Adaptation of suspension-based Adenovirus production process to scale-X™ technology

Case study

E. Gateau[1], C. Meeüs[1], T. Bouckenooghe[1], H. Kallel[2], J. Castillo[2], Marco Soriani[3], Maria Ferraiuolo[3], Alessia Noto[3], Stefano Colloca[3]

Abstract

Adenoviral vector are non-enveloped virus containing double stranded genome engineered to be used for gene therapy and vaccine application. A broaden use of suspension HEK 293 cell line has been observed with the increasing needs for larger scale production. Commonly operated in batch, cell density achieved is low and the implementation of more complex feeding strategy (fed-batch or perfusion) is challenging. In this study, the scale-X™ bioreactor* was shown as an alternative upstream technology to produce a non-replicative Gorilla Adenovirus vector (GRAd, proprietary of ReiThera) currently under clinical development for an anti-COVID19 vaccine using suspension cell benefiting from both a simpler seed train and an increased flexibility in process designed using cells entrapped in the fixed-bed structure. This first proof of concept showed the potential for process adaptation to adherence without major changes and highlighted parameters that could be key in increasing titers and batch yields.

* For more information, please visit www.univercellstech.com

1. Context

Initial Suspension Protocol

Vessel
2L bioreactor

Cell line:
Suspension HEK 293 (property of Reithera)

Process
Cell density at inoculation: $5x10^5$ cells/mL
Growth phase duration: 3 days

Dilution with fresh medium prior to infection
Cell density at infection: $5x10^5$ cells/mL
MOI: 200

Production phase duration: 3 days

Controlled parameters
DO ≥ 40%
pH = 7.00
Temperature = 37°C

Objective

Demonstrate adaptation of the suspension process to scale-x fixed-bed technology

Identify main parameters to be addressed during process development for productivity increase

Identified Challenge

Nutrient limitation observed with batch mode process for the suspension culture with accumulation of metabolic by-product

Target

Specific productivity
> 0.7-$1x10^5$ VP/cells

2. Solution proposed

RUN 1
Process Transfer

Adjust cell densities to an adherent technology while maintaining other parameters constant

Run parameters:
Cell density at inoculation: $1.5x10^4$ cells/cm² (in 750 mL) and infection after 3 days, production duration increased to 3 days

Success criteria:
Population doubling time = 42 ± 5 h

% cells in suspension
< 5 %

RUN 2
Increase production phase duration

Assess if production reaches a plateau after 3 days or can be extended

Run parameters:
Cell density at inoculation: $3x10^4$ cells/cm² (in 750 mL) and infection after 3 days, production duration increased to 4 days

Success criteria:
Population doubling time = 42 ± 5 h

Specific productivity
> 0.7 - $1x10^5$ VP/cells

RUN 3
Increase cell density at infection

Assess the interest of increasing biomass infected

Run parameters:
Cell density at inoculation: $3x10^4$ cells/cm² (in 750 mL) and infection after 4 days, production duration kept at 3 days

Success criteria:
Population doubling time = 42 ± 5 h

Specific productivity
> 0.7 - $1x10^5$ VP/cells

3. Expected cell density in scale-X hydro with higher cell density at inoculation and longer growth phase

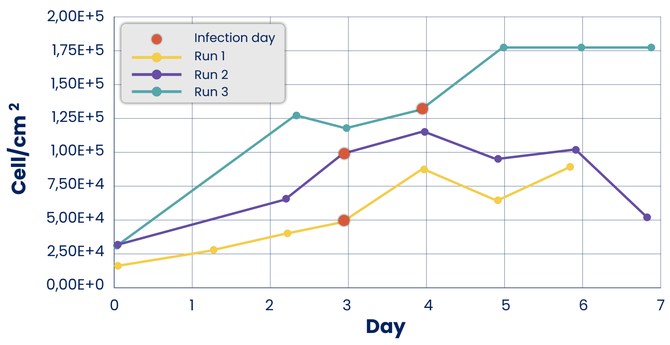

Figure 1: Monitoring of HEK growth curve by total adherent cell density per surface estimation (measured via nuclei count on sampling carriers lysed using Reagent A100, Chemometec, and treated by crystal violet staining).

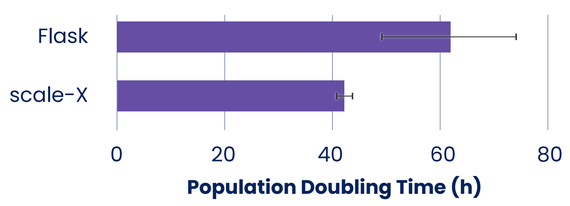

Figure 2: Average population doubling time (n=3) measured in shake flask and scale-X hydro bioreactor between day 0 and day 3.

Observations

- Homogeneous cell growth profile and doubling time decreased in a controlled culture environment
- Adherence properties kept with higher cell density without major changes in process (medium formulation)

➡ **Less than 5%** of the biomass in suspension during the runs

Impact

Successful adaptation of process parameters in scale-X technology without major process change

4. Specific production maintained with adapted process in scale-X hydro

Production of viral particle | scale-X hydro and shake-flask control

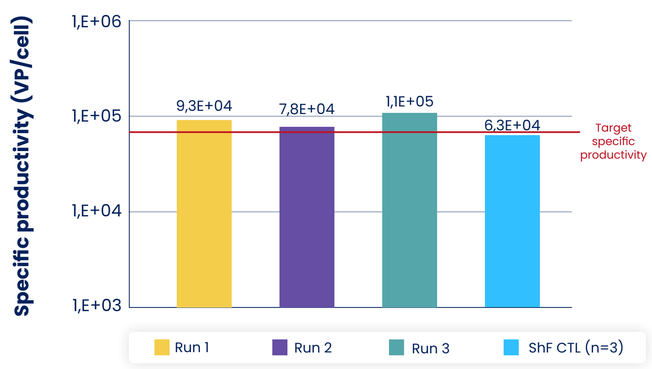

Figure 3: Specific productivity in viral particle per cell measured by qPCR after harvest by cell lysis.

Observations

With a similar process, the specific productivities are maintained when we transferred suspension cells in adherence

Impact

Further process adaptation can be envisionned to increase the biomass inside the bioreactor

5. Higher virus production reachable with longer production phase

Kinetic of viral particle production | scale-X hydro

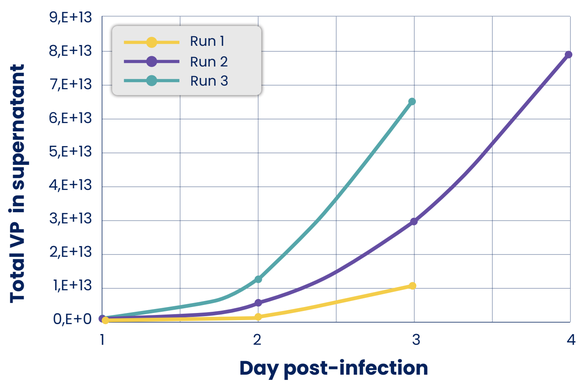

Figure 4: Kinetic of the virus production in the supernatant of the scale-X hydro bioreactor.

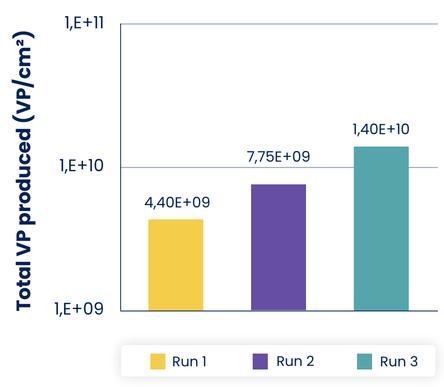

Figure 5: Viral titers (VP/cm²) measured in the pool of cell lysis fraction and supernatant at harvest

Observations

- Extension of cell growth or production phase led to an increase of viral particle concentration in supernatant
- Titers are improved with an increased infected biomass

Impact

Production phase could be extended to **reach higher total viral titers**

6. Successful adaptation and improvement of an adenoviral suspension production process

Key observations and learnings

1. Great adaptation of HEK 293 in fixed-bed technology and identical cell growth
   ➡ **scale-X bioreactor is suitable for HEK 293 growth with initial reference process**
2. Infection method and MOI enable great production of virus with a promising kinetic
   ➡ **Extended production phase and higher cell density at infection can increase significantly the final virus titer**
3. Harvest and rinsing steps managed to successfully recover the viral particle
4. Envisioning scalability in scale-X nitro 600 m² with some process optimization can help reduce **footprint and media used compared to 1000 L stirred tank bioreactor**

Perspective

With current specific productivities, a scale-X nitro 600 m² is foreseen to give equivalent number of doses as a 1000 L stirred tank bioreactor. Targeting similar volumetric titers, a future process development could aim at doubling the number of doses manufactured at larger scale.

Affiliations: [1] Exothera SA, Gosselies, Belgium; [2] Univercells SA, Bruxelles, Belgium; [3] ReiThera Srl, Roma, Italy
Emilie Gateau, e.gateau@exothera.world

exothera.world