

Development of a Scalable Viral Vector Upstream Process for Gene Therapy: rAAV-8 Production by Transient Transfection of HEK-293 Cells in iCELLis® Bioreactor

Pascal Lefebvre¹, Christophe Van Huffel¹, Otto-Wilhelm Merten², Matthias Hebben², Cédric Rousseaux², Simon Arias¹, Moustapha Hohoud¹, Roel Lievrouw¹, Fabien Moncaubeig¹, Paule Nowicki³, Catherine Cancian²

¹Pall Artelis, Rue de Ransbeek 310, B-1120 Brussels, Belgium; ²Généthon, Rue de l'Internationale 1, BP 60, 91002 Evry, France,

³Aktehom, Avenue du Maréchal JOFFRE - 92000 Nanterre, France

INTRODUCTION

Today, gene therapy offers perspectives for a wide range of incurable genetic disorders and industrialization of viral vector production becomes a key challenge for the biotechnology industry. In this context, Généthon and Pall combined their respective expertise to assess the single-use iCELLis fixed bed bioreactor system for viral vector production. The choice of the iCELLis system was driven by process automation and control via single-use sensors, reduced footprint and capital investment, and ease of process scale-up. The iCELLis bioreactor is a fully-integrated, high-cell density bioreactor designed to simplify processes involving adherent cells by combining the advantages of single-use technologies with the benefits of a fixed-bed system. The fixed bed provides a 3D environment with low shear stress resulting in very high cell densities and high productivity. iCELLis technology can be used at bench-scale (0.5 to 4 m²) and manufacturing-scale (66 to 500 m²). Fixed-bed height remains constant as scale increases leading to linear scalability between the iCELLis Nano and iCELLis 500 bioreactors (Figure 1). Généthon is interested to move forward in their clinical trials with a scalable high-capacity and cost efficient process with the intention to commercialize in the coming years affordable gene therapy products for rare diseases.

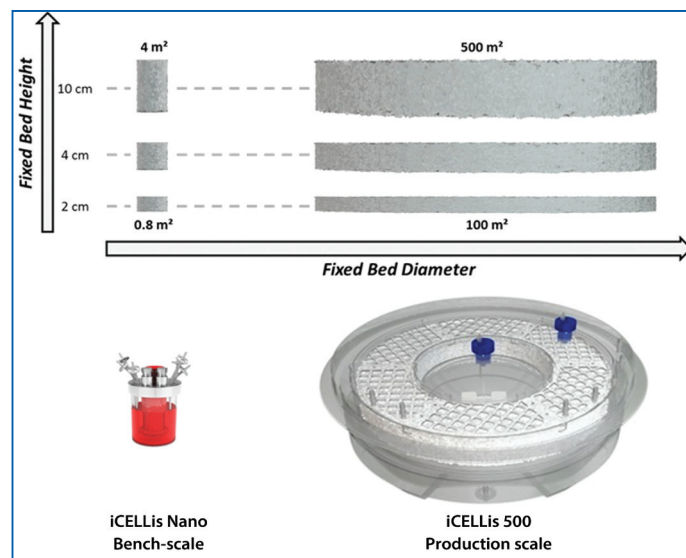
MATERIALS AND METHODS

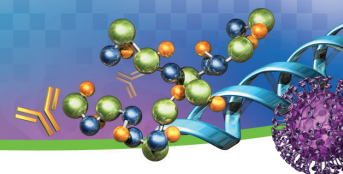
Materials

- ▶ Biological materials: HEK-293 cells (Généthon).
- ▶ Support: iCELLis Nano bioreactor 0.8 m² (Pall, Cat. 810040NS) and 4 m² (Pall, Cat. 810042NS).
- ▶ Growth medium: FreeStyle F17 Expression medium (Thermo Fisher, Cat. A13835-02) supplemented with 4 mM GlutaMAX[®] Supplement (Thermo Fisher, Cat. 35050-083).
- ▶ Transfection reagents: Diluted solutions of PeiPRO[®] transfection reagent (PolyPlus, Cat. 115-100) and mix of proprietary plasmid constructions: pGFP, pRep2Cap8 and pHelper.
- ▶ Production medium: Dulbecco's Modified Eagle Medium (Thermo Fisher, Cat. 31053-028 supplemented with 4 mM GlutaMAX Supplement (Thermo Fisher, Cat. 35050-083).
- ▶ Lysis buffer: Diluted Triton X-100 solution (Merck Millipore, Cat. 1086432500) and NaCl solution (Sigma Aldrich, Cat. S9888). pH adjustment performed with NaOH 0.5 M (Merck Millipore 1.09137.2500).

Figure 1

Linear scalability from iCELLis Nano to iCELLis 500 bioreactors.



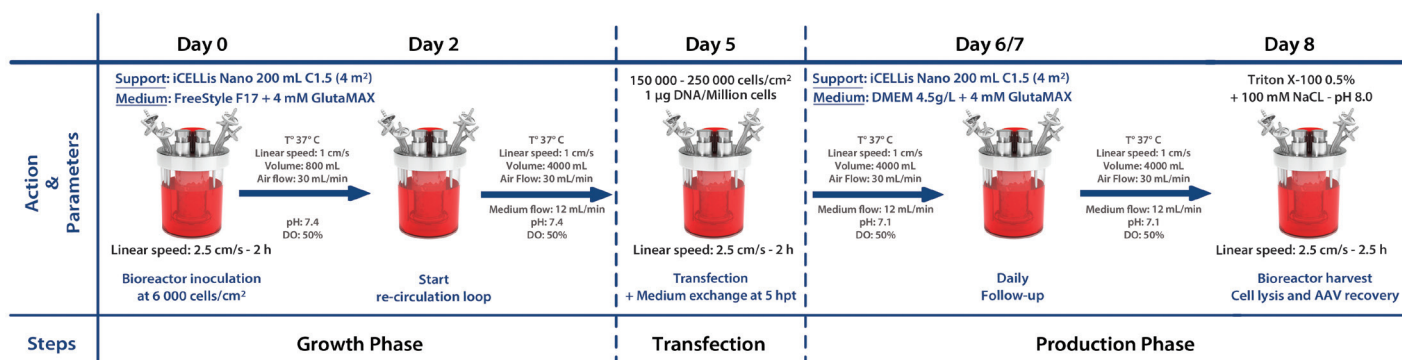


Methods

AAV-8 production process was developed and scaled-up in iCELLis Nano bioreactor. Final upstream process and main parameters are presented in the flow-chart below (Figure 2). AAV productivity was assessed at 72 hours post transfection (hpt) by qPCR performed directly on cell lysate.

Figure 2

Flow-chart of AAV-8 production process by triple-transfection of HEK-293 cells in iCELLis Nano bioreactor.



RESULTS

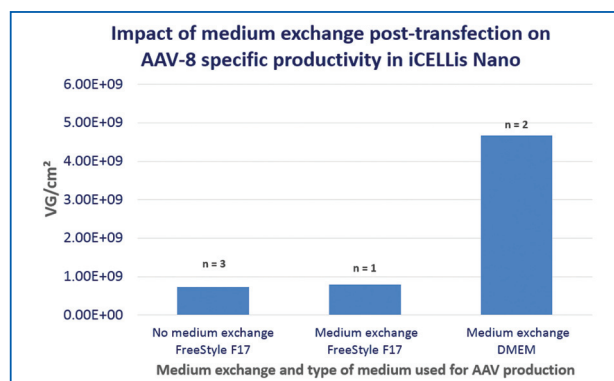
Process transfer and optimization in iCELLis Nano bioreactor 0.8 m²

In order to evaluate the potential of iCELLis technology for AAV-8 production, Généthon's benchmark process was transferred to iCELLis Nano bioreactor. Growth study revealed that HEK-293 cells adhered to the carrier within 6 hours and presented an exponential cell growth profile (data not shown). Growth study was performed in FreeStyle F17 medium complemented with GlutaMAX supplement and no medium optimization was required to adapt the process to adherence in iCELLis bioreactor.

AAV-8 productivity in the iCELLis Nano bioreactor was optimized and significantly improved by moving from FreeStyle F17 medium to Dulbecco's Modified Eagle Medium (DMEM) for the production phase. Up to a 8 fold increase of specific productivity was observed after adding a medium exchange using DMEM post-transfection (Figure 3). Medium exchange with FreeStyle F17 medium post-transfection did not lead to any titer increase. Therefore, medium composition itself and metabolic stress are the main hypotheses to explain this promoting effect.

Figure 3

AAV-8 production in iCELLis Nano bioreactor 0.8 m². Growth phase and triple PEI-mediated transfection in F17 medium using 2.0 µg DNA/Million cells. Medium exchange with F17 or DMEM applied 5 hpt. Data are based on carrier sampling at the top of the bioreactor at 72 hpt and qPCR analysis on cell lysate.



Transfection conditions were optimized. First, impact of cell density at transfection on productivity was evaluated. Results indicated that optimum cell density at transfection was around 200,000 cells/cm² (data not shown). Impact of pDNA quantity used for transfection was also investigated. qPCR analysis revealed that pDNA/cell ratio could be reduced by 50% (so it was halved) (Figure 4). This represents a major achievement in terms of cost-efficiency because pDNA has been identified to be one of the main cost drivers in this process.

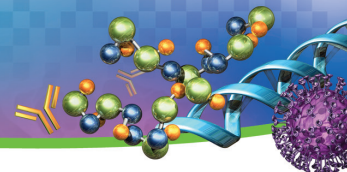
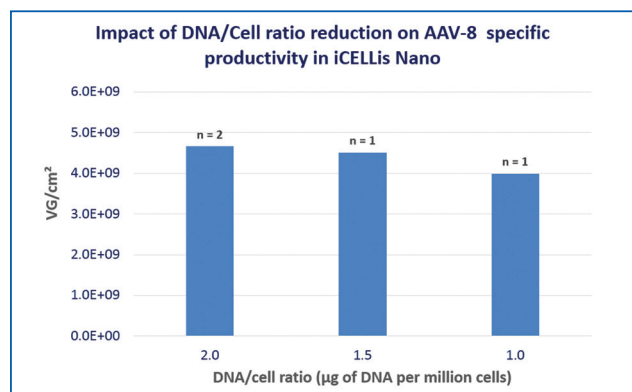


Figure 4

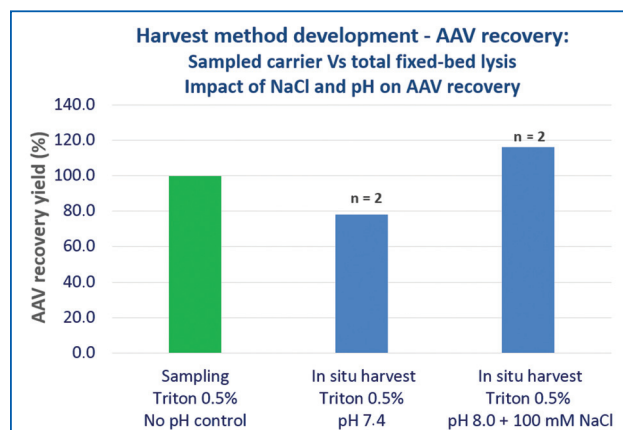
AAV-8 production in iCELLis Nano bioreactor 0.8 m². Triple PEI-mediated transfection using different ratios of pDNA and medium exchange with DMEM applied 5 hpt. Data are based on carrier sampling at the top of the bioreactor at 72 hpt and qPCR analysis on cell lysate.

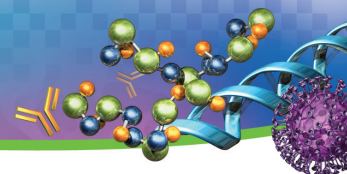


Through a series of screening experiments performed on carriers extracted from the fixed-bed at time of harvest, the cell lysis method was optimized. Then this method was applied for an *in-situ* lysis of the iCELLis Nano fixed-bed that led to > 80% recovery yield compared to individual carrier sampling at the top of the bioreactor. AAV adsorption on carriers was suspected to be the cause of this productivity discrepancy. Hence, pH and salt concentration during lysis were fine-tuned to limit AAV. This led to AAV recovery yields > 100% compared to sampled carriers. Variability from carrier to carrier has not been challenged here.

Figure 5

AAV-8 recovery yield from iCELLis Nano bioreactor 0.8 m². Harvest method: Triton X-100 addition to bioreactor supernatant (0.5% v/v) and incubation for 2.5 hours at 37 °C under stirring. Method optimization led to pH set point increase from 7.4 to 8.0 and NaCl addition after Triton addition. Data are based on qPCR analysis performed on cell lysate.





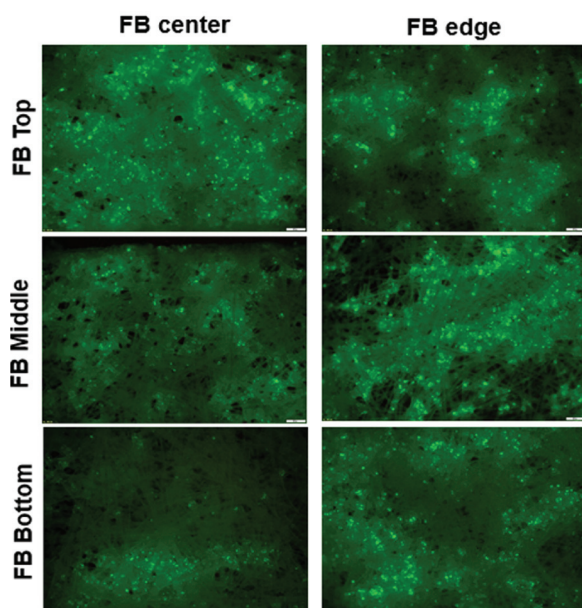
Process scaling-up to iCELLis Nano bioreactor 4 m²

Process scaling up from 0.8 m² to 4 m² involves an increase of the fixed-bed height from 2 to 10 cm. In this context, homogeneity of transfection was evaluated using GFP plasmid. Microscopic observations on sampled carriers revealed similar fluorescence intensity from the bottom to the top of the fixed-bed (Figure 6). These results suggested that transfection conditions applied led to homogeneous distribution of the transfection complex throughout the whole bioreactor. qPCR analysis tended to confirm these observations while revealing similar AAV productivity from the bottom to the top of the bioreactor.

Figure 6

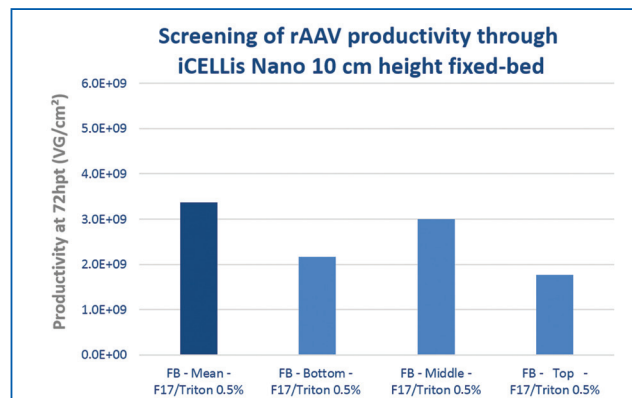
AAV-8 production in iCELLis Nano bioreactor 4 m². Triple PEI-mediated transfection in F17 medium using 2.0 µg DNA/million cells and medium exchange with DMEM applied 5 hpt. The bioreactor was dismantled at 72 hpt and carriers were sampled from different areas of the fixed-bed. Microscopic observation with defined parameters shows GFP-fluorescence on carriers (A) and qPCR analysis performed on cell lysates reveals similar productivities (B).

A



(FB - Fixed Bed)

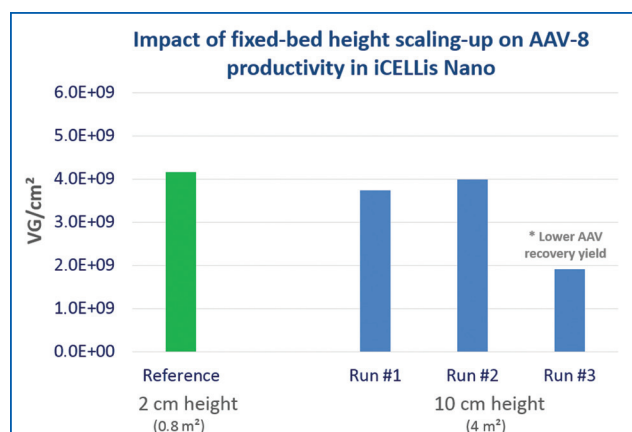
B

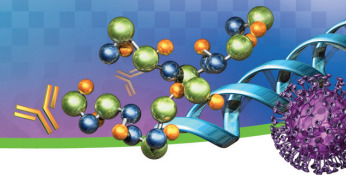


AAV-8 productivity in iCELLis Nano bioreactor 4 m² revealed good process scalability and reproducibility. Results showed that AAV production process could be scaled-up from 2 to 10 cm height while maintaining specific productivity (Figure 7). The three runs showed similar productivities based on carrier sampling (data not shown). Though, run #3 exhibited lower productivity based on *in-situ* cell lysis of iCELLis Nano bioreactor. Fixed-bed clogging by cell debris is highly suspected to be the source of the lower productivity observed.

Figure 7

*AAV-8 production in iCELLis Nano 0.8 m² (Reference) and 4 m². Triple PEI-mediated transfection in F17 medium using 1.0 µg DNA/million cells and medium exchange with DMEM applied 5 hpt. Data are based on *in-situ* cell lysis and AAV recovery at 72 hpt. qPCR analysis was performed on cell lysate.*Lower productivity probably due to fixed-bed clogging by cell debris.*





CONCLUSION AND PERSPECTIVES

iCELLis Nano bioreactors showed promising results as a support for AAV-8 production at high cell density (0.15 to 0.25×10^6 cells/cm²) using PEI-mediated triple transfection. Productivity as well as cost-efficiency - via plasmid amount reduction - were significantly improved and resulted in an average yield of 4×10^{13} VG/ m² before demonstrating scaling-up capability. These results confirmed iCELLis bioreactors to be a promising scalable technology for clinical production of AAV Gene Therapy products. Studies are ongoing to evaluate the quality of the vectors in order to consider a scale up at 500 m².

Corporate Headquarters

Port Washington, NY, USA
+1.800.717.7255 toll free (USA)
+1.516.484.5400 phone
biopharm@pall.com e-mail

European Headquarters

Fribourg, Switzerland
+41 (0)26 350 53 00 phone
LifeSciences.EU@pall.com e-mail

Asia-Pacific Headquarters

Singapore
+65 6389 6500 phone
sgcustomerservice@pall.com e-mail

Visit us on the Web at www.pall.com/biopharm

E-mail us at biopharm@pall.com

International Offices

Pall Corporation has offices and plants throughout the world in locations such as: Argentina, Australia, Austria, Belgium, Brazil, Canada, China, France, Germany, India, Indonesia, Ireland, Italy, Japan, Korea, Malaysia, Mexico, the Netherlands, New Zealand, Norway, Poland, Puerto Rico, Russia, Singapore, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand, the United Kingdom, the United States, and Venezuela. Distributors in all major industrial areas of the world. To locate the Pall office or distributor nearest you, visit www.pall.com/contact.

The information provided in this literature was reviewed for accuracy at the time of publication. Product data may be subject to change without notice. For current information consult your local Pall distributor or contact Pall directly.