Transfer of Hepatic Progenitor Stem Cell Culture Process from Multitray Stacks to the Integrity® Xpansion™ Multiplate Bioreactor

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Introduction

Commercial success for cell-based products is directly linked to the ability of sponsor companies to ensure cell supply at a reasonable cost.

Promethera Biosciences (Mont-St-Guibert, BE) is developing cell therapies to treat serious metabolic liver disorders, such as Crigler-Najjar syndrome. Human heterologous adult liver progenitor cells (HHALPCs) were initially cultivated in standard 2-D cultivation devices. A successful transition from laboratory scale, which is suitable for supplying early clinical trials to an efficient and robust manufacturing (GMP) process, is key and represents a major challenge. Maintaining the cell culture environment is critical for preserving the consistency of cells during process scale-up. The present study investigated the feasibility for cultivating HHALPCs in Integrity[®] Xpansion[™] bioreactors, while meeting the following criteria:

- The process must be closed
- The growth rate and population-doubling level (i.e., the number of times the total cell population has doubled) must be equivalent to the current process in multilayer trays
- The process must comply to the cGMP rules
- The cells must succeed the quality control tests in terms of identity, purity and therapeutic potency

Materials and Methods

Integrity[®] Xpansion[™] Multiplate Bioreactors (ATMI LifeSciences) have been specifically designed to enable an easy transfer from existing multitray stack processes by offering the same cell growth environment (2-D hydrophylized polystyrene plates) in a compact, closed system. Critical cell culture parameters as pH and dissolved oxygen are controlled in Xpansion, and cell density could of parallel cultures in Xpansion be automatically monitored via specific holographic microscopy.

Xpansion

Bioreactor

Xpansion 50

Xpansion 180

pH Set Points

Number of Plates

50

180

Control: Corning® CellBIND® Surface, CellSTACK® cell culture chambers, 10 plates

Stem cell expansion and harvesting:

- Inoculation: 5.000 cells/cm²
- Harvest: 20,000-30,000 cells/cm²
- 10% serum-containing medium

QC testing:

ICC/FACS/Cyp activity are

performed to determine and confirm identity & purity of cells. Urea secretion and bilirubin conjugation are potency tests.

At the level of morphology, cell shape is evaluated (undifferentiated cells display an elongated, mesenchymal cell shape, differentiated cells display a polygonal, cuboidal cell shape).

Results

Cultivation of stem cells in Xpansion 10

Xpansion 10 was used to optimize HHALPCs cell culture parameters. Cell densities were superior to 20,000 cells/cm² and similar to control (*Chart 1*). This was considered an optimal cell density at harvest as cell-to-cell contact at higher confluence may induce differentiation¹. Cell viability was over 97% and the cultures passed the complete set of QC tests (Chart 1).

Scale-up from the Xpansion 10 to the Xpansion 180

As in Xpansion 10, cells reached confluence and cell densities were superior to 20,000 cells/ cm²-similar to control in the Xpansion 50 and Xpansion 180 systems (Chart 1). The Xpansion 180 system generated a final yield of 2.4x10⁹ cells. Cell viability was over 98% and the cultures passed the complete set of QC tests (Chart 1).

Bioreactor regulation and culture reproducibility

Figure 1 (right) is showing the pH and DO regulation profiles 10 and Xpansion 180. Gas diffusion through silicon tubing is efficient and maintained pH and D.O. around set point values. After a certain level of cell confluence due to the cell growth at the end of culture, pH falls progressively to reach val-

Cell Culture Surface (cm²)

Trends of both bioreactors are highly similar, demonstrating ME are media exchanges. reproducibility of the culture at different scales.



Chart 1: Triplicate cultivation of stem cells in XP-10, 50 and 180. Densities and viabilities at harvest compare to the control and the results of QC tests.

D.O.-XP-10 pH-XP-10 Texts: VP.10

Integrity Xpansion bioreactors are suitable for the growth of mesenchymal progenitor cells. The production process for HHALPCs in multitray stacks have been successfully transferred into large-scale Xpansion bioreactor systems in only a few months. Cell densities, viability, identity, purity and cell therapeutic potency were preserved. A final yield of 2.4x10° cells per Xpansion 180 plates has been achieved. Furthermore, several tools are available to record evolution of culture:

- A sampling port that can be used for dosing of nutrients, growth factors, etc.
- On-line pH and D.O. tracking

Homogenous cells distribution

Figure 2 (right) illustrates how

Xpansion bioreactors are homog-

enously colonized (both between

plates and between different

zones of the same plate).

over plates

• Off-line microscopic observations

Xpansion 10 proved to be a useful tool for determining optimal cell culture parameters. Actually, several runs could be performed using this scaled-down system, while sparing time and money and still extrapolating the cell behavior, the pH and DO trends in the Xpansion 50 and Xpansion 180.

Bibliography

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Figure 2: Macroscopic (A) and microscopic (B) pictures of an Xpansion-180, stained with crystal violet. Microscopic pictures were detailed of the plate 105. (C) is showing the 5 zones where the microscopic pictures were taken.

plates of each bioreactor. The microscope software enables an automatic cell counting of the cell confluency². Some representative pictures at different times in Xpansion 50 are presented in Figure 3 (below). The pictures help the operator to correctly evaluate cell confluence. The confluence of picture (D) is visually assessed at roughly 85%. The picture is representative of the cell laver at harvest.



Conclusion

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Figure 1: Regulation parameters in XP-10 (A) or XP-180 (B) in the course of time. pH (green), D.O. (Blue) and T° (red) evolution. Set points (dashed lines) were fixed at 7,5 for pH and D.O.> 50%. T° peaks are due to Xpansion disconnection for microscopic observation or samplings.

ues between 7.0-7.3 (day 7-9).