PALL Biotech

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Scaling-Up and Industrializing the Production of Viral Vectors and Cells for Therapeutic Use

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INTRODUCTION

Cell and gene therapies have the potential to revolutionize medicine. Bioprocessing for these therapies still faces many challenges during scale-up. The utilization of scale-down unit operations from bioreactors to downstream purification is very useful in defining the design space and preventing costly scale-up errors. Two case studies focusing on viral vectors will be presented to demonstrate the challenges and solutions associated with process development of such therapeutics.

Case Study 1: Lentivirus Production-Proof of Concept (POC)

This approach for gene therapy works by genetically modifying patients own cells to shield and strengthen their immune system, using a stable lentivirus producing technology that can enable large scale, low cost production of lentiviral vectors. In this case study of proof of concept experiment, Pall successfully transferred the client's current flatware process into the iCELLis® Nano bioreactor 0.53 m^2, resulting in a 1.62 x fold increase in the average titer yield (TU/cm^2). Total lentivirus yield was reproducible in two consecutive iCELLis bioreactor runs; by increasing surface area by a magnitude of 70, Pall increased the total yield by a magnitude of 90 resulting in total lentivirus of 3.9 x 10^9 TU (Transducing Units). Harvest strategy was also incorporated into the proof of concept iCELLis Nano bioreactor runs to recover the viral vector yield from the carriers.

Case Study 2: Adenovirus Production and Purification

Orgenesis'◆ objective is to commercialize an autologous insulin producing cell line for type I diabetes therapy. The Xpansion® 200 multiplate bioreactor was used for the growth of the primary human liver cells under controlled culture conditions. This process generated the cell mass required for curing a diabetic patient. Results show that 1 - 2 gr (10 - 15 million cells) of a patient's liver biopsy was expanded to approximately 2 billion cells in the Xpansion 200 bioreactor. The 1-2 billion cells are then trans-differentiating in the bioreactor into autologous insulin producing (AIP) cells with adenovirus vectors and then infused them back into the patient for long term amelioration of insulin dependency.

For large scale viral production we used the packed-bed iCELLis 500 disposable bioreactor that provides 3D matrix in a controlled environment for HEK293 cells. In this case study, we have developed optimized manufacturing processes for three adenovirus vectors using the predictive small scale iCELLis Nano bioreactor, and scaled it up to the manufacture scale iCELLis 500 bioreactor. High Yield of 1.04 x 10^{16} total infectious crude virus particles (IFU) was produced in the iCELLis 500 bioreactor (66 m^2) by optimizing various key process parameters. This represents more than the targeted dose requirement of 1 billion cells per patient.

HEK293 cell cultivation, infection and harvest of the virus in an adherent environment proved possible reaching a total virus yield of 1.04 x 10^{16} IFU/batch (iCELLis 500 - 66 m^2 scale bioreactor).

From Bench to Clinical

- Autologous insulin producing cells required.
  - 1.8 billion cells per patient, 1.8 x 10^{10} cells for clinical site Phase I.
- Quantities of purified adenovirus required to transfect 1.8 billion cells.

Adenovirus	Dose Per Patient (IFU virus)	Pre-Clinical Dose (IFU virus, 10 patients)	Crude Virus for Pre-Clinical Site (IFU of viruses for 10 patients)
PDX-1	1.8 x 10^{12}	1.8 x 10^{13}	7.2 x 10^{13}
Neuro-D	4.5 x 10^{11}	4.5 x 10^{12}	1.8 x 10^{13}
Maf-A	9.0 x 10^{10}	9.0 x 10^{11}	3.6 x 10^{12}

MATERIALS AND METHODS

Materials

Case Study 1

- Licensed lentivirus producer cell line technology HEK293 cells.
- Growth media: DMEM (Thermo Fisher Scientific◆ part number 10566) supplemented with 10% FBS (Thermo Fisher Scientific part number 10082147).
- Other reagents: DPBS (Thermo Fisher Scientific part number 10010), TrypLE Select◆ (Thermo Fisher Scientific part number 12563), Zenon Human IgG Labeling Kit-PE (Life Technologies◆ part number Z-25455), Doxycycline (Clonetech part number 631311), Puromycin dihydrochloride (Sigma-Aldrich◆ part number P9620), Zeocin (Invitrogen◆ part number R25001), 2F5 antibody (Polymun Scientific part number 1475).
- Cell culture support: T-175 flask (Corning◆ part number 3290), CellSTACK◆ 2 (Corning part number 3310), CellSTACK 5 (Corning part number 3311), iCELLis Nano 0.53 m^2 bioreactor (Pall part number 810039NS), iCELLis Nano, full starter kit (Pall part number 6415-0537S).

Case Study 2

- Biological materials: Human liver cells and hPDX-1, hNeuroD & hMafA adenoviruses (provided by Orgenesis), HEK293 cells (ATCC).
- Reference adenoviruses amplified in flatware and purified by cesium chloride method (provided by O.D.260 Inc).

- Growth media: DMEM (Thermo Fisher Scientific part number 11965) supplemented with 10% FBS (Thermo Fisher Scientific part number 26140).
- Other reagents: DPBS (Thermo Fisher Scientific part number 10010) and TrypLE Select (Thermo Fisher Scientific part number 12563).
- Cell culture support: CellSTACK 10 (Corning part number 3320), Xpansion 50 plates bioreactor (Pall part number 810122), Xpansion 200 plates bioreactor (Pall part number 810155), iCELLis Nano 0.53 m^2 bioreactor (Pall part number 810039NS), iCELLis Nano 1.07 m^2 bioreactor (Pall part number 810061NS), iCELLis Nano 4 m^2 bioreactor (Pall part number 810042NS), iCELLis Nano 2.65 m^2 bioreactor (Pall part number 810206NS), iCELLis 500-66 m^2 bioreactor (Pall part number 4415-i500V66), iCELLis 500, full starter kit, with Kleenpak® Presto sterile connectors (Pall part number 6415-i500MFHT), iCELLis Nano, full starter kit (Pall part number 6415-i537S).

Methods

Case Study 1

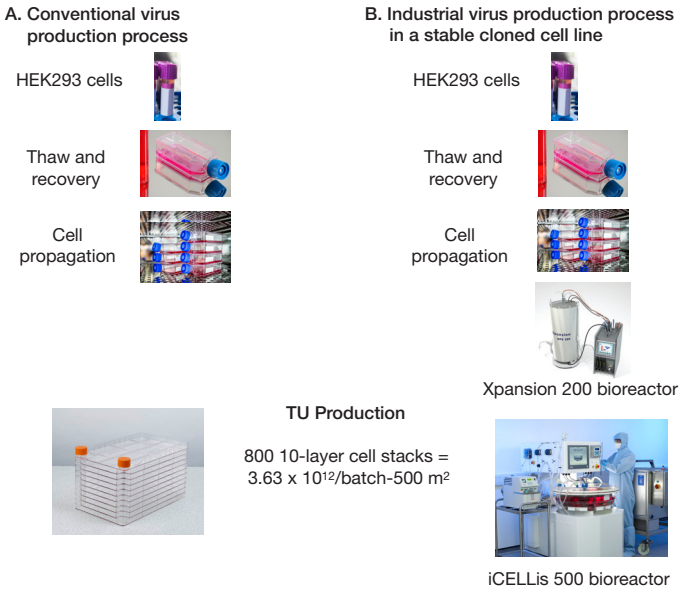
- HEK293 host cells seed train and production: process flow chart.
- The iCELLis Nano bioreactor process development and scalability strategy were performed as described.
- Bioreactor controller set points: High pH-7.2 and DO 50% air saturation.
- Target seeding density: 80000 cells/cm^2.
- HEK293 culture duration for both growth and Lentivirus production: 6 days.
- Analytics: Metabolites using Nova analyzer, pH meter for off-line analysis, and FACS analysis for titer measurement.

Case Study 2

- HEK293 host cells seed train: process flow chart (Figure 4).
- HEK293 cells were used in the iCELLis Nano and iCELLis 500-66 bioreactors at passage # 7&8.
- The iCELLis Nano process development and scalability strategy were performed as described in Figure 5.
- Flatware cultures for adenovirus production were performed in parallel to the iCELLis bioreactors as a production control.
- Bioreactor controller set points: High pH-7.2 and DO 50% air saturation.
- Target seeding density: 7000 cells/cm^2.
- HEK293 culture duration for both growth and adenovirus production: 8 days.
- Analytics: Metabolites using Nova analyzer, pH meter for off-line analysis, Adeno X- infectivity titer (CloneTech), Death curve analysis (performed by Orgenesis on primary culture of liver derived cells) and Trans-differentiation efficiency by Real Time PCR analysis.

Figure 1

Large scale lentivirus production: from current process to industrial process



RESULTS

Figure 2

Translate conventional process into iCELLis Nano bioreactor

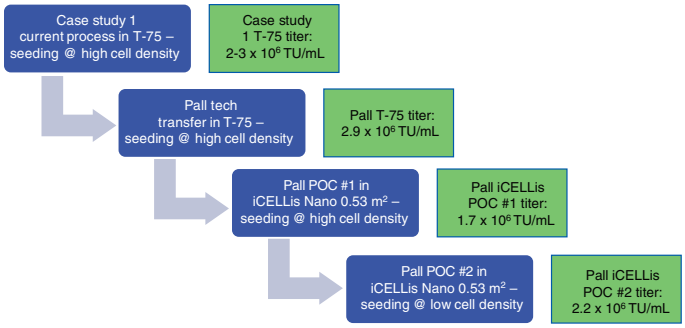
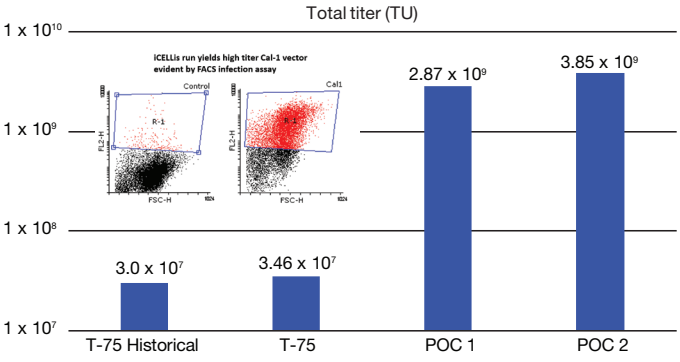


Figure 3

Lentivirus production in stable HEK293 reproducibility between runs

Total Titer (TU)

T-75 Historical (Case Study 1)	T-75 – Pall	POC #1 (iCELLis Nano – 0.53 m² Run 1)	POC #2 (iCELLis Nano – 0.53 m² Run 2)
3.00 x 10^7	3.46 x 10^7	2.87 x 10^9	3.85 x 10^9



The coefficient of variation (CV) for T-75 = 10.1%

The coefficient of variation (CV) for iCELLis Nano = 20.62%

Case Study – Production and purification of infectious Adenovirus

Figure 4

Upstream and downstream for the entire workflow

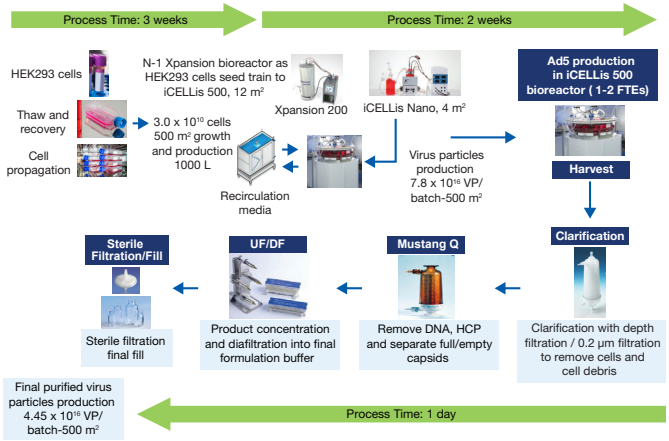
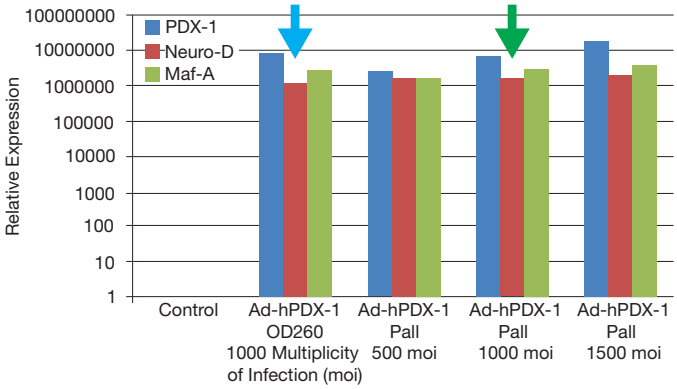


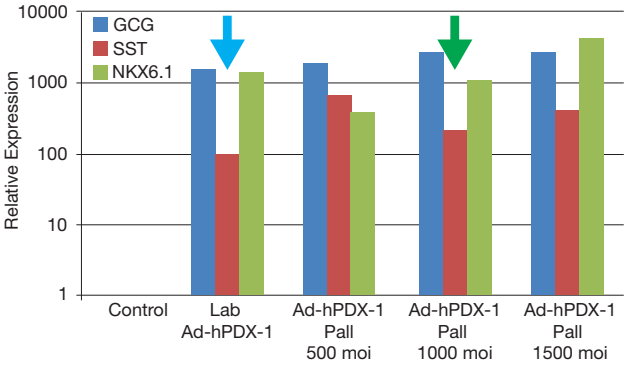
Figure 5

Trans-differentiated efficiency of Ad5-PDX1, Ad5-NeuroD & Ad5-MafA produced and purified by the manufacture scale was analyzed in comparison to the current process reference (produced by optical density (O.D). 260 Inc.)

A. Ectopic expression of three adenoviruses



B. Pancreatic genes expression



The ectopic expression Ad-hPDX1, Ad-hNeuroD & Ad-hMafA and the pancreatic genes amplified and purified by the industrial process (green arrow) were similar and to the control adenovirus produced by the current process (blue arrow). Operational range for Multiplicity of Infection (MOI) for PDX-1 is between 500-1500, Neuro-D is 250, and MafA is 50. No cell death was observed for any of the concentrations of the three adenoviruses amplified and purified by Pall.

CONCLUSIONS

Case Study 1

- The tech transfer into flatware was successfully completed.
- Pall successfully transferred the current flatware process into the iCELLis Nano bioreactor 0.53 m^2, resulting in a 1.62 fold increase in the average titer yield (TU/cm^2).
  - The increase in average titer yield was the result of seeding the bioreactor at a low cell density, allowing cells to grow in the bioreactor, and performing induction at lower cell density enhanced the specific productivity from 2 - 3 TU/cell to 5 TU/cell.
- Harvest strategy was also incorporated into the both POCs to recover the viral vector yield from the carriers.

Case Study 2

- Xpansion bioreactor was successfully used to generate 1.8 billion human liver cells required for one patient.
- Optimization of culture parameters and harvesting method demonstrate good agreement between small scale iCELLis Nano bioreactor and large scale iCELLis 500 bioreactor.
- iCELLis bioreactor successfully generated functional adenoviruses.
- AIP cells were cultured with the Xpansion bioreactor, transduced with viruses produced in 3 iCELLis bioreactors and successfully implanted into SCID mice at both facilities in the USA and Israel.
- Purified adenovirus was used in a TOX study and has demonstrated comparable results to the virus produced by the conventional non-industrial process.
- The iCELLis single-use fixed-bed bioreactors generate fully functional adenovirus that are very comparable to the adenovirus used for the traditional dish process.
- We have demonstrated how to facilitate the AIP cell therapy approach for treating diabetes using the Xpansion bioreactor, the iCELLis bioreactor for virus production, and AIP cell technology.
- Pre-IND submission to Kinexum team for audit and organizing the data for the FDA meeting.

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