Virus-like particle bioprocessing: challenges and opportunities

Rapid advances in the design and manufacture of structured materials will continue to see high levels of innovation, and the commercialization of new matrices tailored to virus-like particle purification.

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Modern virus-like particles (VLPs) are bioengineered structures that lack the infectious viral trait, so are replication incompetent. They are excellent vaccine candidates as these evolved meso-structures are highly immunostimulatory, even in the absence of adjuvant. The first recombinant vaccine approved more than 20 years ago was a VLP (for Hepatitis B), and the most recently approved recombinant vaccine is also (for Hepatitis E, in 2012). In between, Gardasil® and Cervarix® VLPs for cervical cancer were launched, to sales of more than US$1 billion per annum. To-date, all VLP vaccines approved as safe for humans are simple VLPs – they comprise a single recombinant protein that organizes into a nanoparticle able to present authentic immunogenic epitopes. Approved VLPs are manufactured at scale in yeast, Escherichia coli, insect and mammalian cells.

VLPs present interesting and unique challenges for the bioprocess engineer. The usual challenge of attaining high product purity is compounded by the need to maintain the structural organization of the particle, and its colloidal stability. A VLP with the wrong meso-structure will not be functional and may be difficult to distinguish and separate from the desired product. Also, as VLPs are hollow particles, their interior can encapsulate contaminants that might become packaged if the VLPs are assembled in the presence of those contaminants. This can be a particular problem for VLPs packaged within a cell line [1]. While these contaminant-encasing VLPs might be separable in the laboratory using density-gradient ultracentrifugation, this technique is less attractive at full scale. Unsurprisingly, pharmaceutical bioprocessing methods used to make commercial product incorporate steps of VLP disassembly and re-assembly in vitro to remove refractory contaminants and/or to improve structural homogeneity.

The quality-driven incorporation of in vitro VLP disassembly–assembly leads to obvious scope for bioprocess improvement. For simple VLPs where assembly is – or can be – understood, it is attractive to use cell-free approaches. These place control over protein expression and assembly directly into the hands of the bioprocess engineer, and ensure it is not necessary to first undo and then correct the VLP assembly reaction. This simplifies bioprocessing, and is an emerging approach for VLP preparation. For example, a C-terminally truncated Hepatitis B core protein VLP has been produced using fully cell-free approaches [2]. The open reaction format allows for precise redox control [3] as well as the incorporation of non-natural amino acids for click conjugation of antigens [4]. In vitro cell-free assembly of modular VLPs based on a murine polyomavirus backbone has also been reported following bacterial expression at gram-per-liter levels [5]. Murine polyomavirus VLPs prepared in this way have been engineered to display various antigens including from influenza and group A streptococcus [6–8]. The in vitro assembly of other VLPs including for rotavirus [9], cowpea chlorotic...
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mottle virus [10] and bacteriophages MS2 [2] and Qβ [3] has also been examined. Cell-free VLP assembly approaches are expected to mature as understanding of VLP assembly and its control improves, and as bioprocess-relevant mathematical models that capture not only the ideal assembly reaction, but also off-pathway aggregation, are developed [11].

The research pipeline of emerging VLP products is filled with a diversity of VLP mesostructures [12–14]. Many of these have architectures more complex than those approved to date, comprising multiple structural proteins and, in many cases, a cell-derived lipid coat. These VLPs may incorporate host glycoproteins or other adherent contaminants, as well as packaged contaminants. Such structures are difficult if not impossible to efficiently disassemble and then reassemble. Purification challenges abound for these VLPs. Traditional chromatography matrices, for example ion exchange, can play a role. However, these matrices rely on diffusion-limited mass transfer and thus may be extremely inefficient for large macromolecular complexes. A far preferable mode is monolith-based purification. Monolith structures allow for high convective flows and adsorption with planar surfaces, in a reversible and minimally disruptive fashion. Similarly, membrane-based exchange systems offer opportunities to separate VLPs from complex feedstock, although these have not been well researched in the context of VLPs. A highly promising approach for VLPs is the new CaptoCore™ resin, which uses a low-binding size-selective material to exclude VLP interaction from an interior adsorptive matrix that binds small and soluble contaminating proteins. VLPs are thus purified in flow-through mode. Mixed-mode chromatography has also shown promise for VLP purification, notably for the commercial Gardasil vaccine. Rapid advances in the design and manufacture of structured materials will continue to see high levels of innovation, and the commercialization of new matrices tailored to VLP purification.

Enhanced analytical capability will underpin future VLP bioprocess development. As noted earlier, an initial challenge in VLP bioprocessing is to obtain purified protein product. However, purity does not provide information on the VLP mesostructure. Determination of the particulate state is traditionally obtained using slow laboratory methods including analytical ultracentrifugation and transmission electron microscopy. However, high-resolution techniques such as cryo-electron microscopy and atomic force microscopy are less likely to cause structural perturbation and are therefore preferred. Dynamic light scattering is attractive for VLP analysis, however is sensitive to the presence of large material (e.g., aggregates) and has inherently low resolving power. Turbidometric methods have been used to monitor the assembly and aggregation of VLPs [15] and have high relevance for bioprocess development [16]. Quantitative methods such as field-flow-fractionation [17] and electrospray differential mobility analysis [18] combine speed and high resolution, and may play an increasingly important role in underpinning the rapid translation of new VLP products through bioprocess development.

Increasing research into novel VLP modalities will generate new challenges and opportunities for pharmaceutical bioprocessing. As our understanding of fully engineered VLPs increases through combined computational and experimental efforts [6,19] we expect new diseases to be addressed using VLP-based entities, in a rapid and cost-effective manner. New complex VLPs from plants and other hosts demand sophisticated new recovery strategies including new matrices for more efficient purification. There may be considerable scope to revisit older approaches, for example those based on selective precipitation or two-phase extraction, underpinned by screening-based methods of bioprocess development [20]. Elimination of aggregates and adherent and intra-VLP contaminants will require innovative strategies that fully gear the power of emerging separation matrices including those based on monolith, membrane and mixed-mode exchange methods. Methods for eliminating packaged contaminants will still prove elusive and may require innovative upstream solutions for complex VLPs or increasing use of in vitro approaches. The translational pathway, considering the multidimensionality of the challenge, will require new combinations of rational (e.g., computational) and statistical screening approaches, underpinned by high-resolution analysis of product purity and VLP meso-structure. As in the past, we fully expect the pharmaceutical bioprocessing challenge to be met head-on, ensuring that these innovative, efficacious and profitable meso-structured VLP vaccines can be translated to market for the global improvement of human and animal health.
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