Poster Content as Presented at BPI and ISCT 2019

Pall Xpansion® Bioreactor Supports Progenitor Cell Growth To >1 Million Cells/cm² and Proper Cell Differentiation

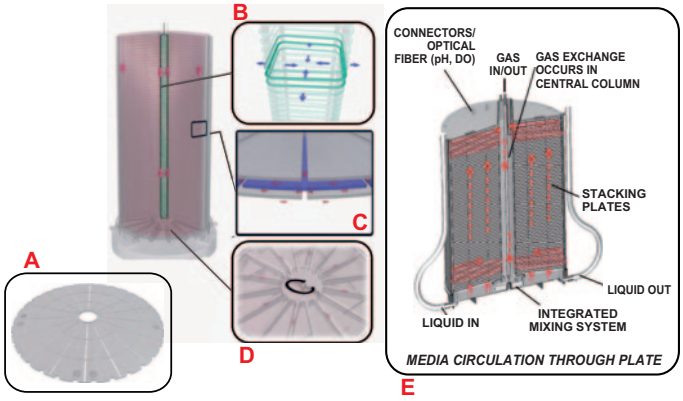
Andrew Laskowski and Siddharth Gupta • Pall Biotech, 20 Walkup Drive, Westborough, MA 01581, USA

INTRODUCTION

- **Problem Statement:** Several cell therapies approved by the FDA or currently in clinical development rely on the expansion of adherent primary cells. The current platforms used to expand these cells are labor-intensive, consist of many open handling steps, have a large manufacturing footprint, and do not provide adequate process control.
- **Gap Addressed by Pall:** The need for a GMP-compliant manufacturing platform for adherent human cells that can be implemented in research labs, allowing for control of the cellular environment, and can be easily scaled up for Phase II, Phase III, and commercial manufacturing
- **Proposed Solution:** Pall's Xpansion single-use bioreactor offers a scalable manufacturing platform with online monitoring and control of critical quality attributes (CQAs) for cell therapy applications
- **Experimental Goals**
  - Successfully demonstrate Xpansion bioreactor plate coating with a proprietary protein solution that enhances cell attachment
  - Expand and differentiate a previously untested proprietary progenitor cell in the 10-plate Xpansion bioreactor (Xpansion 10) using a 16-day and 22-day differentiation protocol. Compare results to T-150 controls
  - Optimize the procedure to harvest and recover cells from the Xpansion bioreactor
  - Develop a post-harvest cell concentration and washing process to handle the larger cell suspension volumes from the Xpansion 10 bioreactor
- **Success Criteria**
  - 2-4x amplification of cells and equivalent harvest cell densities to the control
  - >95% viability post cell washing
  - >90% differentiation to a more mature cell fate
  - Microscopic evidence of cellular self-assembly into characteristic monolayer with cell colony formation

XPANSION BIOREACTOR BACKGROUND

Schematic Diagram of Xpansion Bioreactor Components

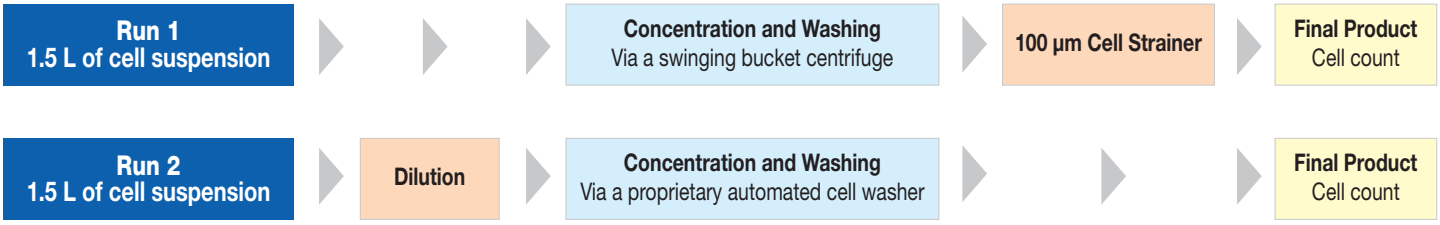


- Single-use multi-plate bioreactor available in 4 sizes (10, 50, 100, and 200 plates; 612 cm² per plate)
- Cell attachment surface is a circular, cell culture-treated polystyrene plate (A)
- Stacking of pates creates a center column containing a coil of gas tubing for CO_2 and O_2 exchange (B)
- Plates have radial slits and are stacked such that these slits are staggered, forcing media flow uniformly over the plate surface (C)
- Mixing is achieved via an impeller located under the bottom plate (D)
- Constant, low shear stress maintained by keeping plate distance, geometry, and media mixing velocity constant
- Head plate contains single-use pH/DO PreSens♦ sensors and sampling port
- Two large liquid addition and removal lines at bottom of reactor (E)

METHODS

	Xpansion Bioreactor Run 1	Xpansion Bioreactor Run 2	T-150 Flask Control
Plate coating (Proprietary protein solution)	Overnight (0.16 mL/cm^2, 2x conc.); Triple rinse with PBS		Overnight (0.33 mL/cm^2, 1x conc.) Triple rinse with PBS
Cell seeding density (proprietary adherent human progenitor cell)	0.75 x 10^6 cells/cm^2	1.2 x 10^6 cells/cm^2	Run 1: 0.75 x 10^6 cells/cm^2 Run 2: 1.2 x 10^6 cells/cm^2
Cell culture media volume (Proprietary media + differentiation-inducing small molecule cocktail)	1.6 L (0.26 mL/cm^2)	1.6 L (0.26 mL/cm^2)	Both runs: 39 mL (0.26 mL/cm^2) & 90 mL (0.6 mL/cm^2)
Media exchange frequency (variable small molecule cocktail)	Every 2 – 3 days Modified to every day	Every 2 – 3 days	Every 2 – 3 days
Cell culture duration	22 days	16 days	Run 1: 22 days; Run 2: 16 days
pH control	Set point = 7.3; Controlled by CO_2 addition		No control possible
Dissolved oxygen (DO) control	Set point = 50% air saturation Controlled by O_2 addition and increased stirring speed		No control possible
Harvest	1. Remove spent media 2. 55 minute incubation with TrypLE at 37 °C (no mechanical agitation) 3. 10 minute incubation with TrypLE at 37 °C	1. Remove spent media 2. Rinse with PBS 3. 55 minute incubation with TrypLE + DNAse at 37 °C 4. Mechanical agitation (~60 “strikes” with Xpansion harvest station) 5. Rinse with fresh basal media	1. Decant media 2. 35 minute incubation with TrypLE at 37 °C 3. Quench with basal media

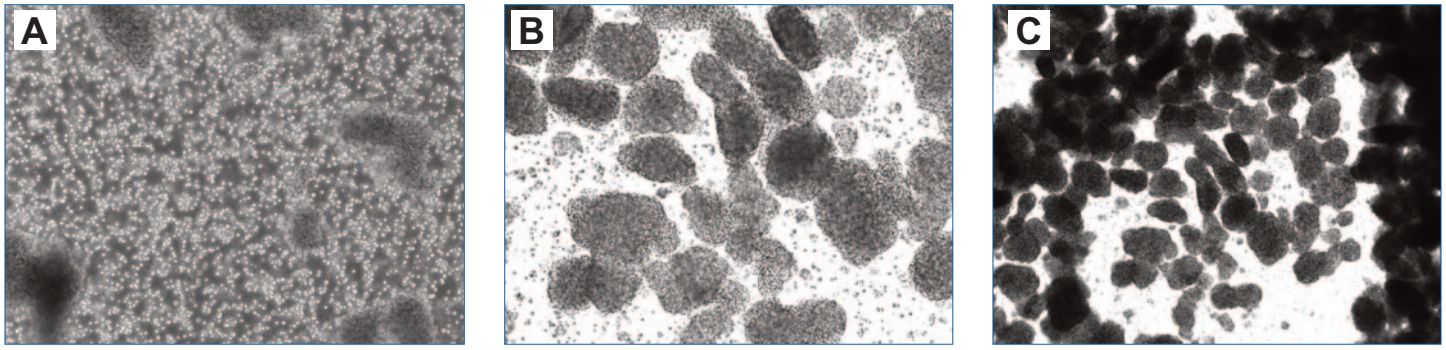
Cell Concentration, Wash, and Fill



RESULTS

1. Harvest Optimization Studies

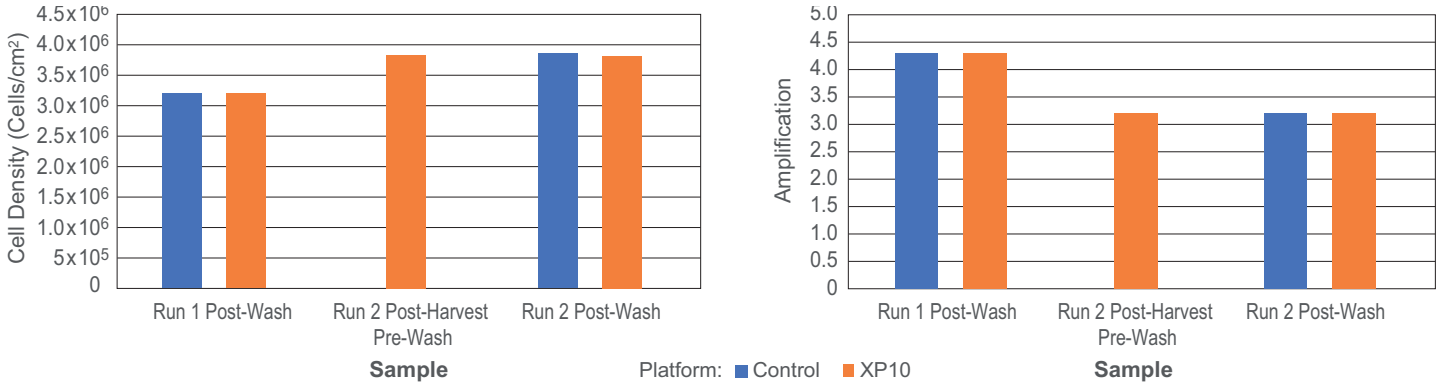
	TrypLE Concentration (%)	Incubation Time (min)	TrypLE Prewarm Temp. (° C)	Incubation Temperature (° C)	Cells in Aggregates (%)	Live Cell Recovery (million)
Test A1	100	55	37	21	15	141
Test A2	100	55	37	37	22	270
Test A3	100	55	40	21	14	90
Test A4	100	55	40	37	2	135
Test A5	100	80	37	21	57	121
Test A6	100	80	37	37	11	252
Test A7	100	80	40	21	47	99
Test A8	100	80	40	37	21	252
Test B1	100	50	37	21	NA	158
Test B2	50	50	37	21	NA	120
Test B3	25	50	37	21	NA	86
“Baseline”	100	35	37	37	NA	370



Cell clumping observed at 100x magnification for the baseline harvest process (A), test case B2 (50% TrypLE, B), and B1 (100% TrypLE, C)

- A TrypLE-based harvest procedure was optimized in T-150 flasks by testing varying dilutions, incubation temperatures, and incubation times to maximize cell recovery and minimize cell aggregation
- 100% TrypLE and incubation temperatures of 37 °C (test case A2, A6, and A8) generally resulted in high cell recoveries and less cell clumping
- Incubation time did not have an impact: 55 minutes preferred to reduce processing time

2. Cell Recovery, Viability, and Morphology



Cells from the Xpansion 10 Run 1 were too aggregated pre-wash to obtain an accurate cell count.
Cell viabilities in all samples counted were above 95% and there was no difference in the viability of cells grown in Xpansion 10 bioreactor versus control at any time point.

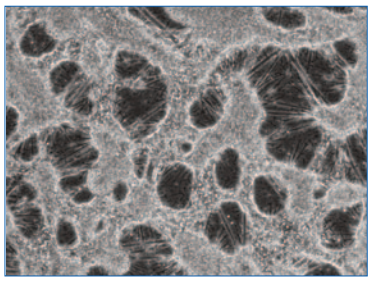
Run 1

- Cell density post-wash is less than estimated cell density pre-harvest and provides opportunities for improvement in the downstream operations:
  - ~7% cell loss in the spent media due to premature cell detachment
  - ~7% of cells did not completely detach from the Xpansion 10 bioreactor and were instead collected in a subsequent harvest step
  - Cell loss in Xpansion 10 bioreactor compared to control due to longer centrifugation process and cell aggregation before straining
  - Cells progressively aggregated during counting, leading to a lower cell count average
  - With an optimized process, 4.6 million cells/cm^2 or a 6x amplification (close to 'gold standard'), would have been recovered from Xpansion 10 bioreactor
- Cell density and viability in the Xpansion 10 bioreactor otherwise equivalent to the flatware control
- Cells self-assembled into a characteristic monolayer with cell colony formation in the Xpansion 10 bioreactor, similar to the flatware controls

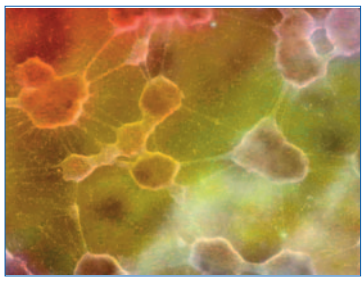
Run 2

- Cell density and viability in Xpansion 10 bioreactor equivalent to controls
- Cells expanded less than Run 1, likely due to the differences between the two differentiation protocols
- Less cell aggregation and yield loss occurred post-bioreactor harvest. The automated cell washing process was more effective than centrifugation at concentrating cells and maintaining a single-cell suspension

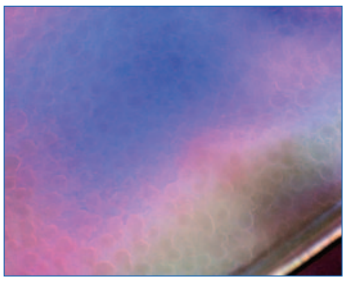
Morphology



Control Flask Run 1 Day 22

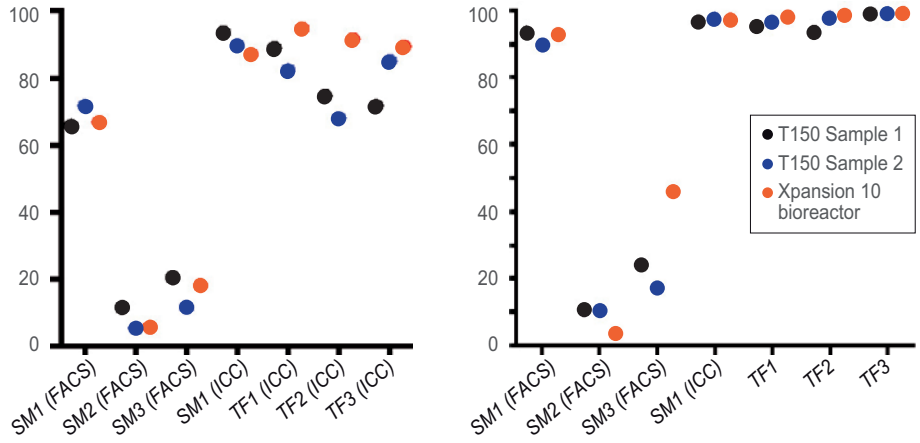


Xpansion 10 Bioreactor Run 1 Day 22



Xpansion 10 Bioreactor Run 2 Day 16

3. Cell Differentiation



Surface marker 1 (SM1) is the key cell surface marker used to indicate cell differentiation.

SM1 is measured both by fluorescence-activated cell sorting (FACS) and Immuno-cytochemistry (ICC) assay. The ICC assay is usually considered the more reliable of the two methods.

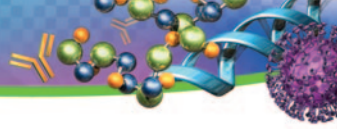
SM2 and SM3 are also cell surface markers used to indicate cell differentiation, although they are typically observed in a much smaller percentage of the cell population.

TF1, TF2, and TF3 are three transcription factors (TF) that are expressed at high levels in differentiated cells.

- Population level cell surface marker expression was equivalent between Xpansion 10 bioreactor and controls for both Run 1 and Run 2
- SM1 was expressed in >90% of the cell population, as indicated by ICC, for both Run 1 and Run 2
- The improvement from Run 1 to Run 2 was due to changes between the 16-day vs. 22-day differentiation protocols

CONCLUSION

- **The Xpansion bioreactor plates were successfully coated with a proprietary protein critical to cell attachment and proliferation**
- **The Xpansion bioreactor can support adherent human cell culture at densities of >1 million cells/cm^2 for extended culture durations**
- **T-flasks can be used to optimize several harvest parameters and the optimized parameters translate successfully when transferred to the Xpansion 10 bioreactor**
- **Using two different protocols, the Xpansion bioreactor facilitated 3-4x amplification of cells and >95% cell viability. This was equivalent to flatware controls**
- **Cells self-assembled into a characteristic monolayer with cell colony formation in the Xpansion 10 bioreactor, similar to the flatware controls**
- **Cells differentiated to the more mature cell fate equivalently in both the Xpansion 10 bioreactor, and the controls, achieving >90% differentiation using both differentiation protocols**
- **The automated washing process was more effective at concentrating and washing larger volumes of cell suspension compared to centrifugation, reducing both cell aggregation and yield loss**



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

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