PRODUCTION AND PURIFICATION OF A VLP BASED HEPATITIS C VACCINE CANDIDATE

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Presentation Outline

01 VLP as Hepatitis C vaccines
02 Baculovirus / insect cell expression platform
03 Challenges in VLP vaccine production and purification
04 VLP production in insect cell culture
05 Clarification of VLP
06 Concentration / Diafiltration of VLP
07 Chromatographic purification of VLP
08 Summary
Motivation

- VLP vaccine candidates have become quite popular of late
- VLP-based processes are, however, currently quite diverse
- We undertook an effort to standardize the process
- We used hepatitis C VLP as a model
- This presentation will explain the approach taken and present the results obtained
Why virus-like particles (VLPs)?

- Contain repetitive high-density displays of viral surface proteins that elicit strong T cell and B cell immune responses
- Non infectious because they do not contain genetic material, thus cannot replicate and are safer
- Their size (40-120 nm diameter) is optimal for uptake by dendritic cells
- Can be produced in a variety of cell culture systems
- Can self assemble in vivo
- Proven technology (Hepatitis B and Human Papilloma Virus vaccines)

**VLPs for hepatitis C vaccine development**

**E1 and E2 glycoproteins from Hep C virus**

**Capsid and structure VLP from retrovirus (murine leukemia virus)**

**Hepatitis C**

- 170 million people infected
- Cirrhosis, liver cancer, death
- Current therapies only partially effective, costly and poorly tolerated
- No vaccine currently exists
Insect cell / baculovirus VLP production platform

Recombinant baculovirus (BV) is used to infect insect cells

**Key features**
- Transient production
- High cell densities
- Regulatory acceptance
  - Cervarix® (GSK)
  - Flublok® (Protein Sciences)
  - Several late-stage clinicals
Challenges in VLP vaccine production

- Low production yields
- Stability of enveloped VLPs
- Difficulties in baculovirus (BV) removal lowers recovery
- No established platform processes for purification
Work carried out in collaboration with iBET

iBET: Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
Typical VLP-based vaccine process

Insect cell / baculovirus VLP production platform
Typical VLP-based vaccine process

Insect cell / baculovirus VLP production platform

- Media and Inoculum Preparation
- Cell growth in Bioreactor and Virus Inoculation
- Primary Clarification
- Bioburden Reduction
- Sterile Filtration
- Polishing Chromatography
- Baculovirus Inactivation
- Purification Chromatography
- UF/DF
Insect cell culture

- Cell culture was carried out in stirred tank glass bioreactor and disposable bioreactor (Mobius® 3L bioreactor)

- Sf9 insect cells and Sf900II cell culture media were used in the process

- Mobius® 3L bioreactor was first operated at same conditions previously used for stirred tank glass bioreactors

- Cell aggregation
- Formation of foam
- Longer lag phase
- Lower viable cell concentration
Insect cell culture conditions improved based on experience with Mobius® bioreactor

- Increased agitation rate
- Increased cell density of inoculation
- Replaced micro sparger with an open-pipe sparger
### Microscopic evaluation of cells

<table>
<thead>
<tr>
<th>Stirred glass bioreactor</th>
<th>Run CR2</th>
<th>Run CR3</th>
<th>Run CR4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45 hrs</td>
<td>70 hrs</td>
<td>95 hrs</td>
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</tbody>
</table>

![Microscopic images](images)

**Mobius® 3L bioreactor**

- Run CR2
- Run CR3
- Run CR4
Western blot analysis of VLPs using three markers

<table>
<thead>
<tr>
<th></th>
<th>GAG-MLV (core protein)</th>
<th>HCV-E1 (envelope protein)</th>
<th>HCV-E2 (envelope protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>STR1</strong></td>
<td>[Image]</td>
<td>[Image]</td>
<td>[Image]</td>
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<tr>
<td><strong>CR2</strong></td>
<td>[Image]</td>
<td>[Image]</td>
<td>[Image]</td>
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<tr>
<td><strong>CR3</strong></td>
<td>[Image]</td>
<td>[Image]</td>
<td>[Image]</td>
</tr>
<tr>
<td><strong>CR4</strong></td>
<td>[Image]</td>
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VLPs pelleted by ultracentrifugation

Sucrose cushion purified VLPs
Successful use of Mobius® bioreactor for VLP production

- Successful growth of Sf9 insect cells and infection with baculovirus for production of VLP vaccine using Mobius® 3L disposable bioreactor

- Comparable cell and VLP properties between disposable and glass bioreactors

- Reproducible performance of the disposable bioreactor was seen with identical results for three separate cell culture runs
Typical VLP-based vaccine process
**Insect cell / baculovirus VLP production platform**

1. **Media and Inoculum Preparation**
2. **Cell growth in Bioreactor and Virus Inoculation**
3. **Bioburden Reduction**
4. **Sterile Filtration**
5. **UF/DF**
6. **Polishing Chromatography**
7. **Purification Chromatography**
8. **Inactivation**
9. **UF/DF**
10. **Purification**
Clarification

Centrifugation
- Lab models used early on
- Well suited for large-scale production
- High capital expense
- Shear

Depth filtration
- Well suited for smaller vaccine batches
- Easier to scale
- Lower cost
- Disposable
- Gentle treatment
- Simpler process development
- Wide choice of depth filters
Clarification: throughput data

Disposable capsule filters
Polygard® CN, nominal pore sizes of 10, 5, 0.6 and 0.3 µm
Pleated, all-polypropylene depth filters
Filter area: 17 cm²; Inlet flux: 988 LMH

![Graph showing volume filtered against inlet pressure for Polygard CN filter trains](image-url)
Unlike centrifugation, depth filtration resulted in ~70% DNA clearance.
Typical VLP-based vaccine process

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Concentration of clarified VLP harvest

**Pellicon® cassettes**

**Two different ultrafiltration membranes**
- 300 kD composite regenerated cellulose (Ultracel® membrane, “CRC”)
- 100 kD polyethersulfone (Biomax® membrane, “PES”)

**Similar process conditions employed**
- 4-5x concentration factor
- Loading: 72 L/m²; Feed flux: 480 LMH; TMP: 1 bar; \( P_{\text{feed}} \): 0.6-0.9 bar; \( P_{\text{retent}} \): 1.1-1.4 bar
Concentration of clarified VLP harvest – results

Both membranes were fully retentive of the VLP.
Polygard® CN depth filters and Pellicon® cassettes with Ultrace® membrane offered best results

Clarification

- Filter-only clarification train can be used without compromising recovery yield of VLPs.
- 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/2° depth filtration
- Moderate DNA removal with depth filtration was seen

UF/DF

- Pellicon® cassette with 300 kD regenerated cellulose membrane offered the best combination of recovery and purification
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Purification Chromatography
Purification strategy

Anion exchange chromatography (AEX) resins used

Identify purification goal
Ensure analytics are available

Batch adsorption
- Resin in multiwell plates
- Vary pH, conductivity
- Measure recovery, purity

Chrom bind/elute
- Prepacked columns
- Confirm batch adsorption

Chrom breakthrough
- Prepacked columns
- Capacity measurements

Scale up

Iterations...
Batch adsorption experiments (bind-elute)

- Fractogel® and two Eshmuno® prototypes approach target of 2 BV LRV
- Yield increases with increasing ligand density for Eshmuno® prototypes
Batch adsorption experiments (flow-through)

- Inadequate performance in pure flow-through mode; Similar trends with ligand density
- **Adopted strategy**: collect the flow-through fraction, then wash/elute the resin to recover more material
Column experiments

Breakthrough curves for dynamic binding capacity

- 10% dynamic binding capacity ranges at 900-1300 ng VLP / mL of packed resin
- The Eshmuno® series has about 30% higher DBC compared to Fractogel®
DOE of flow-through conditions: Fractogel® TMAE

Inputs: load NaCl (100/200/300 mM) and flow rate (100/200/400 cm/hr)

Responses: % VLP recovery and BV LRV

Flow rate (mL/min)

- Higher flow rate
- OR
- Higher load conductivity
  - AND
  - Higher recovery
  - Lower BV LRV
Successful purification of VLPs using Fractogel® and Eshmuno® AEX chromatographic resins

- Successfully purified VLPs using Fractogel® TMAE commercial resins and AEX prototype resins

- Yield of >60% with ~2 LRV baculovirus can be achieved with a flow-through/wash purification strategy for both resins

- Options to increase recovery or purification depending on product value by varying process conditions
Optimum performance achieved

<table>
<thead>
<tr>
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<th>Traditional lab process</th>
<th>New scalable process</th>
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<tbody>
<tr>
<td><strong>Purity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baculovirus clearance</td>
<td>94%</td>
<td>97.6%</td>
</tr>
<tr>
<td>DNA clearance DNA</td>
<td>99.9%</td>
<td></td>
</tr>
<tr>
<td>HCP clearance HCP</td>
<td>82%</td>
<td></td>
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<tr>
<td><strong>Recovery by P30 ELISA</strong></td>
<td></td>
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<tr>
<td>VLP recovery VLP</td>
<td>&lt; 10%</td>
<td>~ 65%</td>
</tr>
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</table>
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5. UF/DF
6. Polishing Chromatography
7. Baculovirus Inactivation
8. Bioburden Reduction
9. Pellicon® Ultrafiltration cassettes UltraceIl® 300 kD membrane
10. Fractogel® AEX resins
Typical VLP-based vaccine process
Insect cell / baculovirus VLP production platform

Mobius® Bioreactor
Polygard®-CN 5.0→0.3 μm filters
Fractogel® AEX resins
Pellicon® Ultrafiltration cassettes
Ultracel® 300 kD membrane
Summary

- Successfully used Mobius® 3L disposable bioreactor for production of VLP-based vaccine in insect cell culture system

- Optimized downstream processing using Polygard® CN 5.0→0.3 µm depth filters followed by UF/DF using Pellicon® cassette with Ultracel® 300 kD membrane

- Purified VLP by using Fractogel® commercial resins and AEX prototypes

- Integrated all the above components to achieve recovery and impurity clearance in line with requirements
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