Scale-up of an adherent rAAV production process from the iCELLis™ Nano to iCELLis 50 and iCELLis 500+ bioreactors

Anne MacIntyre, Sara Krekels, Andrew Laskowski, Kelly Cybulski, Michelle Olson Peabody
Cytiva, 20 Walkup Drive, Westborough, MA 01581, USA

Abstract

Gene therapy is a rapidly growing field with significant advances and successes that have generated the demand for highly productive and scalable manufacturing processes. Cell culture in adherent conditions represents a valuable platform for viral vector biomanufacturing for therapeutic purposes. However, scaling these processes is not always straightforward. Fixed-bed bioreactors offer effective solutions for the efficient production of viral vectors using adherent cell expression systems.

The iCELLis™ bioreactor system is an automated, single-use, fixed-bed bioreactor that provides a robust platform for adherent cell growth and high-titer viral production. Until recently, this bioreactor system has been available in two formats: the iCELLis Nano bioreactor system for process development and small-scale production (up to 4 m^2); and the iCELLis 500+ bioreactor system for industrial-scale manufacturing (up to 500 m^2). The newly launched iCELLis 50 bioreactor system (6 to 50 m^2) effectively addresses the need for an intermediate-scale offering that bridges the gap between process development (PD) and commercial manufacturing.

To confirm the scalable performance of the iCELLis fixed-bed bioreactor platform, we studied recombinant adeno-associated virus (rAAV) production using the three bioreactor formats. Scalability was illustrated by demonstrating similar metabolic performance profiles and rAAV5 titer across all scales. Our results indicate that the iCELLis bioreactor platform is scalable, and the iCELLis 50 bioreactor is a valuable addition to this bioreactor family.

Introduction

The iCELLis fixed-bed bioreactor family (Fig 1) provides a scalable solution for gene therapy production across development, clinical, and commercial applications. Compared to conventional 2D flatware systems, it enables significantly larger batch sizes while reducing both manufacturing labor and facility footprint by 67% using software that is fully compatible with 21 CFR Part 11 standards. The iCELLis 500+ bioreactor is currently used in the production of six approved human gene therapies. For intermediate-scale needs, the iCELLis 50 offers a geometrically consistent scale-down model of the iCELLis 500+ bioreactor, making it suitable for producing small Phase 1 and Phase 2 clinical batches, with surface areas ranging from 6 to 50 m^2.

Fig 1. The iCELLis family of fixed-bed bioreactors: iCELLis Nano, 0.53 to 4 m^2 (left), iCELLis 50, 6 to 50 m^2 (middle), iCELLis 500+, 66 to 500 m^2 (right).

Process parameters

Bioreactor process parameters are listed in Table 1. Experimental work was performed from May 2025 to September 2025 at Cytiva, Westborough.

Table 1. Process parameters for the iCELLis Nano, iCELLis 50, and iCELLis 500+ bioreactor scales used during testing. Seed train expansion was completed in CellSTACK chambers before bioreactor inoculation

Parameter	Specification		
	iCELLis Nano	iCELLis 50	iCELLis 500+
Vessel size (10 cm bed-height, low compaction)	2.65 m^2	33.3 m^2	333 m^2
Seeding density	5×10^3 cells/cm^2		
Temperature	37°C		
pH	≤ 7.4 (controlled with CO_2 only)		
Dissolved oxygen (DO)	80% (before fixed-bed)	50% (after fixed-bed)	
Linear speed (inoculation, transfection)	2.0 cm/s	1.4, 0.6* cm/s	1.3 cm/s
Linear speed (growth and production)	1.0 cm/s	0.7 cm/s	0.7 cm/s
Falling film height (inoculation, transfection)	7 cm†	0, 10 cm†	0 cm
Falling film height (growth and production)	6 cm		
Growth medium	DMEM supplemented with 10% FBS, 2% GlutaMAX, 1% nonessential amino acids		
Production medium (media exchange prior to transfection)	DMEM supplemented with 2% GlutaMAX, 1% nonessential amino acids		
System volume	0.17 mL/cm^2 (2.5 volumes/d recirculation rate)		
Transfection day	Day 5		
pDNA concentration	0.2 µg DNA/cm^2		
Plasmids	pALD-HELP, pALD-ITR-GFP, pALD-AAV5 (Aldevron)		
Transfection reagent	PEI MAX (Kyfora Bio)		
Plasmid DNA to transfection reagent ratio	1:2 mg		
pDNA concentration in transfection complex	40 mg/L		
Complexation time	15 min		
Production phase	Harvest 120 h post-transfection		

* Agitation speed of 222 rpm used, Linear speed determined post-run completion.
† Falling film height was chosen to achieve a volume to surface area ratio similar to the iCELLis 500+.

Bioreactor scalability performance

To evaluate process scalability, adherent HEK 293H cell growth and rAAV5 production was performed in iCELLis Nano, 50, and 500+ bioreactors. Robust and consistent performance in terms of metabolites and titer was observed throughout all production scales (Fig 2 and 3).

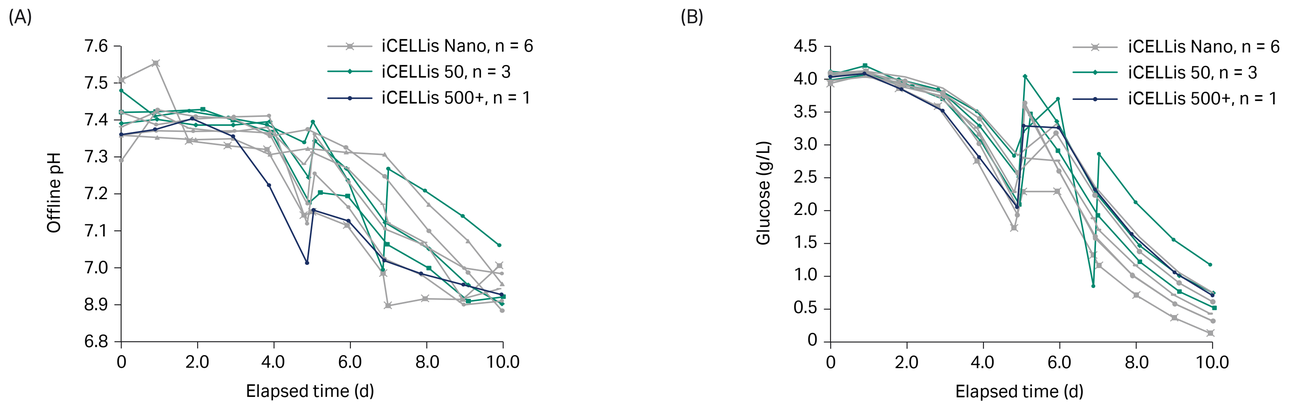

Fig 2. pH (A) and glucose (B) trends for culture growth and production phases in iCELLis Nano, 2.65 m^2 vessel (n = 6), iCELLis 50, 33 m^2 vessel (n = 3), and iCELLis 500+, 333 m^2 vessel (n = 1) bioreactors. Media exchange to serum-free media along with transfection was completed on day 5. Drop in pH and glucose for one iCELLis 50 run around day 7 was due to an overnight media recirculation deviation.

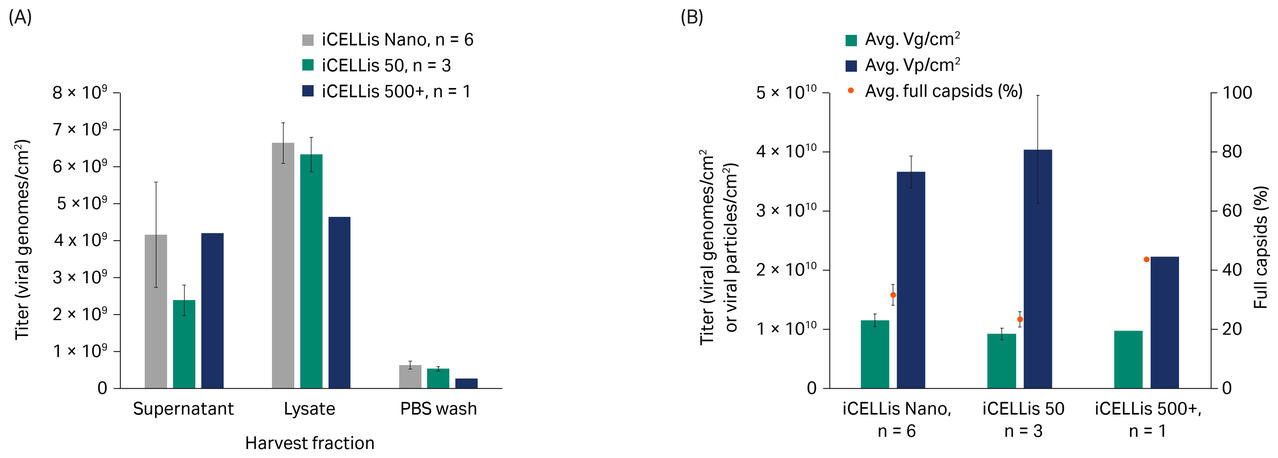

Fig 3. Comparison of (A) rAAV5 titer from harvest fraction samples and (B) rAAV5 titer and packaging yields for final harvest pool samples (supernatant, lysate, and wash fractions) in iCELLis Nano, 2.65 m^2 vessel (n = 6), iCELLis 50 , 33 m^2 vessel (n = 3), and iCELLis 500+, 333 m^2 vessel (n = 1) bioreactors. Titers of recombinant AAV (rAAV) were quantitated using droplet digital PCR (ddPCR). Total capsid levels were measured via ELISA (PROGEN) for all iCELLis 50 runs conducted alongside iCELLis Nano bioreactors, and with the Gyrolab xPlore system (Gyros Protein Technologies) for the iCELLis 500+ run performed in parallel with iCELLis Nano bioreactors. The percentage of full capsids was determined by comparing titer values to total capsid measurements.

Conclusions

- Cell culture runs assessing the scalability across the iCELLis fixed-bed bioreactor family were successfully completed (iCELLis Nano, 2.65 m^2 to iCELLis 50, 33 m^2 to iCELLis 500+, 333 m^2).
- Throughout all scales of the iCELLis bioreactor, the process exhibited strong and reliable performance, maintaining consistent growth, metabolite output, and titer levels.
- Results validate the scalability of the iCELLis platform, positioning the iCELLis 50 bioreactor as a strategic asset for bridging development and commercial manufacturing.

Acknowledgements: Tala Calvi, Chris Ivimey, Mark Duncombe, Kohei Natori, Tim Erlandson, Akshay Gutha Ravichandran.

cytiva.com

CY55906-26Sep25-PO

cytiva