Options for continuous production of cell culture-derived viral vaccines

- Motivation: Influenza virus production
- Upstream processing
  - Host cells
  - Virus strains
  - Cultivations conditions
- Downstream processing
  - Batch versus SMB mode
  - Virus strains
  - Cultivations conditions
  - Summary
- Summary and outlook

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Influenza Virus / Cell Lines

- **Hemagglutinin**
- **Neuraminidase**
- **Lipid Envelope + M2 protein**
- **Ribonucleocapsid**
- **Matrix Protein M1**

**Human Influenza**
- A/Puerto Rico/8/34/ (H1N1)
- Seasonal strains (A/B)
- Attenuated strains
- del NS1 mutant

**Equine / Porcine Influenza**

**MDCK** (Madin Darby Canine Kidney)
**Vero** (African Green Monkey Kidney)
- Epithelial cells, polarized
- **Adherent** => **Microcarrier culture!**

**Process options: Suspension cells**
- MDCK.SUS2, HEK293.SUS
- AGE1.CR, AGE1.HN (ProBioGen)
- CAP (Cevec Pharmaceuticals GmbH)

http://www.virology.net/BigVirology/BVRNAortho.html

**215 µm**

MDCK on Cytodex 1, LSM image
Cell Culture-based Influenza Virus Production

A) adherent cells

- MDCK: dog kidney
- Vero: monkey kidney
- MDCK.sus: dog kidney
- Vero.SUS: monkey kidney
- HEK293.sus: human embryonic kidney
- CAP: human amniocytes
- AGE1.CR: duck retinoblasts
- AGE1.CR.pIX: duck retinoblasts

B) suspension cells

- 1-10 x 10^6 cells/mL
- 160-250 rpm
- 1. high cell density
- 2. dilution

> 1 x 10^7 cells/mL

October 2013 Continuous Production of Influenza Virus
Frensing & Heldt et al. (2013) Continuous Influenza Virus Production in Cell Culture Shows a Periodic Accumulation of Defective Interfering Particles PLOS ONE, 8(9), e72288
Propagation of Influenza Virus A/PR8/34 H1N1 in AGE1.CR (DUCK) Cells

1st continuous cultivation

2nd continuous cultivation
Fluctuations Can Be Explained by Increase and Decrease of Defective Interfering Particles

Influenza virus genome segment

DI genome

Infectious virion

Defective interfering particles

Non-infectious virions

viral proteins
Segment-specific PCR for the Detection of Full-length and Defective Interfering Genome Segments for 2nd cultivation

FL = full-length; DI = defective interfering
Model of Continuous Infection in the Presence and Absence of DIPs

Mathematical model without DIPs

- Feed → Uninfected → Infection → Infected → Virus production → Apoptosis → Degradation
- Growth

Mathematical model with DIPs

- Feed → Uninfected → Infection → STV-infected → DIP-infected → Co-infected → Degradation
- Growth

Frensing & Heldt et al. (2013) Continuous Influenza Virus Production in Cell Culture Shows a Periodic Accumulation of Defective Interfering Particles PLOS ONE, 8(9), e72288
Continuous cultivations are possible but significant reduction of virus yield by formation of defective interfering particles (DI).

Impact of DI particles depends on quality of virus seed, but DI particle formation cannot be completely prevented by conventional methods.

Formation of DI particles is reported for most animal viruses – except poxviruses (MVA), coronaviruses, and parvoviruses.

Productivity of continuous cultivation superior if drop in HA titer is less than 0.6 log units, and we also need to consider genetic stability of product (virus, typically limited to max. 5 passages) and inactivation (14 d) if DSP is not on BSL 2/3.

Continuous cultivations useful

- to investigate mechanisms of DI particle formation
- to perform virus evolution studies, e.g.
  - one strain over time (mutation rate, adaptation, etc.)
  - co-infections
Downstream Processing
Generic Process

Cell Culture
(adh. MDCK cells, A/PR/8/34 (H1N1))

Filtration
(5, 0.65, 0.45 µm)

Inactivation
(β-PL)

Concentration 10x
(750 kDa, cross-flow)

Batch Chromatography

50 kHAU/mL
130 µg/mL protein
9 µg/mL DNA

Batch limited in
- Processing volume
- Throughput
- Scalability

Resin: Sepharose 4FF (GE)
CV: 23 mL, Injection: 0.15 CV

Kalbfuß et al. (2007) B&B 96(5):932
SMB System (Knauer), column switching valve
Columns: Sepharose 4FF, 2 mL CV

Continuous Production of Influenza Virus
SMB: Equipment

- SMB System (Knauer) column switching valve
- Columns: Sepharose 4FF, 2 mL CV

Kröber et al. (2013) accepted
SMB: Design

- Internal and external flow rates determine separation performance
- Dimensionless flow rate ratios (TMB):
  \[ m_z = \frac{Q_z}{Q_s} \quad z=I, II, III; \; s=\text{solid} \]
- For linear adsorption the following inequalities have to be fulfilled to achieve complete separation*
  \[ K_P < m_I \]
  \[ K_V < m_{II} < m_{III} < K_P \]
  \[ (m_{IV}<K_V) \]

*"Triangle theory", Mazzotti et al. (1997) JChrA 769:3
SMB: Operating Points

\[
m_{\text{III}} \quad m_{\text{II}}
\]

- (0.20; 0.32)
- (0.33; 0.47)
- (0.45; 0.62)
- (0.72; 0.93)
SMB: Operating Points

Continuous Production of Influenza Virus

October 2013
Performance: 1 and 2 Columns per Zone

HA yield (Raffinate)

- % of feed vs. $m_{II}$
- Symbols: 1 column per zone vs. 2 columns per zone

Productivity w/o CIP

- kHAU/(mL*h) vs. $m_{II}$

Protein depletion

- % of feed vs. $m_{II}$

Protein contamination

- µg/KHAU vs. $m_{II}$
SMB with Anion Exchange Column

- DNA co-elutes with virus in the raffinate
- Anion exchange (AEX) column to bind DNA (1 mL CaptoQ, GE)
- Virus flows through
Performance: SMB and AEX

**HA yield (Raffinate)**

- % of feed
- $m_\|$

**protein depletion**

- % of feed
- $m_\|$

**DNA depletion**

- % of feed
- $m_\|$

**protein contamination**

- $\mu g/kHAU$
- $m_\|$

Legend:
- 1 column per zone
- 2 columns per zone
- SMB and AEX

Continuous Production of Influenza Virus

Max Planck Institute Magdeburg

October 2013
Productivity: Batch vs. SMB

- Max. flow rate
- HA yield
- Depletion
- Protein contamination
- Productivity

<table>
<thead>
<tr>
<th></th>
<th>Batch</th>
<th>SMB</th>
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<tbody>
<tr>
<td>Max. flow rate (mL/min)</td>
<td>4.0</td>
<td>1.9</td>
</tr>
<tr>
<td>HA yield (% feed)</td>
<td>80</td>
<td>95</td>
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<tr>
<td>Depletion (% feed protein)</td>
<td>60*</td>
<td>47</td>
</tr>
<tr>
<td>DNA (%)</td>
<td>27*</td>
<td>5</td>
</tr>
<tr>
<td>Protein contamination (µg/kHAU)</td>
<td>1.17</td>
<td>1.55</td>
</tr>
<tr>
<td>Productivity (kHAU/(mL*h))</td>
<td></td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>incl. CIP</td>
<td>45</td>
</tr>
</tbody>
</table>

Extrapolation for 100 L concentrated cell culture broth:

<table>
<thead>
<tr>
<th></th>
<th>Batch</th>
<th>SMB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max. flow rate (cm/h)</td>
<td>138</td>
<td>275</td>
</tr>
<tr>
<td>Column dimensions (d_i/L)</td>
<td>0.5 / 0.3</td>
<td>0.4 / 0.1</td>
</tr>
<tr>
<td>Resin volume (L)</td>
<td>59</td>
<td>3x13.4</td>
</tr>
<tr>
<td>Number of runs</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Process time (h)</td>
<td>6.1*</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>incl. CIP</td>
<td>45</td>
</tr>
</tbody>
</table>

* tailing, => conservative cut-off
Summary DSP

- Comparison of batch and SMB
  - Similar separation performance
    - High HA yield
    - Sufficient protein depletion (< 100 µg/strain)
  - Productivity of SMB higher than batch, i.e. when CIP is considered
  - SMB needs lower total resin volume (i.e. validated backup columns)
- Combination of SEC and AEX removes 99% of DNA, however, contamination levels still exceed limits of regulatory guidelines for human influenza vaccines (<10 ng)

Outlook

- Investigation of batch to batch variations and other viral strains
- Benzonase® treatment to further reduce DNA contamination levels
- Use of various other matrices in SMB (SEC, affinity, membranes)
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and

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**Figure 1.** Principle of true moving bed (TMB) chromatography. Left: schematic representation of a TMB apparatus; right: typical internal concentration profiles for successful binary separation.

Seidel-Morgenstern 2008
Mass balances:

- \( Q_I = Q_{IV} + Q_D \)
- \( Q_{II} = Q_I - Q_E \)
- \( Q_{III} = Q_{II} + Q_F \)
- \( Q_{IV} = Q_{III} - Q_R \)

Dimensionless flow rate ratio: \( m_i = \frac{Q_i}{Q_S} \)

- \( m_I > K^B \)
- \( K^A < m_{II} < m_{III} < K^B \)
- \( K^A > m_{IV} \)
SMB Design: TMB Conversion Rules

• TMB → conversion rules → corresponding SMB

\[
t_{sh} = \frac{V_t(1-\varepsilon)}{Q_S} \quad Q_i^{SMB} = Q_i^{TMB} + \frac{\varepsilon \cdot V_t}{t_{sh}} + \frac{V_{i,dead}}{t_{sh}} \quad i = I, II, III, IV
\]

• BUT:
SMB with only few columns doesn’t meet the ideal TMB case
Calculation of productivity

\[ \text{Pro}_{\text{batch}} = \frac{a_{\text{HA,PF}} \cdot V_{\text{PF}} \cdot Q}{V_{\text{cycle}} \cdot V_{\text{col}}} \]  

\[ \text{Pro}_{\text{SMB}} = \frac{a_{\text{HA,R}} \cdot Q_{\text{R}}}{N_{\text{col}} \cdot V_{\text{col}}} \]

for calculation regeneration with NaOH (2 CV) and reequilibration with buffer (3 CV) was considered:

batch: \( V_{\text{cycle}} = V_{\text{inj}} + V_{\text{elu}} + V_{\text{reg}} + V_{\text{reequ}} \)

SMB: \( N_{\text{col}} = 7 \) (column 4 to 7: regeneration and reequilibration)