Challenges and Solutions for Allogeneic Cell Therapy Manufacturing

Sunghoon Jung, Kelly Gray, Hannah Worden, Breanna Borys,
Max Lee, Kenny Cruz, Yas Hashimura, and Brian Lee

PBS Biotech, Camarillo, CA, USA

VERTICAL-WHEEL® BIOREACTORS

Topics for Realizing Robust & Efficient Cell Manufacturing Processes

- Principles of cell therapy manufacturing process optimization/scale-up and a roadmap toward developing robust/efficient processes
- Scale-up challenges for large scale processes
  - **Equipment, Materials and Protocols –** Evaluating and selecting high-performing, truly scalable technologies (e.g., bioreactor) and establishing robust and efficient protocols (including seed train culture processes)
  - **Hydrodynamics –** Maintaining homogeneous hydrodynamic environment with uniform distribution of turbulent energy dissipation and consistently minimal shear forces across various scales
  - **Gas Supply –** Understanding culture scale and achievable cell density via headspace gassing, and finding a new approach for proper gas supply (oxygen) / stripping (carbon dioxide) at large scale in which headspace gassing is not sufficient to maintain oxygen and CO_2 at desired levels
  - **Optimal Protocols of Medium Exchange and Cell Harvest –** Finding a new way for rapid and efficient medium exchange (MX) and harvest for cells that are sensitive to process time – e.g., induced pluripotent stem cells (iPSCs) growing as aggregates that tend to fuse with others to result in larger aggregates when they are settled on the bottom of a bioreactor during MX or harvest.

Process Optimization to Develop a Robust & Efficient Process

Importance of Process Development Strategies: Example Case Studies with Different Outcomes

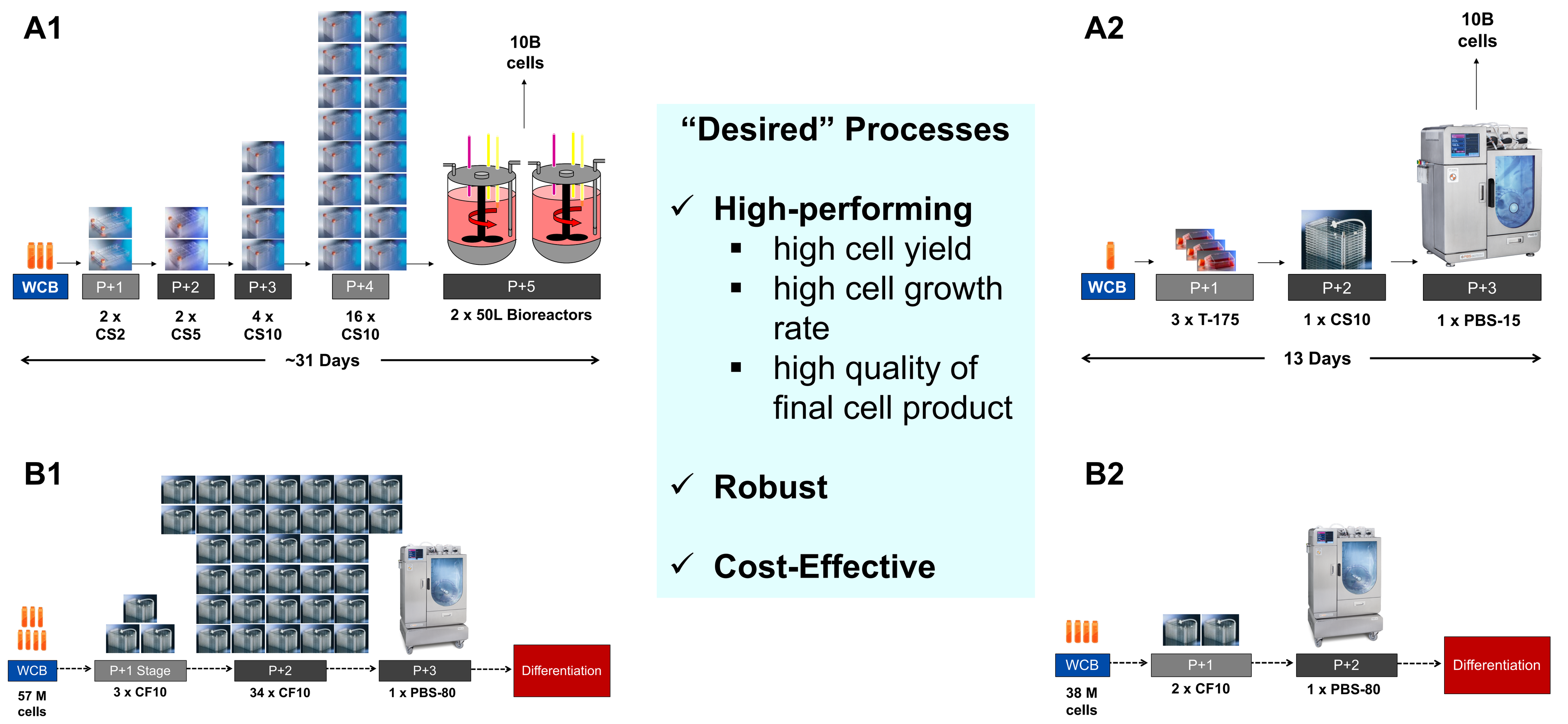


Figure 1. **A1 and A2:** Manufacturing process schemes designed, based on data generated from two different approaches toward developing a process for a target lot size (10 billion cells/lot) of hMSCs. **B1 and B2:** Another example of process development studies for an anchorage cell type and the processes designed, based on data generated from each study.

Principle of Process Development

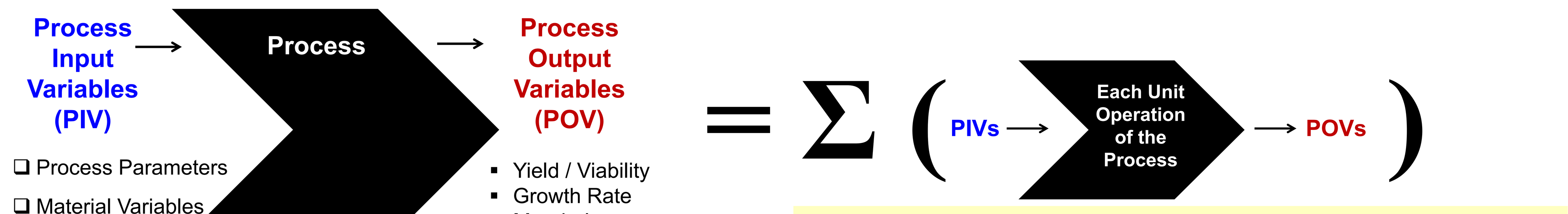


Figure 2. Principle is Process Development – A journey during which one continue to make effort to understand / get to know about your process – specifically the correlations of PIVs and POVs of each unit operation of the process.

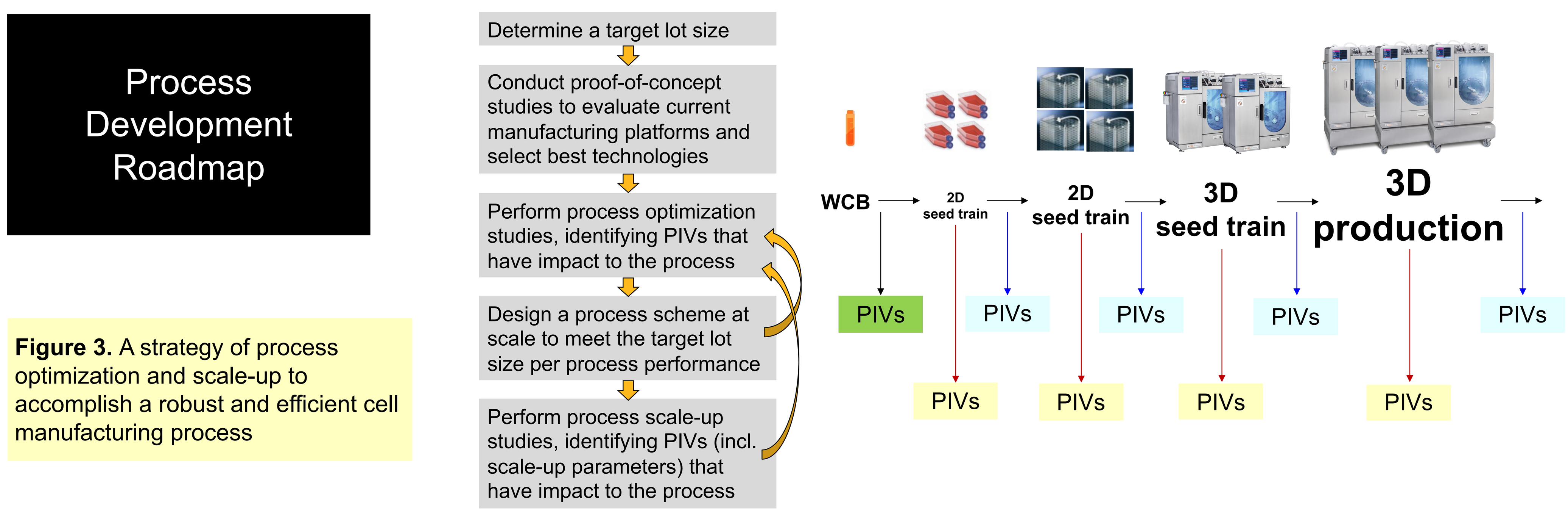


Figure 3. A strategy of process optimization and scale-up to accomplish a robust and efficient cell manufacturing process

A Case Study: hMSC Process Optimization and Scale-Up

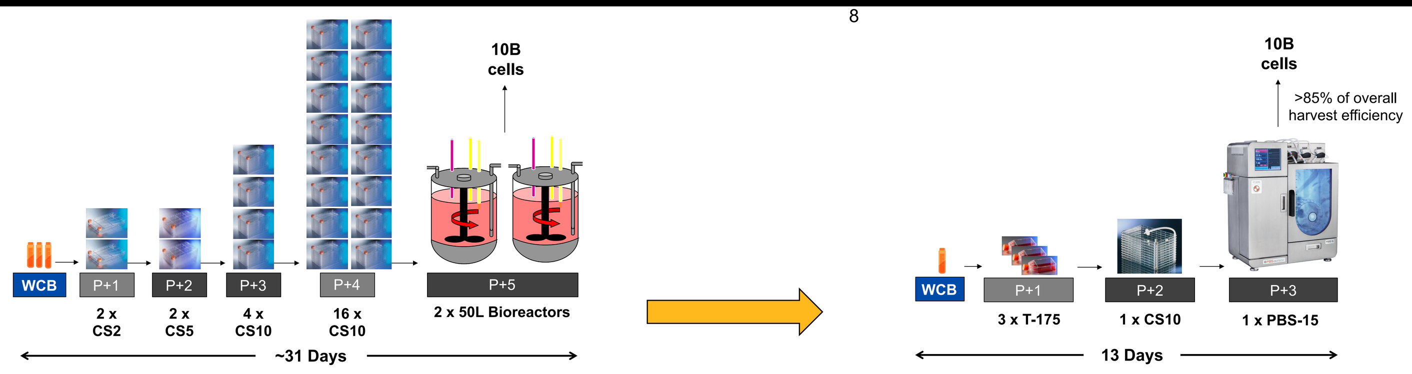


Figure 4. A summary of a case study of process optimization and scale-up carried out and PIVs investigated to develop a clinical-scale (>10 B cells/lot) manufacturing of hMSCs, in comparison with a process designed based on data from a separate study.

- **POC and Process optimization for 2D seed train culture**
  - Cell passage number
  - Growth media
  - Concentration of media components
  - Medium feeding regime
  - Cell seeding density
  - Harvest process time
- **Process optimization of microcarrier culture/harvest in small-scale bioreactors**
  - Microcarriers type and loading density
  - Cell seeding density
  - Medium feeding regime
  - Agitation speed
  - pH control with agitation speed and base addition
  - Dissolved oxygen (DO) control
  - Cell harvest process conditions and operation time
  - Optimization of sampling and cell counting methods
- **Scale-up of cell culture and harvest process**
  - pH control with CO_2 gassing and base addition
  - DO control
  - Agitation speed control
  - Harvest time
  - Optimization of sampling methods for accurate cell counts

Scale-Up Challenges and Potential Solutions

Selecting a Scalable Bioreactor System (with Similar Geometries) and Determining Agitation Speed to Offer Comparable Hydrodynamics

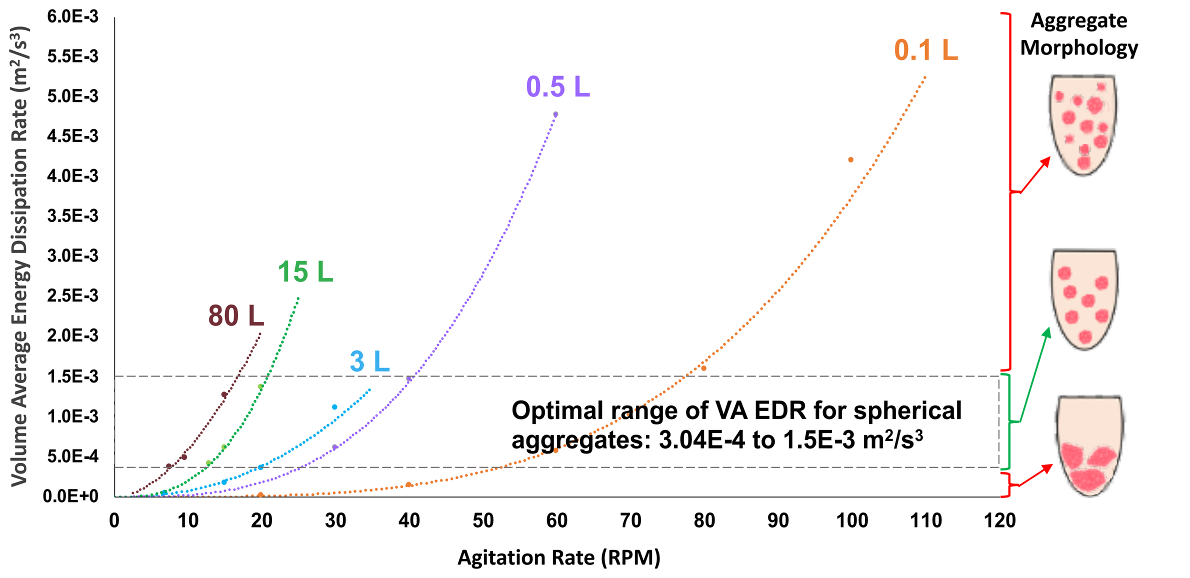


Figure 5. Curves to determine volume average (VA) energy dissipation rate (EDR) based on VW bioreactor working volume and agitation rate. Average EDR can be used to predict aggregate morphology for a particular combination of volume and agitation, with desired spherical aggregates, a consequence of average EDR within an optimal range.

Volume (L)	Agitation Rate (RPM)	VA EDR (m^2/s^3)	Optimal EDR Range	Day 1	Day 3	Day 5	Day 7
0.1	60	6.1E-4	Within				
0.5	30	6.1E-4	Within				
0.5	40	1.4E-3	Within				
0.5	18	1.4E-4	Below				Agglomerated clump of cells too large to suspend
0.5	60	4.8E-3	Above				

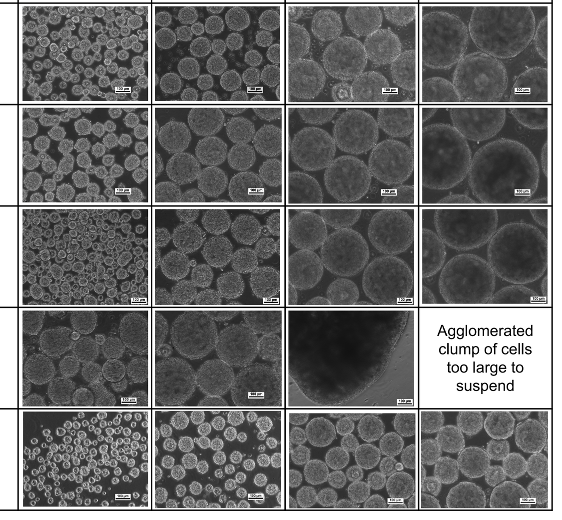
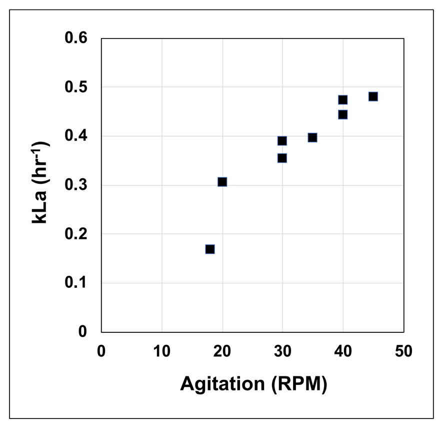


Figure 6. Observed morphologies of iPSC aggregates for different combinations of volume and agitation rate. Uniformly spherical aggregates correspond to VA EDRs that fall within the optimal range. There is also an inverse correlation between average EDR and aggregate size. Photomicrographs were taken at 10X magnification. Scale bars: 100 μm.

Strategies for Optimal Gas Supply for Target Culture Scales and Cell Densities

Understanding culture scales & achievable cell densities via headspace gassing: oxygen transfer rate (OTR) vs uptake rate (OUR)



Agitation (RPM)	k_La (hr^{-1})	Achievable Cell Density at 100% DO set point (cells/mL)	Achievable Cell Density at 50% DO set point (cells/mL)
18	0.168	672,000	756,000
20	0.306	1,224,000	1,377,000
30	0.372	1,488,000	1,674,000
35	0.396	1,584,000	1,782,000
40	0.459	1,836,000	2,065,500
45	0.480	1,920,000	2,160,000

Experimental Condition:
- Vessel: PBS-3 MAG bioreactor
- Working Volume: 3L
- Medium: Water
- Gas inlet: Pure oxygen via headspace (0.5 L/min)
- Agitation speed: 18-45 RPM

Assumptions:
- Specific oxygen consumption rate, q_{O2}: 2E-10 mmol O_2/cell/hr
- Oxygen concentration at 100% DO: 0.2 mmol O^2/L
- Max. oxygen concentration with pure oxygen via headspace: 1.0 mmol O^2/L

Figure 7. kLa values measured in a 3L VW bioreactor under different agitation speeds.

Table 1. Theoretical cell densities achievable under the given experimental conditions and assumptions.

Oxygenation of culture media outside a large bioreactor using an oxygenator

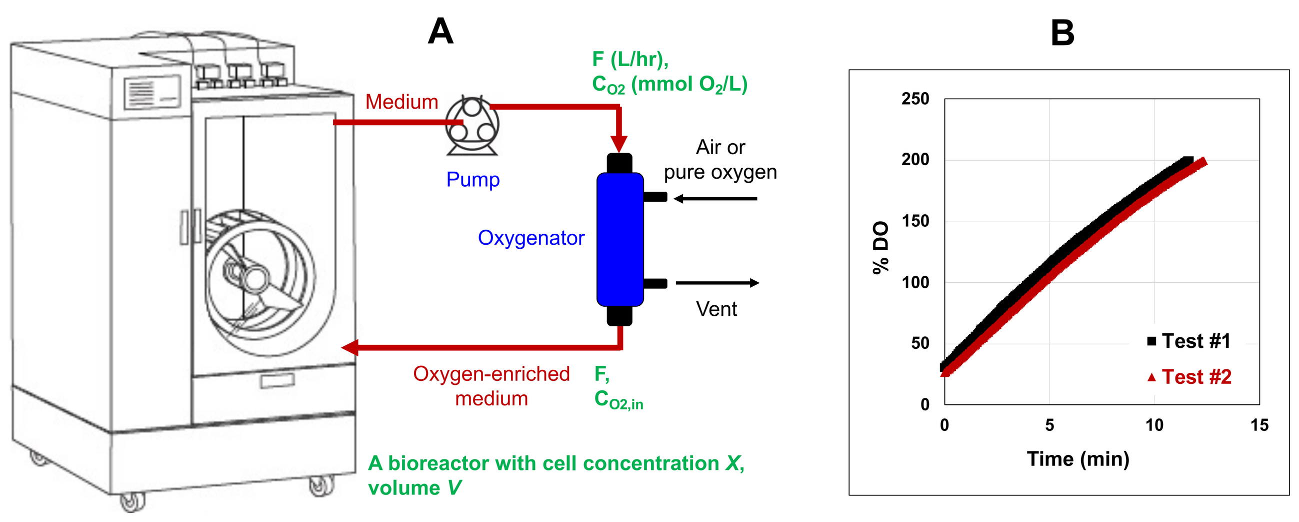


Figure 8. A methodology for using an external oxygenator to facilitate a rapid and efficient oxygenation of medium at a large-scale bioreactor (A). The rapid increase of DO in the test bioreactor demonstrates the proof-of-concept of using an external oxygenator effectively (B).

Assumptions:
- Specific oxygen consumption rate, q_{O2}: 2E-10 mmol O_2/cell/hr
- Oxygen concentration at 50% DO set point, C_{O2}: 0.1 mmol O^2/L
- Oxygen concentration in medium oxygenated (w/ pure oxygen in the oxygenator) entering into bioreactor, $C_{O2, in}$: 1.0 mmol O^2/L

X, cell density (cells/mL)	F/V (hr^{-1})	Flow rate required for different bioreactor working volume (mL/sec)		
		3L	15 L	80L
500,000	0.11	0.09	0.46	2.47
750,000	0.17	0.14	0.69	3.70
1,000,000	0.22	0.19	0.93	4.94
1,250,000	0.28	0.23	1.16	6.17
1,500,000	0.33	0.28	1.39	7.41
1,750,000	0.39	0.32	1.62	8.64
2,000,000	0.44	0.37	1.85	9.88
2,250,000	0.50	0.42	2.08	11.11
2,500,000	0.56	0.46	2.31	12.35

Table 2. Medium flow rate calculated for different bioreactor working volume for its sufficient oxygenation

Developing Optimal Protocols for Medium Exchange and Cell Harvest for Very Sensitive Cells

Current protocol using Adjustable Height Harvest Valve (AHHV) for rapid and efficient medium exchange and cell harvest in single-use VW bioreactors

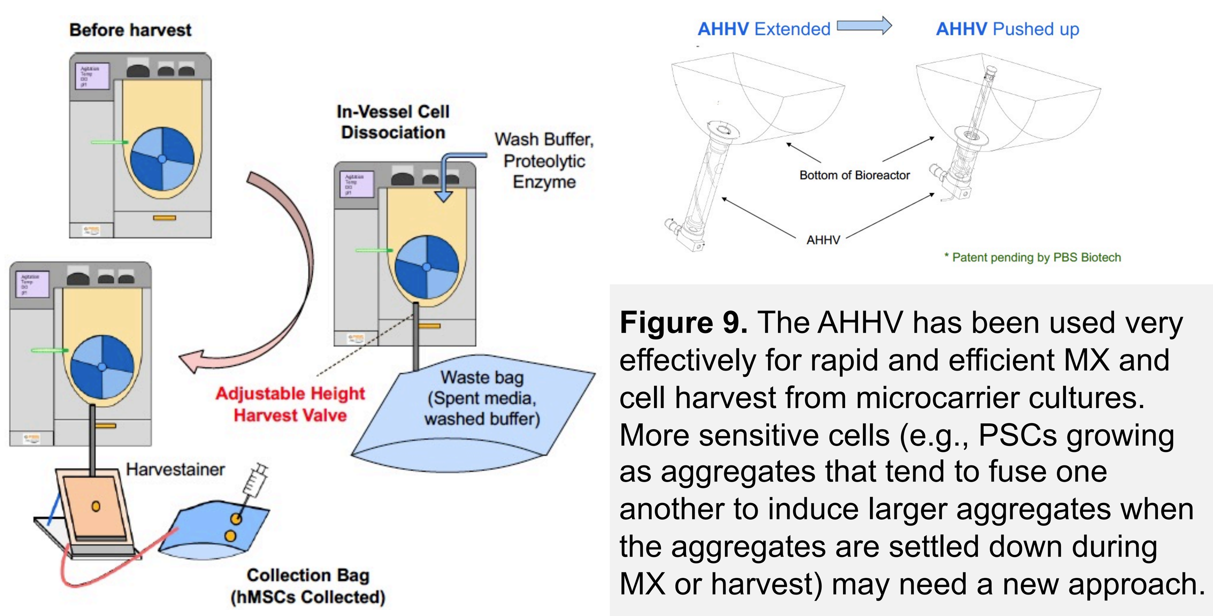


Figure 9. The AHHV has been used very effectively for rapid and efficient MX and cell harvest from microcarrier cultures. More sensitive cells (e.g., PSCs growing as aggregates that tend to fuse one another to induce larger aggregates when the aggregates are settled down during MX or harvest) may need a new approach.

Rapid separation of cells from medium outside a bioreactor to enable rapid and complete medium exchange and return to a 2nd bioreactor that contained pre-conditioned fresh medium

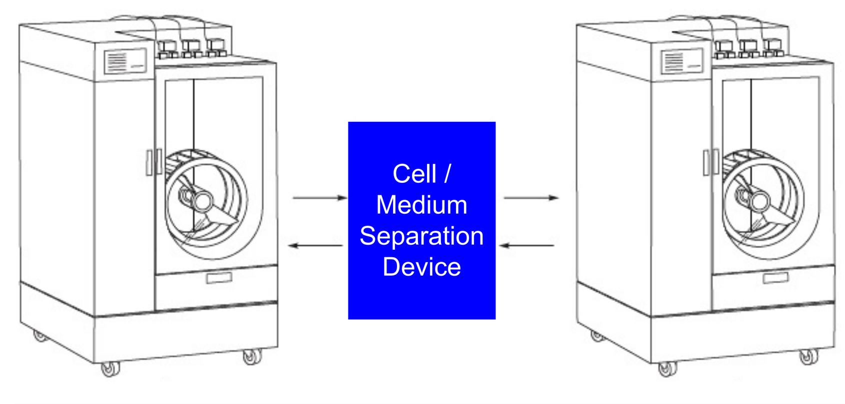


Figure 10. A methodology for using an external separation and retention device in conjunction with multiple bioreactors to facilitate rapid, scalable medium exchange

ACKNOWLEDGMENTS

UNIVERSITY OF CALGARY

SCM Lifescience

CONCLUSIONS

In order to reliably provide PSC-derived allogeneic cell therapies to vast numbers of patients, a series of optimized unit operations at various scales will be needed to meet target manufacturing lot sizes. Numerous manufacturing processes such as cell aggregate expansion and differentiation, medium and gas exchanges, and cell harvesting all need to be developed and optimized for large-scale use. The proper combination of single-use bioreactor technology and methodologies can avoid potential upstream process bottlenecks and enable robust commercial-scale manufacturing of therapeutic cells.