Scalable, high performance single-use bioreactor technology for viral vectors production

Case study for an adeno-associated virus-based gene therapy

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Abstract

The success of gene therapies resulted in a surge in demand for viral vectors exceeding current production capacities. However, producing viral vectors using conventional technologies (e.g. stirred tank, static cell culture) has several limitations including limited scalability, flexibility, and high production cost.

To address these challenges, Univercells developed the single-use fixed-bed scale-X bioreactor for enhanced upstream processing of viral products. The scale-X technology provides scalability by design, eliminating viral production from process development to large-scale commercial production.

Case study for an adeno-associated virus-based gene therapy

In this study, a rAAV process using HEK293 cells and 4DMT vectors is transferred from a static cell culture system to Univercells’ scale-X™ bioreactor. The scale-X™ bioreactor demonstrates homogeneous cell distribution, increased virus titers and the resulting, significantly reduced manufacturing time and costs.

1. The scale-X™ single-use fixed bed bioreactor

The scale-X™ bioreactor system is a single-use bioreactor based on a compact and structured fixed-bed design that allows for easy media and cell sampling.

2. Materials and methods

**scale-X™**
- Scale-X™ hydro bioreactor (2.4 m² growth surface)
- Culture: HEK293 cells from cryopreserved seed bank, seeded in plastic flatware and incubated at 37°C, 5% CO₂.
- Chained TFF: Waterfader with cut-off 100kDa, surface 1.3 m², targeted volume concentration factor (VOC) 10-15

**Controls**
- Conning Cell Stck 1 (OSI) as controls #1
- Operated in a chemically induced scale up at 37°C, 5% CO₂ using the same inoculation density and proportional volume of culture medium

**Method**
- Cell density determined by cytopsinization of a duplicate (for controls)
- scale-X™ bioreactor, cell density measured via nucleic count on carriers by crystal violet staining
- Triple Transfection using PEI
- Virus titer measured using BEC0

**Cell culture and transfection process**

3. Homogeneous cell distribution in scale-X™ hydro bioreactor

- homogeneous axial and radial distribution of HEK293 cells in the fixed-bed demonstrated (Figure 4),
- enabling an optimal usage of the available surface area for cell growth.
- sampling of the scale-X™ fixed-bed is representative of the average cell concentration across the fixed-bed.

4. Cell growth, viral expression and chained concentration

**Proof of concept and optimization objectives**
- Adaptation of reference process to scale-X™ technology.
- Identification and optimization of critical process parameters to achieve high titer yields.
- Implementation of chained-in-line TFF for viral concentration

**Cell growth**
- HEK293 cell growth performance was similar in the bioreactor compared to flatware controls in the four experiments (Figure 5), reaching high cell density (>140 million cells/mm²) 4 days after inoculation.
- No negative impact was observed with the applied changes for the process optimization parameters (pH setpoint 7.1, pH 2-4), and volume per surface ratio reduction in the growth phase (2.25x lower in #3).

**Identification and optimization of critical process parameters to achieve high titer yields**

**Process scale-up**
- Process transfer efficiency (experiment 1) improved up to 45% of the average reference, within the targeted expected range.
- Yield results from #2 demonstrated no impact of a lower pH, permitting lower usage of media throughout the process.
- Experiment #3 resulted in similar yields compared to run #1 and #2, although transfected with a three-fold reduced DNA and PEI concentration based on lower cell density at transfection.
- Experimental conditions #4 resulted in a similar (98%) yield compared to the reference process, even with media volume variation reduction during growth phase.

**Advantages of scale-X™ compared to flatware Bioreactors**
- Significant process intensification leading to shorter production time and cost.
- Reduced footprint leading to reduced material costs.
- Fewer operational steps leading to reduced labor costs.

**Process scale-up and chained TFF concentration**

Experimental results were extrapolated for large scale production, both for the reference process and optimized process (Figure 7). Experimental volumes of scale-X™ hydro bioreactor (2.4 m²) are extrapolated to scale-X™ and nitro bioreactors with 25% total volume reduction based on initial experiments.

- The scale-X™ narrow fixed-bed entraps major impurities and acts as a pre-clarification step allowing direct and a allowing a direct product concentration using TFF.
- Implementation of inline concentration permits desalting and reduction of harvest volume, resulting in 80 L at large scale instead of 1,215 L as in the reference process.
- Such volume reductions positively impact process costs and time.

Conclusion and future steps

- Successful transfer of the cell culture process from static plasticware to fixed-bed bioreactor leading to a low footprint and cost-effective production.
- Preliminary process intensification with few experiments:
  - Decreased cell seeding density with a shorter seed train
  - Three-fold reduced DNA and PEI amounts
  - Reduced media volume during growth phase
  - Reduced material costs
  - The reference process can be combined with chained TFF allowing harvest volume reduction and omit clarification step leading to lower time and material cost.
- Process optimization and cell homogeneity assessment demonstrate high performance and reproducibility of the scale-X™ bioreactor; reaching higher yields than alternative packed-bed bioreactors.

- Next steps will involve scale-up to production level in scale-X™-carbo and nitro bioreactors with in-line TFF for further improvements.

- Unlike other technologies for the culture of adherent cells, the scale-X™ bioreactor allows process intensification leading to significant cost and time reduction.

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