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Minireview

Large-scale production and purification of VLP-based vaccines

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Abstract

Virus-like particles (VLPs) hold tremendous potential as vaccine candidates. These innovative biopharmaceuticals present the remarkable advantages of closely mimicking the three-dimensional nature of an actual virus while lacking the virus genome packaged inside its capsid. As a result, an equally efficient but safer prophylaxis is anticipated as compared to inactivated or live attenuated viral vaccines. With the advent of successful cases of approved VLP-based vaccines, pharmaceutical companies are indeed redirecting their resources to the development of such products. This paper reviews the current choices and trends of large-scale production and purification of VLP-based vaccines generated through the baculovirus expression vector system using insect cells.

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1. Introduction

Virus-like particles (VLPs) are multimeric protein complexes mimicking the organization and conformation of native viruses but lack the viral genome potentially permitting safer vaccine candidates. VLPs fall in the size range of viruses (22–200 nm or more in some cases), with their exact size and morphology depending on the particular viral proteins being incorporated. VLPs offer a promising approach to the production of vaccines against many diseases, because their repetitive, high density display of epitopes is potentially highly effective in eliciting strong immune responses (Bachmann et al., 2004). VLPs therefore constitute a safe and effective approach for the induction of neutralizing antibodies to surface proteins, where soluble forms of their protein subunits have failed. VLPs can also be exploited as “platforms” for the presentation of foreign epitopes and/or targeting molecules on chimeric VLPs. This can be achieved through modification of the VLP gene sequence(s), such that fusion VLP proteins and foreign vaccine...
proteins are assembled into VLPs during de novo synthesis. VLP chimeras have been extensively explored as vaccine candidates over the last 20 years (Pumpens and Grens, 2001).

VLPs can be generated based on a single protein as for the commercially available VLP-based human vaccines GlaxoSmithKline’s (GSK’s) Cervarix™ (human papillomavirus) and Engerix™ (hepatitis B virus) and Merck & Co.’s Recombivax HB™ (hepatitis B virus) and Gardasil™ (human papillomavirus). Envisaging the same success, there is currently a large number of other VLP-based vaccine candidates, including VLPs composed of multiple different proteins (especially when different viral structural proteins are needed for a stable VLP), either in clinical trials or undergoing preclinical evaluation; examples include influenza virus, parvovirus, Norwalk and various chimeric VLPs (Clinicaltrials.gov, 2010). Adding more viral proteins brings nevertheless extra challenges for setting up optimal processing parameters up- and downstream; furthermore, the required quality control methods are inevitably more complex because a mixture of both assembled and non-assembled proteins co-exist. These issues are addressed in more depth in the following sections.

In order to deliver these products in sufficient amounts for them to be evaluated cost-effectively in clinical trials and for eventual widespread use, robust and scalable manufacturing strategies need to be put into place. Even if proof-of-concept has been demonstrated with support from strong pre-clinical data, a VLP-based product candidate could never be competitive in the market if its clinical trial process and eventual commercial manufacturing are not carried out cost-effectively.

In order to deliver these products in sufficient amounts for them to be evaluated cost-effectively in clinical trials and for eventual widespread use, robust and scalable manufacturing strategies need to be put into place. Even if proof-of-concept has been demonstrated with support from strong pre-clinical data, a VLP-based product candidate could never be competitive in the market if its clinical trial process and eventual commercial manufacturing are not carried out cost-effectively.

Table 1 summarizes different VLPs produced using the BEVS/IC. With such a long track record on recombinant protein production and large-scale suspension cultures established in either stirred or rocked bioreactors, the BEVS/IC has been shown to be one of the best cell culture systems for this purpose. The only potential disadvantage of BEVS/IC resides in its pattern of post-translational modifications (Davis and Wood, 1995). Insect cells perform simpler post-translational glycosylation based on enriched mannose in comparison to mammalian cells, which can be a shortcoming for certain applications (Wu et al., 2010). Nevertheless, improved insect cells lines – “humanized” cells that perform mammalian-like post-translation modifications – are becoming available (Hang et al., 2003) (see also van Oers, this volume). Thus, when the insect cell glycosylation pattern and biological functionality of the desired VLP match, BEVS/IC is most likely the strongest candidate tool for VLP-based vaccine development.

The bioprocess challenges associated with large-scale production and purification of VLP-based vaccines are discussed in this review.

2. Upstream processing of VLP-based vaccines

2.1. BEVS/IC-based large scale manufacturing/bioreactor technologies

Depending on the specific target of the VLP vaccine, namely their biological function, different expression systems should be employed for expression of the required subunits: bacteria, yeast, mammalian cells, plant cells, and insect cells can be used. Although all systems have their own advantages and drawbacks, the primary focus within the context of this special issue on insect viruses in medicine is on the insect cell system using recombinant baculoviruses as a major tool. An extensive analysis of the different expression systems is available elsewhere (Roldão et al., 2010).

Insect cells have fast specific growth rates in serum and/or protein-free medium cultures; additionally, these cells are perfectly adapted to suspension conditions allowing for large-scale upstream processes (USPs) (O’Reilly et al., 1994). Running batch and fed-batch bioreactors (from 2 to 2000 L) is straightforward with cell densities usually reaching high levels (over 10 × 10⁶ cells/
mL) and are generally robust as long as the culture conditions are tightly monitored and controlled. These latter features make BEVS/IC especially attractive when compared to mammalian expression systems, which often require tedious cell culture adaptation procedures before suspension. Insect cells are also able to grow in the absence of serum, which is highly desirable because serum may be a cause of adventitious viruses thereby requiring more intensive purification processes in order to remove serum proteins in addition to host cell impurities.

The BEVS/IC system is characterized by a two-phase process, where insect cells are (i) grown to a desired viable cell density and (ii) infected by recombinant baculoviruses for protein expression during the exponential cell growth phase (as opposed to other systems). The second step involves definition of the most suitable multiplicity of infection (MOI), time of infection (TOI) and cell concentration at infection (CCI). The design and production of recombinant baculoviruses carrying the desired gene of interest is typically fast, allowing short turnaround times, critical when producing vaccines based on viruses whose surface proteins rapidly mutate over different seasons (e.g., influenza A virus, Cox and Hashimoto, 2011). Such product development flexibility is essential for a timely response to a potential pandemic outbreak.

One of the most important production issues is the upstream processing (USP) yield dramatically determining the scale needed: VLP yields can vary from 0.2 mg/L to hundreds of mg/L (Table 1). Although other expression systems like bacteria or yeast normally achieve higher yields, they would not match the degree of complexity of VLPs designed with the BEVS/IC (up to five or theoretically even more diverse proteins are possible). These high expression levels are explained by the ability of the virus to shut off the transcription of host genes and allocate the cellular transcriptional and translational machinery for the over-expression of heterologous genes delivered upon infection (Roldao et al., 2007).

Specifically for the influenza virus, BEVS/IC-derived influenza VLPs provide a good match to the wild-type influenza strain (Robinson, 2009). Moreover, the product yields are significantly higher when compared to the egg- or mammalian cell-based VLP production, over one order of magnitude higher in terms of its hemagglutinin (HA) titer; furthermore the production process can be shortened to approximately 12 weeks from identification of the new strain, contrasting with the 20–24 weeks needed for the traditional egg-based manufacturing processes (Robinson, 2009).

Single-use bioreactors have been emerging as a strong alternative to stainless-steel vessels (Ozturk and Hu, 2006). This USP technology presents many benefits, including: (i) single- or campaign-use, thus eliminating cross-contamination; (ii) no cleaning and sterilization costs; (iii) residual validation costs; (iv) increased flexibility for process scale adjustments; (v) lower upfront investment than stainless-steel reactors; (vi) short turnaround time; and (vii) ease of installation and little facility space utilization. Still, there are some drawbacks when going fully disposable, for example: (i) limited extractable and/or leachable substance quality control data from disposable materials and disposable manufacturers; (ii) disposable bag systems are difficult to handle for scales over 1000 L; (iii) pressure limitation; (iv) high temperature limitation. Nonetheless, with the current improvements in USP yields and in some cases early proof-of-concept stages, small to medium scales are sufficient, which make disposable bioreactors extremely appealing. In addition, a given lead biopharmaceutical has generally a much smoother technology transfer to any contract manufacturing organization (CMO) when relying on disposable manufacturing than with stainless-steel equipment due to the flexibility allowed by the former.

2.2. Advanced tools of the ‘ome’ era for yield optimization

Provided that suspension cultures and fast specific cell growth rates are demonstrated, the “envirome” of the insect cell hosts has been examined by identifying the impact of different parameters upon recombinant baculovirus replication and viral protein expression (Ozturk and Hu, 2006; Roldão et al., 2010). These include the bioreactor parameters such as dissolved oxygen concentration, pH, temperature, agitation rate, inlet gas flow and composition, volume or fluid dynamics. All of these parameters may influence VLP production by interfering with the cell growth and metabolic activity or even with protein post-translational modifications. Biological processes are complex and highly interactive responding to a greater or lesser extent to environmental stimuli. Although insect cells are very robust, optimization of bioreactor parameters should be carefully performed.

Table 2 highlights critical steps in the design and optimization of USP. Regarding the molecular/cell biology, the nature of the promoters strongly impacts productivities as early, late or very late promoters can be chosen. Early promoters induce lower yields as a result of the fact that, at such an early stage, most enzymes and transcriptional factors necessary for protein expression are not yet fully active (Roldão et al., 2007). With late or very late promoters, protein expression occurs towards the end of the viral replication cycle when the cells are approaching the stationary and death phases and proteases may start to degrade the newly produced proteins and their post-translational modifications. The MOI, i.e., the average number of viruses per infected cell, the time of harvest (TOH) and the CCI strongly influence protein expression and consequently VLP assembly. The optimal MOI is typically dependent on the target product (single or multiple protein based VLP) and the production strategy (single- or co-infection). For single protein expression, low MOIs (0.01–1 virus/cell) normally induce higher volumetric productivities than high MOIs (>1 virus/cell) as an outcome of higher infected cell concentration – specific productivities at low and high MOIs are comparable (Roldão et al., 2008). In theory, the difference in volumetric productivities could be counterbalanced with an increase in CCI. However, other problems may arise, e.g. the observed cell density effect affecting cell-specific productivity with increasing CCI (Bernal et al., 2009).

Although fed-batch or perfusion culture modes can, at least theoretically, improve specific productivities within a wide range of MOIs, they are often neither practical nor economically attractive. Metabolome/proteome or more recently “fluxome” (Teixeira et al., 2007) tools show great promise for USP optimization as an integrated analysis of the metabolic fluxes can derive online process

Table 2

| Stage | Strategy | Purpose |
|-------|----------|---------|
| **USP** | Clone screening | Optimal VLP yield and VLP quality media screening for improved yields |
| | High-throughput screening/DoE | Media screening for improved yields |
| | Metabolic flux analysis | Evaluation of critical nutrients for fed-batch |
| | Bioreaction engineering/scale-down models | Environment parameter optimization |
| **DSP** | Product characterization | Biophysical characterization |
| | Material/matrix screening | Optimal filtration/ chromatography materials |
| | Scale-down models | Identification of optimal operating conditions |
| | High-throughput screening/DoE | Optimal design space identification |

Abbreviations: USP: upstream processing; DSP: downstream processing; DoE: design of experiments.
control strategies to further improve product yields or productivities. This can be achieved through novel systems biology tools, e.g., metabolic flux analysis coupled with mathematical models to analyze the complex network of metabolite fluxes and identify the limiting factors (Teixeira et al., 2009).

In regard to the assembly of the required protein subunits into the final VLP, thermodynamic studies can prove very useful for identifying the most suited environmental conditions upon production and also for stability maintenance during downstream processing (DSP) and storage. A recent study of in vitro assembly/disassembly of rotavirus VLPs explores how physicochemical parameters affect the assembly and stability of the produced VLPs (Mellado et al., 2009): protein macrostructure stability was shown to be highly dependent on pH, ionic strength and temperature. As a follow-up, our team developed a thermodynamic equilibrium-based model describing, for the first time, the assembly of a triple-layered VLP (Roldão et al., 2010). Simulations suggested that the concentration of correctly assembled particles is maximized if the structural proteins are provided at the exact stoichiometric ratio and with high concentration. Such insights lead to the conclusion that the amount of unassembled/malformed VLPs typically observed in many systems is most likely due to a non-optimal ratio of proteins at the moment of assembly inside the cell host.

3. Downstream processing of VLP-based vaccines

In order to use VLPs as vaccines, efficacy and safety are essential; hence, purity, potency and consistency become crucial. The development of large-scale bioprocesses with high yield, short processing times and reduced total costs – scalability and robustness – is thus critical for large-scale VLP manufacturing.

A clear trend is observed from laboratory purification methods like sucrose or cesium chloride gradient ultracentrifugation procedures towards scalable processes like tangential flow filtration and chromatography (Morenweiser, 2005). Ultracentrifugation-based procedures are non-scalable, tedious and very labor intensive; however, these procedures are still used in order to prepare standards to serve as reference materials for process development. The first downstream processing (DSP) step depends on whether the VLPs are released to the extracellular medium. If the VLP is not efficiently secreted, a cell lysis or other extraction step might be required before the actual clarification step (Fig. 1). However, the current trend is to design a clone compatible with an efficient secretory pathway facilitating or shortening DSP.

The natural tendency of single proteins to adopt incorrect but equally stable macroassembly structures poses serious concerns to the DSP; the controllability of process conditions (e.g., salt and divalent ion concentrations and pH) can be crucial to avoid random protein aggregation. In such challenging cases, the strategy developed has to take into account process(es) capable of differentiating the product-derived impurities (single or other macroassemblies deriving from the actual VLP) besides the process-derived impurities (primarily host-cell protein, host-cell DNA and baculoviruses).

One of the critical drawbacks of the BEVS/IC is the co-production of recombinant baculoviruses (enveloped viruses) during the infection process. As baculoviruses have shown adjuvant activity, if not removed or inactivated they might induce some negative synergistic effects on the target VLP-based immunologic response (Hervas-Stubbis et al., 2007). Obviously, this complicates DSP as recombinant baculoviruses and some VLPs have sizes within the same order of magnitude. Other biophysical features such as electrostatics need to be exploited to allow separation of the two virus particles and this may prove challenging if the VLP is also enveloped (e.g., Influenza VLPs (Wu et al., 2010)). As a last resort, purified VLPs undergo chemical inactivation treatments to eliminate any possible baculovirus infectivity (Rueda et al., 2000).

Given the particular and challenging characteristics of each designed VLP and their final clinical application it is necessary to simplify the downstream process as much as possible. Furthermore, critical issues associated with each VLP production system should be overcome as earlier in the process as possible; e.g., product degradation due to the proteolytic activity or diminished stability due to lack of a given structural protein upon the design of the VLP. Overall, the downstream processes should be designed to accommodate the best compromise between cost, throughput, and purity needs meeting the desired purity and potency. The capacities of the integrated DSP should include delivery of the product in large quantities (scalability), with high quality (purity), in high titer (potency), and doing so in a cost-effective manner.

3.1. Scalable technologies for DSP

Intensification of processes is of utmost importance if one desires a strategy that can later be efficiently transferred to a production site operating under current good manufacturing practices (cGMP). Examples that fit this concept include filtration (by depth-filters) as a clarification step, tangential-flow ultrafiltration/diafiltration as a concentration step, affinity or ion-exchange chromatography as a capture/purification step, and finally, size-exclusion or other type of chromatography as a final polishing step. The typical process is represented in Fig. 1 for the case of rotavirus VLPs (Peixoto et al., 2007).

3.1.1. Clarification

The clarification step is designed to efficiently remove cell debris and large aggregates, while maintaining and protecting the quality of the product. Before the clarification process, especially in the BEVS/IC, due to the presence of host cell derived proteases (Gotoh et al., 2001) anti-proteolytic preservatives are needed during bioreaction or while initiating DSP; this avoids extended degradation of the external glycoprotein layer essential for the final application.

The gold standard in the industry for clarification is centrifugation for cell sedimentation (either continuous or batch).
More recently, however, membrane processes appear as a very attractive alternative: scalability is guaranteed as the membrane area is easily scaled up by stacking more membrane units in parallel, for example. Dead-end disposable depth-filters have been used efficiently (Peixoto et al., 2007). Tangential flow microfiltration, using membrane cassettes or hollow fiber units (Saha et al., 1994) with 0.2 μm retention pore is also possible. The use of depth filters makes the sieving and particulate retention process much more efficient because of the depth-dependent size separation factor; moreover, due to the charged nature of some depth-filter materials (non-propylene) and their three-dimensional arrangement (Prashad and Tarrach, 2006), a higher degree of cell and cell debris retention is achieved while VLPs still permeate. A careful selection of the depth-filter materials is critical as the use of non-inert materials may bind efficiently not only the debris but also the VLPs being sieved (Table 2).

In line with the increasing interest in the use of cleaning- and validation-free disposable technologies, membrane processes represent a clear advantage over centrifugation as the former are easily adaptable to disposable formats; there are indeed a variety of choices from DSP equipment manufacturers. Membrane processes do perform fairly well with recovery yields frequently above 90% (Peixoto et al., 2007; Vicente et al., 2008).

3.1.2. Intermediate purification/concentration

Concentration is a critical step, ubiquitous for the DSP of VLPs. The stream volume should be reduced as early in the DSP as possible in order to reduce the upfront investment in the equipment downstream and overall consumables and buffers. Ultrafiltration/diafiltration using tangential flow mode is the process of choice (see Fig. 1 for an example). Adsorptive chromatography, operated in bind-elute mode, constitutes a good alternative for the concentration/purification step. When chromatography is operated as a capture step at this stage, good concentration factors can be achieved upon elution if the product is dilute in bulk and if the capacity of the chosen matrix is significant for the VLP in question; this is a recurrent option (Kalbfuss et al., 2007; Vicente et al., 2008).

More recently, other chromatographic matrices based on porous membrane layers or monoliths have drawn the attention of DSP engineers. These porous matrices are particularly suited for very large particles, such as VLPs. The specific surface areas (per unit volume) of porous matrices with pore sizes of 0.8 μm (Mustang membrane adsorbers from Pall), 3 μm (Sartobind membrane adsorbers from Sartorius-Stedim Biotech) or >1 μm ( CIM monoliths from BIA Separations) are significantly larger than those of resin beads used in packed-bed chromatography, whose pores are generally 10–20 nm for conventional resins or 400 nm for perfluorine resins (Trilisky et al., 2009). VLPs, ranging from 20 nm to over 200 nm, are hindered from binding onto the internal surface area of the resin, resulting in sub-optimal usage of such matrices. In addition, due to the porous nature of these matrices, diffusional mass transfer resistances are highly reduced. For the case of VLPs that have rather small diffusion coefficients, as compared to smaller biologicals such as proteins, these matrices clearly present an advantage over packed-bed chromatography. Moreover, these porous matrices can be purchased as disposable units eliminating cleaning and sterilization as well as their validation costs.

Other chromatographic chemistries have been efficiently used including hydrophobic interaction (Vedvick et al., 2008), hydroxypatite (Cook, 2001) and sulfated (Wolff et al., 2010) ligands, the latter two considered as “pseudo”-affinity chromatography ligands.

In order to select the best matrix and then the best operating conditions, high-throughput screening and design of experiment (DoE) approaches are implemented using scale-down models (Table 2). Our team has focused on ion-exchange (IEX) chromatography, developing scale-down models based on dynamic light scattering and surface plasmon resonance to address the electrostatic properties of the viral particles and use these insights to optimize the ion-exchange chromatographic step (Vicente et al., 2010a,b).

3.1.3. Polishing

Polishing is a critical step if clinical grade material is envisaged. Residual host-cell protein and host-cell DNA need to be removed to acceptable threshold values, typically below 100 μg/dose and 10 ng/dose (with DNA fragments not exceeding 200 bp), respectively (CBER guidelines, 2007). As BEVS/IC is used for the production of VLPs, one needs to devise a suitable strategy to remove, at least, most of the recombinant baculoviruses. This may be very challenging especially in the case of VLPs with very similar sizes and/or overall surface charge at a given pH.

Affinity or ion-exchange chromatographic processes are in principle suited for polishing. They allow high resolution separations (Morenweiser, 2005). IEX chromatography constitutes a process of choice for removing to the flowthrough pool host-cell proteins or host-cell DNA (Kalbfuss et al., 2007; Vicente et al., 2008; Wu et al., 2007). However, if the VLP and some VLP-derived impurities (such as non-assembled proteins or other macrostructures) have the same electrostatic properties, size-exclusion chromatography (SEC) probably becomes a better alternative if the difference in size is still significant; the polished VLP pool is obtained in the void volume of the gel filtration column (Peixoto et al., 2007).

Ultrafiltration/diafiltration (UF/DF) (usually by tangential flow filtration) constitutes the other alternative at the latest stage for removing lower molecular weight impurities. Both UF/DF and SEC efficiently allow exchange into the final formulation buffer. Tangential flow filtration scales more easily than SEC that is always limited to a process scale chromatography column volume (the load should not exceed 10% of column bed volume).

As in any other biopharmaceutical process, sterile filtration is typically the final step before vialing. Even though the VLPs are in general smaller than 200 nm, depending on the VLPs and their biophysical properties, low adsorption/inert sterile membrane materials should be evaluated in order to minimize product losses due to nonspecific binding.

4. Analyticals: towards a “Quality by Design/Process Analytical Technologies” approach

Analyticals play a crucial and limiting role in the development of a VLP-based vaccine (or other complex biopharmaceutical). Upon USP or DSP development one must be cognizant with the many features required for the effective, biologically useful VLPs. It is typical that a good percentage of the VLP proteins, perhaps even over 80%, are not properly assembled into the desired VLP (Roldão et al., 2010); therefore, it is critical that both qualitative and quantitative analytical methods are developed. This is critical so that process developers can design less “blindly” USP optimization (e.g., by changing the strategy of infection) or selecting the right chromatographic matrix/operating conditions (e.g., for removing a specific/resilient impurity). Moreover, for a cGMP process, product identity, quality, titer, purity and consistency/reproducibility need to be carefully documented. Table 3 shows the basic set of methods that are needed to ensure this manufacturing quality and consistency; in particular, for in-process control, in vitro potency and identity assays as well as methods for estimating the major process-derived impurities such as host-cell protein, host cell DNA and residual baculoviruses are fundamental. The rather extensive wishlist shown in Table 3 stresses the challenges
puriﬁcation development strategies will determine the pace for no-
and productivity/purity. The newer tools from systems biology
processing does not adequately address both high recovery yields
It is clear that there are still many bioprocessing challenges ahead:
able versatility of baculoviruses and their host insect cells are
man papillomavirus VLP now being commercialized, the remark-

5. Conclusions and outlook

and complexity underlining the manufacturing of such complex biopharmaceuticals.

Thus, it is clear that product and process understanding stream-
lines not only the optimization of the overall manufacturing strategy
but converges with the guidelines of the Quality by Design/Process
Analytical Technologies approach of the FDA (Mollerup et al.,

Table 3
A summary of analytical methods for VLP-based vaccines produced using BEVS/IC.

| Attribute          | Method                  | Analyte               |
|-------------------|-------------------------|-----------------------|
| Identity          | ELISA                   | Viral proteins        |
|                   | SPR                     | Assembled             |
|                   | (for antibody based interaction analysis) | VLP                  |
| Other immuno-based methods (e.g., SRID) |                        |
|                   | SDS–PAGE                |                       |
| Quantity          | ELISA                   | Viral proteins        |
|                   | SPR                     | Assembled             |
|                   | SRID                    | VLP                   |
|                   | Total protein (BCA or Bradford) |                       |
| Potency           | In vitro tests (e.g., hemagglutination) | Viral proteins Assembled |
|                   | In vivo pre-clinical tests vaccination and challenge experiments | VLP |
| Process-related impurities | Total protein | HCP |
|                   | ELISA                   | Host cell dsDNA       |
|                   | Immunoblotting         | BVs                   |
|                   | DNA staining (e.g. picogreen) |                       |
|                   | RT qPCR                 |                       |
| Product-related impurities | TEM                   | Protein sequencing | Viral proteins |
|                   | Mass spectrometry       | Viral protein variants |                   |
|                   | Immunoblotting         |                       |
|                   | ELISA                   | Aggregates            |
| Biophysical properties | DLS                   |                       |
|                   | TEM or SEM              | Viroyal proteins      |
|                   | CZE                     | Assembled             |
|                   | Analytical ultracentrifugation |                       |
|                   | Analytical size exclusion | Chromatography        |

Abbreviations: BCA: bicinchoninic acid; BVs: recombinant baculoviruses; CZE: capillary zone electrophoresis; DLS: dynamic light scattering; ELISA, enzyme-linked immunosorbent assay; HCP: host-cell protein; RT-qPCR: real-time quantitative PCR; SEM: scanning electron microscopy; SPR: surface plasmon resonance; SRID: single radial immunodiffusion; TEM: transmission electron microscopy.

5. Conclusions and outlook

VLP-based vaccine candidates produced by means of BEVS/IC are receiving wider attention. With the successful case of the human papillomavirus VLP now being commercialized, the remarkable versatility of baculoviruses and their host insect cells are slowly stepping into the world of marketed medicines as many more products are being tested in pre-clinical and clinical trials. It is clear that there are still many bioprocessing challenges ahead: often, production yields are unsatisfactory and the downstream processing does not adequately address both high recovery yields and productivity/purity. The newer tools from systems biology and a complete set of analytics in conjunction with innovative purification development strategies will determine the pace for novel VLP-based vaccine candidates.

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Conflicts of interest

António Roldão, Tiago Vicente, Cristina Peixoto, Manuel Teixeira Teixeira Carrondo and Paula Marques Alves were employees and students at IBET, Av. da República, Quinta do Marques, P-2784-505 Oeiras, Portugal.

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