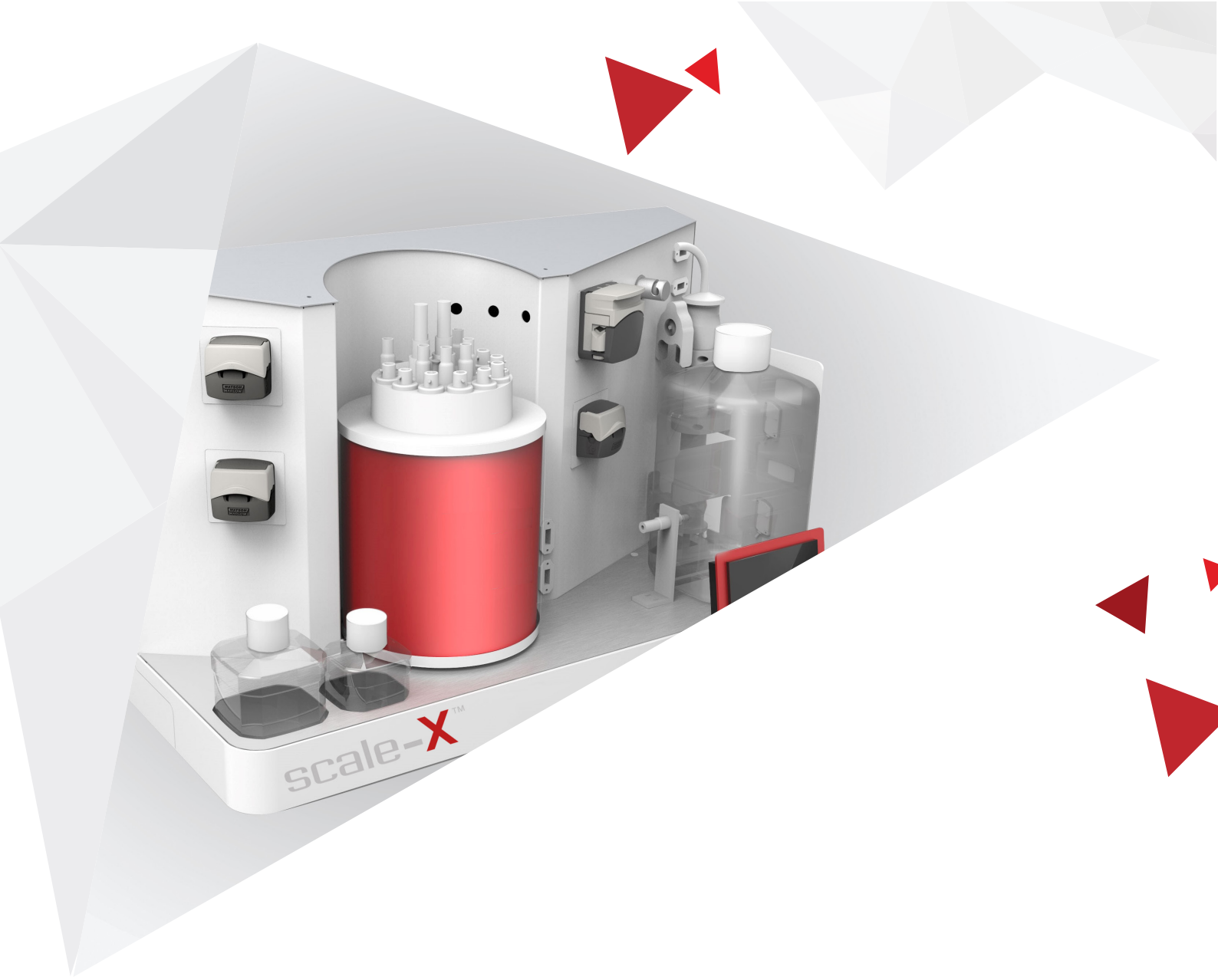

scale-X™ bioreactor for viral vector production

Proof of concept for scalable HEK293 cell growth and adenovirus production

Application note

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Abstract

In this application note, the Univercells team demonstrates successful transfer of the culture of HEK293 cells for adenoviral vector production from static plasticware to the scale-X™ hydro fixed-bed bioreactor system (2.4 m^2 available growth surface). Cell growth, infection and harvest steps are detailed, with results that validate proof of concept. In the study, a cell line is transferred from a static culture support to a fixed-bed bioreactor, with improved cell growth and reliable viral productivity results. A feasibility study was also performed to assess the impact of Serum-Free Medium (SFM) on production in the fixed-bed bioreactor. This application note focuses on the scale-X hydro bioreactor. Such culture methods are scalable to pilot (30 m^2) and production (600 m^2 and above) scales with the full range of scale-X bioreactors. This includes the hydro, carbo, nitro and oxo bioreactors for R&D, clinical and commercial supply of adenovirus-based gene therapies.

Introduction

Adherent cells for viral vector production are widely used in the development and commercialization of gene therapies, and this demand is expected to continue to grow in the future[1]. Traditional processes for adherent cell culture use static methods (e.g. multi-tray plastic ware) for process development and industrial production, but these suffer from several limitations. Static methods lack precise environmental monitoring and control (pH, Dissolved Oxygen (DO), media composition), are heavily dependent on manual operations, and can only be scaled-out as opposed to scaled-up. Additionally, use of serum-based mediums in static processes create contamination risk and raise ethical and traceability concerns. Since gene therapies typically require large doses of active viral agents per patient, global demand is expected to rise [2] and the market is seeing an increasing need for automated, scalable, and serum-free solutions. Our team worked to develop a solution with a novel bioreactor technology portfolio that enables quality production in an affordable manner [3] [4] [5].

A novel single-use, fixed-bed bioreactor portfolio

The scale-X hydro bioreactor system consists of a single-use fixed-bed bioreactor providing 2.4 m^2 of surface for the adherence of cells in the form of spiral-wound, non-woven polyethylene terephthalate fabric layers. The system provides automated pH, DO and temperature control; media and cell sampling as well as pumps allowing further media addition through a re-circulation loop or perfusion (Figure 1). A magnetic centrifugal impeller located inside the bioreactor provides two functions: good mixing ensuring even availability of nutrients throughout the fixed-bed, and aeration through the creation of a "falling film" via the vessel headspace. The latter increases the surface area available for gas exchange thereby ensuring an adequate volumetric mass transfer coefficient (kLa) in the system. The scale-X hydro system is part of a portfolio of bioreactors allowing process development and pilot scale cultures (scale-X carbo, 10 - 30 m^2), medium-to-large scale industrial production (scale-X nitro, 200 - 600 m^2, typically suitable for vaccine production) and larger scale industrial production (scale-X oxo, >2000 m^2, to meet the needs of gene therapy).

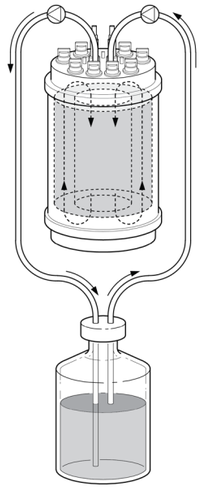

Figure 1: Schematic representation of the scale-X carbo bioreactor and recirculation loop

Scaling principles:

Scaling-up from the bench to the plant in the scale-X bioreactor range is based on the principle of constant linear velocity by providing more surface area; the diameter of the fixed-bed is increased while the total height remains the same across scales. This keeps the linear velocity of liquid media travelling through the fixed-bed constant, meaning that residence time also remains the same across the scales. Figure 2 illustrates how this is achieved.

Key features	Benefits
Low-footprint, high growth surface area	Delivering high titers at bench scale
Structured, stackable fixed-bed ensuring even linear velocity	Linear scalability from R&D to clinical batches in one system
Ultra-low shear stress	Gentle process conditions adapted to fragile products
Fixed-bed sampling for cell analysis	Direct & reliable control improving process robustness
Single-use, pre-assembled components (bioreactor & tubing manifolds)	User-friendly installation and efficient change-over
Sterilization via autoclave or gamma-irradiation	Adapted to process and operational needs
Optional automated in-line concentration of product	Enhancing product recovery & overall efficiency

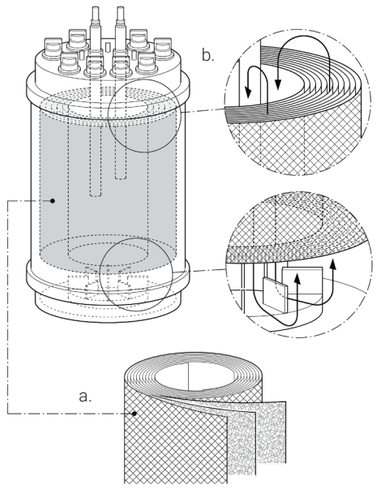

Figure 2: Schematic details of the scale-X carbo bioreactor
(a) Structured fixed-bed, (b) Fluid flow patterns within the fixed-bed

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Application note

Material, Methods & Equipment

Cells & medium

HEK293 cells from a cryopreserved cell bank (Momotaro-Gene Inc., Okayama, Japan) were thawed at 37°C. A cell culture flask (Corning™ U-Shaped Cell Culture Flasks, Fisher Scientific, Asse, Belgium) was inoculated at a seeding density of 3.0 x 10^4 cells.cm^{-2} using fetal bovine serum-enriched medium (10%) and incubated at 37°C, 10% CO_2 in a humidified incubator until ≥80% confluency was observed using an optical microscope. Harvested cells formed the inoculum for experiments. Additionally, a direct adaptation was performed on HEK293 cells used for Serum-Free Medium (SFM) experiments. In this condition, an expected decrease of growth rate was observed. Therefore, seeding densities were adjusted to 4.0 x 10^4 cells.cm^{-2}. Cells were grown up to target density.

Assays

Cell Density – Pre-cut sample strips of the fixed-bed material are incorporated into the fixed-bed during fabrication and can be manually extracted in a biosafety cabinet at set times during the culture to assess cell growth. After removal, the strips are vortexed in a cell lysis buffer and nuclei in the lysis solutions were stained using crystal violet. Cell density was derived from the resulting nuclei count, the latter of which was performed manually using an optical microscope.

Virus Titer – Measured using the Adeno-X Rapid Titer Kit (Clontech, Terra Bella, USA), which makes use of adeno-specific viral hexon proteins for detection of infected cells. Upon fixing and staining, infected cells are visually counted using an optical microscope. Supernatant and harvest samples were used to this effect.

Metabolites Concentration – Glucose and lactate concentrations were measured from supernatant using an off-line metabolite analyser (Vi-CELL MetaFLEX Bioanalyzer, Beckman Coulter, Suarlée, Belgium).

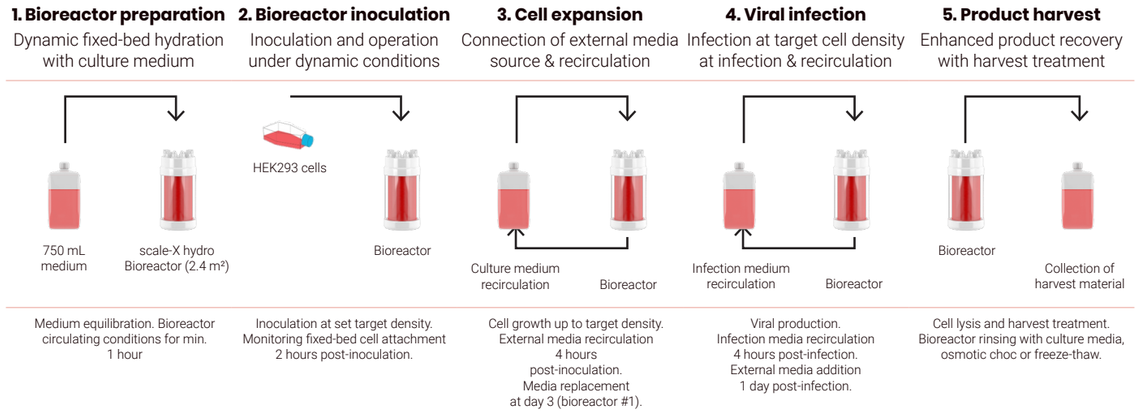

Figure 3: Process flow diagram of the experimental setup

Experimental setup and culture parameters

The cell culture process in the scale-X hydro system takes place in 5 steps: (1) bioreactor preparation, (2) inoculation and cell attachment, (3) cell expansion, (4) viral infection and (5) harvest (Figure 3).

During bioreactor preparation, DO calibration was achieved under non-regulated aerated conditions (100% set point) before starting regulation (>50%). In all experiments an external media source was connected during cell expansion and recirculated through the bioreactor shortly after inoculation (Figure 3). For bioreactor #1, this media source was replaced at day 3 to allow further cell growth and the process was stopped thereafter. Cell expansion was performed for 2 days (bioreactor #2) and 3 days (bioreactors #3-5) in batch mode, subsequent to which the cells were infected. Cell lysis and harvest treatment were conducted to recover product.

Experimental control

For each bioreactor experiment plastic flatware cultures were operated in a humidified incubator using the same inoculation density and proportional volume of culture medium as experimental controls:

- Corning CellBIND CS2 cell culture chambers as controls #1-4
- Corning T225 vented cap cell culture flasks as control #5

Cell density was determined by trypsinization of a duplicate.

Culture condition summary

Bioreactor Parameters	Bioreactors #1-4	Bioreactor #5 (SFM)
Bioreactor vessel effective working volume	750 mL	750 mL
Agitation speed	740 rpm (1 $cm.s^{-1}$ vertical velocity)	740 rpm (1 $cm.s^{-1}$ vertical velocity)
Falling film height	~5 cm	~5 cm
Medium flow	10 $mL.min^{-1}$	10 $mL.min^{-1}$
Temperature	37°C	37°C
pH	Growth: 7.2; Production: 7.0	Growth: 7.1; Production: 7.0
Dissolved oxygen	50%	50%
Culture		
Inoculation density	30,000 cells.cm	40,000 cells.cm
Recirculation Volume	4.05 L	4.05 L
Culture time	3 – 6 days	3 days
Viral Production		
Adenovirus stock	1.7 x 10^9 IFU.mL^{-1}	6.3 x 10^9 IFU.mL^{-1}
CDI	150,000 ± 50,000 cells.cm^{-2}	150,000 ± 50,000 cells.cm^{-2}
Recirculation volume	4.05 L	4.8 L
Production time	4 days	4 days
Harvest		
Harvest treatment	Triton X-100, Benzonase and $MgCl_2$	Triton X-100, Benzonase and $MgCl_2$
Harvest volume (incl. treatment)	5.04 L	5.83 L

Results

Cell growth

Figure 4 shows the cell density measured in five separate experiments. Transferring the serum-based culture from multi-tray plasticware to the bioreactor yielded higher cell densities under the same conditions, both at day 2 or 3 (average 134%, n=3) and day 6 (197%, n=1).

In order to achieve the high cell density seen at day 6 (bioreactor #1), 85% of the medium was exchanged at day 3 in the plastic flatware, while an external bottle containing 4.2 L of fresh culture medium was connected and circulated (10 mL.min-1) through the bioreactor, corresponding to the same ratio of medium exchange.

Higher cell density observation in scale-X bioreactor (128%, n=1) has been reproduced with serum-free process. Transition from serum-based to serum-free medium did not prevent cells from adhering to the fixed-bed, and targeted cell density after 3 days was reached regardless of the expected decrease in growth rates.

The higher cell densities obtained in the bioreactor are most likely a reflection of the better environmental control (pH and DO) in the system which promotes a stable growth environment. The potential to reach very high cell densities within a small bioreactor volume highlights the system's capability for high levels of production in a much reduced footprint, using automated process step.

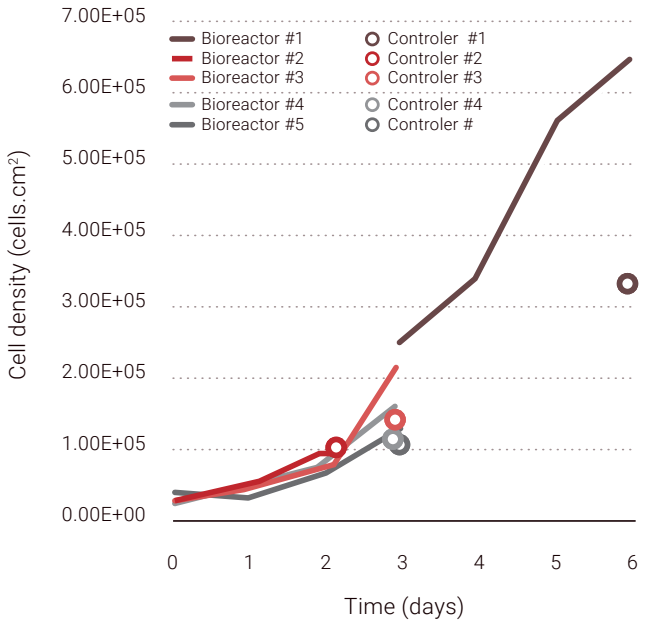

Figure 4: Comparative growth curves of HEK293 cells grown in scale-X hydro bioreactor and in plastic flatware controls, showing: Experiments #1-4 with a serum-based process, experiment #5 with SFM. Bioreactor #1 was operated with an external medium circulation loop replaced at day 3 (see material and methods for further details). Bioreactor #2 was infected at day 2, bioreactors #3-5 at day 3. Cell density post-infection is not shown.

Viral expression

Infection was performed at a target cell density in the cell cultures presented here.

Cells were lysed 3 days post-infection, using detergent treatment to recover more product (see Materials, Methods & Equipment). The same harvest protocol was performed on the plastic flatware control experiments and both are presented in Figure 5.

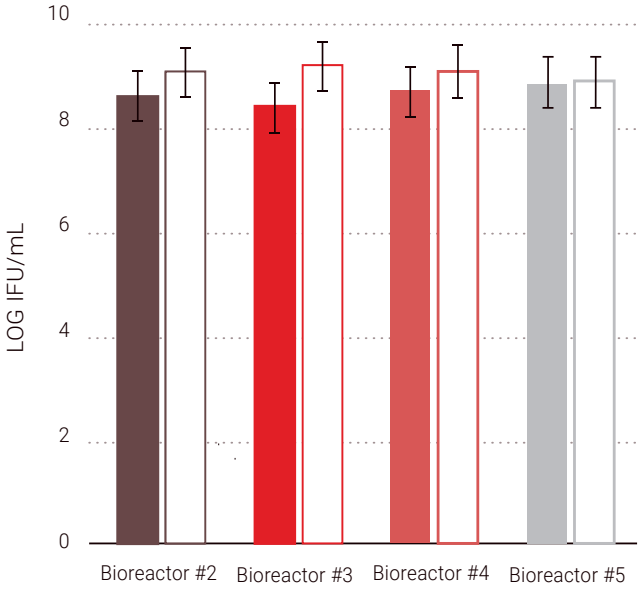

Figure 5: Adenovirus viral titers from scale-X hydro bioreactors #2 to #5 and in plastic flatware control, showing: Experiments #2-4 with a serum-based process, experiment #5 with SFM. Infection was not carried out in bioreactor #1. The error bar represents the range of the virus titer analytical assay measurement.

For the serum-based process, the viral titer is in average 0.53 log (IFU) less in the bioreactor than in the control plastic flatware, which is a promising result for a direct transfer of culture conditions without process development work. Good reproducibility is shown between all bioreactors, with similar promising titer obtained with the serum-free process. The differences in yield observed between the bioreactors and their plastic flatware controls are not significant due to the analytical assay sensitivity and could be enhanced with further process development.

Conclusion and perspectives

An HEK293 process producing an adenovirus for gene therapy based on the use of static plasticware was successfully transferred to the scale-X bioreactor system. Cell densities were shown to be higher in the scale-X hydro bioreactor, while promising viral yields open the door for further developments. Based on the experiment in the bioreactor using serum-free medium, both cell growth and productivity data showed that a serum-free process also offered a good alternative for Adenovirus production in scale-X bioreactor. To mitigate risks during transfer additional assessment of medium exchange either after inoculation or infection were performed without loss in titers. These results (not shown here) demonstrated possible flexibility in the timings for medium exchange along the process. Medium exchange strategy must be defined according to regulatory and cost constraints. Further process development and determination of critical process parameters for the optimization of the culture conditions should complete this study before demonstrating the scalability of the process to larger-scale bioreactors.

Bibliography

[1] J. van der Loo and J. Fraser Wright, "Progress and challenges in viral vector manufacturing," Human Molecular Genetics, vol. 15, pp. 42-52, 2016. **[2]** N. Faust, "Addressing the Challenges of Commercial-Scale Viral Vector Production," Cell & Gene Therapy Insights, vol. 4, pp. 31-36, 2018. **[3]** R. Rajendran, R. V. S. Lingala, B. Bandi, E. Manickam, S. Macherla, S. Dubois, N. Havelange and K. Maithal, "Assessment of packed bed bioreactor systems in the production of viral vaccines," AMB Express, vol. 4, 2014. **[4]** A. Valkama, H. Leinonen, E. Lipponen, V. Turkki, J. Malinen, T. Heikura and L. H. Ylä-Herttuala S, "Optimization of lentiviral vector production for scale-up in fixed-bed bioreactor," Gene Therapy, vol. 25, pp. 39-46, 2017. **[5]** D. Ventini-Monteiro, S. Dubois, R. Astray, J. Castillo and C. Pereira, "Insect cell entrapment, growth and recovering using a single-use fixed-bed bioreactor. Scaling up and recombinant protein production," Journal of Biotechnology, vol. 216, pp. 110-115, 2015.

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