Poster Content as Presented at ISCT 2020 Virtual, May 2020

Overcoming Adherent Seed Train Biomass Limitations: Pall Xpansion® Bioreactor

Michelle Olson, Odette Becheau, Ly Truong & Todd Sanderson • Pall Biotech, 20 Walkup Drive, Westborough, MA 01581, USA

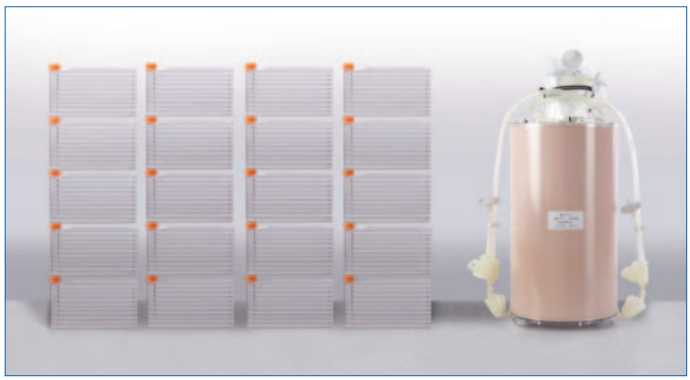
OBJECTIVE

Produce HEK293T high cell density seed train in the Xpansion multiplate bioreactor system for adherent iCELLis® 500+ fixed-bed bioreactor inoculation. Additionally, demonstrate high cell growth in adherent iCELLis Nano fixed-bed bioreactor.

INTRODUCTION

The Xpansion bioreactor is a time and space-saving alternative to traditional flatware seed trains (Figure 1). Previous studies at Pall Corporation have shown high HEK293 cell growth in the Xpansion 200 bioreactor for iCELLis 500+ bioreactor inoculation1,2. Here, we address the issue of high seed train HEK293T biomass demands for large scale adherent manufacturing in iCELLis 500+ bioreactors. HEK293T cells are often used for lentiviral vector production and current manufacturing processes are limited by seed train availability and biomass demands for transient transfection processes.

Figure 1
*Xpansion 200 bioreactor surface area (122,400 cm²) compared to CellSTACK♦ flatware equivalent (20*CS10 = 127,200 cm²).*



MATERIALS AND METHODS

Table 1
Bioreactor operational parameters

	Xpansion Seed Train Bioreactor	iCELLis Nano Production Bioreactor
Bioreactor size	Xpansion 10 and 200 bioreactors	iCELLis Nano 4 m² bioreactors (high compaction fixed-bed)
Seeding density	10,000 cells/cm²	20,000 cells/cm²
Medium composition	DMEM + 10% FBS	DMEM + 5% FBS
pH setpoint	7.25 – 7.48	7.2 ± 0.05
Dissolved oxygen (DO) % setpoint	50%	50%
Duration	4 days	5 days
Agitation rate	2 mm/s	1.2 cm/s
Viable cell count method	Vi-CELL♦	Cell carrier nuclei counts
Control flasks	T75, harvested day 1–4	T75, harvested day 1–4

Figure 2

Xpansion 200 seed train bioreactor for iCELLis Nano production bioreactor inoculation as a proof of principle for iCELLis 500+ bioreactor operation. For this experiment, a previous version of the Xpansion 200 bioreactor control tower was used

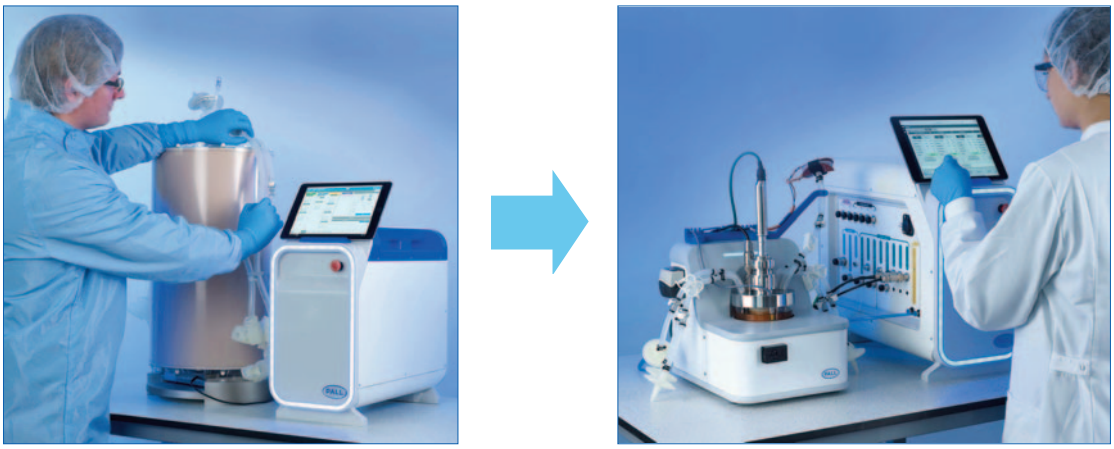
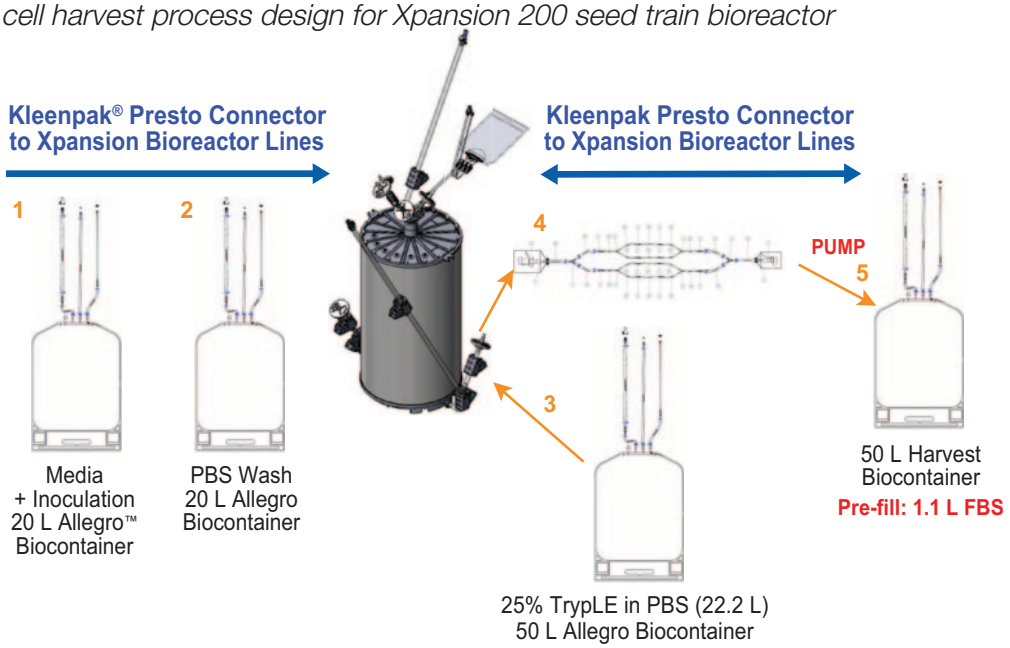


Figure 3

Inoculation and cell harvest process design for Xpansion 200 seed train bioreactor



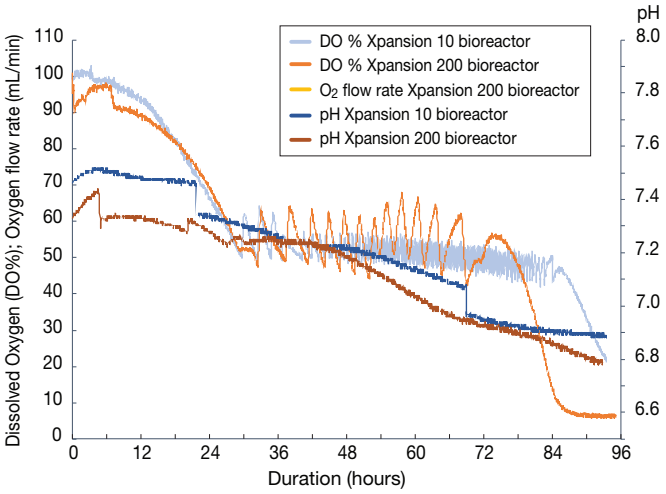
1. Fill Xpansion bioreactor with media + inoculum. After 4 days, pump spent media back into biocontainer
2. Wash Xpansion bioreactor with PBS, pump PBS wash back into biocontainer (pump speed = 2 L/min)
3. Fill Xpansion bioreactor with 25% TrypLE♦ reagent in PBS, wait 30 minutes
4. Harvest cells by pumping through harvest manifold (series of small-size tubing and reducers)
5. Collect harvest cells into harvest biocontainer with FBS supplementation (5% final concentration)

- Harvest manifold reduced cell aggregation while draining bioreactor
- Total harvest duration = 1.5 hours, significant reduction in time compared to multiple CellSTACK♦ harvests
- Cells were concentrated via centrifugation for iCELLis Nano bioreactor inoculation

RESULTS & DISCUSSION

Figure 4

Dissolved oxygen levels, oxygen flow rate, and pH profiles in the Xpansion 10 and Xpansion 200 bioreactor with HEK293T adherent cells.



- pH profile similar between Xpansion 10 and 200 bioreactors, and was also comparable with the T75 control flasks (data not shown)
- Maximum oxygen flow in last 24 hours of Xpansion 200 bioreactor cell culture growth
- Oxygen limitation in Xpansion Bioreactor with Mycontrol Tower seen at very high HEK293T cell density in the Xpansion Bioreactor with Mycontrol Tower, >200,000 cells/cm^2
- Despite oxygen oxygen limitation at very high cell density, cells recover well in flatware and iCELLis Nano 4 m^2 bioreactors (Figure 6)

Figure 5

HEK293T high cell density harvest in Xpansion 10 and 200 bioreactors compared to TF75 control flasks. 4 days growth post-inoculation.

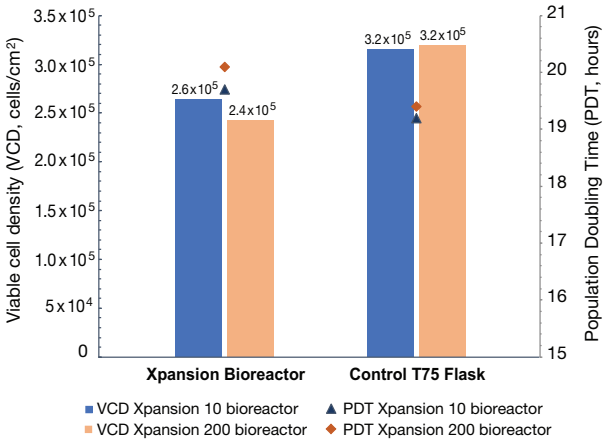
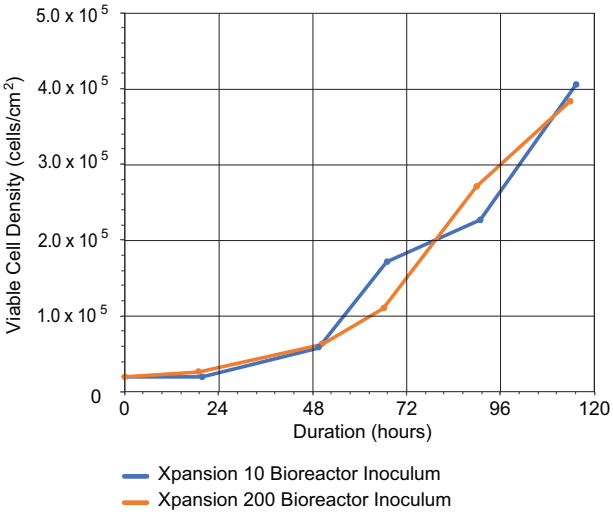


Figure 6

Comparison of HEK293T cell growth profiles in iCELLis Nano 4 m^2 bioreactor from two different size Xpansion bioreactors. Xpansion bioreactors were sed to seed iCELLis Nano 4 m^2 bioreactors at 20,000 cells/cm^2 in DMEM + 5% FBS. Recirculation bottle media exchange (3 L) at day 3



- Previous results showed similar HEK293T cell growth profile between 5% and 10% FBS concentration in the iCELLis Nano 0.8 m^2 bioreactor (n=1)
- Similar growth profile of HEK293T in iCELLis Nano 4 m^2 bioreactor from Xpansion 10 and Xpansion 200 bioreactor inoculum cell sources
- Good HEK293T cell growth after Xpansion bioreactor harvest and iCELLis Nano 4 m^2 bioreactors reached a high cell density of >3.8 x 10^5 cells/cm^2
- After 3 days, reached the target cell density needed for viral transfection

CONCLUSION

- This work demonstrates the feasibility of HEK293T adherent cell culture seed train scale up in the Xpansion multiplate bioreactor systems for large scale bioreactor inoculation
- Successful Xpansion HEK293T seed train bioreactor harvest with minimized cell aggregation by using harvest manifold and FBS supplementation into harvest cell solution
- Use of the new mPath™ control tower may address oxygen limitation seen in the Xpansion bioreactor, as this controller allows for more flexibility in parameter control
- Further work can be done with Xpansion bioreactor parameter optimization for HEK293T cells such as increase DO% set point, agitation, and gas flow rate
- Successful production of HEK293T viable cell harvest and seed train from Xpansion bioreactors for future possible inoculation of adherent iCELLis 500+ bioreactors

References

1. *"An end-to-end process for large-scale adenovirus manufacturing for gene therapy." Pall Biotech, Port Washington, NY, USA. 2018.*
2. *Kaspar, B. et al. "Assessment of an adherent HEK293 cell transfection process for scalable AAV production in the iCELLis fixed-bed bioreactors." ASGCT. Pall Biotech, Westborough, MA, USA. 2019.*

PALL Biotech

Corporate Headquarters
Port Washington, NY, USA
+1 800 717 7255 toll free (USA)
+1 516 484 5400 phone

European Headquarters
Fribourg, Switzerland
+41 (0)26 350 53 00 phone

Asia-Pacific Headquarters
Singapore
+65 6389 6500 phone

Filtration. Separation. Solution.SM

Visit us on the Web at www.pall.com/biotech
Contact us at www.pall.com/contact

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

6/20, PDF GN20.10340