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Industrialization of a Cell-based Autologous Therapy Targeting Diabetes: Industrialization of a Liver Cell Proliferation Process from Petri Dish to the Xpansion® Multiplate Bioreactor

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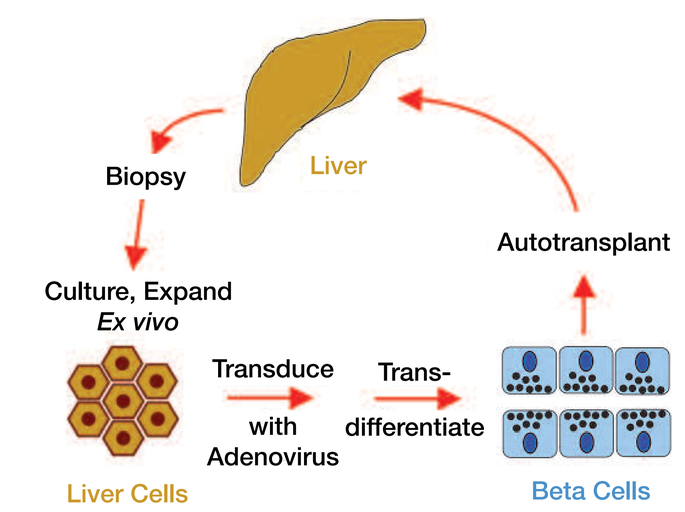
INTRODUCTION

Orgenesis is a development-stage company with a novel therapeutic approach in the treatment of diabetes by correcting malfunctioning pancreatic insulin producing cells with new functional tissues generated from the patient's own existing organs. Orgenesis employs a molecular and cellular approach directed at converting human liver derived cells into functional insulin-producing cells by transcription factors induced transdifferentiation (Figure 1). This new therapeutic approach generates Autologous Insulin Producing (AIP) cells, overcoming the shortage in tissue availability from donors.

Figure 1
Liver cell-based autologous cell therapy schema, adapted from Castellano et al

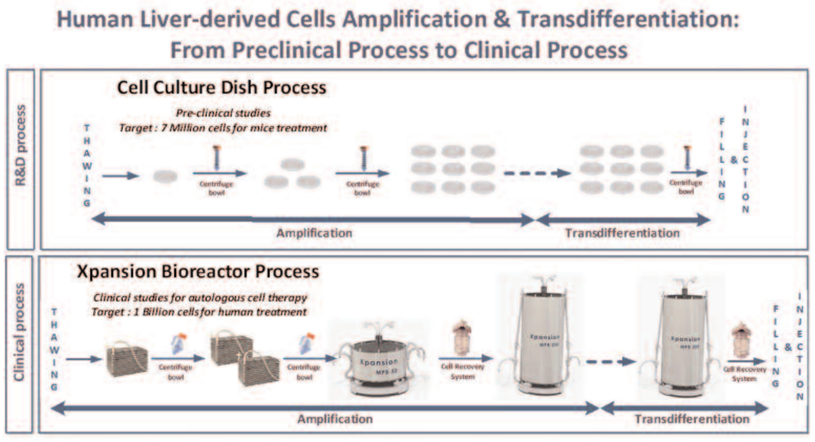


Pall Life Sciences provides process intensification technologies and services for the biotech industry. This includes single-use high-cell-density bioreactor solutions and cell culture process development services. Pall has designed and developed the Xpansion bioreactor platform: a multi-plate bioreactor offering up to 122,000 cm² of growth surface - specifically developed for shear sensitive adherent cells such as stem cells.

PURPOSE

Orgenesis developed a bioprocess in cell culture dishes for preclinical applications that includes 2 main steps: liver cell proliferation followed by liver cell transdifferentiation into insulin producing cells. For treatment of patients in human clinical trials, Orgenesis anticipates a dose requirement of 1 billion cells per patient to ameliorate hyperglycemia in Type 1 diabetes. Such a production scale would require large culture surface area, this manufacturing strategy does not provide an efficient solution for treatment of the predicted patient population. In this context, Orgenesis and Pall combined their respective expertise to industrialize this cell based therapy using the Xpansion platform.

Figure 2
Human liver-derived cell amplification & transdifferentiation process industrialization



The choice of the Xpansion system as the production platform for clinical and early commercial phase was driven by several considerations: cost efficiency (by minimization of operation time), contamination risk reduction (by using a closed system), minimization of footprint and capital investment, and ease and speed of process scale-up.

This application note details the successful scale-up of the human liver-derived cell amplification phase from Petri dishes to the Xpansion 200 bioreactor. The industrialization of the transdifferentiation process will be presented in further application notes.

MATERIALS AND METHODS

Materials

- Biological materials: Human liver-derived cells (provided by Orgenesis).
- Growth medium: Dulbecco's Modified Eagle Medium (DMEM; Life Technologies Cat. 21885-025) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Life Technologies Cat. 10500-064), 1% Penicillin-Streptomycin-Amphotericin B (100X) (Lonza Cat. 17-745E) and 5 nM Exendin-4 (Sigma-Aldrich Cat. E7144)
- Other reagents: Dulbecco's Phosphate Buffered Sales (DPBS; Lonza Cat. 17-512Q) and TrypLE◆ Select (Life Technologies Cat. 12563-029).
- Cell culture support: CellBIND◆ CellSTACK◆ 2-, 5- & 10- chamber (Corning Cat. COS-3310, COS-3311 & COS-3320), Xpansion 50 plates (XP-50) bioreactor (Cat. XPAN050000000) and Xpansion 200 plates (XP-200) bioreactor (Cat. 810155).

Methods

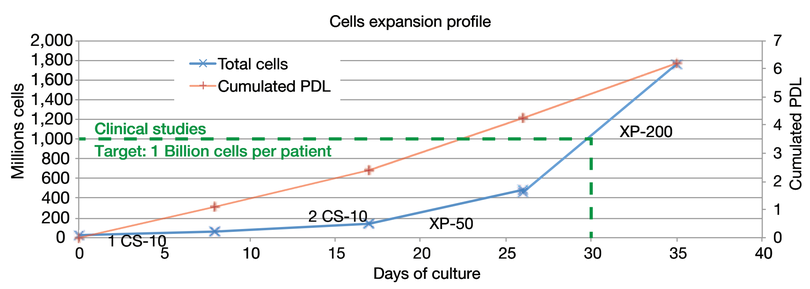
- Seed train: Process Flow chart (Figure 2)
- Cells were used in the Xpansion bioreactor(s) at Passage 14 & 15
- Multitray cultures were performed in parallel to the Xpansion culture as a cell growth control
- Controller set points: p: 7.3-7.6, DO: maintained above 50%
- Target seeding density: 4,000 cells/cm² at each passage
- Culture duration: 7-9 days
- Medium exchange applied every 2-4 days (XP-50: Days 4, 6 and 8 – XP-200: Days 4 and 7)

RESULTS

Cell Growth

The cell expansion profile (Figure 3) clearly demonstrates that cells are in exponential phase of growth from the first pre-cultures steps to the final XP-200 culture. Within 4 passages, cells were amplified from 25 million to ~1.8 Billion, representing a 72-fold biomass increase. Therefore, feasibility of large-scale production of human liver-derived cells has been clearly demonstrated, and Orgenesis' target of 1 billion cells/ patient, per XP-200 was achieved.

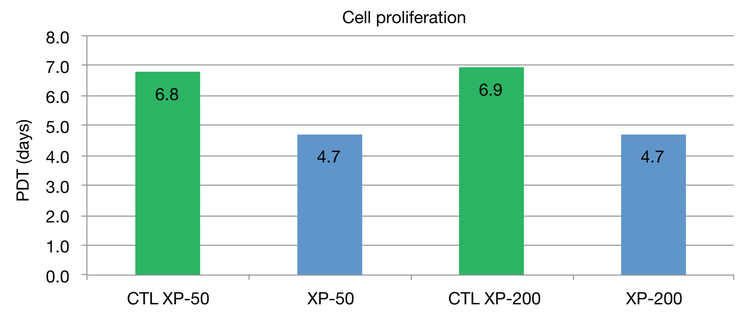
Figure 3
Seed train and cell expansion profile of human liver-derived cells from multitray 10 plates to XP-200 bioreactor.



Dotted lines in green represent Orgenesis' target in term of cell numbers required per patient.

Population doubling time (PDT) comparison revealed that the human liver-derived cells proliferated faster in the Xpansion bioreactor than in the traditional multitray system (Figure 4). Harvested cell densities were around 15,000 cells/cm² in the 50-plates bioreactor, and 14,000 cells/cm² in the 200-plates bioreactor. These densities represent ~160% of their respective multitray controls. Better control of the culture environment (pH, DO) is the main hypothesis to explain this result.

Figure 4
Population doubling time in XP-50 (blue), XP-200 (blue) and their control in classic multitray support (green). Data based on harvested cell densities.



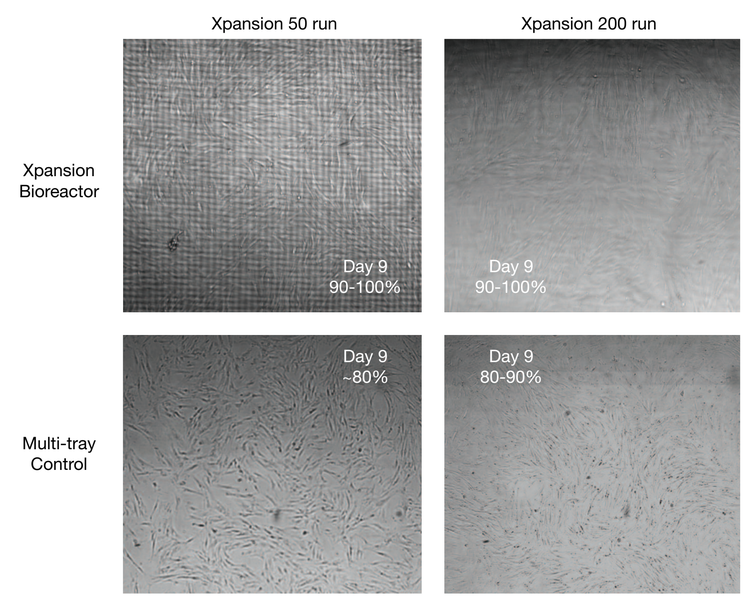
RESULTS *(continued)*

Microscopic Observation using the Ovizio Holographic Microscope

Cell confluence and morphology are key parameters to monitor in cell therapy processes. To this end, Ovizio Imaging Systems and Pall co-developed a microscope that allow observation of the top ten plates in the Xpansion bioreactor.

Images in Figure 5 confirm the homogeneous distribution of human liver-derived cells throughout the Xpansion plates. Cell confluence was determined to be approximately 90% after 9 days of culture, and estimated to be equivalent in both the XP-50 and XP-200 bioreactors. At both the 50- and 200- plate scale, confluence observed in the Xpansion system was slightly higher than confluence observed in the control multitray system. These images also demonstrate that the cell morphology was not affected by successive culture in the Xpansion system. Results from early QC assay demonstrate that neither the cell ability to undergo in transdifferentiation, nor the insulin secretion profiles after transdifferentiation are altered by cell proliferation in Xpansion bioreactor.

Figure 5
Microscopic observations in the Xpansion bioreactor and control multitray system before harvest (Day 9) during both the Xpansion 50 bioreactor run (left) and the Xpansion 200 bioreactor run (right)



CONCLUSION AND PERSPECTIVES

Xpansion bioreactors were successfully used to scale-up the human adult liver-derived cells proliferation process. By using the Xpansion platform, Orgenesis now has a reliable process to amplify their cells from 1 million up to 1.8 billion cells/ patient (vs. 7 million in their previous process that used Petri dishes) that preserves cell viability and potential for transdifferentiation. As a result of this successful co-development partnership, Orgenesis is moving forward with their process to large-scale clinical studies for GMP-compliant commercial manufacturing of AIP cell for transplantation.

Pall and Orgenesis are now collaborating on scaling up the transdifferentiation process, including the generation of suitable quantities of viral vectors to transfect the required 1 billion cells per patient. This achievement will enable Orgenesis to move forward in their clinical trials and intention to commercialize their Autologous Insulin Producing (AIP) cell transplantation.

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