the envelope provides an additional structural classification for VLPs (Figure 1b). Frequently enveloped VLPs contain a matrix

Table 1: Vaccines against infectious diseases based on VLP: clinical trial or FDA-approved.

Trade name	Infectious agent	Target disease	Status	Company	Antigen(s)	Expression system
Gardasil®	(HPV)	HPV, Types 6, 11, 16, 18, 31, 33, 45, 52, and 58	Approved	Merck	Major capsid protein L1 epitope of HPV types 6, 11, 16, and 18	Yeast
Gardasil9R	(HPV)	HPV, Types 6, 11, 16, 18, 31, 33, 45, 52, and 58	Approved	Merck	Major capsid protein L1 epitope of HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58	Yeast
CervarixR	Virus (HPV)	HPV (Types 16 and 18)	Approved	Glaxo-smith Kline Inc	Major capsid protein L1 epitope of HPV types 16 and 18	Insect cells
Sci-B-Vac™	HBV	HBV	Approved	VBI vaccines	The 3 epitopes of HBV surface antigen: S, Pre-S1, and Pre-S2	Eukaryotic cells (Chinese hamster ovary (CHO) cells)
Mosquirix™	Plasmodium falciparum	Malaria	Approved	Glaxo-SmithKline Inc	Plasmodium falciparum circumsporozoite protein fused to HBV surface antigen, combined with HBV surface antigen (S)	Yeast
Still no trade name	Influenza Virus	Seasonal Flu Clinical trial Phase 3	(Clinical trial No.: NCT03301051)	Medicago	A mix of recombinant H1, H3, and 2 B hemagglutinin Medicago proteins	Plant (<i>Nicotiana benthamiana</i>)
Still no trade name	Influenza Virus	Pandemic Flu	Clinical trial Phase 2	Medicago	Hemagglutinin protein	Plant
COVID-19	Still no trade name SARS-CoV-2	Virus clinical trial Phase 1	(Clinical trial No.: NCT04450004)	Medicago	SARS-CoV-2 spike protein	Plant (<i>Nicotiana benthamiana</i>)

protein located immediately inside the host-derived lipid membrane in which the viral glycoproteins embedded. The requirement for generation of a lipid envelope and the targeting of virus proteins to the lipid bilayer for some VLPs places requirements on the choice of production system that can be used for their generation (Chroboczek *et al.*, 2014).

When compared to the lipid envelope seen in naturally infectious virus particles, the lipid layer surrounding the capsid structure of other VLPs, like those generated from HIV-1 and influenza virus, contains viral surface antigens. A further structural categorization of VLPs is based on whether or not they have an envelope. The viral glycoproteins in enveloped VLPs are often encased in a matrix protein that is localized just inside the host-derived lipid membrane. Some VLPs have specific criteria for the production system that can be employed for their development, such as the formation of a lipid envelope as well as the targeting of viral proteins to the lipid bilayer.

Manufacturing, purifying, and developing a VLP vaccine
In general, three primary components comprise the

generic manufacturing procedure for a VLP-based vaccine: (A) production-related upstream processing, (B) purification-related downstream processing, and (C) formulation. Cloning the viral structural genes of interest is the initial move in VLP production. Viral structural proteins capable of self-assembly are subsequently expressed in eukaryotic (baculovirus/insect cell, mammalian cell, and plant) or prokaryotic (bacteria, yeast) expression systems. Following the harvesting and lysis of the cells, a clarification phase is executed to guarantee the elimination of contaminating cell debris and aggregates. In order to obtain VLPs that are more intact and purified, additional purification procedures are required, including ion-exchange chromatography as well as ultracentrifugation (Peixoto *et al.*, 2007; Vicente *et al.*, 2011; Hillebrandt *et al.*, 2020).

Polishing is the last stage in purification and is utilized to get rid of any lingering host cell proteins or nucleic acids. Sterile filtering and formulation are the last stages of the VLPs vaccine production process, resulting in a vaccine that is free of contaminants and highly effective. In the last step of manufacturing

process of VLPs vaccine development, sterile filtration and formulation is done to finally achieve a safe, efficient and effective product (Figure 2) (Vicente *et al.*, 2011).

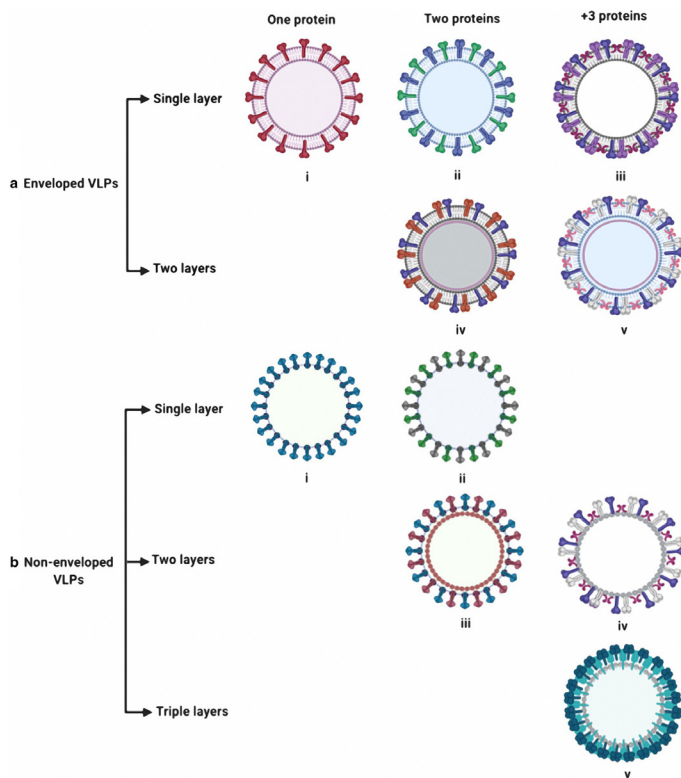


Figure 1: Synopsis of the structure of VLPs.

a As illustrated by the expression of influenza virus hemagglutinin alone or the co-expression of hemagglutinin and neuraminidase, expressed in enveloped VLPs, a single layer can be formed by the expression of 1 (i) or 2 glycoproteins. A single-layer VLP will also result from the expression of over three glycoproteins (iii). 2 (iv) or more than 3 glycoproteins (v) may be present on the surface of double-layered enveloped VLPs, which are capable of forming such structures. *b* A single protein (i) or 2 proteins (ii) may be utilized in the assembly of single layered non-enveloped VLPs. 2 proteins (iii) or greater than 3 proteins (iv) may be utilized in the construction of double-layered non-enveloped VLPs. In excess of 3 proteins, triple-layered VLPs (v) have been constructed.

Expression platforms for producing VLPs

Various expression platforms including prokaryotic, and eukaryotic systems can be used for producing VLP vaccines (Syomin and Ilyin, 2019; Lünsdorf *et al.*, 2011). Eukaryotic systems that have been used include the baculovirus/insect cell (B/IC) system (Syomin *et al.*, 2004), mammalian cell culture (Ren *et al.*, 1997) and plants (Scotti and Rybicki, 2013). In addition cell-free expression systems have also been used successfully. Choosing an appropriate expression

system to produce VLPs is a crucial factor to ensure proper protein folding and post-translational modifications (PTM) (Fuenmayor *et al.*, 2017). The quaternary structure of viral capsid proteins can differ in different expression systems due to protein PTMs such as glycosylation and phosphorylation and this may affect the immunogenicity of the vaccine as PTMs are often necessary to stimulate an appropriate immune response (Donaldson *et al.*, 2015). Each expression system has benefits and drawbacks which are briefly highlighted below.

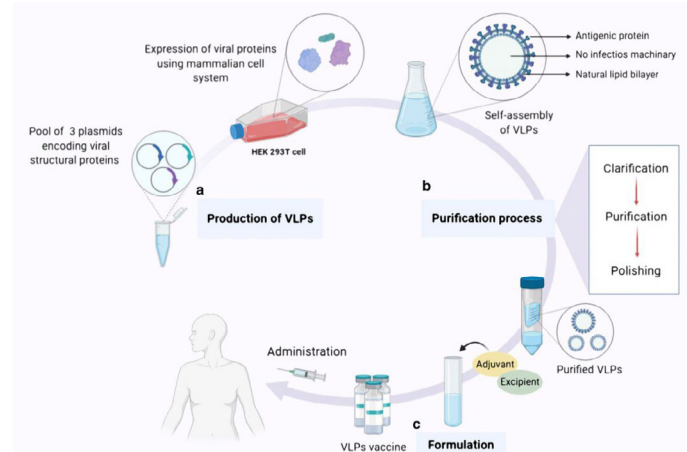


Figure 2: An overview of the expression, purification, and formulation of vaccines based on VLPs. There are typically three steps involved in producing a vaccination based on VLPs. The first step is called “Production,” and it entails cloning the viral structural genes of interest and then expressing the viral proteins that have the potential to self-assemble in an appropriate expression platform (in this case, the HEK293T cell line, a mammalian expression system). The final product of this process is a collection of VLPs in the form of noninfectious particles. *b* Purification phase, which includes downstream processes such as clarification, purification, and refining, culminates in the acquisition of intact purified VLPs devoid of any residual host detritus. *c* The formulation stage, during which adjuvants as well as supplementary ingredients are incorporated into the vaccine formulation to attain a final product that is not only secure but also efficient and effective for vaccination purposes.

Bacteria: Many virus-like particles (VLPs) are manufactured using bacteria, an expression system that is also commonly utilized for the synthesis of recombinant proteins. However, they are not appropriate platforms for manufacturing enveloped VLPs because of variables as a shortage of PTM system, insufficient disulfide bond formation, and protein solubility issues (Huang *et al.*, 2017).

Non-enveloped VLPs (Naskalska and Pyrc, 2015) containing only 1 or 2 viral structural proteins may be easily produced in bacteria, making this organism an ideal expression system (Hu *et al.*, 2014; Duffy and Patrick Gorres, 2020; De Filette *et al.*, 2006).

A major barrier to vaccination use, especially in underdeveloped nations, is the elevated price of the product. Since a prokaryotic-based expression system has the potential to provide safe and cost-effective vaccinations for worldwide usage, it is frequently considered the best for creation of VLPs vaccines (Lacson *et al.*, 2005).

Most VLPs are made in *Escherichia coli*, a kind of bacterium. The advantages of employing an *E. coli* expression system include high protein expression levels, quick cell growth, low production costs, and easy scalability. For the mass production of low-PTM, tiny proteins, the *E. coli* expression method is often recommended. Clinical studies using many different VLP vaccines against infectious and non-infectious disorders have begun, all of which were produced utilizing *E. coli* expression systems. Xiamen's p239 VLP-based Heculin Hepatitis E vaccine was the first *E. coli*-expressed vaccine authorized for use against Hepatitis E virus (HEV) (Nardin *et al.*, 2004).

Yeast: Yeast cells have been utilized to make virus-like particles (VLPs) in addition to expressing recombinant proteins. Due to their many benefits, including fast cell growth, a high yield of expression proteins, scalable, cost-effective production, and a degree of post-translational modification (PTM) processes (Dai *et al.*, 2018), yeast expression platforms, particularly *Saccharomyces cerevisiae* and *Pichia pastoris*, are the most popular options. Using yeast expression systems (Keating and Noble, 2003; Block *et al.*, 2006) researchers have developed two vaccines depend on VLPs that have since been authorized by the Food and Drug Administration (FDA). It was recently discovered that *Pichia pastoris* can be used to manufacture Chikungunya virus-like particles (CHIK-VLPs). Despite these advances, the use of yeast expression systems for VLP generation is restricted due to the absence of complicated PTM pathways (Saraswat *et al.*, 2016). Other possible problems include increased mannose glycosylation, plasmid loss, and decreased protein outputs contrasted to the bacterial expression method.

Therefore, non-enveloped VLPs are often produced using yeast-based methods. HIV-1 Gag protein VLPs and, on the other hand, have been effectively engineered in yeast systems (Liu *et al.*, 2010).

Baculovirus/ Insect cells (B/IC)

For the most part, enveloped and non-enveloped VLPs are produced using the B/IC expression method. This technique is well-suited to the production of vaccines against viruses like influenza that often alter their surface antigens between outbreaks because of the ease and speed with which baculovirus-based VLP (Dai *et al.*, 2018) expression may be accomplished. The benefits of insect cell expression methods for VLP generation include a protein output that is on par with that of bacterial or yeast systems, the existence of complicated PTM pathways, and the formation of multi-protein VLPs. *Spodoptera frugiperda* (Sf9/Sf21) and *Trichoplusia ni* (Tn5) are the most common insect cell lines utilized in the production of recombinant proteins (Fuenmayor *et al.*, 2017).

Cervarix, the HPV vaccine authorized by the FDA, was manufactured utilizing this expression system and comprises VLPs (Dai *et al.*, 2018) based on the L1 protein of HPV16 and HPV18. Prophylactic vaccine candidates against a variety of infectious diseases, including HIV 1, influenza virus A, Chikungunya virus, severe acute respiratory syndrome (SARS), Ebola virus, dengue fever virus, RVFV, Norwalk virus, as well as HCV, have also been obtained using the Baculovirus/insect cell platform (Liu *et al.*, 2010).

Plant cells

In contrast to conventional methods, plant expression systems offer a number of benefits, such as fast expression processing, minimal refining expenses, and the ability to produce up to eighty percent of total soluble protein. By leveraging the capabilities of MagnICON as well as CPMV-HT technology, a platform for protein production derived from plants has emerged as a versatile and auspicious invention, enabling the synthesis (Gleba *et al.*, 2005; Shirbaghaee and Bolhassani, 2016) of numerous proteins as well antibodies utilized in the veterinary as well as individuals pharmaceutical sectors for less than \$50 per gram. The extensive examination of TMV (Harrison and Wilson, 1999) (Figure 3), which has included landmark developments in the field of recombinant plant-based vaccines, has included genome analysis, the analysis of the viral particles' three-dimensional

(3D) structure, as well as the locating of surface areas that are visible thanks to structural research (Rybicki, 2020).

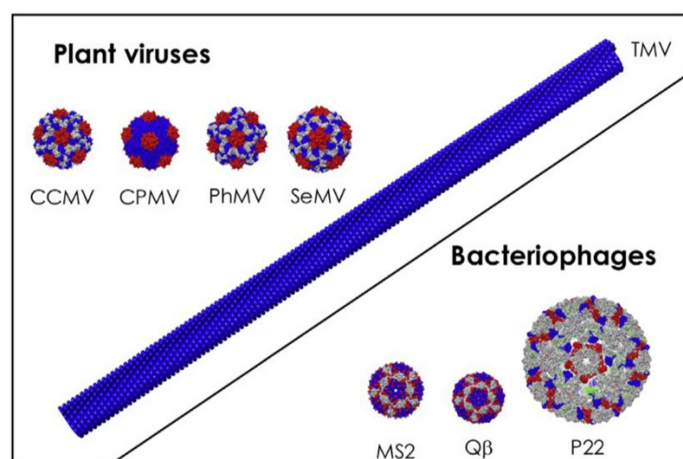


Figure 3: Numerous experimental vaccines have been effectively developed using noninfectious plant VLPs as their foundation. A substrate for antigen expression on the surfaces of over fifty-five distinct plant viruses has been established, which includes but is not limited to the potato X virus (PVX), cucumber mosaic virus (CMV), alfalfa mosaic virus (AIMV), cowpea mosaic virus (CPMV), and papaya mosaic virus (PapMV). TMV, PVX, CPMV, and CCMV are significantly more stable at elevated temperatures as well pH levels, and they are abundantly expressed in native plant hosts.

Purification of VLP based vaccines

It is critical to perform downstream processing for VLP purification in order to guarantee adequate efficacy and safety for clinical application. The initial phase of the purification process, which occurs subsequent to cell retrieval, is determined by the capacity of the VLPs to be liberated into the extracellular medium. Although there have been reports of influenza VLPs produced in insect cell culture being released into medium without special procedures, disruption of the cells may be necessary if the VLP cannot be released effectively. In such cases, cell lysis or an altar-native extraction method may be necessary. The prevailing methodology entails the development of a cloned gene that encodes a protein harboring a potent signal peptide that facilitates release by being recognized by the secretory pathway. In an effort to cut down on the amount of stages and expenses associated with the purification procedure, aggregates and whole cell debris are eliminated from primary VLP preparations via a clarification phase. Critical to the process, VLP concentration as well as capture substantially decrease aggregate volume as well elevate the proportion

of VLP concentration to other cellular impurities. Clarification can be achieved through the utilization of diverse techniques, such as cell sedimentation, depth filtration, and micro-filtration TFF. Ultrafiltration/diafiltration (UF/DF) and TFF using membranes or hollow fibers are often employed to separate the VLPs from host cell contaminants such cell debris, digested DNA, or components of the medium. It is also possible to capture VLPs using a bind-and-elute strategy, which involves utilizing the techniques of affinity chromatography, ion-exchange chromatography, plus hydrophobic interaction chromatography. Reducing DNA and endotoxin levels often necessitates further purification procedures, including IEC, HIC, and Super centrifuge.

To improve the stability, homogeneity, and immunogenicity of the final VLPs product, disassembly and reassembly can be conducted as a standalone process. This stage may involve titration or cross-flow filtration. All remaining impurities from VLP processing must be eliminated in the last purification stage, known as the polishing phase. The IEC, SEC, and UF/DF (often the crossflow approach) are frequently used to do this. Filtration using 0.22 μm sterile-grade filters is used to sanitize the preparations before they are used in the final formulation (Figure 4).

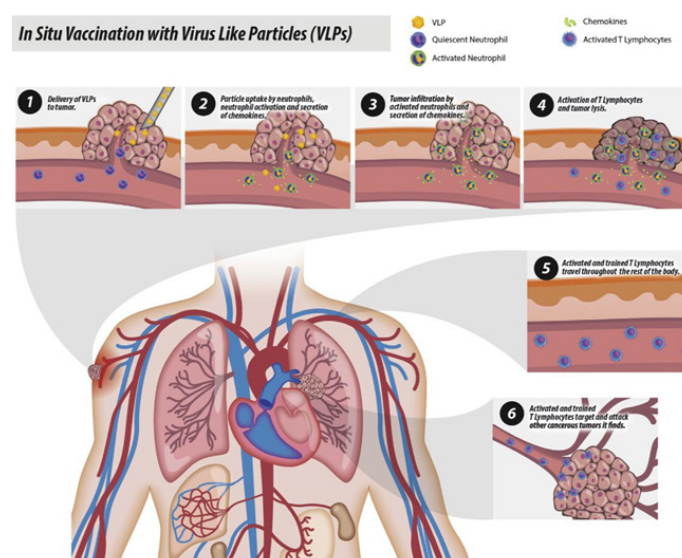


Figure 4: Formulation of VLP-based vaccines.

The objective of vaccine formulation is to enhance the safety, effectiveness, as well as stability of the vaccine from the time it is stored and transported until it is administered. In order to enhance the effectiveness of VLPs, adjuvants as well authorized excipients are commonly incorporated into vaccine

formulations. These excipients serve the purpose of safeguarding VLPs against physical and chemical instability, as well as enzymatic degradation. In the final product formulation, excipients consist of buffers, preservatives, and other stabilizing chemical compounds. In the formulation of VLP-based vaccines, additives including 2-phenoxyethanol, sodium borate/phosphate, polysorbate 80, and L-histidine are frequently employed as buffering agents, surfactant stabilizers, and preservatives, respectively.

Increased stability in liquid suspension of Norwalk and rotavirus VLPs has been seen after adding carbohydrate preservatives including glycerol, sucrose, and trehalose to vaccination formulations. All polyanions studied were able to stabilize CHIKV VLPs against aggregation, as determined by studying the results of adding a polyanion to the CHIKV VLPs that are unstable at neutral pH.

There is no requirement for adjuvants when using many VLPs since their molecular and structural features can raise the body's defenses on their own. However, adding adjuvants to VLPs vaccine formulations has the potential to boost the vaccine's immunogenicity and elicit a more targeted immune response. Different types of adjuvants, including aluminum salt-based (Alum) adjuvant, liposome/virosome adjuvant, agonist adjuvant for pattern recognition receptors (PRRs), emulsion adjuvant, IL12, chitosan, and bacterial toxin, have all been used for VLP vaccinations.

All licensed VLPs vaccines have been formulated with aluminum salts, the most common adjuvant utilized in vaccine production. Alum-based vaccines are made with insoluble aluminum salts like aluminum phosphate, aluminum hydroxyl-phosphate, and aluminum hydroxide. Commercially available VLP-based vaccinations that include aluminum salts adjuvant include Engerix-B (hepatitis B virus vaccine), Gardasil (HPV vaccine), Cervarix (HPV vaccine), and Hecolin (HEV vaccine). Two commercially available examples of VLPs vaccines using virosomal adjuvants are Inflexal (influenza vaccine) and Epaxal (HEV).

Culture mode utilized for the production of multimeric VLP

The VLPs were produced using 3 distinct cultivation methods: Batch, fedbatch, and continuous. The yield, productivity, and end product concentration

may all be affected by the process design, making it of paramount importance. Keeping your yield as near to the maximum theoretical value as possible might help you save money on your raw materials. The cost of downstream processing can be lowered and manufacturing equipment can be used more efficiently if its use is concentrated on periods of high product concentration and productivity. Many industrial procedures choose the batch mode because of its ease of use and minimal contamination risk. When using a bioreactor for batch culture, all of the media ingredients are introduced at once, and no new nutrients are added throughout the growth phase. However, this procedure seldom reaches its full potential. Major issues with the batch approach include substrate and product inhibition, low viral titers, susceptibility to temperature, low productivity (Balke and Zeltins, 2019), and inefficient utilize of time among batches. Maximum yield of the final product should be the goal, and all nonproductive procedures (bioreactor cleaning and filling, medium formulation, etc.) should be reduced for optimal performance of batch fermentation (Lee *et al.*, 2018). Several VLPs, such as those for HIV (Wang *et al.*, 2017), chikungunya (D'Aoust *et al.*, 2010), and Ebola (Tregoning, 2020), have been evaluated using this method. Substrate inhibition may be avoided in a fed-batch process by shortening the lag period, and the product concentration and yield can be improved by the strategic feeding of concentrated nutrients. The concentration of the limiting ingredient in the feed medium controls the response rate. This method of culture was evaluated in a baculovirus/insect cell expression system for the generation of parvovirus like particles (Rockman *et al.*, 2020) as well as recombinant HBsAg (Baglivo and Polack, 2019).

Continuous mode allows for maximum output as the culture is in the log phase at this point. Here, the conditioned medium is drained and replaced with new media. This approach allows for continuous production, but necessitates temperature and humidity controlled storage throughout the manufacturing process. In order to generate rabies VLPs, HEK293 cells have been employed to do so (Keech *et al.*, 2020). Continuous modes come in a wide variety, including the chemostat, turbidostat, stressostat, and morbidostat. When it comes to continuous cultivation, chemostat is by far the most popular (O'Donnell and Marzi, 2020).

Changing the concentration of the limiting substrate for a given strain allows one to regulate growth rates and product production in a chemostat. Low product concentrations, vast volumes of low-concentration broth produced by the system, and higher potential for contamination are all issues (Proffitt, 2012). When choosing a fermentation method, it's important to weigh the benefits and drawbacks of each option. Minimum investment required per unit of product yield is the primary consideration when choosing a bioreactor or fermentation process. For instance, measles virus is three hundreds nm-1 mm in size and causes lysis of host cells, making it more difficult to develop a continuous manufacturing process for Host cell proteins (HCPs) and cellular debris rapidly accumulate in the media when a cell is lysed. As a result, debris or HCPs clog the filter pores, making cultivation filtering difficult. This virus is better suited for continuous filtering or repeated

batch mode (Grein *et al.*, 2017; Weiss *et al.*, 2015). Table 2 summarizes the yields obtained from the various culturing methods. Vaccines based on viral enveloped particles (VLPs) are produced in stainless steel bioreactors. Single-use technology, on the other hand, is rising in prominence since it cuts down on contamination, expenses, and investment by doing away with the requirement for on-site cleaning and sterilization. However, disposable containers aren't as effective (Fuenmayor *et al.*, 2017). There are benefits and drawbacks to all three types of cultural practices (Table 3).

Characterization of VLPs

Purified VLPs must be characterized biochemically, biophysically, and biologically before their functionality, potency, and stability can be determined during manufacturing of vaccines based on VLPs (Lua *et al.*, 2014).

Table 2: VLP-based vaccines against different cancers.

VLP type	Cancer type	Antigen	Clinical phase	References
MS2	Cervical L2	L2	Preclinical	Zhai <i>et al.</i> , 2017
AP205	Cervical (and placental malaria)	HPV RG1 epitope (and VAR2CSA PM antigen)	Placental	Janitzek <i>et al.</i> , 2019
AP205	Breast	HER-2	Preclinical	Pouyanfard <i>et al.</i> , 2018
MS2	Breast	xCT	Preclinical	Rolih <i>et al.</i> , 2020
MS2	Breast	xCT	Preclinical	Bolli <i>et al.</i> , 2018
SHIV	Pancreatic	hMSLN	Preclinical	Zhang <i>et al.</i> , 2013
SIV	Pancreatic	mTrop2	Preclinical	Cubas <i>et al.</i> , 2011
eCPMV	Melanoma	Empty	Preclinical	Cubas <i>et al.</i> , 2011
Cucumber mosaic VLPs (CMV)	Melanoma	LCMV-gp33	Preclinical	Mohsen <i>et al.</i> , 2019
Bacteriophage Q β	Melanoma	PMEL17, MTC-1, Calpastatin, ZFP518, TRP-2, Caveolin2, Cpsf3l and Kif8b	Preclinical	Mohsen <i>et al.</i> , 2019

Table 3: Application of VLPs as drug delivery.

VLP	Cargo (Drug, Nucleic acids, Proteins)	Application	References
Adenovirus (AdV)	Bleomycin (BLM), Paclitaxel (PTX), mRNA cap analog	Tumor therapy	Zochowska <i>et al.</i> , 2015
Bacteriophage MS2	Doxorubicin, Cisplatin, 5-fluorouracil, siRNA, Ricin toxin A-chain (RTA)	Tumor therapy	Ashley <i>et al.</i> , 2011
Rotavirus [66]	Doxorubicin (DOX)	Tumor therapy	Zhao <i>et al.</i> , 2011
Cowpea mosaic virus (CPMV)	Doxorubicin (DOX)	Tumor therapy	Aljabali <i>et al.</i> , 2013
Cucumber mosaic virus (CMV)	Doxorubicin (DOX)	Tumor therapy	Zeng <i>et al.</i> , 2013
Hepatitis B virus (HBV)	siRNA	Tumor therapy	Choi <i>et al.</i> , 2013 ,
Polyomavirus	Methotrexate (MTX)	Tumor therapy	Abbing <i>et al.</i> , 2013
Filamentous (fd or M13)	Chloramphenicol	Antimicrobial drug	Yacoby <i>et al.</i> , 2006
Bacteriophage Q β	Azithromycin/clarithromycin	Antimicrobial drug	Crooke <i>et al.</i> , 2019

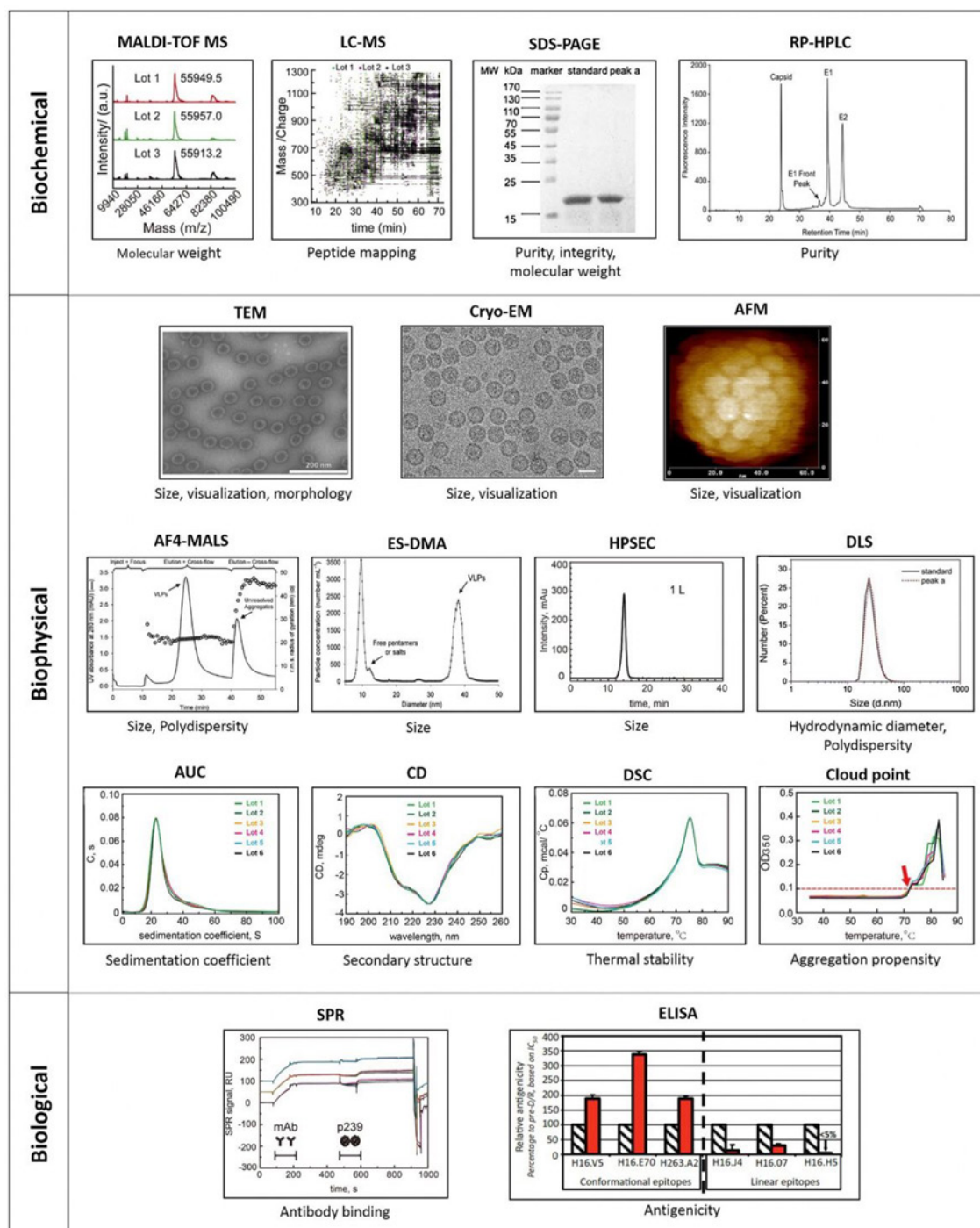


Figure 5: Analytical techniques for characterization of VLPs. A series of analytical tools has been used for biochemical, biophysical and biological characterization of VLPs. Biochemical method: Matrix assisted laser desorption ionization time of flight-mass spectrometry (MALDI-TOF MS), liquid chromatography-mass spectrometry (LC-MS), sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and reverse phase-high performance liquid chromatography (RP-HPLC). Biophysical method: Transmission electron microscopy (TEM), cryo-electron microscopy (Cryo-EM), atomic force microscopy (AFM), asymmetric flow field-flow fractionation coupled with multiple-angle light scattering (AF4-MALS), electrospray differential mobility analysis (ES-DMA) and high performance size exclusion chromatography (HPSEC), dynamic light scattering (DLS), analytical ultracentrifugation (AUC), Circular dichroism (CD), differential scanning calorimetry (DSC), Cloud point. Biological characterization: Surface plasmon resonance (SRP), enzyme-linked immunosorbent assay (ELISA). MALDI-TOF MS and LC-MS images are reprinted with permission from Elsevier. SDS- PAGE and DLS images are reprinted with permission from Elsevier, RP-HPLC image is reprinted with permission from Elsevier, TEM, A4-MALS and ES-DMA images are reprinted with permission from John Wiley and Sons. Cryo-EM image is reprinted with permission from Elsevier. AFM is reprinted with permission from Elsevier, HPSEC, AUC, CD, DSC, Cloud point and SPR images are reprinted with permission from Elsevier.

Amino acid sequence, molecular mass, isoelectric point, and purity are only some of the biochemical characteristics of VLPs that may be determined by characterization. The formed proteins' molecular mass, protein sequences, and amino acid content may all be examined by mass spectrometry (MS). Matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF MS) can be utilized to assess the molecular weights of VLPs (Figure 5).

Molecular mass, disulfide bond links, chemical changes, and PTMs are only some of the structural details made available by the combination of mass spectrometry and liquid chromatography. Therefore, the LC-MS method is an effective characterization tool for use in the manufacture of VLP-based vaccines (Sharma *et al.*, 2020).

For determining the molecular weight, purity, as well as integrity of VLPs, reverse phase-high performance liquid chromatography (RP-HPLC) (Yang *et al.*, 2015) as well as SDS-PAGE are the most frequently employed techniques. The primary drawback associated with the SDS-PAGE method is its arduous as well as time-intensive nature.

RP-HPLC methods are esteemed for quantifying the mass and purity of VLP-based vaccines on account of their exceptional sensitivity and reproducibility. Moreover, the RP-HPLC technique can be employed to characterize post-translational modifications in viral glycoproteins as well as facilitate product purity monitoring at both phases.

VLP-based vaccine as an inducer of adaptive immunity

Virus capsids commonly comprise a protein structure which is repetitive in nature and has the ability to activate innate immunity by directly stimulating B cells to generate neutralizing antibodies. As the intermediary between innate and adaptive immunity, DCs are among the most vital components of APC. VLPs typically range in size from ten to two hundred nm. Given that (Manolova *et al.*, 2008). DCs have the ability to ingest particles as small as one hundred to five hundred nm via phagocytosis as well as macropinocytosis, VLPs are highly suitable for acquisition prior to the immune system's (Al-Barwani *et al.*, 2014; Win *et al.*, 2011) presentation of critical epitopes. DCs engage in interactions with VLPs via the identical PRRs that detect natural viruses, (Buonaguro

et al., 2006; Bournazos *et al.*, 2017) namely TLRs as well as C-type lectin receptors (CLRs). Following administration via the parenteral or mucosal route, VLP-based vaccines are acknowledged by APCs, including DCs, which subsequently transport them to secondary lymphoid tissues such as the spleen. The process of DC maturation is initiated by the recognition as well as absorption of VLPs, which stimulates the production of pro-inflammatory factors such as TNF- α and IL-1 β (Fiebiger *et al.*, 2001). The pro-inflammatory factors enhance the recruitment of lysosomal proteolysis in the DCs as well as augment the activity of APCs. This results in transformation of VLP-based vaccines into small peptides, which are then presented on the dendritic cell surface as an MHC-peptide complex. Concurrently, costimulatory molecules for lymphocytes (e.g., CD80, CD86) manifest on the surface of DCs in order to stimulate B and T cells (Sallusto and Lanzavecchia, 2000). CD4+ T-helper cells are stimulated by the MHC class II peptide and costimulatory proteins. T-helper cells are indispensable for the proliferation and differentiation (Morón *et al.*, 2003) of both B and T cells. Under certain conditions, humoral immunity can be activated directly and independently of innate immunity or T helper cells when B cells detect VLPs. Plasmacytoid DCs (pDCs) can produce antibodies in response to an HPV16-based VLP, which stimulates the production of IFN- α as well as IL-6. Generally, the ability of VLP-based vaccines to serve as effective substrates for stimulating cellular and humoral immunity is one of their greatest advantages. generation of antibodies is induced by plasmacytoid DCs (pDCs) through the stimulation of IFN- α as well as IL-6 secretion by a VLP based on HPV16. VLP-based vaccines are, in general, effective substrates for inducing humoral and cellular immunity, which is one of their most significant advantages (Figure 6) (Bessa *et al.*, 2013).

Influenza virus A VLP based vaccine

In order to produce influenza VLPs, the B/IC expression system has been utilized. Three distinct baculoviruses, each expressing a gene for a different component of the influenza virus, were used to infect Sf9 cells. These viruses encoded for the hemagglutinin (HA), neuraminidase (NA), and matrix (M1) proteins, respectively. Major antigens of the virus are the HA and NA glycoproteins. When these three proteins are expressed together, a VLP is formed that may be collected from the culture supernatant. In comparison to inactivated virus or recombinant hemagglutinin

protein alone, the immune response elicited by these VLPs is more widespread. Preclinical outcomes for influenza VLPs produced utilizing transgenic plant technologies have been encouraging (Yuen *et al.*, 2016).

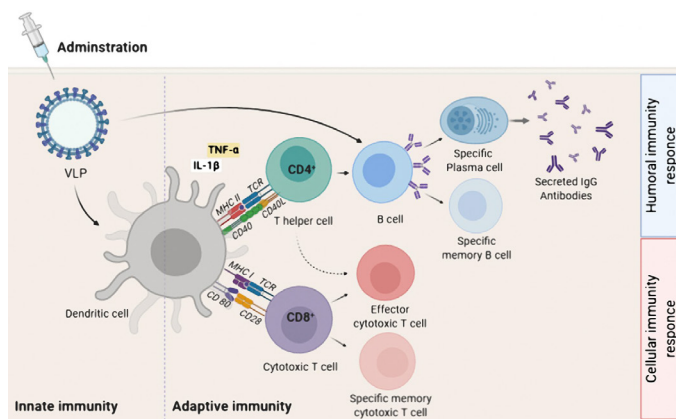


Figure 6: Adaptive immune activation induced by VLP-based vaccine. After administration, a VLP-based vaccine is taken up by APC such as dendritic cells. The phagocytosed VLP-based vaccine is processed and presented by both MHC-II and MHC-I for detection by CD4+ and CD8+ T cells, respectively. For induction of humoral immune responses, B cells interact with CD4+ T helper cell (TH) to uptake VLP-based vaccine by B cell receptor. The interaction between CD4+ TH cells and B cells occurs for sufficient secretion of IgG antibodies by plasma cells as well as the generation of B memory cells. For induction of cellular immunity responses, immature CD8+ cytotoxic T lymphocytes (CTL) proliferate and differentiate into effector and specific memory CTL. Effector CD4+ TH cells, increase antigen presenting by APC by secreting cytokines, and also assist activated CTL.

HIV VLP based vaccine

Multiple expression systems have been utilized to produce HIV VLPs. HIV VLPs made from *S. cerevisiae* have made it to the clinical trials, and they contain the structural proteins p17 and p24. Using either transient transfection or stably transfected cell lines, researchers have been able to create HIV VLPs based on the Gag and envelope (env) glycoproteins proteins in a variety of mammalian cell lines. In addition to baculovirus methods, gag-env VLPs have been produced using insect cells stably expressing the HIV proteins (Doan *et al.*, 2005; Pulcini *et al.*, 2013).

Coronaviruses VLP based vaccine

Diverse animal coronaviruses have been implicated in the development of severe human diseases. These involve SARS-CoV-1, MERS-CoV, as well

as SARS-CoV-2, which has caused a worldwide pandemic most recently. Four structural proteins comprise coronavirus particles: the spike (S) protein, the envelope (E) protein, the membrane (M) protein, and the nucleoprotein (N). Experiments on SARS-CoV-1 VLPs demonstrated that when the SARS-CoV-1 M and E proteins were expressed in a BVES, a smooth VLP devoid of spikes was produced, whereas when M, E, and S were expressed simultaneously, a structure resembling native SARS was generated. These have yet to undergo evaluation regarding their protective capacity (Mortola and Roy, 2004).

Drug delivery

In an ideal world, drug delivery vehicles would be non-toxic, encapsulate their payload, elude the immune system, zero in on the cells or tissues they need to reach, and then release their contents in a measured fashion. Since viruses already serve as delivery vehicles for nucleic acids, VLPs are well-suited to achieving these goals. Furthermore, VLPs are adaptable structures that can be genetically or chemically modified in predictable, well-defined, and homogeneous ways. These properties have sparked a lot of interest in employing VLPs as drug delivery vehicles, but there are still obstacles to overcome, such as the VLPs' intrinsic immunogenicity, their cell/tissue targeting selectivity, and the cargo release mechanism. We'll talk about how to solve these problems through design and what's been accomplished recently. In addition, VLPs may be rapidly cleared from the host due to memory activation of the immune response due to preexisting immunity or recurrent doses, resulting in decreased circulation time and diminished drug delivery effectiveness. Consequently, the inherent immunogenicity of VLPs is a major roadblock to their utilization as drug delivery vehicles, and adjustments are required to promote immune evasion. PEGylation, the process of attaching polyethylene glycol (PEG) to the surface of a delivery vehicle in order to conceal it from the host immune system, is the most well-known and commonly used method of immunological stealthing (Zdanowicz and Chroboczek, 2016).

Targeting specificity

Vaccine-like particles (VLPs) can be used to deliver therapeutic payloads to specific tissues or organs since they frequently preserve the inherent tropism of the wild-type virus. JC polyomavirus VLPs, for instance, have been demonstrated to specifically target xenografted human bladder tumor nodules, whereas

rotavirus VLPs have been found to specifically target intestinal cells in mice. When it is preferable to target other areas, the inherent tropism of VLPs might be a drawback. Displaying cell-specific targeting ligands allows the VLPs to be retargeted in these situations. One of the most prevalent targets for drug delivery is cancer cells, which frequently overexpress growth-promoting receptors such as folate, epidermal growth factor, as well as transferrin. As a result, VLPs presenting corresponding ligands have been widely employed for targeted delivery and absorption by various cancer cells. To a lesser extent, these receptors are also present on healthy cells, which can lead to off-target delivery and cytotoxicity, as well as a decrease in delivery efficiency owing to competition with naturally occurring ligands in the bloodstream. Other targeting ligands have been used to enhance targeting specificity and delivery efficiency.

Application of VLPs in drug delivery

Despite VLPs' widespread recognition for their immunogenic features, researchers have just lately begun to examine the possibility of using these nanoparticles for medication delivery and gene therapy. It is possible for VLPs to entrap proteins, nucleic acids, or other tiny molecules as well as transporting peptides/proteins or other active compounds shown on the surface of the VLPs. This makes them a viable option for transporting the chemicals in question to the target cells, tissues, or organs.

The absorption of VLPs into cells occurs via receptor-mediated endocytosis. Endocytosis occurs when the plasma membrane encloses the VLPs, forming a vesicle that buds off inside the cell. Once the vesicle has been released from the membrane, it will drop into the cytosol. Once the vesicles have been freed, they travel down the cytoskeleton to merge with primary endosomes. Vesicles mature into the final endosome and fuse with pre-lysosomal vesicles harboring acidic hydrolase to create lysosomes after separating from the main endosome. Lysosomes are responsible for digesting foreign substances and releasing them for cellular use. Around forty percent of newly developed medications are rejected because of inadequate bio-availability, however this is largely attributable to lysosomal degradation, which limits effective drug delivery.

To get over this problem, the usage of medication nanocarriers is highly recommended. Several

nanocarriers, utilizing a variety of approaches, have been created thus far. VLPs' capacity to evade lysosomal degradation makes them particularly well-suited for use as drug delivery nanocarriers. VLPs are well-suited for precise medication delivery due to their many desirable properties.

These NPs have the potential to improve treatment results by delivering materials in a targeted and intracellular fashion, therefore increasing medication accumulation and bioavailability at target areas (such as tumor tissues) while reducing drug dosage requirements. Some VLPs exhibit innate tropism for a given tissue, which is often attributable to the virus of origin. For instance, HBV-derived VLPs can specifically target liver cells due to the virus's common mode of infection there. Rotaviruses also have a marked preference for intestinal tissue, and the VLPs that are generated from them can be employed to deliver drugs specifically to that organ.

By exposing the receptor-binding region on the VLP surface, more precise targeting can be achieved. The therapeutic benefits of medications can be improved by attaching target domains to the surface of VLPs, which allows the VLPs to preferentially bind to cancer cells that express a certain receptor.

Nucleic acids can also be transported via VLPs

A known gene silencer, miR-146a, was shown to be an effective therapy for decreasing inflammatory cytokines in mice vulnerable to systemic lupus erythematosus when it was delivered systemically using bacteriophage MS2 -derived VLPs. In [Table 2](#), we can see some of the ways in which VLPs have been successfully used for drug delivery.

Drug release

VLPs follow the same life cycle as their host viruses during their transit into and inside cells. Upon first entering a cell, enveloped viruses normally fuse their lipid membrane with the plasma membrane or the endosomal membrane. Regardless of the exact process, the payload of encapsulated viruses is released very immediately and enters the cytoplasm. The entrance of non-enveloped (i.e. capsid) viruses into cells often occurs by receptor-mediated endocytosis, but they are unable to exit the endosome via membrane fusion, necessitating other, non-fusion mechanisms for release of their molecular payload into the cell. As a result, capping VLPs have been modified to

exploit the distinct endosomal environment, which is typically acidic and reducing, in order to induce drug release. An alternative approach involves displaying fusogenic peptides on the surface of the capsid VLP in order to introduce them into the lipid bilayer of the host cell membrane, thereby avoiding endocytosis and entering the cytosol directly. This strategy could prove advantageous for delicate cargoes, such as siRNA, which exhibit instability when exposed to the challenging conditions of the endosomal environment.

Pharmacological release can only be marginally regulated through activation strategies that incorporate in situ stimuli such as endosomal pH change, proteases, and redox molecules. Photosensitive chemistries, in which the activation and/or release of drug molecules from nanoparticles can be more precisely and spatiotemporally regulated through the application of light irradiation, have garnered attention in recent times. Light therapy exhibits favorable characteristics for biomedical applications due to its profound tissue penetration, minimal cellular toxicity, and substantial tunability in terms of wavelength, intensity, beam diameter, location, and duration. As an advantageous method for regulating the release of drugs, light-activated release of molecular payload from synthetic nanoparticles, viruses, and VLPs has been documented. Photoactivation serves as an additional efficacious approach to stimulate photosensitive therapeutics, in addition to instigating drug release.

Delivery of small molecule drugs for anti-microbial therapy

Antimicrobial resistance is a growing problem as bacterial populations adapt to and avoid standard treatments. Drug delivery strategies are a viable therapeutic alternative, and VLPs have been developed to this end. Phage display methods may be used to choose peptide binders that are unique to a certain microorganism, allowing for their targeted elimination. co-delivery of the antibiotic chloramphenicol allowed for tailored medication administration; phage display on filamentous fd phages was used to extract and display peptides unique to the gram-positive bacteria *Staphylococcus aureus*. When compared to chloramphenicol alone, the fd phages conjugated with chloramphenicol significantly slowed the growth of bacteria by a factor of Twenty. Azithromycin and clarithromycin are two examples of the macrolide antibiotics that have

recently been conjugated onto Q β particles for the management of pulmonary macrophage-dwelling bacteria like *Mycobacterium TB* and *Legionella pneumophila*. The uptake of the VLPs was evaluated both *in vitro* in RAW264.7 macrophages in addition to *in vivo* in lung tissue from mice. It was discovered that the VLPs were able to traffic into the RAW264.7 cells in addition to the lungs in a much larger amount than the negative control tolyl-labeled Q β particles. No reports of effectiveness trials have been made available as of yet (Figure 7).

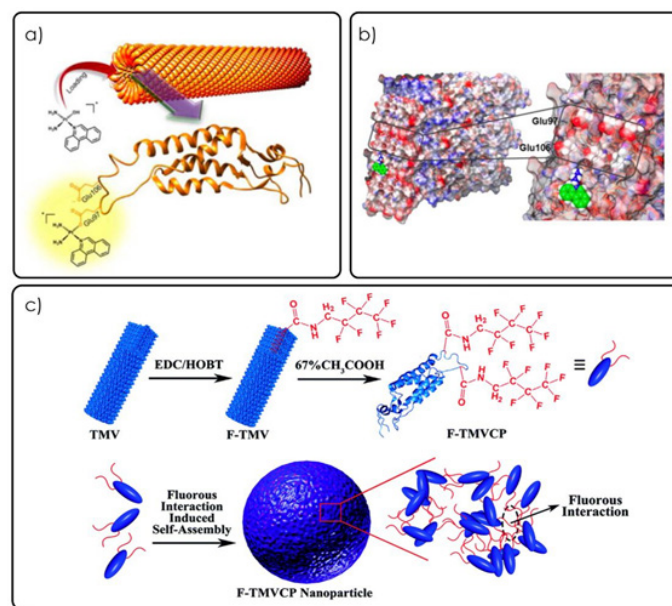


Figure 7: Different mechanisms of loading platinum-based drugs such as phen Pt and cisplatin into TMV a) Graphical abstract from Lippard, S.J., et al. showing the loading schematic for phenPt loading into wild-type TMV particles (reproduced with permission from ref (Vernekar et al., 2018b) Graphical illustration of the phenPt docking onto the Glu97 and Glu106 residues of TMV as discovered through matrix-assisted laser desorption/ionization–mass spectrometry and nuclear magnetic resonance spectroscopy c) Graphical schematic of the assembly of TMV spherical nanoparticles using a fluororous ponytail interaction (F-TMVCP). Cisplatin was loaded into the F-TMVCP using the same glutamic residues as in b (not shown) (reproduced with permission from ref (Gao et al., 2018).

Delivery of genes and nucleic acids

Gene transfer by virus is a natural process. Due to their ability to efficiently encapsulate nucleic acids, VLPs have found widespread application in the transport of genes and therapeutic nucleic acids. In reality, viruses are used as vectors to carry genetic material in more than sixty-seven percent of gene therapy

clinical studies across the world. While mammalian viral vectors have long been the foundation of gene delivery systems, more recently plant viruses and phages have been included into the mix. Plant VLPs are proteinaceous in nature, allowing for better cell penetration and end lysosomal escape than some synthetic nanoparticle systems, according to some reports. However, displaying peptides that facilitate cellular absorption and trafficking is a typical method for increasing delivery efficiency. Intracellular vesicles can be a bottleneck for plant viruses and phages since they have not adapted to move freely across mammalian cells.

Cell penetration ligands like TAT peptide have been added to bacteriophages like PP7 and MS2 for microRNA delivery in an effort to tailor cell trafficking features. Successful i.v. and i.t. delivery of GFP silencing RNA (siRNA) into GFP-expressing hepatocellular carcinoma tumors (MHCC97-H/GFP) *in vivo* in Balb/c-nude mice was achieved using this designed TMV-TAT system, which also showed improved internalization and acquired endo/lysosomal escape capability. Q β VLPs loaded with RNAi were modified with cell-penetrating peptides and apolipoprotein E (ApoE) to help them traverse the blood-brain barrier and target malignant brain tumors.

Nude mice with intracranial U87 brain tumors were treated with a combination of temozolomide and engineered Q β VLPs administered intravenously. Our lab has developed a method for delivering siRNA to mammalian cells using CCMV VLPs that are tagged with a cell-penetrating peptide (M-lycotoxin peptide L17E).

mRNA can also be delivered via plant VLPs. Single research looked at using CCMV to transfer the gene for enhanced yellow fluorescent protein (EYFP) from bacteria to mammalian BHK-21 cells. Evidence was presented that encapsulation of the RNA cargo into the plant viral capsid stabilized it, and that transfection of cells with lipofectamine resulted in the nucleic acid being transported and introduced into the cytoplasm of the BHK-21 cells by the VLPs, where it facilitated the expression of EYFP.

Challenges moving forward and future directions

Significant advancements have been made in recent years within the domain of viral drug delivery. These

involve the utilization of plant viruses to treat canine cancer cases, the implementation of CpG-laden bacteriophages in clinical trials, and the ongoing phase 2 clinical testing of adenoviral COVID-19 vaccine candidates. These instances underscore the fact that the discipline has exited its nascent stage. The science of viral medication delivery has made significant progress, but there are still certain obstacles to overcome. The main problem is figuring out if repeated delivery causes side effects or decreases efficacy due to preexisting or newly developed adaptive immunity against the viral carrier. It's possible that this will be difficult for conventional drug delivery strategies to overcome when aiming at sick cells and tissues, but surface passivation and immune modification techniques have showed promise. It is less expected that carrier immunogenicity will be an issue when it comes to immunotherapy applications.

Conclusions and Recommendations

In this study, we have shown how plant viruses and bacteriophages may be used as nanomachines, and how they are now widely acknowledged as a type of nanotechnology (Figure 8). VLPs have specialized in drug delivery due of their ability to transport and release cargo.



Figure 8: Bubble diagram of the proposed drug delivery and imaging/theranostic platforms discussed in this review by plant viruses, bacteriophages, mammalian viruses, and protein cages.

Novelty Statement

This article presents a novel, comprehensive analysis of the plant virome using a recently adopted phylogenomic taxonomy. We explore the evolutionary origins of plant viruses, tracing their lineages back to

primordial mobile genetic elements.

This combined approach sheds new light on the intricate coevolution of plant and viral genomes, offering valuable insights for plant biologists and virologists.

Author's Contribution

All authors have equally contributed to the manuscript. Specifically, each author has been involved in the following aspects of the research:

- Conception and design of the review
- Analysis and interpretation of the literature
- Drafting and revising
- Final approval of the version to be published

Abbreviations

VLPS: Virus-like particles; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; HEV: Hepatitis E virus; CHIK-VLPs: Chikungunya VLPs; B/IC: Baculovirus/Insect cells; SARS: Severe acute respiratory syndrome; RVFV: Rift Valley fever virus; TMV: Tobacco mosaic virus; PVX: Potato X virus; CPMV: Cowpea mosaic virus; AIMV: Alfalfa mosaic virus; TFF: Tangential flow filtration; SEC: Size exclusion chromatography; PRRs: Pattern recognition receptors; IL12: Interleukin 12; HEV vaccine: He Colin vaccine; SDS-PAGE: Sodium dodecyl sulphate - polyacrylamide gel electrophoresis; Naase: Neuraminidase; MERS-CoV: Middle east respiratory syndrome CoV; PEG: Polyethylene glycol; TAT: Transacting activation transduction; Apo E: Apo lipoprotein E.

Conflict of interest

The authors have declared no conflict of interest.

References

- Abbing, A., Blaschke, U.K., Grein, S., Kretschmar, M., Stark, C.M., and Thies, M.J., 2004. Efficient intracellular delivery of a protein and a low molecular weight substance via recombinant polyomavirus-like particles. *J. Biol. Chem.*, 279(26): 27410–27421. <https://doi.org/10.1074/jbc.M313612200>
- Al-Barwani, F., Young, S.L., Baird, M.A., Larsen, D.S., and Ward, V.K., 2014. Mannosylation of virus-like particles enhances internalization by antigen presenting cells. *PLoS One*, 9(8): e104523. <https://doi.org/10.1371/journal.pone.0104523>
- Aljabali, A.A., Shukla, S., Lomonosoff, G.P., Steinmetz, N.F., and Evans, D.J., 2013. Cpmvdox delivers. *Mol. Pharm.*, 10(1): 3–10. <https://doi.org/10.1021/mp3002057>
- Ashley, C.E., Carnes, E.C., Phillips, G.K., Durfee, P.N., Buley, M.D., Lino, C.A., *et al.* 2011. Cell-specific delivery of diverse cargos by bacteriophage MS2 virus-like particles. *ACS Nano*. 5(7): 5729–5745. <https://doi.org/10.1021/nn201397z>
- Baglivo, S.J., and Polack, F.P., 2019. The long road to protect infants against severe RSV lower respiratory tract illness. *F1000Research*. pp. 8. <https://doi.org/10.12688/f1000research.18749.1>
- Bai, B., Hu, Q., Hu, H., Zhou, P., Shi, Z., Meng, J., *et al.* 2008. Virus-like particles of SARS-like coronavirus formed by membrane proteins from different origins demonstrate stimulating activity in human dendritic cells. *PLoS One*, 3(7): e2685. <https://doi.org/10.1371/journal.pone.0002685>
- Balke, I., and Zeltins, A., 2019. Use of plant viruses and virus-like particles for the creation of novel vaccines. *Adv. Drug Deliv. Rev.*, 145: 119–129. <https://doi.org/10.1016/j.addr.2018.08.007>
- Balke, I., and Zeltins, A., 2020. Recent advances in the use of plant virus-like particles as vaccines. *Viruses*, 12(3): 270. <https://doi.org/10.3390/v12030270>
- Baumert, T.F., Ito, S., Wong, D.T., and Liang, T.J., 1998. Hepatitis C virus structural proteins assemble into virus like particles in insect cells. *J. Virol.*, 72(5): 3827–3836. <https://doi.org/10.1128/JVI.72.5.3827-3836.1998>
- Bayer, M.E., Blumberg, B.S., and Werner, B., 2018. Particles associated with Australia antigen in the sera of patients with leukaemia. *Down's Syndr. Hepat. Nat.*, 218(5146): 1057–1059. <https://doi.org/10.1038/2181057a0>
- Bessa, J., Zabel, F., Link, A., Jegerlehner, A., Hinton, H.J., Schmitz, N., *et al.* 2012. Low-affinity B cells transport viral particles from the lung to the spleen to initiate antibody responses. *Proc. Natl. Acad. Sci.*, 109(50): 20566–20571. <https://doi.org/10.1073/pnas.1206970109>
- Block, S.L., Nolan, T., Sattler, C., Barr, E., Giacoletti, K.E., Marchant, C.D., *et al.* 2006.

- Comparison of the immunogenicity and reactogenicity of a prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in male and female adolescents and young adult women. *Pediatrics*, 118(5): 2135–2145. <https://doi.org/10.1542/peds.2006-0461>
- Bolli, E., O'Rourke, J.P., Conti, L., Lanzardo, S., Rolih, V., Christen, J.M., *et al.* 2018. A virus-like-particle immunotherapy targeting epitope-specific anti-xCT expressed on cancer stem cell inhibits the progression of metastatic cancer *in vivo*. *Oncoimmunology*, 7(3): e1408746. <https://doi.org/10.1080/2162402X.2017.1408746>
- Bournazos, S., Wang, T.T., and Ravetch, J.V., 2017. The role and function of Fcγ receptors on myeloid cells. *Myeloid cells in health and disease: A synthesis*. pp. 405–427. <https://doi.org/10.1128/9781555819194.ch22>
- Buonaguro, L., Tornesello, M., Tagliamonte, M., Gallo, R., Wang, L., Kamin-Lewis, R., *et al.* 2006. Baculovirus-derived human immunodeficiency virus type 1 virus-like particles activate dendritic cells and induce ex vivo T-cell responses. *J. Virol.*, 80(18): 9134–9143. <https://doi.org/10.1128/JVI.00050-06>
- Chang, G-D., Chen, C-J., Lin, C-Y., Chen, H-C., and Chen, H., 2003. Improvement of glycosylation in insect cells with mammalian glycosyltransferases. *J. Biotechnol.*, 102(1): 61–71. [https://doi.org/10.1016/S0168-1656\(02\)00364-4](https://doi.org/10.1016/S0168-1656(02)00364-4)
- Charland, N., 2012. Plant-made influenza virus-like particles for Pandemic and Beyond. <https://doi.org/10.1016/j.vaccine.2017.06.084>
- Choi, K., Kim, K., Kwon, I.C., Kim, I.S., and Ahn, H.J., 2013. Systemic delivery of siRNA by chimeric capsid protein: Tumor targeting and RNAi activity *in vivo*. *Mol. Pharm.*, 10(1): 18–25. <https://doi.org/10.1021/mp300211a>
- Chroboczek, J., Szurgot, I., Szolajska, E., 2014. Virus-like particles as vaccine. *Acta Biochim. Pol.*, 61(3). https://doi.org/10.18388/abp.2014_1875
- Chung, Y.H., Cai, H., and Steinmetz, N.F., 2020. Viral nanoparticles for drug delivery, immunotherapy, and theranostic applications. *Adv. Drug Deliv. Rev.*, 156: 214–235. <https://doi.org/10.1016/j.addr.2020.06.024>
- Crooke, S.N., Schimer, J., Raji, I., Wu, B., Oyelere, A.K., and Finn, M., 2019. Lung tissue delivery of virus-like particles mediated by macrolide antibiotics. *Mol. Pharm.*, 16(7): 2947–2955. <https://doi.org/10.1021/acs.molpharmaceut.9b00180>
- Cubas, R., Zhang, S., Li, M., Chen, C., and Yao, Q., 2011. Chimeric trop 2 virus-like particles: A potential immunotherapeutic approach against pancreatic cancer. *J. Immunother.*, 34(3): 251–263. <https://doi.org/10.1097/CJI.0b013e318209ee72>
- D'Aoust, M.A., Couture, M.M.J., Charland, N., Trepanier, S., Landry, N., Ors, F., *et al.* 2010. The production of hemagglutinin-based virus-like particles in plants: A rapid, efficient and safe response to pandemic influenza. *Plant Biotechnol. J.*, 8(5): 607–619. <https://doi.org/10.1111/j.1467-7652.2009.00496.x>
- Dai, S., Wang, H., and Deng, F., 2018. Advances and challenges in enveloped virus-like particle (VLP)-based vaccines. *J. Immunol. Sci.*, 2(2). <https://doi.org/10.29245/2578-3009/2018/2.1118>
- De Filette, M., Fiers, W., Martens, W., Birkett, A., Ramne, A., Löwenadler, B., *et al.* 2006. Improved design and intranasal delivery of an M2e-based human influenza A vaccine. *Vaccine*, 24(44–46): 6597–6601. <https://doi.org/10.1016/j.vaccine.2006.05.082>
- Dennis, S.J., Meyers, A.E., Guthrie, A.J., Hitzeroth, I.I., and Rybicki, E.P., 2018. Immunogenicity of plant-produced African horse sickness virus-like particles: Implications for a novel vaccine. *Plant Biotechnol. J.*, 16(2): 442–450. <https://doi.org/10.1111/pbi.12783>
- Doan, L.X., Li, M., Chen, C., and Yao, Q., 2005. Virus-like particles as HIV-1 vaccines. *Rev. Med. Virol.*, 15(2): 75–88. <https://doi.org/10.1002/rmv.449>
- Donaldson, B., Al-Barwani, F., Young, V., Scullion, S., Ward, V., and Young, S., 2015. Viruslike particles, a versatile subunit vaccine platform. *Subunit Vaccine Delivery*. Berlin: Springer. pp. 159–180. https://doi.org/10.1007/978-1-4939-1417-3_9
- Donaldson, B., Lateef, Z., Walker, G.F., Young, S.L., and Ward, V.K., 2018. Virus-like particle vaccines: Immunology and formulation for clinical translation. *Expert. Rev. Vaccines*, 17(9): 833–849. <https://doi.org/10.1080/14760584.2018.1516552>
- Duffy, P.E., and Patrick, G.J., 2020. Malaria vaccines

- since 2000: progress, priorities, products. NPJ Vaccines, 5(48): 1–9. <https://doi.org/10.1038/s41541-020-0196-3>.
- Fiebiger, E., Meraner, P., Weber, E., Fang, I.F., Stingl, G., Ploegh, H., *et al.* 2001. Cytokines regulate proteolysis in major histocompatibility complex class II-dependent antigen presentation by dendritic cells. J. Exp. Med., 193(8): 881–892. <https://doi.org/10.1084/jem.193.8.881>
- Fuenmayor, J., Gòdia, F., and Cervera, L., 2017. Production of virus-like particles for vaccines. New Biotechnol., 39: 174–180. <https://doi.org/10.1016/j.nbt.2017.07.010>
- Fuenmayor, J.G. and Cervera. 2017. Production of virus-like particles for vaccines. N. Biotechnol., 39(Pt B): 174–180.
- Gao, S.X., Liu, Z.W., Jiang, S.M., Wu, Y.T. and Niu, Z., 2018. Fluorous interaction induced self-assembly of tobacco mosaic virus coat protein for cisplatin delivery. Nanoscale, 10: 11732–11736. <https://doi.org/10.1039/C8NR03748E>
- Garg, A., and Dewangan, H.K., 2020. Nanoparticles as adjuvants in vaccine delivery. Crit. Rev. Ther. Drug Carr. Syst., 37(2). <https://doi.org/10.1615/CritRevTherDrugCarrierSyst.2020033273>
- Gleba, Y., Klimyuk, V., and Marillonnet, S., 2005. Magniffection a new platform for expressing recombinant vaccines in plants. Vaccine, 23(17–18): 2042–2048. <https://doi.org/10.1016/j.vaccine.2005.01.006>
- Goelet, P., Lomonossoff, G., Butler, P., Akam, M., Gait, M., and Karn, J., 1982. Nucleotide sequence of tobacco mosaic virus RNA. Proc. Natl. Acad. Sci., 79(19): 5818–5822. <https://doi.org/10.1073/pnas.79.19.5818>
- Grein, T.A., Weidner, T., and Czermak, P., 2015. Concepts for the production of viruses and viral vectors in cell cultures. In: Gowder SJT editor. (London, UK: IntechOpen) New insights into cell culture technology (2017). 31. <https://doi.org/10.5772/66903>
- Grein, T.A., Weidner, T., and Czermak, P., 2017. Concepts for the production of viruses and viral vectors in cell cultures. In: Gowder SJT editor. (London, UK: IntechOpen) New insights into cell culture technology. <https://doi.org/10.5772/66903>
- Harrison, B., and Wilson, T., 1999. Milestones in research on tobacco mosaic virus. Philos. Trans. R. Soc. Lond B Biol. Sci., 354(1383): 521–529. <https://doi.org/10.1098/rstb.1999.0403>
- Herzog, C., Hartmann, K., Kunzi, V., Kursteiner, O., Mischler, R., Lazar, H., *et al.* 2009. Eleven years of inflexal V-a virosomal adjuvanted influenza vaccine. Vaccine, 27(33): 4381–4387. <https://doi.org/10.1016/j.vaccine.2009.05.029>
- Hillebrandt, N., Vormittag, P., Bluthardt, N., Dietrich, A., and Hubbuch, J., 2020. Integrated process for capture and purification of virus-like particles: enhancing process performance by cross-flow filtration. Front. Bioeng. Biotechnol., 8: 489. <https://doi.org/10.3389/fbioe.2020.00489>
- Hu, Y., Huang, S.J., Chu, K., Wu, T., Wang, Z.Z., Yang, C.L., *et al.* 2014. Safety of an *Escherichia coli*-expressed bivalent human papillomavirus (types 16 and 18) L1 virus-like particle vaccine: An open-label phase I clinical trial. Hum. Vaccines Immunother., 10(2): 469–475. <https://doi.org/10.4161/hv.26846>
- Huang, X., Wang, X., Zhang, J., Xia, N., and Zhao, Q., 2017. *Escherichia coli* derived virus-like particles in vaccine development. NPJ Vaccines, 2(1): 1–9. <https://doi.org/10.1038/s41541-017-0006-8>
- Huang, Z., Chen, Q., Hjelm, B., Arntzen, C., and Mason, H., 2009. A DNA replicon system for rapid high-level production of virus-like particles in plants. Biotechnol. Bioeng., 103(4): 706–714. <https://doi.org/10.1002/bit.22299>
- Janitzek, C.M., Peabody, J., Thrane, S., Carlsen, P.H., Theander, T.G., Salanti, A., *et al.* 2019. A proof-of-concept study for the design of a VLP-based combinatorial HPV and placental malaria vaccine. Sci. Rep., 9(1): 1–10. <https://doi.org/10.1038/s41598-019-41522-5>
- Keating, G.M., and Noble, S., 2003. Recombinant hepatitis B vaccine (Engerix-BR). Drugs, 63(10): 1021–1051. <https://doi.org/10.2165/00003495-200363100-00006>
- Keech, C., Albert, G., Cho, I., Robertson, A., Reed, P., Neal, S., *et al.* 2020. Phase 1–2 trial of a SARS-CoV-2 recombinant spike protein nanoparticle vaccine. N. Engl. J. Med., 383(24): 2320–2332. <https://doi.org/10.1056/NEJMoa2026920>
- Lacson, E., Teng, M., Ong, J., Vienneau, L., Ofsthun, N., and Lazarus, J.M., 2005. Antibody response to Engerix-BR and Recombivax-HBR hepatitis B vaccination in end-stage renal disease. Hemodial. Int., 9(4): 367–375. <https://doi.org/10.1111/j.1492-7535.2005.01155.x>
- Langeveld, J.P., Brennan, F.R., Martínez-

- TorreCuadrada, J.L., Jones, T.D., Boshuizen, R.S., Vela, C., *et al.* 2001. Inactivated recombinant plant virus protects dogs from a lethal challenge with canine parvovirus. *Vaccine*,19(27): 3661–3670. [https://doi.org/10.1016/S0264-410X\(01\)00083-4](https://doi.org/10.1016/S0264-410X(01)00083-4)
- Lee, Y.T., Ko, E.J., Lee, Y., Kim, K.H., Kim, M.C., Lee, Y.N., *et al.* 2018. Intranasal vaccination with M2e5x virus-like particles induces humoral and cellular immune responses conferring cross-protection against heterosubtypic influenza viruses. *PLoS One*, 13(1): e0190868. <https://doi.org/10.1371/journal.pone.0190868>
- Lee, Y.T., Ko, E.J., Lee, Y., Kim, K.H., Kim, M.C., Lee, Y.N., *et al.* 2018. Intranasal vaccination with M2e5x virus-like particles induces humoral and cellular immune responses conferring cross-protection against heterosubtypic influenza viruses. *PLoS One*, 13(1): e0190868. <https://doi.org/10.1371/journal.pone.0190868>
- Liu, W., Jiang, H., Zhou, J., Yang, X., Tang, Y., Fang, D., *et al.* 2010. Recombinant dengue virus-like particles from *Pichia pastoris*: efficient production and immunological properties. *Virus Genes*, 40(1): 53–59. <https://doi.org/10.1007/s11262-009-0418-2>
- López-Macías, C., 2012. Virus-like particle (VLP)-based vaccines for pandemic influenza: Performance of a VLP vaccine during the 2009 influenza pandemic. *Hum. Vaccines Immunother.*, 8(3): 411–414. <https://doi.org/10.4161/hv.18757>
- Lua, L.H., Connors, N.K., Sainsbury, F., Chuan, Y.P., Wibowo, N., and Middelberg, A.P., 2014. Bioengineering virus-like particles as vaccines. *Biotechnol. Bioeng.*, 111(3): 425–440. <https://doi.org/10.1002/bit.25159>
- Lünsdorf, H., Gurramkonda, C., Adnan, A., Khanna, N., and Rinas, U., 2011. Virus-like particle production with yeast: Ultrastructural and immunocytochemical insights into *Pichia pastoris* producing high levels of the Hepatitis B surface antigen. *Microb. Cell Fact.*, 10(1): 1–10. <https://doi.org/10.1186/1475-2859-10-48>
- Manolova, V., Flace, A., Bauer, M., Schwarz, K., Saudan, P., and Bachmann, M.F., 2008. Nanoparticles target distinct dendritic cell populations according to their size. *Eur. J. Immunol.*, 38(5): 1404–1413. <https://doi.org/10.1002/eji.200737984>
- Mohsen, M.O., Heath, M.D., Cabral-Miranda, G., Lipp, C., Zeltins, A., Sande, M., *et al.* 2019. Vaccination with nanoparticles combined with micro-adjuvants protects against cancer. *J. Immunother. Cancer*, 7(1): 1–12. <https://doi.org/10.1186/s40425-019-0587-z>
- Morón, V.G., Rueda, P., Sedlik, C. and Leclerc, C., 2003. *In vivo*, dendritic cells can cross-present virus-like particles using an endosome-to-cytosol pathway. *J. Immunol.*, 171(5): 2242–2250. <https://doi.org/10.4049/jimmunol.171.5.2242>
- Mortola, E., and Roy, P., 2004. Efficient assembly and release of SARS coronavirus like particles by a heterologous expression system. *FEBS Lett.*, 576(1–2): 174–178. <https://doi.org/10.1016/j.febslet.2004.09.009>
- Namba, K., and Stubbs, G., 1986. Structure of tobacco mosaic virus at 3.6 Å resolution: Implications for assembly. *Science*, 231(4744): 1401–1406. <https://doi.org/10.1126/science.3952490>
- Nardin, E.H., Oliveira, G.A., Calvo-Calle, J.M., Wetzel, K., Maier, C., Birkett, A.J., *et al.* 2004. Phase I testing of a malaria vaccine composed of hepatitis B virus core particles expressing *Plasmodium falciparum* circumsporozoite epitopes. *Infect. Immun.*, 72(11): 6519–6527. <https://doi.org/10.1128/IAI.72.11.6519-6527.2004>
- Naskalska, A., and Pyrc, K., 2015. Virus like particles as immunogens and universal nanocarriers. *Polish J. Microbiol.*, 64(1): 3–13. <https://doi.org/10.33073/pjm-2015-001>
- O'Donnell, K., and Marzi, A., 2020. The Ebola virus glycoprotein and its immune responses across multiple vaccine platforms. *Expert. Rev. Vaccines*, 19(3): 267–277. <https://doi.org/10.1080/14760584.2020.1738225>
- Peixoto, C., Sousa, M., Silva, A., Carrondo, M., and Alves, P., 2007. Downstream processing of triple layered rotavirus like particles. *J. Biotechnol.*, 127(3): 452–461. <https://doi.org/10.1016/j.jbiotec.2006.08.002>
- Pouyanfard, S., Spagnoli, G., Bulli, L., Balz, K., Yang, F., Odenwald, C., *et al.* 2018. Minor capsid protein L2 polytope induces broad protection against oncogenic and mucosal human papillomaviruses. *J. Virol.*, 92(4). <https://doi.org/10.1128/JVI.01930-17>
- Proffitt, A., 2012. First HEV vaccine approved.

- Berlin: Nature Publishing Group; 2012. <https://doi.org/10.1038/nbt0412-300a>
- Pulcini, C., Massin, S., Launay, O., and Verger, P., 2013. Factors associated with vaccination for hepatitis B, pertussis, seasonal and pandemic influenza among French general practitioners: A 2010 survey. *Vaccine*, 31(37): 3943–3949. <https://doi.org/10.1016/j.vaccine.2013.06.039>
- Pushko, P., Pumpens, P., and Grens, E., 2013. Development of virus-like particle technology from small highly symmetric to large complex virus-like particle structures. *Intervirology*, 56(3): 141–165. <https://doi.org/10.1159/000346773>
- Ren, J., Bell, G., Coy, D., and Brunicardi, F., 1997. Activation of human somatostatin receptor type 2 causes inhibition of cell growth in transfected HEK293 but not in transfected CHO cells. *J. Surg. Res.*, 71(1): 13–18. <https://doi.org/10.1006/jsre.1997.5097>
- Rockman, S., Laurie, K.L., Parkes, S., Wheatley, A., and Barr, I.G., 2020. New technologies for influenza vaccines. *Microorganisms*, 8(11): 1745. <https://doi.org/10.3390/microorganisms8111745>
- Rolih, V., Caldeira, J., Bolli, E., Salameh, A., Conti, L., Barutello, G., *et al.* 2020. Development of a vlp-based vaccine displaying an XCT extracellular domain for the treatment of metastatic breast cancer. *Cancers*, 12(6): 1492. <https://doi.org/10.3390/cancers12061492>
- Rybicki, E.P., 2020. Plant molecular farming of virus-like nanoparticles as vaccines and reagents. *Wiley Interdiscipl. Rev.*, 12(2): e1587. <https://doi.org/10.1002/wnan.1587>
- Sallusto, F., and Lanzavecchia, A., 2000. Understanding dendritic cell and T-lymphocyte traffic through the analysis of chemokine receptor expression. *Immunol. Rev.*, 177: 134–140. <https://doi.org/10.1034/j.1600-065X.2000.17717.x>
- Santi, L., Batchelor, L., Huang, Z., Hjelm, B., Kilbourne, J., Arntzen, C.J., *et al.* 2008. An efficient plant viral expression system generating orally immunogenic Norwalk virus-like particles. *Vaccine*, 26(15): 1846–1854. <https://doi.org/10.1016/j.vaccine.2008.01.053>
- Saraswat, S., Athmaram, T., Parida, M., Agarwal, A., Saha, A., and Dash, P.K., 2016. Expression and characterization of yeast derived chikungunya virus like particles (CHIK-VLPs) and its evaluation as a potential vaccine candidate. *PLoS Negl. Trop. Dis.*, 10(7): e0004782. <https://doi.org/10.1371/journal.pntd.0004782>
- Scotti, N., and Rybicki, E.P., 2013. Virus-like particles produced in plants as potential vaccines. *Expert. Rev. Vaccines*, 12(2): 211–224. <https://doi.org/10.1586/erv.12.147>
- Sharma, V.K., Sharma, I. and Glick, J., 2020. The expanding role of mass spectrometry in the field of vaccine development. *Mass Spectrom Rev.*, 39(1–2): 83–104. <https://doi.org/10.1002/mas.21571>
- Shirbaghaee, Z., and Bolhassani, A., 2016. Different applications of virus-like particles in biology and medicine: Vaccination and delivery systems. *Biopolymers*. 105(3): 113–132. <https://doi.org/10.1002/bip.22759>
- Steinmetz, N.F., 2010. Viral nanoparticles as platforms for next-generation therapeutics and imaging devices. *Nanomedicine*, 6(5): 634–641. <https://doi.org/10.1016/j.nano.2010.04.005>
- Syomin, B., and Ilyin, Y., 2019. Virus-like particles as an instrument of vaccine production. *Mol. Biol.*, 53(3): 323–334. <https://doi.org/10.1134/S0026893319030154>
- Syomin, B., Pelisson, A., Ilyin, Y.V., Bucheton, A., 2004. Expression of the retrovirus gypsy Gag in *Spodoptera frugiperda* cell culture with the recombinant baculovirus. *Doklady Biochemistry and Biophysics*; Springer. <https://doi.org/10.1023/B:DOBI.0000046646.32174.84>
- Tregoning, J.S., 2020. First human efficacy study of a plant-derived influenza vaccine. *Lancet*, 396(10261): 1464–1465. [https://doi.org/10.1016/S0140-6736\(20\)32010-9](https://doi.org/10.1016/S0140-6736(20)32010-9)
- Vernekar, A.A., Berger, G., Czapar, A.E., Veliz, F.A., Wang, D.I., Steinmetz, N.F., and Lippard, S.J., 2018. Speciation of phenanthriplatin and its analogs in the core of tobacco mosaic virus. *J. Am. Chem. Soc.*, 140: 4279–4287. <https://doi.org/10.1021/jacs.7b12697>
- Vicente, T., Roldão, A., Peixoto, C., Carrondo, M.J., and Alves, P.M., 2011. Large-scale production and purification of VLP-based vaccines. *J. Invertebr. Pathol.*, 107: S42–48. <https://doi.org/10.1016/j.jip.2011.05.004>
- Wang, C., Beiss, V., and Steinmetz, N.F., 2019. Cowpea mosaic virus nanoparticles and empty virus-like particles show distinct but overlapping immunostimulatory properties. *J. Virol.*, 93(21). <https://doi.org/10.1128/JVI.00129-19>
- Wang, C., Zheng, X., Gai, W., Wong, G., Wang,

- H., Jin, H., *et al.* 2017. Novel chimeric virus-like particles vaccine displaying MERS-CoV receptor-binding domain induce specific humoral and cellular immune response in mice. *Antiviral Res.*, 140: 55–61. <https://doi.org/10.1016/j.antiviral.2016.12.019>
- Weiss, K., Gerstenberger, J., Salzig, D., Mühlebach, M.D., Cichutek, K., Pörtner, R., *et al.* 2015. Oncolytic measles viruses produced at different scales under serum-free conditions. *Eng. Life Sci.*, 15(4): 425–436. <https://doi.org/10.1002/elsc.201400165>
- Win, S.J., Ward, V.K., Dunbar, P.R., Young, S.L., and Baird, M.A., 2011. Cross-presentation of epitopes on virus-like particles via the MHC I receptor recycling pathway. *Immunol. Cell Biol.*, 89(6): 681–688. <https://doi.org/10.1038/icb.2010.161>
- Wu, C.Y., Yeh, Y.C., Yang, Y.C., Chou, C., Liu, M.T., Wu, H.S., *et al.* 2010. Mammalian expression of virus-like particles for advanced mimicry of authentic influenza virus. *PLoS One*, 5(3): e9784. <https://doi.org/10.1371/journal.pone.0009784>
- Yacoby, I., Shamis, M., Bar, H., Shabat, D., and Benhar, I., 2006. Targeting antibacterial agents by using drug-carrying filamentous bacteriophages. *Antimicrob. Agents Chemother.*, 50(6): 2087–2097. <https://doi.org/10.1128/AAC.00169-06>
- Yang, C.D., Liao, J.T., Lai, C.Y., Jong, M.H., Liang, C.M., Lin, Y.L., *et al.* 2007. Induction of protective immunity in swine by recombinant bamboo mosaic virus expressing foot-and-mouth disease virus epitopes. *BMC Biotechnol.*, 7(1): 1–11. <https://doi.org/10.1186/1472-6750-7-62>
- Yang, Y., Li, H., Li, Z., Zhang, Y., Zhang, S., Chen, Y., and Su, Z., 2015. Size-exclusion HPLC provides a simple, rapid, and versatile alternative method for quality control of vaccines by characterizing the assembly of antigens. *Vaccine*, 33(9): 1143–1150. <https://doi.org/10.1016/j.vaccine.2015.01.031>
- Yuen, C.Y., Dodgson, J.E., and Tarrant, M., 2016. Perceptions of Hong Kong Chinese women toward influenza vaccination during pregnancy. *Vaccine*, 34(1): 33–40. <https://doi.org/10.1016/j.vaccine.2015.11.032>
- Zdanowicz, M., and Chroboczek, J., 2016. Virus-like particles as drug delivery vectors. *Acta Biochim. Pol.*, 63(3): 469–473. https://doi.org/10.18388/abp.2016_1275
- Zeng, Q., Wen, H., Wen, Q., Chen, X., Wang, Y., Xuan, W., *et al.* 2013. Cucumber mosaic virus as drug delivery vehicle for doxorubicin. *Biomaterials*, 34(19): 4632–4642. <https://doi.org/10.1016/j.biomaterials.2013.03.017>
- Zhai, L., Peabody, J., Pang, Y.S., Schiller, J., Chackerian, B., and Tumban, E., 2017. A novel candidate HPV vaccine: MS2 phage VLP displaying a tandem HPV L2 peptide offers similar protection in mice to Gardasil-9. *Antivir. Res.*, 147: 116–123. <https://doi.org/10.1016/j.antiviral.2017.09.012>
- Zhang, S., Yong, L.K., Li, D., Cubas, R., Chen, C., and Yao, Q., 2013. Mesothelin virus-like particle immunization controls pancreatic cancer growth through CD8+ T cell induction and reduction in the frequency of CD4+ foxp3+ ICOS– regulatory T cells. *PLoS One*, 8(7): e68303. <https://doi.org/10.1371/journal.pone.0068303>
- Zhang, T., Breitbart, M., Lee, W.H., Run, J.Q., Wei, C.L., Soh, S.W.L., *et al.* 2005. RNA viral community in human feces: Prevalence of plant pathogenic viruses. *PLoS Biol.*, 4(1): e3. <https://doi.org/10.1371/journal.pbio.0040003>
- Zhao, Q., Chen, W., Chen, Y., Zhang, L., Zhang, J., and Zhang, Z., 2011. Self-assembled virus-like particles from rotavirus structural protein VP6 for targeted drug delivery. *Bioconjug Chem.*, 22(3): 346–352. <https://doi.org/10.1021/bc1002532>
- Zochowska, M., Piguet, A.C., Jemielity, J., Kowalska, J., Szolajska, E., Dufour, J.F., *et al.* 2015. Virus-like particle-mediated intracellular delivery of mRNA cap analog with *in vivo* activity against hepatocellular carcinoma. *Nanomedicine*, 11(1): 67–76. <https://doi.org/10.1016/j.nano.2014.07.009>