Enveloped Virus-Like Particle (VLP) Process Development

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Introduction

Hepatitis C virus (HCV) infection is a major public health problem, causing more than 350.000 deaths every year, according to the World Health Organization. Currently, there is no Hepatitis C vaccine and the standard treatment for acute HCV infections has several limitations, including its low efficacy[1, 2]. 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 to incorporate and display proteins. In this work, we highlight these advances for a chimeric retro VLP displaying E1/E2 HCV proteins, produced in an insect cell expression system. This VLP is a candidate vaccine for Hepatitis C. Furthermore, rational tools are being developed to understand the purification processes of enveloped VLPs[3].

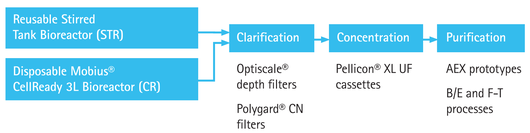
In this study, the production and purification of VLP-HCV was investigated and improved. In particular disposable bioreactor technology was evaluated and compared with glass stirred tank bioreactor culture in terms of viable cell concentration, % viability, growth kinetics and stability.

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) prototype resin yielding 70% recovery and a satisfactory baculovirus (BV) Log reduction (LRV) in bind and elute mode (B/E). Moreover, negative (flow-through, F-T) mode was also investigated, yielding a comparable BV clearance. Appropriate depth filtration and ultrafiltration (UF) devices were selected, leading to a GMP-compliant process that allows easy transfer to pilot scale level.

Aim

Move towards the development of effective vaccine candidates for infectious diseases based on enveloped VLPs, using disposable and scalable technologies.

Strategy



Materials and Methods

Cell lines and culture conditions. *Spodoptera frugiperda* Sf9 cells (Life Technologies) were routinely cultivated in SF-900II SFM medium (Life Technologies) in shake-flasks at 27 °C and a stirring speed of 100 rpm.

Determinations of hydrodynamic parameters. Determination of the hydrodynamic parameters for the two bioreactors was performed as described by Cruz *et. al.* 1998 and is shown in Table 1[4].

Bioreactor cultivations. For VLP-HCV production in stirred tank bioreactor, Sf9 cells were cultured in 2 L working volume reusable stirred tank bioreactor (Biostat® B-DCU, Sartorius Stedim Biotech) or disposable Mobius® CellReady 3L Bioreactor (Merck Millipore). Cultivations were carried out at 27 °C, pO_2 of 30% and an aeration rate of 0.01 vvm. 24h after inoculation ($1x10^6$ cells/ml) cells were co-infected at an MOI of 2 for BV-Gag and BV-HCV, and culture supernatant was collected every day until bioreactor bulk harvesting at 96 hpi.

Western-blot analysis. rVLP-HCV samples were separated on 4-12% acrylamide NuPAGE gels (Invitrogen). After transference, the PVDF membranes were incubated with anti-MLV-Gag (produced by hybridoma R187-ATCC CRL-1219), anti-HCV-E1 (Acris) or anti-HCV-E2 (Austral biologicals). The anti-mouse-HRP (GE Healthcare) or anti-RAT Alkaline Phosphatase (GE Healthcare) secondary antibodies were used and detection was performed using the ECL kit (GE Healthcare) or NBT/BCIP reagent (Thermo scientific), respectively.

Clarification/Concentration/Purification of bioreactor bulk. Bioreactor was clarified using different Optiscale® filter capsules with Polygard® CN filters (Merck Millipore). Concentration of the clarified bulks was performed with 50 cm^2 Pellicon® XL ultrafiltration cassettes (Merck Millipore). Six different ion-exchange prototypes (Merck Millipore) were evaluated.

Upstream Processing of VLP-HCV

I. Optimization of Sf9 growth in Mobius® CellReady 3L Bioreactor

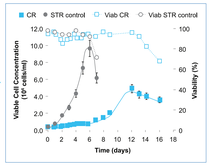
CR Conditions:
27 °C, pO_2 = 30%
Agitation rate: 70-200 rpm
Impeller: 1 marine
Aeration rate: 0.01 vvm
Working volume = 2 L

STR Conditions:
27 °C, pO_2 = 30%
Agitation = 70-250 rpm
Impeller: 2 six-blade rushton
Aeration rate: 0.01 vvm
Working volume = 2 L

Sf9 cells in CR

Sf9 cells in STR

Observation of cell aggregates for unoptimized CR culture



Long lag-phase observed for CR culture

Table 1. Shear stress in the two bioreactors

Agitation Rate (rpm)	Shear stress (N/m^2)	
	STR	CR
70	0.14	0.05
90	0.20	0.07
110	0.27	0.09
130	0.34	0.12
150	0.43	0.15
170	0.51	0.18

CR match STR hydrodynamic parameters for agitation rates above 150 rpm

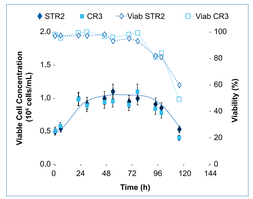
New CR Conditions:
Agitation rate: 150 rpm

	μ_{max} (h^{-1})	T_d (h)
CR	0.20	35
STR control	0.20	34

✓ Enhanced cell growth in CR
✓ CR and STR show similar growth profiles

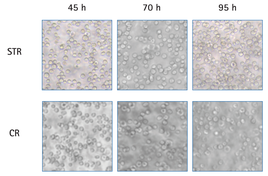
II. VLP-HCV production in bioreactor: reusable versus disposable vessels

Sf9 growth profile in the two bioreactors



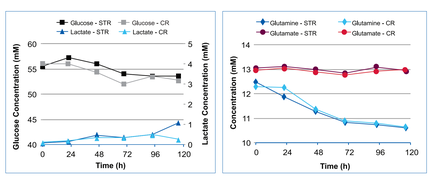
Similar growth profile for CR and STR

Images of Sf9 cell during culture in CR or STR



No formation of cell aggregates

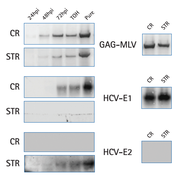
Metabolite concentration in culture supernatants



Comparable metabolite profiles in STR and CR

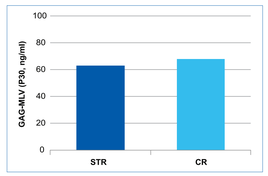
VLP-HCV Productivity in the two bioreactors

WB analysis of VLP-HCV production kinetics



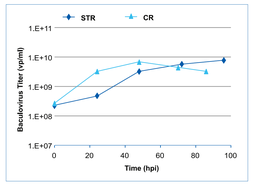
Similar GAG and HCV-E1E2 expression kinetics

Gag-MLV titer (P30) in the bioreactor harvested bulk



Equivalent productivity in STR and CR culture bulks

Baculovirus replication kinetics



Comparable baculovirus replication kinetics

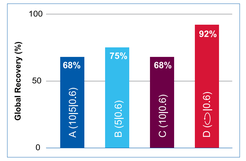
Downstream Processing

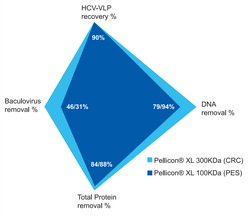
III. Clarification and Concentration Steps

Different clarification strategies with Opticscale® filter capsules with Polygard® CN filters were evaluated:

- Processes A to C use a combination of filters with different pore sizes;
- Process D couples bulk centrifugation to 0.6 µm device.

✓ Removal of 70% of DNA with strategy D.

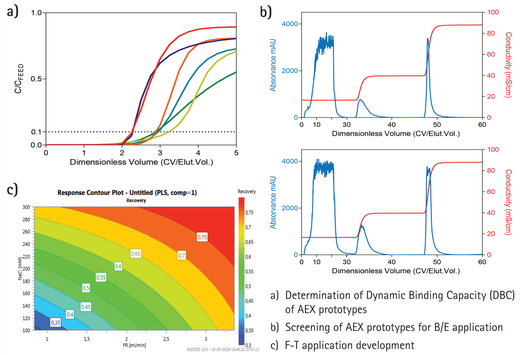




Screening of Pellicon® XL UF cassettes with 100 and 300 KDa cut-offs with PES and CRC membranes respectively

✓ 90% of yield in concentration steps;
✓ More than 80% of total protein and DNA removal.

IV. Purification Step



a) Determination of Dynamic Binding Capacity (DBC) of AEX prototypes
b) Screening of AEX prototypes for B/E application
c) F-T application development

✓ $DBC_{10\%}$ of tested prototypes 1.1-1.3 µg HCV-VLP;
✓ Prototype A and B yielded a LRV reduction of 1.2 and a recovery of 65% and 70% respectively, for a B/E operation;

Final Remarks

- Similar Sf9 growth profile was observed for the reusable STR and the disposable CR bioreactors;
- Comparable VLP-HCV production kinetics, as well as final productivity was obtained in the two types of bioreactors used;
- Development of a clarification and concentration train yielding 80% of recovery;
- Identification of the best IEX prototypes for B/E and F-T applications;

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References

[1] Manns, M.P., et al. (2006). Gut, 55(9): p. 1350-9.

[2] Swadling L, Klenerman P, Barnes E. Ever closer to a prophylactic vaccine for HCV. Expert Opin Biol Ther. Aug 2013; 13(8): 1109-1124.

[3] Vicente, Tiago, et al. Analysis of adsorption of a baculovirus bioreaction bulk on an ion-exchange surface by surface plasmon resonance. J Biotech 148.4 (2010): 171-181

[4] Cruz PE, Peixoto CC, Moreira JL, Carrondo MJ (1998). Biotechnol Bioeng, 60:408-18.

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