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MINI-REVIEW

The application of virus-like particles as vaccines and biological vehicles

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Abstract Virus-like particles (VLPs) can be spontaneously self-assembled by viral structural proteins under appropriate conditions in vitro while excluding the genetic material and potential replication probability. In addition, VLPs possess several features including can be rapidly produced in large quantities through existing expression systems, highly resembling native viruses in terms of conformation and appearance, and displaying repeated cluster of epitopes. Their capsids can be modified via genetic insertion or chemical conjugation which facilitating the multivalent display of a homologous or heterogeneous epitope antigen. Therefore, VLPs are considered as a safe and effective candidate of prophylactic and therapeutic vaccines. VLPs, with a diameter of approximately 20 to 150 nm, also have the characteristics of nanometer materials, such as large surface area, surface-accessible amino acids with reactive moieties (e.g., lysine and glutamic acid residues), inerratic spatial structure, and good biocompatibility. Therefore, assembled VLPs have great potential as a delivery system for specifically carrying a variety of materials. This review summarized recent researches on VLP development as vaccines and biological vehicles, which demonstrated the advantages and potential of VLPs in disease control and prevention and diagnosis. Then, the prospect of VLP biology application in the future is discussed as well.

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Introduction

Virus-like particles (VLPs) are composed of one or more structural proteins/capsid proteins of viruses by self-assembling into a particular spatial conformation. In terms of appearance, VLPs are very similar to a live virus without genetic components (Chroboczek et al. 2014; Kushnir et al. 2012). The high density of the epitopes on its surface can be recognized and presented to the immune system by antigen-presenting cells (APC), thus stimulating humoral and cellular immunity effectively through similar pathways as the original pathogens do (Keller et al. 2010). At the same time, none of the viral genetic materials will participate in the formation process of VLPs, which means that there is no risk of viral replication or proliferation. Therefore, VLPs are considered as one of the safest candidate vaccines.

In the 1960s, some empty viral particles without nucleic acid were identified as the capsid protein of hepatic B virus (Blumberg et al. 1965). This finding was considered as the first recorded instance of the natural existence of VLPs. Subsequently, hepatic B virus VLPs were detected that they can induce the host immune responses to eliminate the invasion of the authentic hepatic virus (Bayer et al. 1968). The phenomenon is a clue to understand the relationship between the VLPs and the host immune system. With the progress in genetic engineering technology, the expression and purification of the major capsid protein of human papillomavirus (HPV) was achieved easily in vitro through experiments (Hagensee et al. 1993; Kirnbauer et al. 1992; Li et al. 1997). Thus, models for understanding the assembly of VLPs in vitro were obtained (Brady and Consigli 1978; Li et al. 1997). In

the 1980s, the antigenicity and immunogenicity mechanism of HBsAg VLPs were interpreted. Hence, VLP-based vaccines gained further attention from researchers (Edman et al. 1981; McAleer et al. 1984; Valenzuela et al. 1982).

The application of VLPs in vaccines is just one of its major biological applications. As a nanoscale material, VLPs have similar characteristics as nanomaterials and have the ability to mediate more biomedical functions through biotechnological methods. Therefore, VLPs, a new biological tool, have a significant function in drug delivery, genetic therapy, cellular targeting, and cancer treatment. This article will discuss the research development of VLPs in its biomedical application.

VLP-based vaccines

The use of vaccines is one of the most effective strategies in the prevention against pathogenic infection. Since the smallpox vaccination performed by Edward Jenner in the eighteenth century, which was the birth of the concept of vaccines, various inactivated or attenuated vaccines for both human and animals have emerged. In fighting against infectious diseases, these vaccines have made significant contributions, particularly in preventing and eliminating poliovirus, measles, mumps, rubella, influenza, and hepatitis A (Amanna and Slifka 2009; Lua et al. 2014; Plotkin 2005). However, several deficiencies still exist in the use of inactivated and attenuated vaccines. For example, there is a security issue that attenuated vaccines may enhance pathogenicity by reverting to a wild-type phenotype in vaccined individuals, although effective immune responses can be induced by such vaccines (Burns et al. 2014; Esteves 1988; Fachinger et al. 2008; Horaud 1993; Sanders et al. 2015; Wang et al. 2014). Inactivated vaccines cannot replicate in vivo after inoculation; however, acquiring full protection after a single immunization by such vaccines will be difficult (Bright et al. 2007). In addition, there is an urgent practical need to find a candidate vaccine that could be produced in an efficient, scalable, and inexpensive way and with a high degree of safety for some viruses that are difficult to culture in vitro (Chackerian 2007; Moon et al. 2014).

However, VLPs, mimicking the organization and conformation of authentic native viruses but lacking the viral genome, have been used as immunogenic molecules in several recombinant vaccines in the last few years and even used as a therapeutic vaccine to induce the production of specific antibodies against endogenous molecules with a preponderant role in chronic diseases (Andrade et al. 2013; Roldao et al. 2010; Speiser et al. 2010; Spohn et al. 2008). Some VLP vaccines have been licensed and commercialized, and a large number of VLP-based vaccine candidates have also been undergoing clinical evaluation (see Table 1); therefore, VLPs have provided delivery systems that combine good safety profiles with strong immunogenicity and constituted a safe alternative to inactivated vaccines or attenuated vaccines.

Prophylactic VLP vaccine

As a novel type of vaccine, VLPs offer a solution to the above problem, primarily because of its biological properties (Young et al. 2006). First, VLPs have a high level of safety without concerns of biosecurity since no viral genetic components are introduced during its production. Second, VLPs present conformational epitopes, which are arranged repeatedly on the surface. With such an arrangement, VLPs are more similar to the native virus; thus, VLPs can easily induce strong B cell responses in the absence of adjuvants (Ramqvist et al. 2007). Third, VLP vaccine can rapidly cope with epidemic viral diseases because of its short time required for proceeding from design to expression. For example, the development and preparation of VLP vaccines was only 8 weeks after the outbreak of influenza, but more than 5 months for attenuated vaccines (Cox 2005). Finally, VLPs, as pathogen-associated molecular patterns (PAMPs), can be recognized by pattern recognition receptors (PRRs) such as the Toll-like receptors (TLRs) of host cells and captured by antigen-presenting cells (e.g., dendritic cells) (Fakruddin et al. 2007), then can be taken up and processed via the MHC class I pathway (cross-presentation) for activation of $CD8^+$ T cells, which are essential for the clearance of intracellular pathogens such as viruses. In addition, VLPs are inherent in a suitable size, and VLPs can also be taken up by dendritic cells (DCs) as exogenous antigens for processing and presentation by MHC class II and for directly promoting DC maturation and migration, essential for stimulation of the innate immune response, whose stimulate immunity patterns are similar as in the original virus (Grgacic and Anderson 2006; Keller et al. 2010; Ponterio et al. 2013; Raghunandan 2011) (Fig. 1). In this way, VLPs may have the advantages over the cognate live viruses for immune activation because some viruses that replicate in DCs are known to block activation and maturation of the cell through expression of particular viral proteins, while some VLPs which can resemble infectious viruses and retain their receptor binding regions are able to be taken up by antigen-presenting cells for class I presentation systems.

The advance in molecular biology improved the expression system of the VLP-based vaccines. The number of reports on newly obtained VLPs has grown proportionally with the systems developed for the expression of these particles, and VLPs have been successfully used as a vaccine platform to which additional components of the virus or other virus or pathogen are attached or inserted and shown to stimulate both cellular and humoral immunity no matter how much the capsid protein the VLPs is composed of (Fig. 2). With the advantages in safe production process, short production time, and many available expression systems, there are numerous VLP-

Table 1 List of partial virus-like particle (VLP) vaccines derived from different viruses

Family	Virus	Composition	Expression system	Ref.
Picornaviridae	FMDV (type O1)	VP0, VP1, and VP3	B/IC	Mohana Subramanian et al. (2012)
	FMDV (Asia1)	VP0, VP1, and VP3	*E. coli*	Guo et al. (2013)
	Enterovirus 71	VP0, VP1, and VP3	B/IC	Chung et al. (2006)
	Enterovirus 71	VP0, VP1, and VP3	B/IC	Chung et al. (2008)
	Enterovirus 71	VP0, VP1, and VP3	Yeast	Li et al. (2013a)
Circoviridae	PCV2	Cap	*E. coli*	Wu et al. (2012)
	PCV2	Cap	Mammalian cells	Chi et al. (2014)
	PCV2	Cap	*E. coli*	Yin et al. (2010)
	PCV2	Cap	B/IC	Bucarey et al. (2009)
	PCV2	Cap	B/IC	Fort et al. (2008)
	PCV2	Cap	B/IC	Martelli et al. (2011)
Papillomaviridae	HPV16	L1	Mammalian cells	Pastrana et al. (2001)
	HPV	L1 and L2	Yeast	Bazan et al. (2009)
	HPV	L1 and L2	Plant	Pineo et al. (2013)
	HPV16	L1	B/IC	Vidyasagar et al. (2014)
Filoviridae	EBV/MBV	VP40, GP, and NP	B/IC	Warfield et al. (2007b)
	EBV	VP40, NP, and GP	B/IC	Warfield et al. (2007a)
	EBV	GP and VP40	Mammalian cells	Warfield et al. (2003)
	MARV	VP40 and GPs	Mammalian cells	Swenson et al. (2004)
	MARV	VP40 and GPs	Mammalian cells	Swenson et al. (2005)
	ZEBV	GP and Fc	Mammalian cells	Konduru et al. (2011)
	PEMCV	P1, 2A, 3C	B/IC	Jeoung et al. (2010)
	PEMCV	P1, 2A, 3C	B/IC	Jeoung et al. (2011)
Paramyxoviridae	NDV	NP, M, HN, F	Avian cells	McGinnes et al. (2010)
	NDV	F and M1	B/IC	Park et al. (2014)
Bunyaviridae	RVFV	Nucleocapsids	Mammalian cells	Naslund et al. (2009)
	RVFV	N, Gn, and Gc	B/IC	Liu et al. (2008)
	RVFV	L, N, and M	Mammalian cells	Habjan et al. (2009)
	UUK virus	GN and GC	Mammalian cells	Overby et al. (2006)
	Hantaviruses	N, Gn, Gc glycoproteins	Mammalian cells	Acuna et al. (2013)
Orthomyxoviridae	H5N1	HA, NA, M1, and M2	Mammalian cells	Wu et al. (2010)
	H5N1	HA, NA, and M1	B/IC	Kang et al. (2009)
	H5N1	HA and NA	B/IC	Bright et al. (2008)
	H5N1	Gag, HA, and NA	B/IC	Haynes et al. (2009)
	H1N1	NA and M1	Mammalian cells	Easterbrook et al. (2012)
	H1N1	HA and M1	B/IC	Quan et al. (2010)
	H1N1	HA, NA, and M1	B/IC	Pyo et al. (2012)
	H9N2 (AIV)	HA and M1	B/IC	Lee et al. (2011)
	H3N2	HA and M1	B/IC	Lee et al. (2013)
	H7N9	HA, NA, and M1	B/IC	Smith et al. (2013)
Reoviridae	Rotavirus	VP2, VP6, and VP7	B/IC	Kim et al. (2002)
	Rotavirus	VP2, VP6, and VP7	B/IC	Vieira et al. (2005)
	Rotavirus	VP2, VP6	B/IC	Agnello et al. (2006)
	Rotavirus	VP2, VP6	B/IC	Mena et al. (2005)
	Rotavirus	VP2, VP6, and VP7	B/IC	Clark et al. (2009)

Table 1 (continued)

Family	Virus	Composition	Expression system	Ref.
	BTV	VP2, VP3, VP5, VP7	B/IC	Stewart et al. (2013)
Parvoviridae	CPV	VP2	*E. coli*	Xu et al. (2014a)
	GPV	VP2	B/IC	Chen et al. (2012)
	GPV	VP1, VP2, VP3	B/IC	Ju et al. (2011)
	PPV	VP2	Mammalian cells	Chen et al. (2011)
	HPB19 virus	VP2	*E. coli*	Sanchez-Rodriguez et al. (2012)
	HPB19 virus	VP1, VP2	Yeast	Chandramouli et al. (2013)
Polyomaviridae	GHPV	VP1	B/IC	Zielonka et al. (2006)
	GHPV	VP1 and VP2	Yeast	Zielonka et al. (2006)
	JC polyomavirus	VP1	B/IC	Goldmann et al. (1999)
	JC polyomavirus	VP1	Yeast	Sasnauskas et al. (2002)
	Polyomavirus	VP1	*E. coli*	Shin and Folk (2003)
	MCV	VP1	B/IC	Touze et al. (2010)
Flaviviridae	JEV	Envelope protein	Mammalian cells	Chiou et al. (2008)
	JEV	prM and envelope proteins	B/IC	Yamaji and Konishi (2013)
	Dengue virus	prM and envelope proteins	Mammalian cells	Zhang et al. (2011)
	Dengue virus-2	prM and envelope proteins	Yeast	Liu et al. (2010)
	West Nile virus	Envelope glycoprotein	*E. coli*	Spohn et al. (2010)
	West Nile virus	prM and envelope proteins	Mammalian cells	Ohtaki et al. (2010)
	HCV	Core protein	*E. coli*	Lorenzo et al. (2001)
	HCV	Core protein	B/IC	Li et al. (2013b)
	HCV	E1 and E2 proteins	Mammalian cells	Garrone et al. (2011)
	HCV	E1, E2 protein	B/IC	Murata et al. (2003)
Caliciviridae	RHDV	VP60	B/IC	Gromadzka et al. (2006)
	RHDV	VP60	B/IC	Nagesha et al. (1995)
	RHDV	VP60	B/IC	Young et al. (2006)
	NV	NV1	Mammalian cells	Harrington et al. (2002)
	NV	Capsid protein	Plant	Lai and Chen (2012)
	NV	Capsid protein	Plant	Santi et al. (2008)
	FCV	VP1	B/IC	Di Martino et al. (2007)

FMDV foot and mouth disease virus; *B/IC* baculovirus/insect cells, namely cloned genes into baculovirus constructs and infect insect cells to generate related protein for forming VLPs; *PCV2* porcine circovirus type 2, *CP* capsid protein, *ORF* open reading frame, *HPV 16* human papillomavirus 16, *EBV* Ebola virus, *MBV* Marburg virus, *ZEBV* Zaire Ebola virus, *MARV* Marburg virus, *GPs* glycoprotein, *PEMCV* porcine encephalomyocarditis virus, *NDV* Newcastle disease virus, *RVFV* Rift Valley fever virus, *UUK virus* uukuniemi virus (Bunyaviridae), *HA* hemagglutinin, *NA* neuraminidase, *M1* matrix 1 protein, *M2* matrix 2 protein, *BTV* Bluetongue virus, *CPV* canine parvovirus, *GPV* goose parvovirus, *PPV* porcine parvovirus, *HPBV19* human parvovirus B19 virus, *GHPV* goose hemorrhagic polyomavirus; *MCV* a new human polyomavirus, known as Merkel cell polyomavirus; *JEV* Japanese encephalitis virus, *HCV* hepatitis C virus, *RHDV* rabbit hemorrhagic disease virus, *NV* Norwalk virus, *FCV* feline calicivirus

based vaccines under development and some are even currently in the market. Further examples of VLPs as vaccine candidates are shown in Table 1.

As shown in some studies, the VLPs can be used as an ideal carrier platform for foreign B cell and/or T cell epitopes to display practically any antigen in a highly immunogenic, multivalent format in vaccination experiments. In addition that some viral epitopes are relatively conserved, the epitope genes can be obtained through genetic engineering and subcloned into the plasmid vector. Thereafter, the genes are genetically incorporated into the gene sequence of coat/capsid protein of VLPs, and then VLPs can present the exogenous antigens by fusion expression (Arora et al. 2012; Kawano et al. 2013, 2014; Tyler et al. 2014; Ye et al. 2014). A simpler mean to produce such chimeric vaccines can be achieved by genetical insert rather than by chemical linking the peptide epitopes to the VLPs, and chimeric epitope peptides can be repeatedly arranged on the surface of VLPs, which have potential prospects (Peabody et al. 2008).

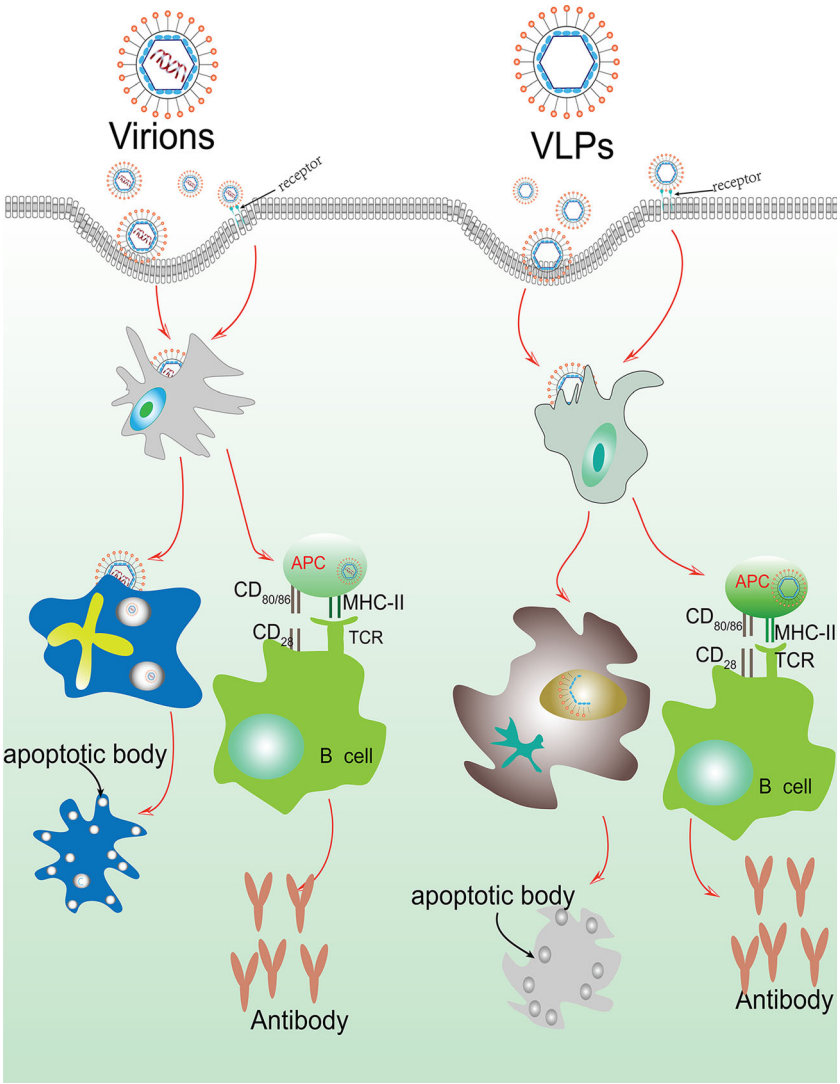

Fig. 1 Virus-like particles (*VLPs*) mimic the overall structure of virus particles, are recognized readily by the immune system, and present viral antigens in a similar pathway to authentic conformation inducing strong immune responses

As proven by numerous experiments, the insertion of a foreign peptide sequence into the upstream or downstream of the viral structure proteins genes has little effect on the assembly of viruses such as HPV (Teunissen et al. 2013), hepatitis B virus (HBV) (Brandenburg et al. 2005; Mihailova et al. 2006), AMCV (Arcangeli et al. 2014), Rotavirus (Cortes-Perez et al. 2010), RNA bacteriophage Qβ (Tissot et al. 2010), and canine parvovirus (CPV) (Gilbert et al. 2004). Therefore, the insertion of foreign genes into VLPs through gene recombination technology renders VLPs suitable as an antigen presentation tool. HBc VLPs are the first reported VLPs that present exogenous antigens, and HBc protein dimers have a highly symmetrical and relatively stable icosahedral structure. Several sites to which exogenous antigen epitopes can be incorporated into the sequences of their proteins include N- and C-terminal and major immunodominant region (MIR) (Jegerlehner et al. 2002). Except viral epitopes, VLPs can also present immune-related factors such as CD40 ligand (Zhang et al. 2010) and cytotoxic T cell epitopes (Tartour et al. 2002), as well as immune-stimulating factors, such as antimicrobial peptides, interferons (IFN), proinflammatory cytokines, and chemokines when recognizing PAMPs in the early period of innate immunity (Liu et al. 2000; Vacher et al. 2013) (Table 2).

VLP-based therapeutic vaccines

As an epitope vector, VLPs can present not only foreign antigens but also self-antigens. Several VLPs have been studied as vehicles for use in immune therapy especially as therapeutic vaccine to treat chronic diseases and cancer (Ramqvist et al. 2007). For immunotherapeutics, which represent a new promising class of vaccines developed to treat chronic diseases (Fulurija et al. 2008; Rohn et al. 2006; Sonderegger et al. 2006; Spohn et al. 2007), therapeutic vaccination has demonstrated that it involves active clearance of an infectious agent, infected cells, and especially tumor cells through breaking immune tolerance or bypassing the mechanisms by which

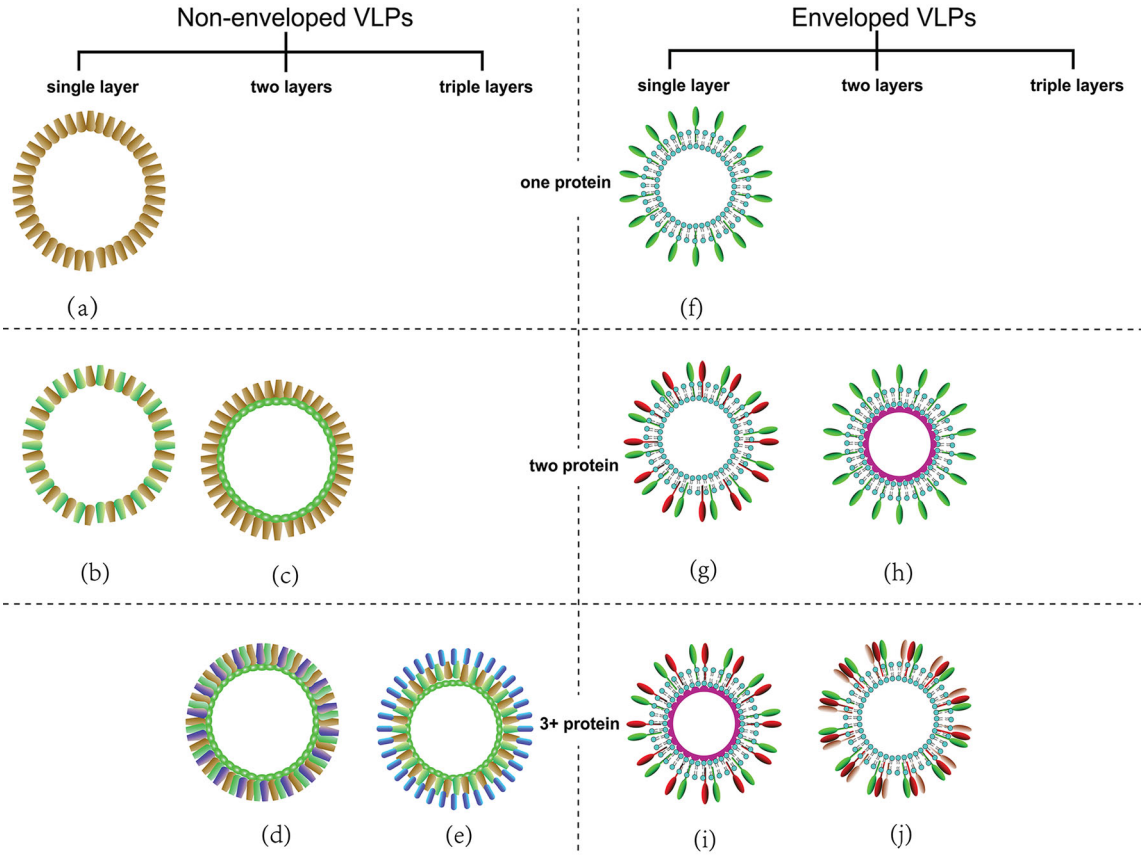

Fig. 2 A schematic diagram of the classification of different virus-like particles based on the number of viral surface proteins and the existence of lipid envelopes or not (adapted from Lua et al. 2014). For non-enveloped VLPs: (*a*) the single layered non-enveloped VLPs assembled by one protein (e.g., hepatitis B core antigen VLPs (Roose et al. 2013) and CPV VP2-VLPs (Xu et al. 2014)); (*b*) The single-layered non-enveloped VLPs assembled by two proteins (e.g., SARS coronavirus VLPs (Mortola and Roy 2004)); (*c*) Two-layered non-enveloped VLPs assembled by two proteins (e.g., papillomavirus L1 and L2 VLPs (McKee et al. 2015)); And (*d*) twolayered non-enveloped VLPs assembled by multiple proteins (e.g., FMDV-VLPs (Guo et al. 2013)); (*e*)The triple-layered VLPs assembled by multiple proteins (e.g., bluetongue virus (Stewart et al. 2013) and rotavirus VLPs (Parez et al. 2006)). For enveloped VLPs: (*f*) single-layered VLPs consisted of one protein (e.g., influenza virus ectodomain of matrix protein 2 (M2e) VLPs (Lee et al. 2014)); (*g*) Single-layered VLPs consisted of two protein (e.g., hantaviruses VLPs (Acuna et al. 2013)); (*h*) Two-layered VLPs consisted of two protein (e.g., hepatitis C VLPs (Bellier and Klatzmann 2013)), and (*i*) Two-layered VLPs consisted of multiple proteins (e.g., SARS coronavirus VLPs (Ho et al. 2004))

the disease has evolved the immune system. It has been known that the $CD8^+$ T lymphocytes (CTL) have played an important role in the control of tumor growth, so stimulation of specific CTL therefore represents one major goal in the design of cancer vaccines (Al-Barwani et al. 2014; Speiser et al. 2010; Zamarin and Postow 2015). VLPs can be good antigen delivery systems that efficiently introduce exogenous molecules into the MHC class I pathway, which showed to be important mediators of antitumor immunity in various animal models because of its ability to elicit tumor-specific CTL (Jegerlehner et al. 2002).

In addition, VLPs have the potential to become therapeutic vaccine to treat chronic diseases through molecular clone technology, thus overcoming the natural tolerance of the immune system and stimulating the immune system to create specific antibodies toward self-proteins (Spohn et al. 2005). With the identification of the pathophysiology of several chronic diseases, a few key pathogenic determinants have been deciphered. Such determinant-related proteins can be presented via VLPs by means of chemically cross-linking them to the surface of virus-like particles or genetic recombination to display. As a result, specific autoantibodies are produced, which are of considerable significance in ameliorating or even curing these chronic diseases (Tissot et al. 2010).

For example, to avoid a wide range of inflammatory reactions involving amyloid-β (Aβ)-specific B cells, $Aβ_{1-6}$ peptide (DAEFRH) and RNA phage Qβ-VLPs (VLPs) are covalently linked when treating Alzheimer's disease (AD) with VLP vaccines causing the production of antibodies specific to Aβ. The aforementioned strategies for the design of Aβ vaccine have been the subject of clinical trials (Chackerian et al. 2006; Wiessner et al. 2011). Other vaccines have been designed through similar methods, such as NGFQβ-VLPs, which are specific to nerve growth factors (NGF) for treating chronic pain (Rohn et al. 2011); bacteriophage Qβ-C-TNF_{4-23} VLPs, which are specific to TNF-α for rheumatoid arthritis

Table 2 The examples of VLP-based delivery for exogenous antigen delivery or epitope

Virus	Expression system	Modification strategies	Chimeric antigen(s)	Ref.
Phage AP205	*E. coli*	Chemical conjugation	α-Helic regions of HIV gp41	Pastori et al. (2012)
HBV	*E. coli*	Fusion expression	CFP-10 of tuberculosis	Cortes-Perez et al. (2010)
HBV	*E. coli*	Fusion expression	B and T cell epitopes of HCV	Mihailova et al. (2006)
HBV	HEK 293T	Fusion expression	VP4N20 of EV71	Cheong et al. (2009)
HBV	*E. coli*	Fusion expression	CTL epitope	Takeda et al. (2004)
HBV	*E. coli*	Fusion expression	MAGE-3	Kazaks et al. (2008)
HBV	*E. coli*	Fusion expression	CTL epitopes of HBV and HCV	Sominskaya et al. (2010)
HBV	*E. coli*	Fusion expression	Rubella virus E1 glycoprotein	Skrastina et al. (2013)
HBV	*E. coli*	Fusion expression	4 HBx-derived epitopes*	Ding et al. (2009)
HBV	*E. coli*	Fusion expression	SP55 or SP70 epitope of EV71	Ye et al. (2014)
HBV	*E. coli*	Fusion expression	(EDIII) of dengue viruses-2	Arora et al. (2012)
HEV	B/IC	Fusion expression	B cell epitope	Niikura et al. (2002)
Retrovirus	293T cells	Fusion expression	E1 and E2 envelope GP	Huret et al. (2013)
Qβ bacteriophage	*E. coli*	Chemical conjugation	CCR5	Hunter et al. (2009)
Qβ bacteriophage	*E. coli*	Chemical conjugation	V3 and ECL2 of HIV	Peabody et al. (2008)
Qβ bacteriophage	*E. coli*	Chemical conjugation	Nicotine	Cornuz et al. (2008)
Bacteriophage P22	*E. coli*	Chemical conjugation	Nucleoprotein of influenza	Patterson et al. (2013)
Bacteriophage MS2	*E. coli*	Fusion expression	L2 peptide of HPV16 and HPV31	Tyler et al. (2014)
FHV	B/IC	Fusion expression	ANTXR2 VWA	Manayani et al. (2007)
BPV	B/IC	Fusion expression	CCR5	Chackerian et al. (1999)
BPV	B/IC	Fusion expression	CTL epitopes	Liu et al. (2000)
Hepatitis E	B/IC	Fusion expression	p18 peptide	Jariyapong et al. (2013b)
HPV-16	Plant	Fusion expression	$M2e_{2\text{-}24}$, $M2e_{2\text{-}9}$	Matic et al. (2011)
HPV-16	Yeast	Conjugation	M2 from influenza A	Ionescu et al. (2006)
RSV	Insect	Fusion expression	*N. cani* surface protein	Deo et al. (2013)
SV40	Insect	Fusion expression	CTL epitope from influenza A	Kawano et al. (2014)
Rotavirus	B/IC	Fusion expression	14 amino acid epitope	Peralta et al. (2009)
HaPyV	Yeast	Fusion expression	GP33 CTL epitope of LCMV	Mazeike et al. (2012)
Influenza virus A	B/IC	Fusion expression	ESAT-6	Krammer et al. (2010)
IBDV	Yeast	Fusion expression	HPV-16 E7	Martin Caballero et al. (2012)
PPV	HEK-293 cells	Fusion expression	$Residues_{165\text{-}200}$	Pan et al. (2008)

B/I baculovirus-insect cell system, *CFP-10 Mycobacterium tuberculosis* antigen culture filtrate protein 10, *CCR5* the most important coreceptors that HIV used in the early stages of infection, *BPV* bovine papillomavirus virus, *CP* coat protein, *HVJ* hemagglutinating virus of Japan, *SV* simian virus 40, *HBVc-VLP* hepatitis B virus core protein virus-like particles, *PA* protective antigen of anthrax; *ESAT-6* early secreted antigenic target-6, an important *Mycobacterium tuberculosis* T-cell antigen; *p18 peptide* V3 loop of HIV-1 gp120, *VP4N20* the first 20 amino acids at the N-terminal of VP4 of EV71 genotype C4, *MAGE-3* cancer-germline gene, *FHV* flock house virus; *ANTXR2 VWA* protective antigen-binding von Willebrand A domain of the ANTXR2 cellular receptor, CTL epitopes including human PV16 E7 protein, HIV IIIB gp120 P18, Nef, and reverse transcriptase (RT) proteins, and an HPV16 E7 linear B epitope; *4 HBx-derived epitopes** HBx(52–60), HBx(92–100), HBx(115–123) and a novel subdominant cytolytic T lymphocyte (CTL) epitope HBx(140–148); *HaPyV* hamster polyomavirus, *LCMV* lymphocytic choriomeningitis virus, $M2e_{2\text{-}24}$ ectodomain of the M2 protein (M2e), $M2e_{2\text{-}9}$ a shorter version of M2e containing the N-terminal highly conserved epitope, *N. cani Neospora caninum*, *RSV* rous sarcoma virus, $residues_{165\text{-}200}$ residues 165-200 from the Porcine circovirus 2 (PCV2) virus nucleoprotein, *IBDV* infectious bursal disease virus

* Four different dominant sequence derived from hepatitis B virus epitopes protein

(Spohn et al. 2007); and Qβ-EC1 VLPs specific to CCR5 for preventing HIV infection (Hunter et al. 2009). Another design strategy for the development of chimeric VLP vaccines is the production of corresponding antibodies by stimulation from immunity-related antigens present on the surface of VLPs. This is achieved by inserting pathogen-related gene expression proteins into the epitopes of particular viral structure proteins (Ogasawara et al. 2006; Pumpens and Grens 2001; Yao et al. 2004). For example, Cubas et al. insert surface glycoprotein-Trop2 expressing excessively on the surface of

tumor cells into the SIV gag gene, which produced a chimeric vaccine to suppress the growth of tumor, because immunization with chimeric Trop2 VLPs can break tolerance to this self-protein and generate a specific cellular and humoral immune response significantly increasing the population of $CD4^+$ and $CD8^+$ T cells as well as natural killer (NK) and natural killer T cells (NKT) inside the tumor tissue, and these effects translated into a significant reduction in tumor growth (Cubas et al. 2011). Schiller et al. inserted an extracellular loop zone of chemokine receptor CCR5 of mice into bovine papillomavirus L1 capsid protein to produce autoantibodies to block CCR5. This step prevented HIV from entering cells and proliferation in cells (Chackerian et al. 1999). This approach is also employed to block B cells' tolerance to autoantigens for the treatment of some chronic diseases (e.g., rheumatoid arthritis, osteoporosis, experimental autoimmune encephalitis, systemic lupus erythematosus, myocarditis, atherosclerosis, hypertension, Alzheimer's disease, and obesity) by inducing therapeutically effective neutralizing autoreactive autoantibodies and is recognized as a potential treatment option for chronic diseases (Jennings and Bachmann 2008).

Chronic diseases are primarily caused by multiple pathogenic factors which make them difficult to cure; nevertheless, these therapeutic vaccines based on VLPs can produce antibodies aiming at autoantigens such as amyloid-β (Zamora et al. 2006), angiotensin II (Ang II) (Tissot et al. 2008), nerve growth factor (Rohn et al. 2011), allergens (Jegerlehner et al. 2002), and ghrelin (Andrade et al. 2013), which can help reduce the risk or cure certain diseases that had been at different stages of clinical trials (Jennings and Bachmann 2009; Roldao et al. 2010).

VLPs as versatile delivery vehicles

Ideal biological vectors should have the following biological characteristics: biocompatibility, solubility, and uptake efficiency, with targeted delivery and high drug loading. As a nanoscale material, VLPs have high potential in drug delivery (Boisgérault et al. 2002; Schott et al. 2011) (Table 3). VLPs fit the aforementioned demands in a certain degree among plenty of studies. First, VLPs are easy to be produced in large-scale quantities using the existing expression systems either as enveloped or nonenveloped VLPs (Fig. 3). Second, VLPs are capable of targeting the corresponding cell transport with its surface ligands by modification on the gene level (gene insertion) (Ungaro et al. 2013) or the protein level (chemical coupling) (Wei et al. 2009). Third, VLPs have good carrying capacity because of its large surface area and numerous amino acid residues on the surface (Patel and Swartz 2011). Fourth, VLPs are self-assembled by viral structure proteins under proper conditions which looks more like a protein cage with a large cavity space that can encapsulate numerous biological molecules, and as a result, expanded these molecules' applications (Wang et al. 2011; Yang and Burkhard 2012). Finally, VLPs have thermodynamic stability because of its dodecahedral or icosahedral structure. To sum, VLPs have emerged as multifunctional platform systems for the development of bioderived nanomaterials and have good potential for application in drug delivery, genetic therapy, and other fields (Fig. 4).

Drug carriers

The greatest adverse effect of chemotherapy in cancer treatment is toxicities to normal tissues, which severely limits the therapeutic effects of anticancer drugs. Anticancer drugs are tethered via the amino acid residues on the surface of VLPs, particularly the icosahedral VLPs, such as HBV, bacteriophage MS2, bacteriophage Qβ, and some dodecahedral VLPs, such as adenovirus (Ad) VLPs (Zochowska et al. 2009) with thermodynamic stability. Through mild chemical coupling reagents, anticancer drugs such as adriamycin and aleomycin can be loaded onto the surface of the aforementioned VLPs with hydrazone bonds created by amino acid residues. In addition, anticancer drug molecules can also be encapsulated into VLPs by the reversible process of self-assembly with changes of the external conditions, such as specific ionic concentration (Huhti et al. 2013). Moreover, VLPs have a proper particle size, good distribution, and biocompatibility as well as ligands on its surface for invasion into special cells. These ligands bind with receptors on the cell surface to help different VLPs to specifically deliver the drugs to various target cells mimicking the native virus. Common ligands are RGD motif (Marelli et al. 2013), transferrin (Singh et al. 2006), and so forth. At the same time, the anticancer drug bioavailability can be improved, that is, the improvement of the ability of the targeted transport and accumulation in target cells. Therefore, VLPs can be acceptable as effective biological vectors for carrying drugs.

VLPs as research surrogates

Studies on highly infective viruses and those without good cell culture systems in vitro or model animals, e.g., Ebola and Marburg viruses (Warfield et al. 2005) and human norovirus (Souza et al. 2013), must be performed under good experimental conditions. VLPs of these virus reserve the conformation of viral capsid protein, presenting the same ligands as those in the invading natural virus, which makes VLPs suitable as surrogates for basic research of these biosafety level 4 restricted viruses (Buonaguro et al. 2013). Therefore, VLPs are a

Table 3 VLPs derived from viral structural proteins as vehicle systems for biomedical applications

Virus	Expression system	VLP composition	Structural information	Cargo	Ref.
Bacteriophage					
φCb5	Yeast	Coat protein	Icosahedral	tRNA, nanoparticles, mRNA	Freivalds et al. (2013)
MS2	Yeast	Capsid protein	Icosahedral	mRNA	Li et al. (2014)
MS2	Yeast	Capsid protein	Icosahedral	Nonmethylated CG motifs	Storni et al. (2004)
MS2	*E. coli*	Capsid protein	Icosahedral	HIV-1 gag mRNAs	Sun et al. (2011)
MS2	*E. coli*	Coat protein	Icosahedral	IgG-binding Z domain	Brown et al. (2009)
MS2	*E. coli*	Coat protein	Icosahedral	Antisense ODNs	Wu et al. (2005)
Qβ	*E. coli*	Coat protein	Icosahedral	CelB glycosidase	Patterson et al. (2012)
Rotavirus	*E. coli*	VP6	Icosahedral	DOX	Zhao et al. (2011)
Rotavirus	B/IC	VP2, VP4, VP6	Icosahedral	GFP	Charpilienne et al. (2001)
Rotavirus	B/IC	VP2	Icosahedral	GFP	Cortes-Perez et al. (2010)
JC PyV	*E. coli*	VP1	Icosahedral	GFP or tk gene	Chen et al. (2010)
JC PyV	*E. coli*	VP1	Icosahedral	Exogenous plasmid DNA	Lin et al. (2014)
JCPyV	Yeast	VP1	Icosahedral	IL-10 shRNA	Chou et al. (2010)
HaPyV	*E. coli*	VP1	Icosahedral	Plasmid DNA	Voronkova et al. (2007)
CPV	B/IC	VP2	Icosahedral	EGFP	Gilbert et al. (2004)
HBV	*E. coli*	tHBcAg	Icosahedral	preS1 ligand	Lee et al. (2012)
HBV	B/IC	HBcAg protein	Icosahedral	DNA fragment	Brandenburg et al. (2005)
MrNv	*E.coli*	Capsid protein	Icosahedral	Plasmid DNA	Jariyapong et al. (2013a)
HCV	B/IC	Core protein	Icosahedral	RGD peptide and IFN-α2a	Li et al. (2013b)
CCMV	Plant cell	Capsid protein	Spherical	RNA derived from SINV	Azizgolshani et al. (2013)
Simian virus 40	*E. coli*	VP1	Spherical	Quantum dots	Li et al. (2009)
HIV-1	Mammals	Nef7	Spherical	HSV-1 TK gene	Peretti et al. (2005)
HPV	B/IC	L1, L2	Icosahedral	Plasmid DNA	Malboeuf et al. (2007)

VP6 rotavirus capsid protein; *DOX* doxorubicin, an anticancer drug; *ONDs* antisense oligodeoxynucleotides, *GFP* green fluorescent protein, *EGFP* enhanced green fluorescent protein, *RCNMV* red clover necrotic mosaic virus, *CPV* canine parvovirus, *liver-specific ligand* a liver-specific ligand, *tHBcAg* truncated HBcAg, *MrNv Macrobrachium rosenbergii* nodavirus, *DNA* a small hepatitis B virus surface antigene (SHBs)-specific sequence, *RGD* Arg-Gly-Asp, *PyV* polyomavirus, *HaPyV* hamster polyomavirus, *CCMV* cowpea chlorotic mottle virus (a plant virus), *RCNMV* red clover necrotic mosaic virus, *SINV* a mammalian virus-Sindbis virus, *HSV-1 TK gene* herpes simplex virus-1 thymidine kinase gene

good surrogate for simulating cell infection and to interact with host cells and viruses by labeling VLPs through the appropriate modification of the VLPs' surface in chemical or genetic ways (Tscherne et al. 2010).

So far, significant progress has been made in the study of characterizing virus-specific surface receptors, pathways of virus entry, and the mechanisms of virus assembly by utilizing VLPs as a surrogate. The mechanism of the infectivity of HuNoV has demonstrated that amino acid residues in the P domain of the VP1 protein are responsible for the specificity of receptor binding, and this data comes from the study of surrogates—HuNoV virus-like particle (VLP) (Tan et al. 2003, 2008; Tan and Jiang 2005). The interaction of capsid ORF2 protein of HEV with heparan sulfate proteoglycans (HSPG) has been proven by Kalia et al., and the researchers observed these results through the expression of hepatitis E virus ORF2 capsid protein in the insect cell Tn5 and the self-assembly of HEV-LP in vitro (Kalia et al. 2009). To observe the process of absorption, entrance, and intracellular transport of the virus, HIV-VLPs were assembled in vitro by Jouvenet et al. using green fluorescent protein (GFP) and Gag, the major structural component of HIV-1, and HIV-VLPs have a very similar morphology compared with the VLPs assembled by the Gag protein (Jouvenet et al. 2008). The single VLPs are employed to simulate a single virus. This method avoids the interference caused by the intracellular replication of the natural virus to the infection of a single virus (Pokorski et al. 2011; Wei et al. 2011). Through the aid of an advanced confocal microscope, Goreliket et al. observed the cellular surface morphology and fluorescent signals during the period of viral entry by a single Cy3-labeled polyoma VLP. This technique can explain viral endocytosis and establish a model

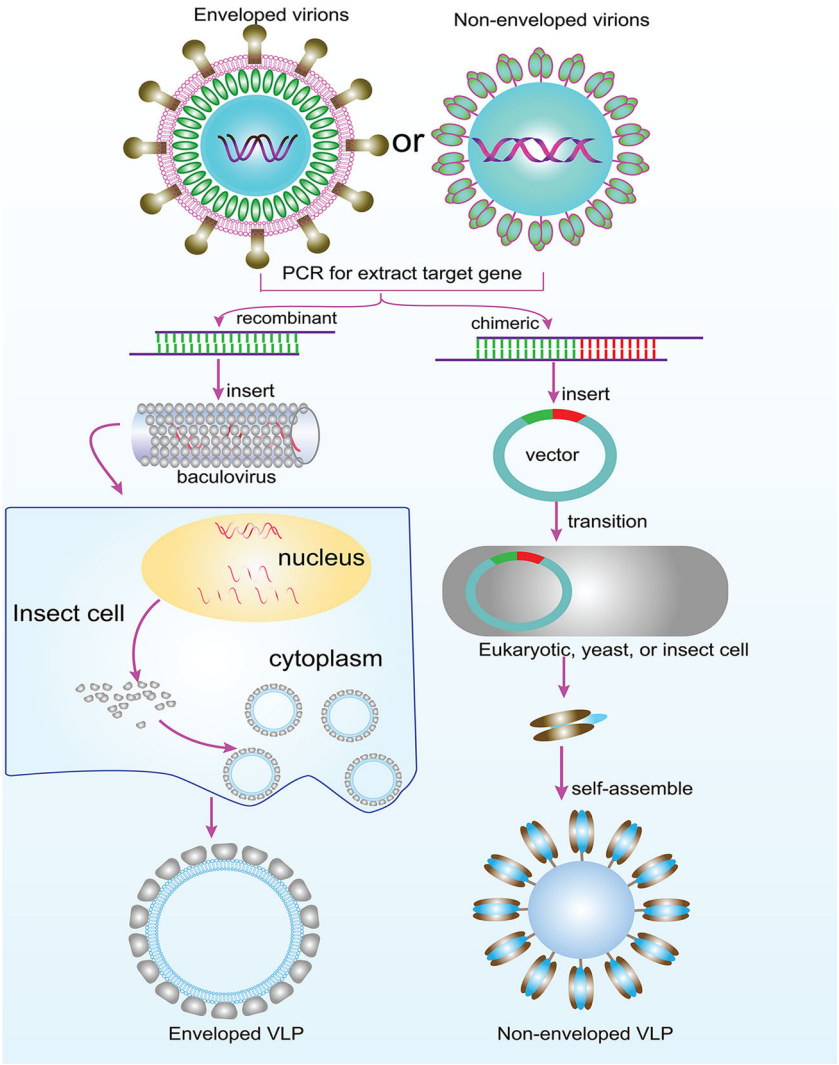

Fig. 3 Production process of VLPs derived from enveloped or nonenveloped through spontaneous self-assembling

for the observation of endocytosis of other nanoparticles (Gorelik et al. 2002). Buonaguro et al. studied the interaction between virus and cellular receptors through HIV Pr55 gag-VLPs with the embedded HIV gp140 envelope proteins (i.e., embedded with extracellular functional regions gp120 and gp41) by double-transfected bacillus in vitro. Tscherne et al. created BlaM1 VLPs through the recombinant expression of beta-lactamase reporter protein (Bla) and influenza matrix protein-1. When the VLPs adsorbed on the target cells, Bla was dissociated from BlaM1 VLPs and entered the cells. The released Bla could then be detected by flow enzyme-linked immunosorbent assay (ELISA) or positive ELISA, but it could not be detected intracellularly in the presence of antibodies of influenza virus because Bla cannot enter the cell. Likewise, this method is employed to detect Ebola (EBOV) and Marburg (MARV) viruses. A VLP was created in vitro by inserting a GFP protein into the N-terminal of the VP2 protein of CPV. This VLP is considered as a probe that tracks the interaction between CPV and host cells. With morphological, biophysical, and antigenic properties similar to those of putative virions, VLPs represent a novel model for the study of virus-host interactions and virus assembly.

Conclusion and future perspectives

In the past 30 years, VLPs have been widely used in the biology field. The VLPs assembled by viral envelope glycoprotein or capsid proteins have been proven to have the ability to induce humoral and cellular immune responses in experimental mice, nonprimates, and even humans (Raghunandan 2011). VLPs, whatever the form of prophylactic vaccine (recombinant VLP vaccine or chimeric VLP vaccine) or the form of therapeutic vaccines is, have the potential to be used as a new generation of vaccine candidates against various viral infections (Guillen et al. 2013).

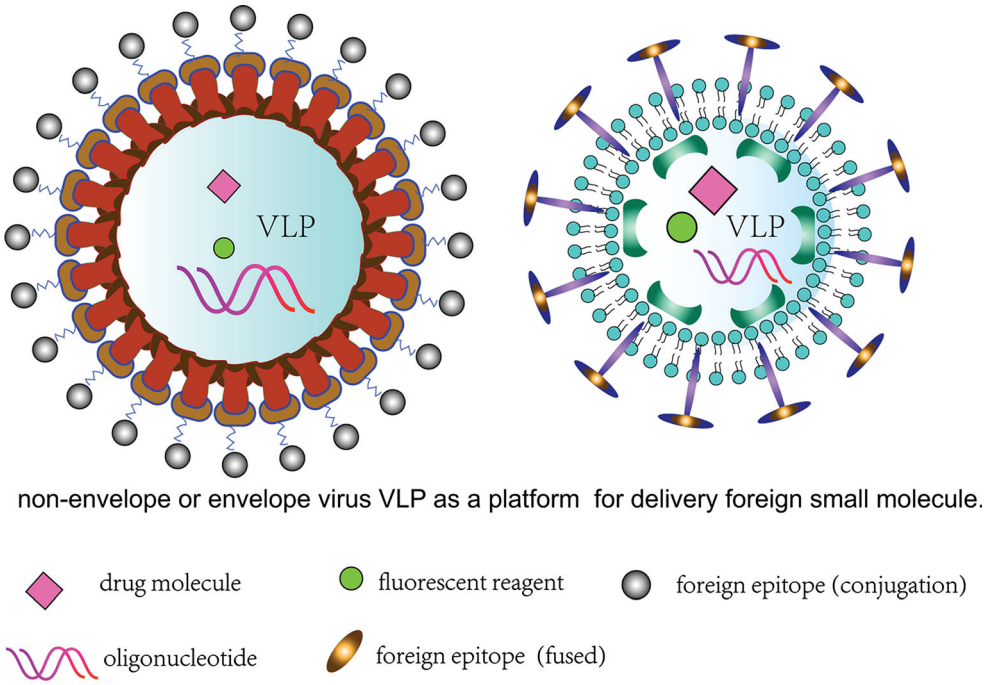

Fig. 4 A schematic representation of assembly VLPs derived from enveloped or nonenveloped viruses which are efficient nanocarriers for cargo delivery

At present, virology, molecular biology, protein chemistry, inorganic chemistry, and materials science are being highly integrated in the pursuit of new materials with controlled physical properties. VLPs have shown to be amenable to both chemical and genetic modifications of their inner cavities as well as their outer surfaces. Owning to their versatile hierarchical assembly, VLPs have been providing a new approach for the targeted transportation of therapeutic drugs and other biological materials. Overall, in this article, we reviewed the development and current situation of VLPs as vaccines and carriers, thereby contributing to the understanding and discovery of new biological applications for VLPs.

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Conflict of interest The authors declare that they have no competing interests.

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