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), erratic 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.

Keywords  Virus-like particles · VLPs · Vaccine · Drug delivery · Diagnostic technology

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; Kimbauer 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 vaccinated 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-
| 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             | Pino 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)          |
|                 | UUK virus              | GN and GC   | Mammalian cells   | Habjan et al. (2009)       |
|                 | Hantaviruses           | N, Gn, Gc glycoproteins | Mammalian cells | Overby et al. (2006)       |
| 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)          |
| Reoviridae      | Rotavirus              | VP2, VP6, and VP7 | B/IC              | Smith et al. (2013)        |
|                 | Rotavirus              | VP2, VP6, and VP7 | B/IC              | Kim et al. (2002)          |
|                 | Rotavirus              | VP2, VP6    | 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)        |
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).

Table 1 (continued)

| Family         | Virus       | Composition                  | Expression system | Ref.                  |
|----------------|-------------|------------------------------|-------------------|-----------------------|
| Parvoviridae   | BTV         | VP2, VP3, VP5, VP7           | B/IC              | Stewart et al. (2013) |
|                | 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   | Chiu 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   | Ohataki 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_ Epstein-Barr virus, _MBV_ Marburg virus, _ZEBV_ Zaire Ebola virus, _MARV_ Marburg virus, _GP_ glycoprotein, _PEMCV_ porcine encephalomyocarditis virus, _NDV_ Newcastle disease virus, _RVFV_ Rift Valley fever virus, _UUK_ uukuniemi virus (Bunyaviridae), _H4_ hemagglutinin, _N4_ neuraminidase, _M1_ matrix 1 protein, _M2_ matrix 2 protein, _BTV_ Bovine tongue 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.
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
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 vaccines 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, Aβ1-40 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-TNF4-23 VLPs, which are specific to TNF-α for rheumatoid arthritis.

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**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) two-layered 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)).
and anti-β-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

| 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                | MAG-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                                    | Cormuz 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                | M2e2-24, M2e2-9                             | 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                | Residues165-200                             | Pan et al. (2008) |

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, M2e22-24 ectodomain of the M2 protein (M2e), M2e2-9 a shorter version of M2e containing the N-terminal highly conserved epitope, N. cani Neospora caninum, RSV rous sarcoma virus, residues165-200 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.
tumor 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, arthritis, 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 nanoparticle, 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 hydrazine 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
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.

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 | Stormi 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 | CellB glycosidase | Patterson et al. (2012) |
| Rotavirus | E. coli | VP6 | Icosahedral | DOX | Zhao et al. (2011) |
| Rotavirus | B/IC | VP2, VP4, VP6 | Icosahedral | GFP | Charnpilienne et al. (2001) |
| 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 | Net7 | 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; ODNs 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 antigen (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
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).
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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