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Review

Challenges for the production of virus-like particles in insect cells: The case of rotavirus-like particles

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ABSTRACT

Virus-like particles (VLP) are formed when viral structural proteins are produced in an heterologous expression system. Such proteins assemble into structures that are morphologically similar to native viruses but lack the viral genome. VLP are complex structures with a wide variety of applications, ranging from basic research and vaccines to potential new uses in nanotechnology. Production of VLP is a challenging task, as both the synthesis and assembly of one or more recombinant proteins are required. This is the case for VLP of rotavirus (RLP), which is an RNA virus with a capsid formed by 1860 monomers of four different proteins. In addition, the production of most VLP requires the simultaneous expression and assembly of several recombinant proteins, which – for the case of RLP – needs to occur in a single host cell. The insect cell baculovirus expression vector system (IC-BEVS) has been shown to be a powerful and convenient system for rapidly and easily producing VLP, due to several convenient features, including its versatility and the short time needed for construction of recombinant baculovirus. In this review, the specific case of rotavirus-like particle (RLP) production by the IC-BEVS is discussed, with emphasis on bioprocess engineering issues that exist and their solutions. Many culture strategies discussed here can be useful for the production of other VLP.

Contents

1. Virus-like particles: from successful applications to promising possibilities

Virus-like particles (VLP) are structures identical to viruses, but devoid of genetic material. VLP are obtained when viral structural proteins are produced in recombinant expression systems, or even in cell free systems [1]. Recombinant structural viral proteins of several viruses can spontaneously assemble into VLP in the absence of the viral genetic material and other viral proteins. Most VLP produced to date are icosahedral and not enveloped, although enveloped VLP of influenza virus have been obtained [2].

VLP have a wide range of important applications. In basic research, VLP have been used for studying viruses and their structure, for determining the role of structural and non-structural proteins, and for examining virus–host interactions, among others [3–6]. VLP are excellent vaccine candidates, as they are not infectious and their size and structure result in excellent immunostimulating properties [7–9]. Humoral and cellular responses have

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Table 1
Examples of virus-like particles produced in the insect cell-baculovirus expression vector system, or by stably-transfected insect cells.

Virus	Recombinant proteins	Structure	Cell line	Reference
Adeno-associated	VP1, VP2, VP3	20–25 nm sl particle	Sf9	[20]
Blue tongue	VP2, VP3, VP5, VP7	sl or dl (69 nm) particles	*S. frugiperda*[a]	[21]
Bursal disease	VP2, VP3, VP4	60 nm sl capsid	Sf9	[22]
Hepatitis B	Surface and core antigens	sl 20–25 nm (HBcAg) or 30 nm (HBsAg) particles	*S. frugiperda*[a] cells, *T. ni* larvae, and *Drosophila* S2 cells[b]	[23,24]
Hepatitis C	C, E1 and E2 proteins	Enveloped 40–60 nm sl particles	Sf9	[25]
Hepatitis E	50 kDa structural protein	sl 24 nm particles	High Five®	[26]
Herpes simplex	UL26.5	sl 90 nm particles	Sf21	[27]
Human papilloma type 11	L1 protein	sl 50 nm particles	Sf9	[28]
Influenza	Hemagglutinin, neuraminidase, M1 and M2 proteins	80–120 nm enveloped sl particles	Sf9	[29]
Human calciviruses	Capsid protein	30–38 nm sl particles	High Five®	[30]
Parvovirus B19	VP1, VP2 and VP3	22 nm sl particles	Sf9	[31]
Poliomavirus	VP0, VP1 and VP3	50 nm sl particle	Sf 21	[32]
Rotavirus	VP2, VP4, VP6, VP7	sl (45 nm), sl (70 nm) or tl (75 nm) particles	SF9, High Five®	[4,33]
HIV	$Pr55^{gag}$ and gp160	90–120 nm sl and dl particles	Sf9	[34]

sl: single-layered; dl: double-layered; tl: triple-layered.
[a] No specific cell line was reported.
[b] Stable recombinant cell line.

been efficiently obtained. VLP size facilitates uptake by dendritic cells, and their structure, which has a large number of repeating epitopes, is ideal for activating B cells. Structural epitopes can be efficiently displayed, and polyvalent VLP can be obtained if various epitopes are simultaneously present in a single particle. This is important for generating multivalent vaccines. In addition, it is possible to insert foreign chimeric epitopes, or even complete proteins, in VLP, which results in highly immunogenic structures [8]. The first VLP vaccine to be produced and characterized contained the surface antigen of the hepatitis B virus (HBsAg), which coassembles with cellular membranes (lipids and carbohydrates) into spherical particles with diameters that range from 17 to 25 nm [10]. Recombinant HBsAg was successfully introduced in 1987 as the first human recombinant vaccine, and its production is commonly based on yeast expression systems. Recently, VLP of human papilloma virus, which are constituted by the major structural protein L1, were approved as vaccines [7]. Two variants of this vaccine exist. Gardasil is produced by Merck in yeast, and is a quadrivalent vaccine that contains types 6, 11, 16 and 18. Cervarix is produced by GlaxoSmithKline using the insect cell baculovirus expression vector system (IC-BEVS) and is a bivalent vaccine against types 16 and 18. Several vaccines based on VLP are in development and in clinical trials, including vaccines against hepatitis C, malaria (as antigenic peptides presented in VLP), SARS, Ebola, Marburg virus, Rift Valley fever, rotavirus, influenza, Norwalk virus, and HIV [7,11]. Recombinant production of VLP also allows the study of viruses that cannot be cultured *in vitro*, for example Bertolotti-Ciarlet et al. [12] have cloned and expressed VLP of the nonculturable norovirus in such a way. The application of VLP goes beyond vaccination against infectious viral diseases. For instance, engineered chimeric VLP have been proposed as therapeutic vaccines for cancer and autoimmune diseases [8].

The interest in VLP is rapidly expanding beyond vaccines, as new and interesting potential applications are starting to appear. The absence of the viral genome leaves space for encapsulating a wide variety of compounds, such as nucleic acids, drugs, or other compounds [13–16]. VLP loaded with a relevant cargo can be used as convenient delivery agents (or vehicles) to target organs or cells for applications that include therapy and diagnostics [15]. VLP have also been used for viral persistence studies in water [17]. Their nanometric dimensions make VLP ideal materials for nanotechnology [18,19]. Plascencia-Villa et al. [19] have explored the use of VLP as nanomaterials by conferring to them new functionalities through coverage with different metal nanoparticles, including gold, silver, platinum and palladium. Such new and unique materials can find novel applications as diagnostic, antimicrobial or targeted cytotoxic agents.

2. The insect cell-baculovirus expression vector system: a workhorse for the production of VLP

A large number of VLP of different viruses have been produced (some examples can be found in Grgacic and Anderson [7] and Table 1). Several VLP are formed by a single protein assembled into one layer, and are often produced in bacteria or yeast [7,10], whereas others are formed by various proteins, which assemble into one or several layers. VLP formed by several proteins require the simultaneous recombinant expression of more than one gene. The IC-BEVS is ideal for this task, as it is a versatile and efficient system for the production of recombinant proteins. Detailed reviews of the main bioengineering challenges and issues of the IC-BEVS can be found elsewhere [35–37]. A brief summary of such issues is given below.

Recombinant proteins in the IC-BEVS are expressed when an insect cell culture is infected with one or several recombinant baculovirus that contain the gene or genes of interest (Fig. 1). The construction of recombinant baculovirus is simple and fast, which provides a high versatility to the expression system. A detailed review of the different alternatives for constructing recombinant baculoviruses can be found elsewhere [36]. Such flexibility is very important when producing vaccines for rapidly changing viruses, as it is a fundamental requirement in an efficient approach to contend with potential pandemics in a timely manner. For instance, an influenza vaccine production campaign based on IC-BEVS can be completed with only 1.5 months after having identified the particular circulating viral strain, whereas an egg-based or other cell culture based platform would require 7–9 months [38–40]. A process flow diagram for the production of recombinant proteins using the IC-BEVS is shown in Fig. 1. Master insect cell and recombinant baculovirus banks should be maintained. Recombinant baculovirus stocks need to be amplified and titered. Several titration methods exist, ranging from the plaque assay, which allows the physical visualization of viral plaques but requires highly specialized personnel and has a high variability, to high-throughput assays, as the viability assay shown in Fig. 1 [41]. High-throughput assays are automated, and do not require extensive training. Baculovirus stocks should have titers at or above 10^8 plaque forming units (pfu)/mL. Viral stocks should be retitered every 6 months and should have been passaged less than five times, to avoid the presence of defective