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rAAV Production in Suspension CAP GT® Cells in BioBLU® 3c and 10c Single-Use Vessels

Simon Fradin[1], Kerstin Hein[1], and Helmut Kewes[1]
[1]Cevec® Pharmaceuticals GmbH, Cologne, Germany
Robert Glaser[2], Ulrike Rasche[2], and Ma Sha[3]
[2]Eppendorf Bioprocess Center Juelich, Germany; [3]Eppendorf Inc., Enfield, CT, USA Contact: rasche.u@eppendorf.com

Abstract

In pharmaceutical and biotech industry there is an increasing interest in gene therapy-based applications. This leads to an increasing demand in scalable production systems for viral vectors. Cell culture in suspension instead of monolayer culture simplifies scale-up, because it allows bioprocessing in scalable, stirred-tank bioreactors. Researchers at Cevec Pharmaceuticals adapted the small scale shake flasks rAAV production process to stirred-tank bioreactors and scaled it up in BioBLU Single-Use Vessels from a working volume of 2 L to 10 L. Scale-up in bioreactors was based on constant power input/volume. It led to comparable cell growth and virus production at both scales and to the original shake flask process.

Introduction

Bioprocess development is usually carried out at small working volumes. This helps saving time and resources, because at small scale several experiments can be conducted in parallel, costs for media are kept low, and not much lab space is required to operate small-scale bioreactors. But in the course of biopharmaceutical development more material is needed for characterization, trial runs, and finally for commercialization. To maintain product yield and product quality while scaling up, bioprocess engineers usually aim to keep one or more process parameters constant across scales.

Stirred-tank bioreactors with similar vessel geometries and capabilities across scales simplify scale-up, as they allow keeping critical parameters constant. Parameters to describe the vessel geometry include impeller diameter, vessel diameter, liquid height and ratios thereof. Key engineering parameters related to scale-up include the tip speed, mixing time, volumetric mass transfer coefficient (k_La), and the power input/volume ratio (P/V). It depends on the process, which of these parameters is most important.

Cevec Pharmaceuticals uses a unique human cell–based expression system (CAP Technology) in two product portfolios. One is the glyco-optimized CAP Go® cell line for tailor-made production of N- or O-glycosylated proteins, the other is the CAP GT cell platform for stable and transient industrial-scale production of recombinant adeno-associated viruses (rAAV), lentiviral, and adenoviral gene therapy vectors. In this study, researchers at Cevec aimed at scaling-up a rAAV transient production process using CAP GT cells. When scaling up, they maintained constant P/V between vessels, which is one of the most prevalent strategies for scale-up.

Material and Methods

Cell line and medium
Human CAP GT cells were cultivated in a chemically defined, animal component-free medium compatible with transient transfection.

The thawing, seedtrain and amplification of the CAP GT cells was performed in shake flasks in suspension, to generate sufficient biomass for seeding the final production vessel (stirred-tank bioreactor).

The cell culture in the bioreactor was inoculated to an initial cell density of 5 x 10^5 cells/mL.

Bioprocess system and process parameters
The bioprocess engineers at Cevec used a BioFlo® 320 bioprocess control station equipped with BioBLU 3c or 10c Single-Use Vessels (Figure 1). BioBLU Single-Use Vessels have an industrial, rigid-wall design. They provide fast and efficient mixing with magnetically coupled overhead drives. The vessel geometry is similar across scales, among others in terms of the ratio of impeller diameter to vessel inner diameter and the ratio of maximum liquid height to vessel inner diameter. This makes it easy to use the BioBLU vessels at small scale during process development and then scale up the process to larger working volumes.

The researchers set the temperature to 37 °C. The pH was regulated with CO_2 (acid) and sodium bicarbonate (base). The vessels were equipped with a macrosparger and one pitched-blade impeller. The gassing strategy was an automatic gas mix, which automatically controls air, oxygen, nitrogen, and CO_2, depending on the pH and dissolved oxygen (DO) set points. The agitation speed of the BioBLU 3c vessel with a 2 L working volume was set to 200 rpm (corresponding to a tip speed of 0.69 m/s); for the BioBLU 10c with a 10 L working volume, it was 175 rpm (corresponding to a tip speed of 0.84 m/s).

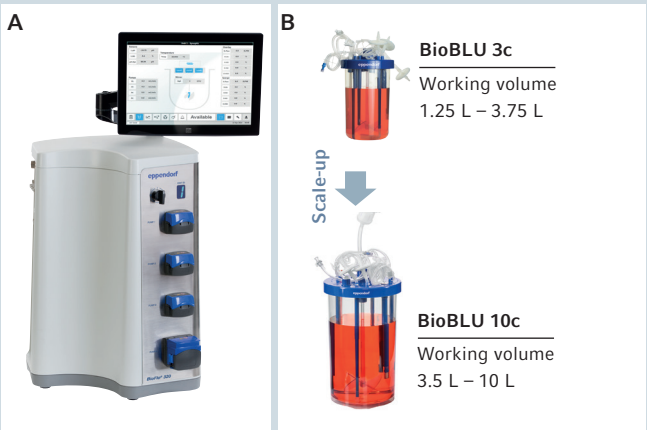

Fig. 1: Bioprocess equipment used in this study.
A: BioFlo 320 bioprocess control system
B: BioBLU c Single-Use Vessels

Virus production process
The bioprocess for rAAV production was divided into four phases.

1. Expansion phase: CAP GT cells were expanded in suspension in BioBLU Single-Use Vessels.
2. Transient transfection: At 72 hours into the process, cells were transiently transfected with a two-plasmid-system from PlasmidFactory® encoding for rAAV-GFP. Transfection was mediated by Polyethylenimine (PEI). With the transfection the production phase was started.
3. Production phase: During this phase the cells produced rAAV-GFP.
4. Harvest phase: The culture medium was collected and processed to harvest the virus particles. Phase 4 is not further described in this application note.

Power number
The impeller power number is a dimensionless number associated with different types of impellers. The impeller power number is used to calculate P/V using the following equation:

$$P/V = (Np \times \rho \times N^3 \times d^5)/V$$

with Np being the impeller power number, ρ being the density of water (1000 kg/m^3), N being the agitation speed (rps), d being the impeller outer diameter (m), and V being the full working volume (m^3).

To calculate P/V, the bioprocess engineers at Cevec used impeller power numbers which were experimentally determined in the Eppendorf applications laboratory. Experimental determination of power numbers using a rotational torque sensor requires a direct drive motor. To be able to measure the power numbers for BioBLU Single-Use Vessels - which have a magnetic drive -, the Eppendorf application scientists modified the vessels to remove the magnet from the magnetic drive coupling. They then connected the torque sensor directly to the impeller shaft. Power numbers were measured at tip speeds between 0.3 and 1 m/s and then the average was calculated. For the

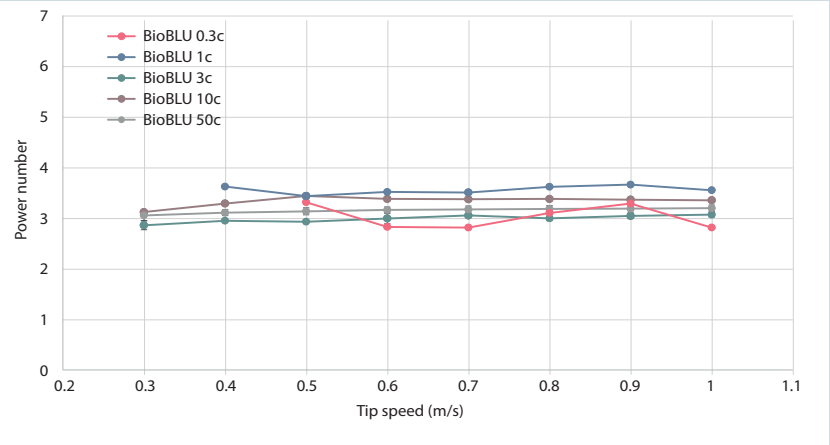

Fig. 2: BioBLU c Single-Use Vessel power numbers.
BioBLU Single-Use Vessels with one impeller were modified to remove the magnet from the magnetic drive coupling and the torque sensor was directly connected to the impeller shaft. Torque data were collected according to DECHEMA® recommendations [1]. The power number was calculated from the impeller torque as described in [2].

BioBLU 3c the mean was 2.98; for the BioBLU 10c the mean was 3.33, and for the 50c the mean was 3.15 (Figure 2).

Scale-up strategy

The scientists optimized the agitation speed during process development. Scale-up was based on similar power input at both scales. The agitation speed of the BioBLU 3c vessel with a 2 L working volume was set to 200 rpm (corresponding to a tip speed of 0.69 m/s); for the BioBLU 10c with a 10 L working volume, it was 175 rpm (corresponding to a tip speed of 0.84 m/s). This results in comparable power inputs of around 62 W/m^3 at both scales.

Analytics

The bioprocess engineers determined the viable cell density and cell viability offline using a NucleoCounter® NC-3000™ (ChemoMetec®, Denmark).

They analyzed the transfection efficiency by measuring GFP fluorescence with a NucleoCounter NC-3000.

Productivity was measured by quantification of the viral genome titer by qPCR.

Results

The researchers compared the performance of the initial shake-flask process with bioprocess performance at

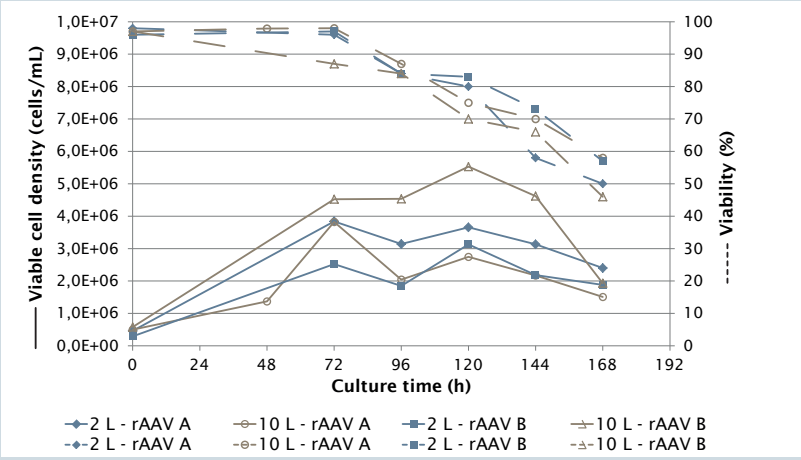

Fig. 3:
Cell growth and viability.
At several time points culture samples were taken and the viable cell density (solid lines) and cell viability (dashed lines) were analyzed offline.

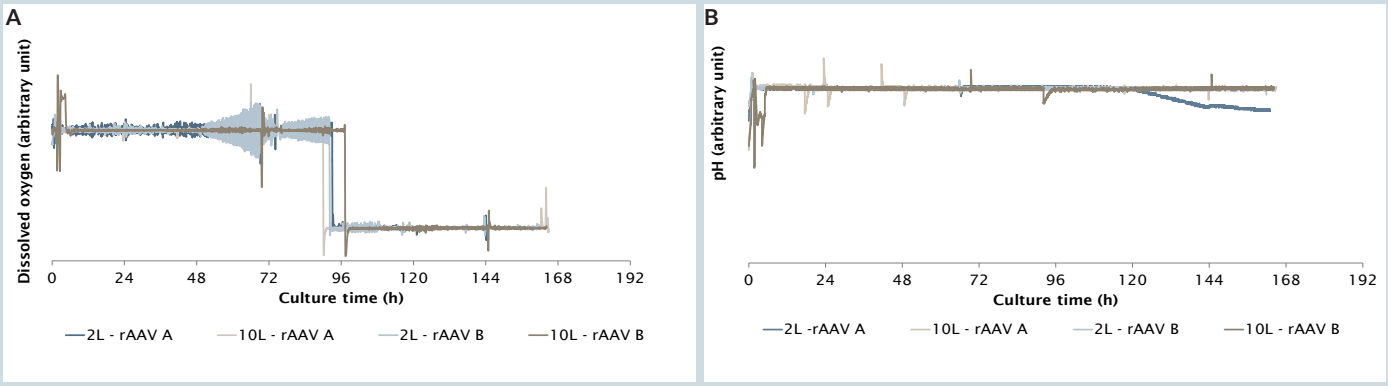

Fig. 4: Control of process parameters.
A: DO was controlled with an automatic gas mix as described in the Material and Methods section. The DO was regulated correctly at both scales. **B:** The pH was controlled with CO_2 (acid) and sodium bicarbonate (base). At both scales the pH was kept at setpoint.

working volumes of 2 L and 10 L. They conducted the same production process in two duplicate runs runs at the 2 L scale (2 L rAAV A and rAAV B) and two duplicate runs at the 10 L scale (10 L rAAV A and rAAV B).

For the two duplicate runs at 2 L and 10 L, the bioprocess engineers plotted the viable cell density and viability over time (Figure 3). Similar growth patterns were achieved. The viable cell density at the point of transfection was similar to the original shake flask process (data not shown). Post-transfection at 72 h, viability dropped in a similar manner in all runs. This was expected, as virus production is cytotoxic.

The DO control was very tight at both scales, with similar profiles for all four runs (Figure 4A). All four runs showed similar pH profiles within the regulation parameters (Figure 4B).

The researchers determined transfection efficiency based on green fluorescent protein (GFP) fluorescence measured with a NucleoCounter NC-3000. Similar transfection efficiencies of around 70 % were achieved at both scales. The transfection efficiency was comparable to that of the

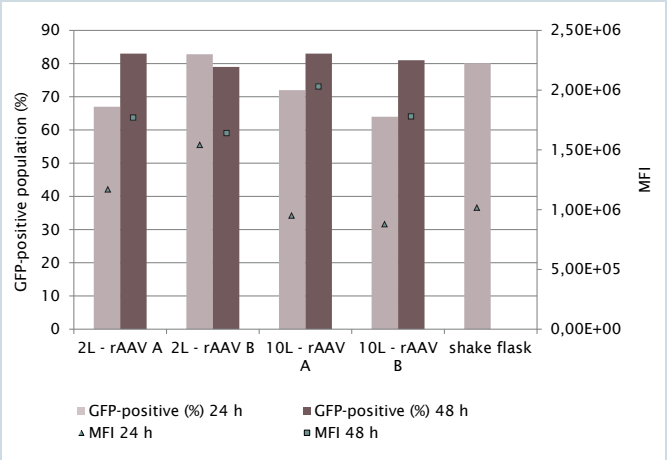

Fig. 5: Transfection efficiency
To determine the transfection efficience, the GFP-positive population and the mean fluorescence intensity (MFI) were measured 24 hours and 48 hours post transfection.

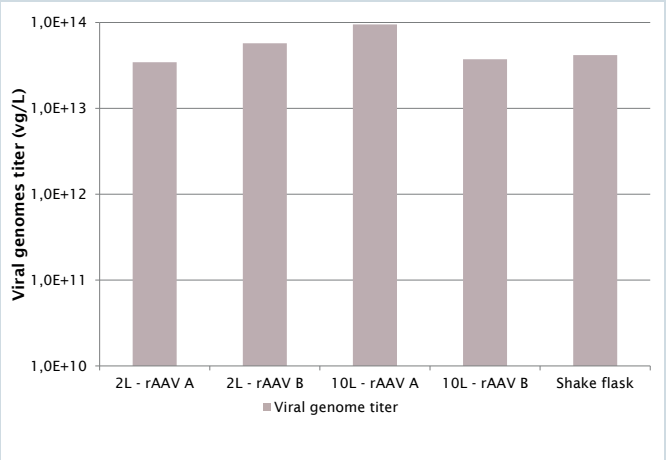

Fig. 6: Productivity
To analyze productivity, the viral genome titer was quantified by qPCR at the end of the bioprocess run.

original shake flask process (Figure 5).

The bioprocess engineers at Cevec measured productivity by quantification of viral genome titer by qPCR. The viral genome titer was similar in the bioprocess runs with a working volume of 2 L and 10 L and again comparable to the original shake flask process (Figure 6).

Conclusion

Bioprocess engineers at Cevec scaled-up a rAAV production process from BioBLU 3c Single-Use Vessels with a working volume of 2 L to BioBLU 10c Single-Use Vessels with a working volume of 10 L. Cell growth, transfection efficiency, and productivity were comparable at both scales. This process development enables then the production of higher amounts of rAAV gene therapy vectors by transient transfection. Moreover, these results give an example for successful bioprocess scale-up based on constant P/V using differently sized BioBLU Single-Use Vessels.

Literature

[1] DECHEMA Expert Group Single-Use Technology. *Recommendations for process engineering characterisation of single-use bioreactors and mixing systems by using experimental methods*. 2016.

[2] Li B, Sha M. Scale-Up of Escherichia coli Fermentation from Small Scale to Pilot Scale Using Eppendorf Fermentation Systems. *Eppendorf Application Note 306*. 2016.

Ordering information

Description	Order no.
BioFlo® 320	
Base control station: All configured units include the same base control station.	1379963011

BioBLU® Single-Use Vessels

Description	Application	Working volume	Sparger	Impeller	pH	Quantity	Order no.
BioBLU® 0.3c	Cell culture	100 – 250 mL	Open pipe	1x pitched blade	Standard	4-pack	78903508
BioBLU® 0.3c	Cell culture	100 – 250 mL	Open pipe	1x pitched blade	Optical	4-pack	78903507
BioBLU® 1c	Cell culture	320 mL – 1.25 L	Open pipe	1x pitched blade	Standard	4-pack	1386110000
BioBLU® 1c	Cell culture	320 mL – 1.25 L	Open pipe	1x pitched blade	Optical	4-pack	1386110400
BioBLU® 1c	Cell culture	320 mL – 1.25 L	Open pipe	2x pitched blade	Standard	4-pack	1386110100
BioBLU® 1c	Cell culture	320 mL – 1.25 L	Open pipe	2x pitched blade	Optical	4-pack	1386110500
BioBLU® 3c	Cell culture	1.25 L – 3.75 L	Microsparger	1x pitched blade	Optical	1-pack	1386000100
BioBLU® 3c	Cell culture	1.25 L – 3.75 L	Macrosparger	1x pitched blade	Optical	1-pack	1386000300
BioBLU® 3c	Cell culture	1.25 L – 3.75 L	Microsparger	2x pitched blade	Optical	1-pack	1386120000
BioBLU® 3c	Cell culture	1.25 L – 3.75 L	Macrosparger	2x pitched blade	Optical	1-pack	1386121000
BioBLU® 5p	Cell culture	3.75 L	Microsparger	Packed bed	Optical	1-pack	M1363-0119
BioBLU® 5p	Cell culture	3.75 L	Macrosparger	Packed bed	Optical	1-pack	M1363-0133
BioBLU® 10c	Cell culture	3.3 L – 10 L	Microsparger	1x pitched blade	Optical	1-pack	1386140000
BioBLU® 10c	Cell culture	3.3 L – 10 L	Macrosparger	1x pitched blade	Optical	1-pack	1386141000
BioBLU® 50c	Cell culture	18 L – 40 L	Microsparger	1x pitched blade	Optical	1-pack	M1363-0131
BioBLU® 50c	Cell culture	18 L – 40 L	Macrosparger	1x pitched blade	Optical	1-pack	M1363-0129

For more information on Cevec Pharmaceuticals GmbH visit www.cevec.com.

Your local distributor: www.eppendorf.com/contact
Eppendorf AG · Barkhausenweg 1 · 22339 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com