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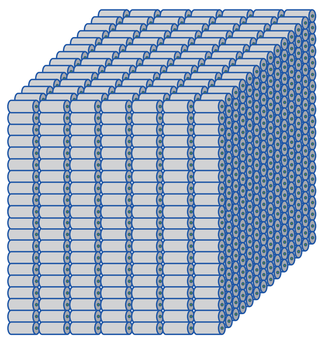
Case Study: Development and Scale-up of a Helper-Dependent Adenovirus (HDAd) Process Using the iCELLis® Bioreactor Platform

Brian Gardell, Scott Gatto & Denis Kole, PhD • *Process Development Services, Pall Corporation, 20 Walkup Drive, Westborough, MA 01581, USA*

INTRODUCTION

Viral vectors are currently the preferred gene-delivery vehicle for most cell and gene therapies, and clinical trials require high titer virus preparations to adequately deliver the therapeutic transgenes to clinical subjects or target cells. For this reason, technologies that enable the industrialization of these processes in a safe, robust, and cost-effective way are necessary to support the demands of the patient population. However, complexity around developing and scaling viral vector processes to commercial manufacturing scale, and the lack of standardized approaches remain as challenges that can impact therapy development timelines and productivity. Selection of the appropriate production platform plays a key role on the successful implementation of a process that meets the commercialization timelines and manufacturing costs. We will share a case study that demonstrates the successful steps taken to develop and scale up a customer process for a HDAd, leveraging Pall's process expertise and the iCELLis bioreactor platform, to help accelerate the development timelines. Critical process parameters for development and manufacturing, such as seeding density, infection density, and harvest strategy, were first tested at the flatware stage, and continually optimized over the course of the iCELLis Nano bioreactor development stage. The optimized parameters were used successfully in duplicate scale-up batches at the iCELLis 500+ bioreactor scale to demonstrate a process ready for tech transfer.

Adherent Process Production Scale Options Yielding Same Surface Area



1960 x Roller Bottles

OR

1 x 333 m² iCELLis 500+ Bioreactor

MATERIALS & METHODS

- Host cell line: HEK293
- Base medium: MEM + FBS + glucose + glutamine
- Virus: HDAd, Helper virus
- Lysis buffer: Buffered surfactant
- Rinse buffer: 10 mM Tris HCl
- Nuclease: Benzonase♦

Flatware Screening to Provide Confidence in Range of Parameters to Test in iCELLis Nano Bioreactor

Tech Transfer and Screening

- Reproduce results at Pall Process Development Services (PDS) labs
- Screen seeding density and define cell growth rate
- Define passage cadence and growth duration
- Define media ratio and cell dissociation volumes

Co-Infection Optimization

- Define acceptable infection density range
- Screen cell lysis reagents – select one for future use
- Establish minimum cell lysis duration

Optimization and Scale-Up Parameters in iCELLis Bioreactors

iCELLis Nano Bioreactor Dev. Round #1

- Seeding density
- Infection time
- Multiplicity of infection (MOI)

iCELLis Nano Bioreactor Dev. Round #2/3

- Infection density
- Media ratios
- Compaction
- Lysis Method

iCELLis 500+ Bioreactor Engineering

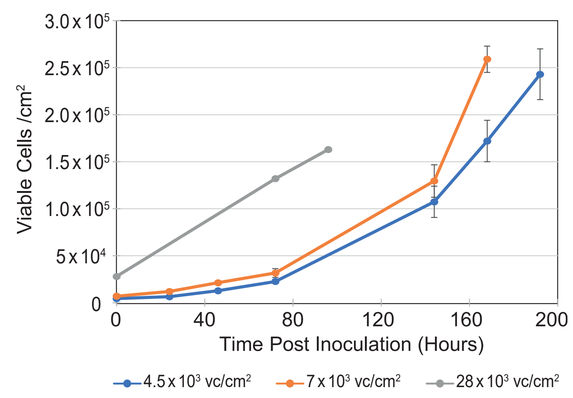
- Obtain comparable results at scale
- Parallel iCELLis Nano bioreactor control

Parameters	iCELLis Nano Bioreactor	iCELLis 500+ Bioreactor
Fixed-bed size (m²)	0.53 – 4.00	333
Growth medium ratio (mL/cm²)	0.23	0.23
Infection medium ratio (mL/cm²)	0.16 – 0.23	0.16
Infection cell density (x10⁵ vc/cm²)	2.1 – 2.7	2.6 – 2.7
Recirculation loop volume (L)	1.2 – 6.4	700
MOI	Constant	
Lysate and rinse buffer volumes	Vessel working volume	

RESULTS

Seeding Density Screening to Reduce Seed Train Burden

Figure 1
Seeding density optimization in flatware

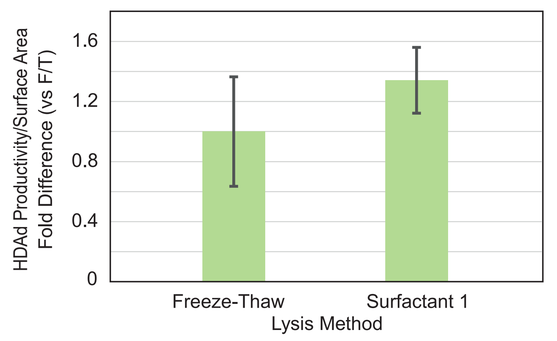


- Lower seeding density and extended growth duration screened in flatware
- Reduced seed density to 4.5 x 10³ cells/cm² to minimize required number of N-1 passage vessels
- Target infection density increased to 2–3 x 10⁵ cells/cm² in the iCELLis Nano Bioreactor

Seeding Density (vc/cm²)	4,500	7,000	28,000
Growth post-inoculation (days)	8	7	4
CellSTACK♦ 10 layers required to inoculate 333 m² iCELLis 500+ Bioreactor	14	22	86

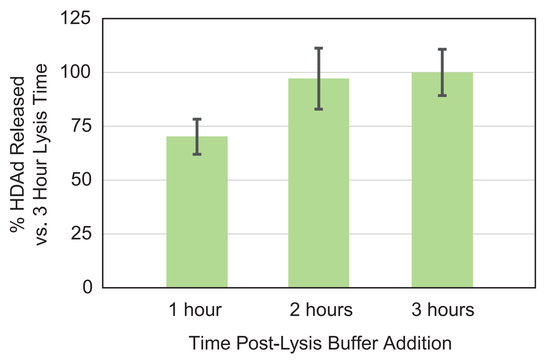
Lysis Process Modified for Manufacturability and Reduced Step Duration

Figure 2
Lysis buffer optimization in flatware



- Selected lysis buffer ameliorated manufacturing process with improved yields to freeze thaw lysis
- Alternative surfactant eliminated due to high viscosity (data not shown)

Figure 3
Lysis duration optimization in iCELLis Nano bioreactor



- Maximal virus recovery achieved after 2 hour lysis time
- Reduced lysis time enables same day harvest clarification process

Virus Recovery Improved with Rinse Implementation

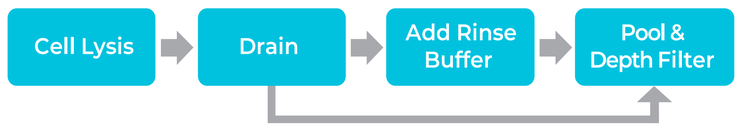
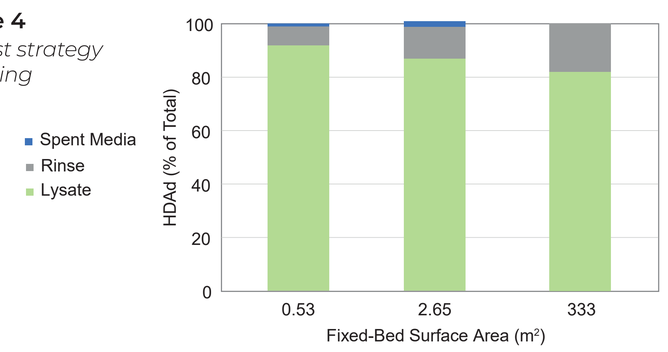


Figure 4
Harvest strategy screening



- Negligible virus in supernatant prior to lysis
- Recirculation media not harvested: 87% reduced harvest volume
- Fixed-bed rinse improves recovery about 20%

Development Parameters Scaled Successfully to iCELLis 500+ Bioreactor

Figure 5
Glucose (——) and lactate (-----) profiles

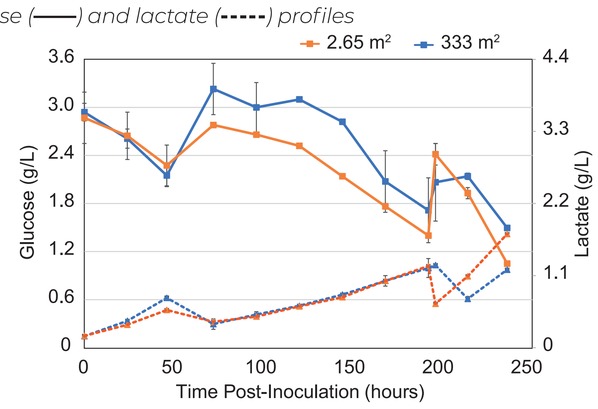
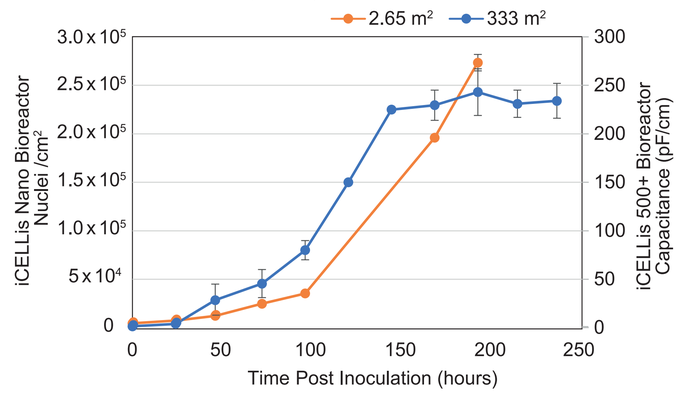


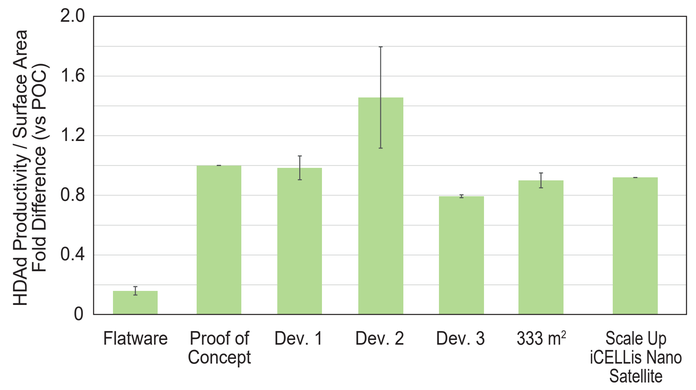
Figure 6
Cell growth profile (iCELLis Nano bioreactor carrier counts vs iCELLis 500+ bioreactor biomass probe)



- Side-by-side iCELLis Nano and 500+ bioreactors using optimized process parameters yielded similar metabolite and cell growth profiles (N=2)

Improved Manufacturability and Process Flexibility Throughout Development

Figure 7
HDAd titer during process development workflow



- Significant increase in productivity compared to flatware production
- Productivity of iCELLis Nano and iCELLis 500+ bioreactors were comparable

CONCLUSIONS

The process was successfully optimized for scalability and manufacturability in the iCELLis Nano bioreactor then scaled to the iCELLis 500+ bioreactor. Screening studies in flatware and several development runs in the iCELLis Nano bioreactor led to a simplified seed train, identification of an efficient lysis buffer resulting in reduced lysis time, and a decrease in the harvest volume to be processed during downstream processing. The process demonstrated improved productivity compared to the flatware process and comparable virus productivity, cell growth and metabolite trends between the bioreactor scales. This data demonstrates that a scalable, manufacturing process for HDAd can be successfully developed using the iCELLis Nano bioreactor then scaled to commercial manufacturing scale using the iCELLis 500+ bioreactor.

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+1 800 717 7255 (USA) • +41 (0)26 350 53 00 (Europe) • +65 6389 6500 (Asia/Pacific) • Email: www.pall.com/contact • Web: www.pall.com/biotech