Production and purification of a virus-like particle (VLP) based Hepatitis C vaccine candidate

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Abstract

Hepatitis C virus (HCV) infection is a major public health problem, causing more than 350,000 deaths every year. Currently, there is no Hepatitis C vaccine and the standard treatment for acute HCV infections has several limitations, including its low efficacy. There is thus a clear need for the development of a vaccine with both preventive and therapeutic roles. Virus-like particles (VLPs) have received increased attention as vaccine candidates, since some of those already marketed have been very successful. VLP titers have been helped by advances in systems biotechnology, as well as the flexibility and versatility of retroviral-like particles in incorporating and displaying proteins. In collaboration with Instituto de Biologia Experimental e Tecnológica (IBET), Oeiras, Portugal, a chimeric retrovirus-like particle (VLP) displaying E1/E2 HCV proteins was produced in an insect cell expression system. This VLP is a candidate vaccine for Hepatitis C. We investigated the production and purification of VLP-HCV to improve the process. In particular, single-use bioreactor technology was evaluated and compared with glass-stirred tank bioreactor culture in terms of viable cell concentration, % viability, growth kinetics and stability. Cell culture supernatants from both processes were then harvested and purified to understand how production affects the downstream process and product quality. Downstream train was improved by selecting an appropriate anion exchange (AEX) resin yielding 70% recovery and a satisfactory baculovirus (BV) Log reduction (LRV). Appropriate depth filtration and ultrafiltration (UF) devices were selected, leading to a GMP-compliant process that allows easy transfer to pilot scale level.

Methods

Insect Cell Culture: Two distinct baculoviruses, one expressing MLV-GAG and the other expressing HCV-E1E2, to transfect Sf9 cells at MOI 2. Cell concentration at infection was 10^6 cells/mL (normally at 24 hrs of culture). Sf900II cell culture media were used in the process. Cell culture was carried out in stirred-tank glass bioreactor and disposable bioreactor (Mobius® Bioreactor 3L). Bioreactor harvested between 80-96 hrs post-infection.

Clarification: Polygard® CN filters of nominal pore sizes of 10, 5, 0.6 and 0.3 µm (all pleated polypropylene depth filters) were evaluated, all operated at inlet flux of 988 LMH.

Ultrafiltration/Diafiltration: Pellicon® cassettes of two different membranes, 300 kD composite regenerated cellulose (Ultracel® membrane) and 100 kD polyethersulfone (Biomax® membrane), were evaluated. No diafiltration but 4-5x concentration was carried out at 72 L/m^2 loading; 480 LMH feed flux; 1 bar TMP with P_{feed}: 0.6-0.9 bar and P_{retent}: 1.1-1.4 bar.

Anion exchange chromatography: Fractogel® TMAE resin, Fractogel® DMAE resin, and several Eshmuno® resin prototypes were evaluated. All the resins were tested for bind and elute as well as flow-through mode using 50mM HEPES buffer, pH 7.2 at varying NaCl concentration (150-1000 mM). Flow-through purification was evaluated at 100/200/300 mM NaCl and a flow rate of 100/200/400 cm/hr. Dynamic binding capacity was measured at 200 cm/hr linear velocity, corresponding to a residence time of 0.6 min.

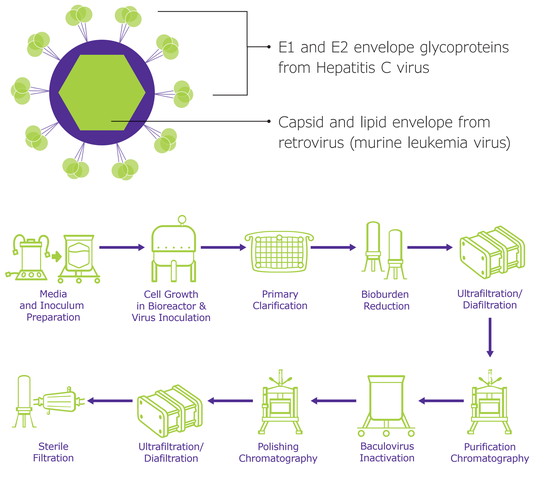
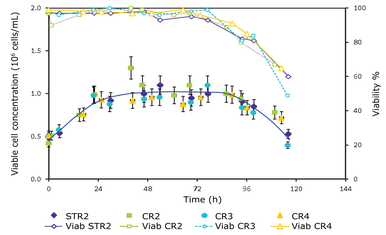


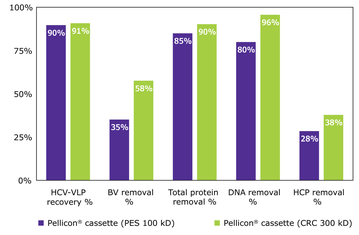
Figure 1.
Typical VLP-based insect cell/baculovirus-expressed vaccine production platform

Results

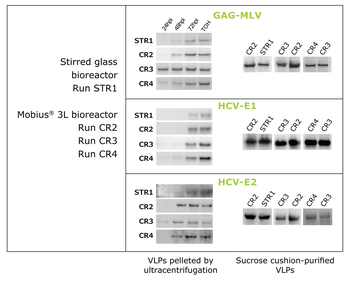
Cell growth obtained using Mobius® 3L Bioreactor



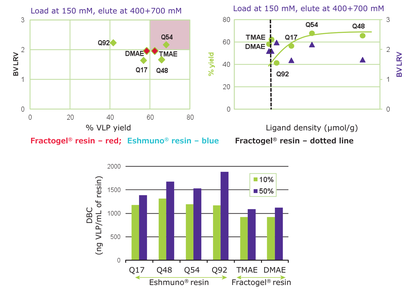
Comparative evaluation of TFF membrane



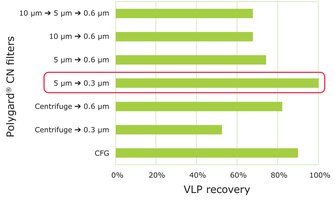
Western blot analysis of VLPs using three markers



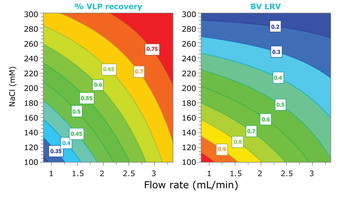
IEX media performance in bind-elute mode



VLP recovery using different methods of clarification



DOE of flow-through conditions using Fractogel® TMAE resin



Conclusion

- Cell cultures resulted in comparable cell and VLP properties between disposable and glass bioreactors.
- Increased agitation rate, increased cell density of inoculation and replacing the micro-sparger with an open-pipe sparger improved the performance of the single-use bioreactor.
- Reproducible performance of the disposable bioreactor was seen with identical results for three separate cell culture runs.
- A filter-only clarification train can be used without compromising VLP recovery yield. Unlike centrifugation, depth filtration resulted in ~70% DNA clearance.
- A filter cascade composed of a Polygard® CN 5 µm filter followed by a 0.3 µm depth filter showed the highest recovery of HCV-VLP, improving on centrifugation/secondary depth filtration.
- Clarification resulted in moderate DNA removal with depth filtration.
- PES 100kD and CRC 300kD membranes were fully retentive of the VLP. Pellicon® cassette with 300 kD regenerated cellulose membrane offered the best combination of recovery and purification.
- Chromatography resulted in >60% yield with ~2 LRV baculovirus with a flow-through/wash purification strategy.
- 10% dynamic binding capacity ranged from 900-1300 ng VLP/mL of packed resin. The Eshmuno® resin series has about 30% higher DBC compared to Fractogel® resin.
- Higher flow rate OR higher load conductivity resulted in higher VLP recovery and lower baculovirus removal. Yield increased with increasing ligand density for Eshmuno® resin prototypes.

Summary

- **A Mobius® 3L single-use bioreactor was successfully used to produce a VLP-based vaccine in an insect cell culture system**
- **Downstream processing was optimized using Polygard® CN 5.0→0.3 µm depth filters, followed by UF/DF using a Pellicon® cassette with an Ultracel® 300 kD membrane**
- **VLPs were purified using Fractogel® TMAE resins and Eshmuno® resin prototypes**
- **All components were integrated in a templatable and scalable process that made it possible to achieve the desired yield and recovery**

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