Utilizing capacitance measurement to improve vaccine and viral vector production processes

from Aber Instruments

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Housekeeping

• Length – about 30 minutes, followed by 10 minutes for questions.
• Please mute yourselves, just in case.
• Any questions during the webinar, please feel free to type them.
• We will try and address as many questions as possible. Any that are not addressed, we will respond via email.
• Sit back and enjoy the webinar!
Structure

Introduction
• Viral vector/vaccine development
• Challenges/Opportunities

Capacitance measurement
• How does it work?

Utilizing capacitance
• Benefits to the process

Take home points
• Summary
Viral vector/vaccine development and production
Vaccine development and production

• CAGR for global vaccine market is 8%.
• 95% of the world’s vaccines are developed for humans. Among these, 90% are prophylactic vaccines and 10% are therapeutic.
• Significant proportion of development/manufacturing strategies are based around *viral vector or virus platforms*. 

Typical process flow

1. Grow cells
2. Infect/Transfect
3. Viral multiplication
4. Cell lysis & Viral release
5. Product recovery & purification
In process challenges

- Obtain fingerprint
- Optimize infection/transfection
- Identify successful infection
- Optimize harvest time
- Simplify DSP
- Reduce/eliminate sampling
- Improve productivity
- Achieve consistency
- Effective scale up strategy

Capacitance technology

- Speed up development
- Effective manufacturing
- Produce more
- Waste less
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www.aberinstruments.com
Capacitance Technology by Aber

Capacitance technology
• Invented in 1988 by Aber Instruments, Aberystwyth UK
• Extensive knowledge

Experience
• Mature technology
• Over 30 years of focused & dedicated experience in biomass measurement

Worldwide customer base
• Customers in over 140 countries
• Major pharmaceutical companies

Complete portfolio
• Installations from R&D through to Manufacturing
• From mini & small glass bioreactors to Stainless Steel vessels
• Single use capacitance measurement technology
The theory

How does it work?
Principle of measurement

• Capacitance and Conductivity
• The Cell Membrane
  • Bilayer
  • Is impermeable to ions (insulator)
  • Under the influence of an electric field, gets polarised
• When membrane is undamaged, each live cell acts as a capacitor
Influence of electric field to ‘polarise’ viable cells

Electronics controlling the electric field

Direction of the field

Positive electrode

Cell

Cell plasma membrane

Negative electrode
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- Measure the build up of charge around live cell membranes
- Capacitance reflection of cell density AND cell size + electrical properties of cell membrane
- Aber capacitance is a measure of the viable biovolume
Failure of electric field to ‘polarise’ dead and ruptured cells

Electronics controlling the electric field

Positive electrode

Cell with badly ruptured membrane

Plasma membrane fragments

Direction of the field

Negative electrode
Influence of gas bubbles and solid media particles on capacitance measurements.

Electronics controlling the electric field

Gas bubble

Solid particle impermeable to ions

Solid particle permeable to ions

Direction of the field
How does Aber detect this capacitance?

• The Aber probe and electronics creates and monitors the electric field

• The probe’s
  • Outer electrodes create the field
  • Inner electrodes measure the voltage

• The electric field is in the Radio Frequency spectrum, typically between 50kHz and 20MHz

*NB: the field projects approximately 30 – 40 mm*
Monitoring a Fed-Batch Culture

- Notice how cells have consumed critical nutrients before feed occurs!
Key points

• Live cells with intact membranes become polarised and thus measured.

• Dead cells with leaky membranes with respect to charge are not measured.

• The Resulting capacitance is directly proportional to the total membrane bound volume of the cells
  
  • The number of cells, and the size of the cells.
Applications

Where has the technology been utilized?
Applications - Platforms

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Diverse application tree

PAT
Continuous manufacturing
Process intensification

Monitor (Fingerprint)
Automatic control
Harvest point detection
Perfusion (ATF)
Seed transfer (N-1)
Microcarrier
Cell therapy
Regen medicine

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Viral vector/vaccine development or production
## Publications utilizing capacitance measurement

<table>
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<tr>
<th>Cells</th>
<th>Viral vector</th>
<th>Publication using capacitance measurement</th>
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Typical real-time fingerprint - viral vector or vaccine production process

- Monitor growth of cells
- Identify/optimize infection/transfection point
- Identify successful infection
- Identify peak infection point
- Cell lysis (viral release)
- Identify/Optimize harvest time
Monitoring Sf-9 cells infected with a virus

Tracking adenovirus production process (HEK293)

Tracking adenoviral vector production kinetics – HEK293

Viral protein synthesis phase: Mean cell radius of HEK293 – 14 micron to 16 micron – 50% increase in cell volume

Tracking adenoviral vector production kinetics – HEK293

Cap peaks 18 h after OUR and offline cell concentration peaks. Can be closely linked to virus concentration

Perfusion
Cell concentration control
An inline biomass sensor continuously controls the cell bleed

Cell bleed: You need:
• Biomass sensor
• PID controller
• Bioreactor scale

Cell concentration control using Aber

Sergeant D., Moser M., Carvell JP., Measurement and control of viable cell density in a mammalian cell bioprocessing facility: Validation of the software (2007), European Society for Animal Cell Technology
Scale up – key performance indicator
Scalability evaluation

Scalability determined over different scales:
5L (bench scale), 200 & 315 L (pilot scale) and 15000 L (manufacturing scale)

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Moore, B. et al., 2019; Case study: The characterization and implementation of dielectric spectroscopy (biocapacitance) for process control in a commercial GMP CHO manufacturing process, Biotech Prog
Scale up (single use systems)

- Cultivations in RM 50 (2x), RM 200, STR 50 (2x), STR (2x) and STR 1000 performed
- Scale Up was successful
  - Good agreement in capacitance trend Flexsafe® RM to STR and throughout scales

![Graphs showing capacitance over time for different systems]
Measuring cells on microcarriers
Monitoring Microcarrier Culture Using Aber

On-line monitoring of the biomass for 20 days in a 10 l fermentor. Cultivation of BHK cells attached to macroporous microcarriers (data courtesy of Novo)

Data courtesy of Novo (Denmark)
Monitoring Microcarrier Culture Using Aber

Cell adhesion

Both conductivity and capacitance were used to determine the adhesion time. Typically, it was seen that once the Vero cells were fully spread, as determined by microscopy, the conductivity remained constant whereas the capacitance correlated well with the offline cell concentration. Even with constant experimental parameters, the time needed for the Vero cells to adhere to the microcarrier varied from 3.8 to 7.3 h post inoculation, thus making an online real time tool to determine adhesion time even more useful.

Quantification of Vero cells during Measles virus infection using a Cytodex 1 microcarriers

Benefits and Summary
Typical real-time fingerprint - viral vector or vaccine production process

- Monitor growth of cells
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- Cell lysis (viral release)
- Identify/optimize infection/transfection point
- Identify peak infection point
- Identify/optimise harvest time
Benefits of capacitance measurement

- Speed to market
- Improve productivity
- Titer consistency
- Process Intensification
- Effective Scale Up
- Automation
- Troubleshoot
- Simplify DSP
Benefits

Speed to market

- Faster & detailed information
- Quicker development
Benefits

**Benefits of capacitance measurement**

- Speed to market
- Improve productivity
- Titer consistency
- Process Intensification
- Effective Scale Up
- Automation
- Troubleshoot
- Simplify DSP

**Improve productivity**

- Harvest time optimization
- Viral titer closely linked to max capacitance
- Grein *et al.* (2017): Capacitance based method increased max virus concentration by more than 3 orders of magnitude
- Can produce one dose/patient in 1 x 500 ml bioreactor as opposed to 20 L bioreactor

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Benefits

- Speed to market
- Improve productivity
- Titer consistency
- Process Intensification
- Effective Scale Up
- Automation
- Troubleshoot
- Simplify DSP

Titer consistency

- Optimal time of harvest can drift significantly in identical bioreactors and similarly run processes.
- Real time capacitance-based control can allow for consistent virus titer to be obtained, despite process changes.
Benefits

- Speed to market
- Improve productivity
- Titer consistency
- Process Intensification
- Effective Scale Up
- Automation
- Troubleshoot
- Simplify DSP

Process Intensification

- Continuous manufacturing
- Automated perfusion process
Effective Scale Up

- Capacitance can be used across different scales
- Can be used as key performance indicator to determine successful scale up
Benefits

- Speed to market
- Improve productivity
- Titer consistency
- Process Intensification
- Effective Scale Up
- Automation
- Troubleshoot
- Simplify DSP

Automation

- Automate
  - ✔ Seed Transfer
  - ✔ Infection
  - ✔ Harvest
Benefits

Troubleshoot

- Real time, robust and detailed fingerprint
- Compare with historical trend
- Troubleshoot much quicker to save or abandon the process in time
- Catch contamination sooner
Benefits

Simplify DSP

- DSP closely linked to time of harvest
- If harvest time is optimized, purification can become less complicated
Thank you for listening

Questions?