scale-X™ bioreactor for viral vectors production

Proof of concept for scalable HEK293 cell growth & adenovirus production

Application note

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Abstract

This note demonstrates the culture (cell growth, infection and harvest steps) of HEK293 cells producing an adenoviral vector in the Univercells scale-X™ hydro fixed-bed bioreactor system (2.4 m^2 available growth surface). Such culture methods are scalable to both pilot (30 m^2) and production (600 m^2 and above) scales using the Univercells scale-X carbo, nitro and oxo ranges of bioreactors for both clinical and commercial supply of adenovirus-based gene therapies. The following set of results demonstrate the first steps in a proof-of-concept study, where a cell line is successfully transferred from a static culture support to a fixed-bed bioreactor.

Introduction

Adherent cells for the production of viral vectors are widely used in the development and commercialization of gene therapies and will become even more so 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 a number of limitations. Static methods lack precise environmental control (pH, Dissolved Oxygen (DO), media composition), are heavily dependent on manual operations and can only be scaled-out as opposed to scaled-up. The need for improved, automated and scalable production tools is two-fold: gene therapies typically require large doses of active viral agents per patient, and the global demand for the latter is expected to rise significantly in the near future [2]. The use of scalable fixed-bed bioreactor technology offers the opportunity to supply the market with the required quantities in an affordable manner [3] [4] [5].

scale-X hydro single-use, fixed-bed bioreactor system

The scale-X hydro bioreactor system consists of a single-use (SU) 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 (PET) fabric layers. The system provides automated pH, DO and temperature control, automated inoculum addition, media and cell sampling as well as pumps allowing further media addition through a re-circulation loop (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).

Scaling principles:

Scaling-up from the bench to the plant in the Univercells scale-X bioreactor range is based on the principle of constant linear velocity: to provide more surface area, the diameter of the fixed-bed is increased while the total height remains the same across scales. Therefore, keeping the linear velocity of liquid media travelling through the fixed-bed constant means that the residence time also remains the same. In this way, the conditions experienced by the cells across scales remain very similar. Figure 2 illustrates how this is achieved.

Key features	Benefits
Low-footprint, high growth surface area	Delivering highly concentrated product 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

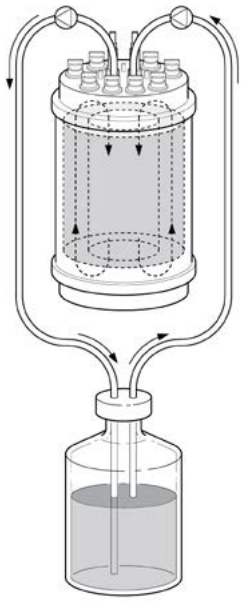

Figure 1 : Schematic representation of the scale-X carbo bioreactor and recirculation loop

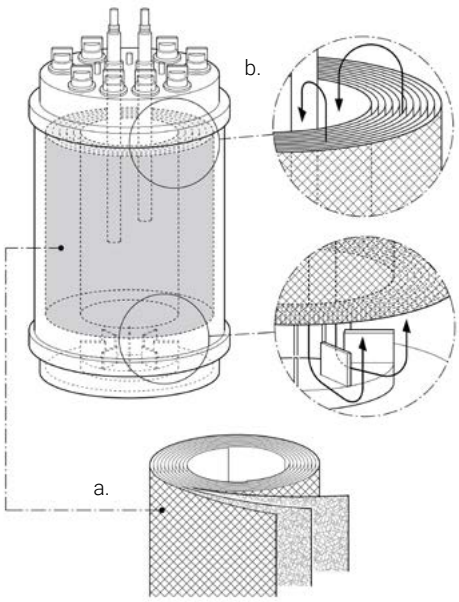

Figure 2 : Schematic details of the scale-X carbo bioreactor
(a) Structured fixed-bed, (b) Fluid flow patterns within the fixed-bed.

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 3.0×10^4 cells.cm^{-2} using fetal bovine serum-enriched medium (10%) and incubated at 37°C, in a humidified incubator until ≥80% confluency was observed using an optical microscope. Harvested cells formed the inoculum for experiments.

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 then 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 titre: TCID50 was used to measure viral titres using the Adeno-X Rapid Titre 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 / harvest samples were used to this effect.

Metabolites concentration: glucose and lactate were measured from supernatant using an off-line metabolite analyser (Vi-CELL MetaFLEX Bioanalyzer, Beckman Coulter, Suarlée, Belgium).

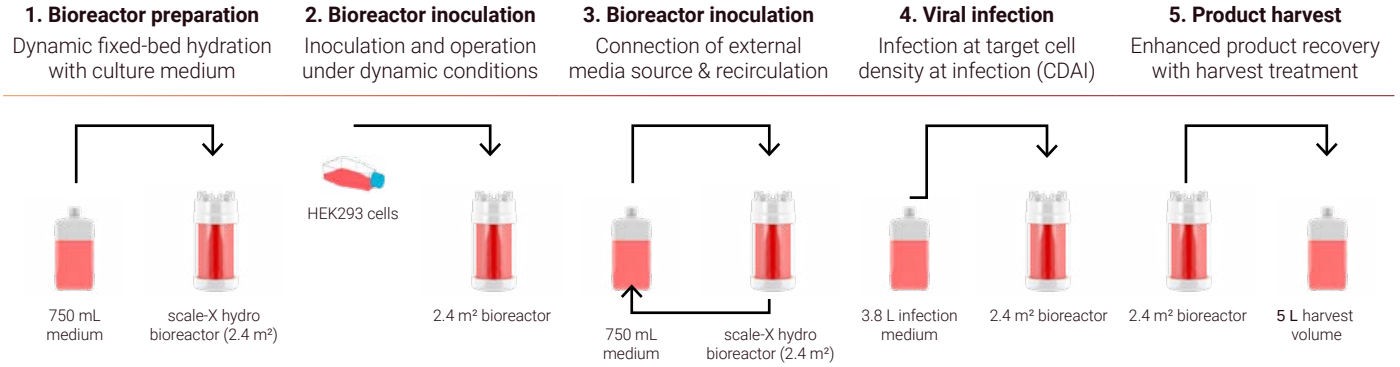

Figure 3: Process flow diagram of the experimental setup

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. For bioreactor #1, this media source was replaced at day 3 to allow further cell growth and the process was stopped thereafter. For bioreactors #2 and #3 cell expansion was performed for 2 and 3 days respectively in batch mode, subsequent to which the cells were infected. Although the cells naturally lyzed upon infection, product recovery was enhanced using detergent.

Experimental control

For each bioreactor experiment, plastic flatware cultures (Corning T225 vented cap cell culture flask, Fisher Scientific, Asse, Belgium) were operated in a humidified incubator using the same inoculation density and proportional volume of culture medium as experimental controls.

Culture condition summary

Bioreactor vessel effective working volume:	750 mL
Recirculation volume:	4.2 L
Agitation speed:	740 rpm (1 cm.s^{-1} vertical velocity)
Falling film height:	~5 cm
Innoculation density:	30,000 cells.cm^{-2}
pH:	7.2
Dissolved oxygen:	50%
Harvest treatment:	Triton X-100, Benzonase and MgCl
Culture time:	3 – 6 days

Results

Cell growth

Figure 4 shows the cell growth curves in scale-X hydro compared to the plastic flatware controls. The cell density reached in the fixed-bed bioreactors is higher under the same conditions than in the control experiment, both at day 3 (2.1×10^5 vs. 1.3×10^5 cells.cm^{-2}) and at day 6 (6.5×10^5 vs. 3.2×10^5 cells.cm^{-2}). In order to achieve the high cell density seen at day 6, 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.

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 promote 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, automated process step.

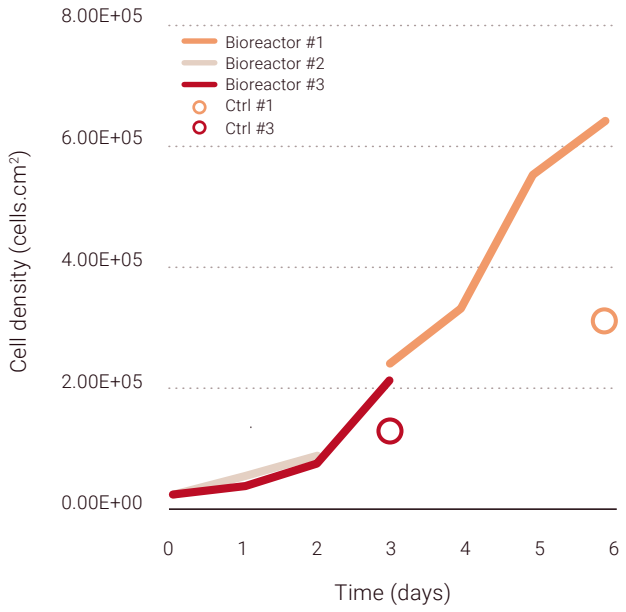

Figure 4: Comparative growth curves of HEK293 cells grown in scale-X hydro bioreactor and plastic flatware controls.

All bioreactors were operated with an external medium circulation loop of 4.2L from inoculation (see material and methods for further details). At day 3, the external media circulation loop of bioreactor #1 was replaced with fresh medium thus allowing further cell growth until day 6.

Bioreactors #2 and #3 were operated in batch mode and then infected (respectively at day 2 and 3). Cell density post-infection is not shown. Control cell density for bioreactor #2 was not available.

Viral expression

Infection was performed at a target cell density in the cell cultures presented here. All cells were observed to be lysed 3 days post infection, upon which it was decided to recover more product using the detergent treatment (see Materials, Methods & Equipment). The same harvest protocol was performed on the plastic flatware control experiments and both are presented in Figure 5. The viral titre is approximately 1 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. Further work will focus on determining critical process parameters (CPCs) and optimizing them in the bioreactor; a feat which would not be possible in traditional static processes due to the lack of control on process parameters such as pH and DO.

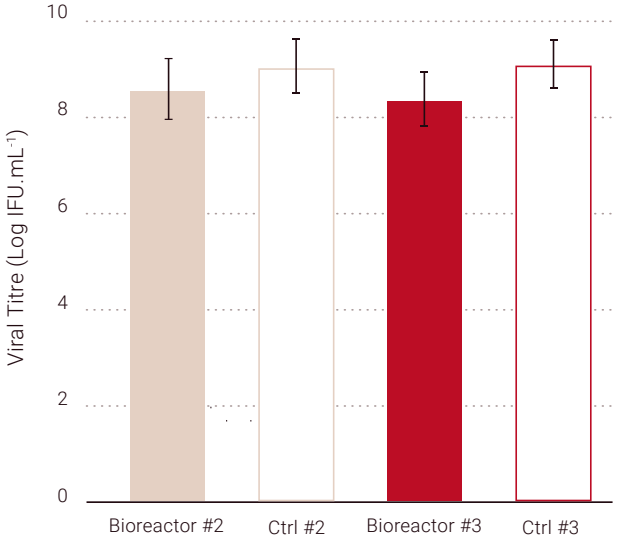

Figure 5: Adenovirus viral titres from scale-X hydro bioreactors #2 and #3 experiments and in plastic flatware controls.

Infection was not carried out in bioreactor #1. Bioreactor #2 was infected at day 2 whereas bioreactor #3 was infected at day 3. The error bar represents the range of the TCID50 analytical assay measurement. Good reproducibility is shown between both bioreactors, and the difference in yield compared to their plastic flatware experiments could be improved 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 Univercells scale-X bioreactor system. Cell densities were shown to be higher in scale-X hydro, while promising viral yields open the door for further process development and the determination of critical process parameters for the optimisation of the culture conditions. Further experiments will demonstrate the scalability of the process to larger-scale bioreactors.

7. Bibliography

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