NOVEL VERTICAL-WHEEL BIOREACTORS FOR THE SCALABLE, ROBUST AND EFFICIENT PRODUCTION OF SHEAR-SENSITIVE CELL THERAPY PRODUCTS

Human Pluripotent Stem Cells (Aggregate Culture) And Mesenchymal Stromal Cells (Microcarrier Culture)

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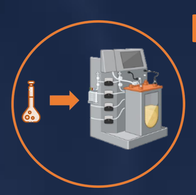
Abstract

- Vertical-Wheel (VW) bioreactors enable **linear scale-up** of induced pluripotent stem cell (iPSC) and mesenchymal stromal cell (MSC) expansion **up to 10L and 50L working volume** with aggregate and microcarrier expansion modalities, respectively.
- Computational fluid dynamic (CFD) modeling and experimental power measurement validation demonstrate that the VW bioreactor family provides thorough mixing and **maintains a consistent hydrodynamic environment across scales**, allowing for consistent cell quality and yield.
- Systematic process optimization at the PBS-Mini scale was performed to develop **robust, reproducible, efficient, and scalable cell expansion processes**.
- The PBS platform for the scale-up of stem cell expansion is an efficient way to **meet dosage demands** for allogeneic therapies that can treat a variety of human diseases.



Key considerations for bioreactor scale-up

- Maintain consistent aggregate size and shape
- Maintain energy dissipation profiles and averages
- Provide adequate mixing and mass transfer

PBS VW Family of Bioreactors Enables Consistent Hydrodynamic Conditions for Process Scale-Up

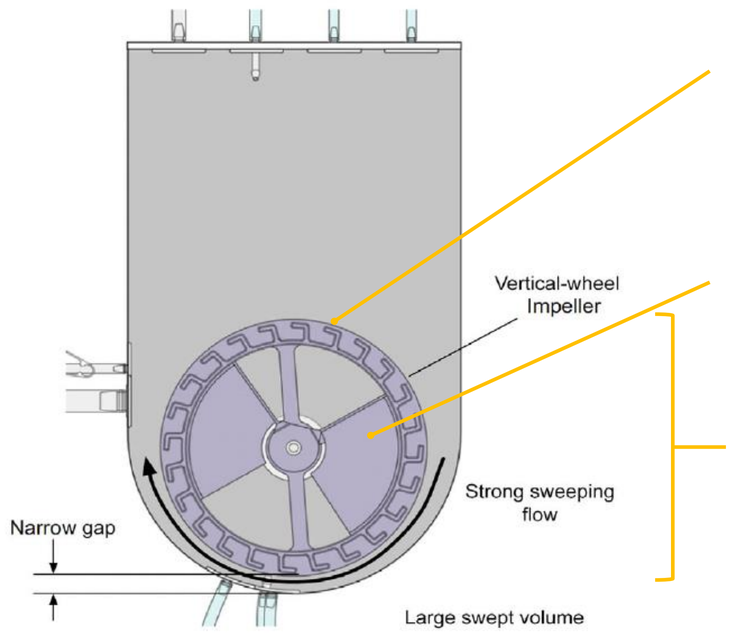


Figure 1. Three key design features of the Vertical-Wheel bioreactor. The efficient mixing and narrowly distributed shear forces – which are desirable in scalable manufacturing technologies – are a result of these geometric features.

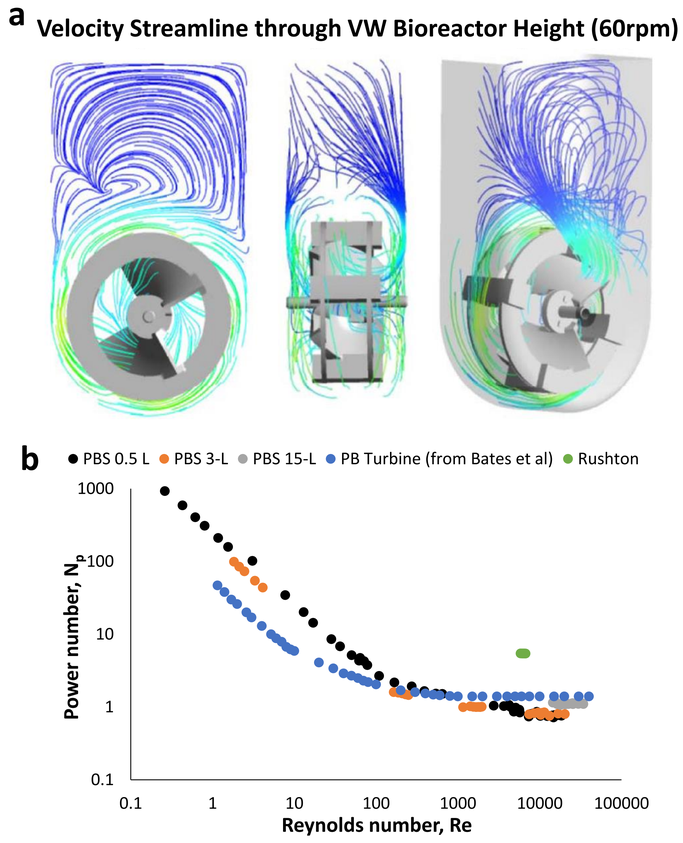


Figure 2. Characterization of VW bioreactors through computational simulations and experimental measurements [1]

(a) Velocity streamlines throughout PBS-0.1 Mini vessel at 60rpm generated by CFD modelling. This image reflects the impact of the key geometrical features discussed in Figure 1. The streamlines show thorough mixing in a lemniscate (figure-eight) profile which allows fluid and particles to move through the entire vessel volume.

(b) Power number versus Reynolds number for the PBS-0.5, PBS-3, and PBS-15 Vertical-Wheel bioreactors, a pitched-blade (PB) turbine in a baffled stirred tank (from Bates et al.), and the Rushton impeller in a baffled stirred tank (Figure 12 in Croughan et al.) [2]. Data illustrates consistency of the power number in the turbulent regime across three scales of the PBS VW bioreactor family. Power number is related to scale-up quantities like volume average energy dissipation rate and can indicate the ability of a reactor to dissociate cells from microcarriers and maintain aggregate size at different scales [3].

Scale-Up of iPSC Aggregate Expansion in PBS VW Bioreactor Platform

The PBS platform for iPSC aggregate culture uses a PBS Mini Vessel (ex. PBS-0.1) as a validated scale-down model to optimize process conditions. The optimized process is then scaled up to PBS-3 and PBS-15 controlled bioreactors with perfusion medium exchange for at-scale process validation and manufacturing.

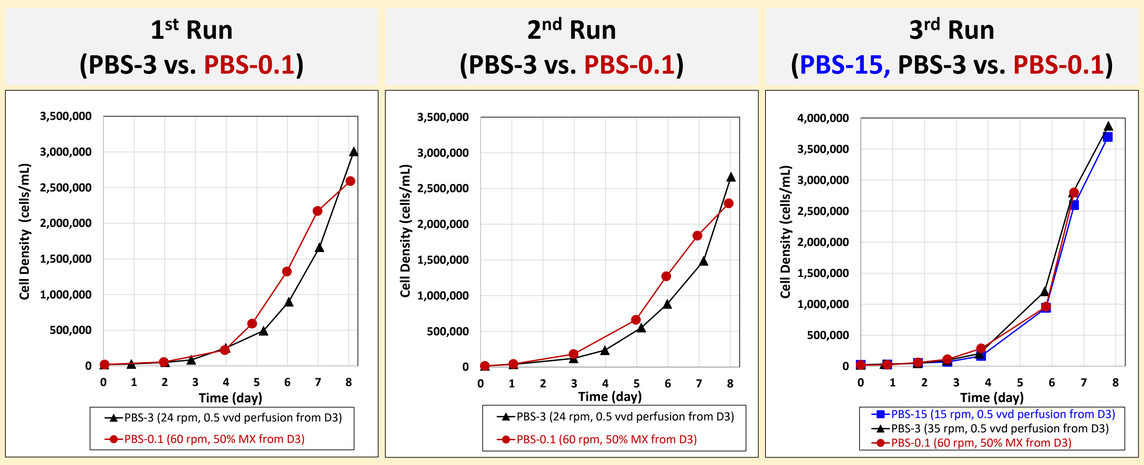


Figure 3. Cell growth profiles showing robustness and scalability of the PBS process for iPSC expansion in aggregates. All runs show reproducibility and robustness within and across scales of the PBS VW bioreactor. Runs 1 and 2 were performed by different operators and at different times yet had comparable growth patterns. Run 3 demonstrates the reproducibility, robustness, and linear scalability of the expansion process across 3 scales of the PBS VW bioreactor – PBS-0.1, PBS-3, and PBS-15.

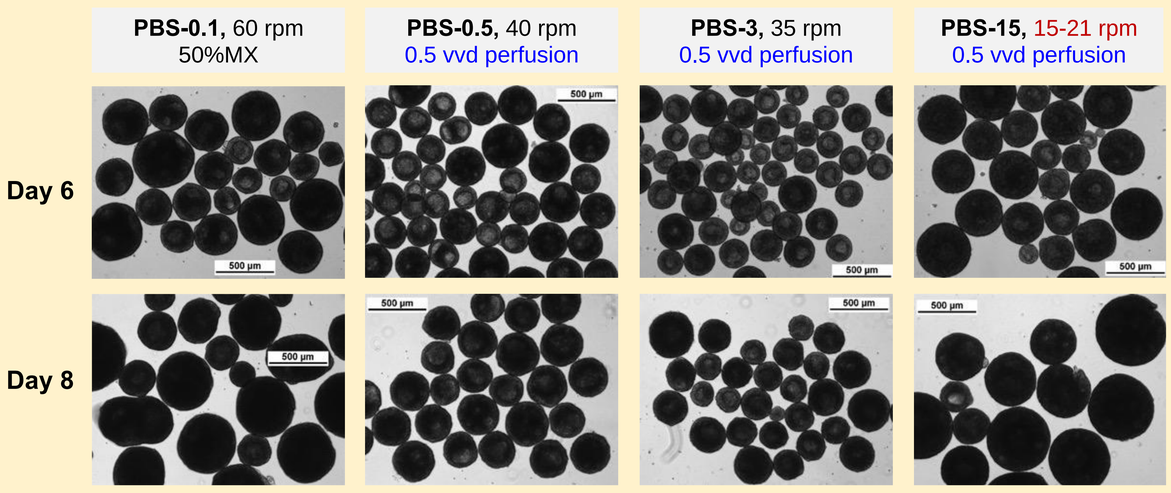


Figure 4. The PBS iPSC aggregate expansion platform maintains the critical quality attribute of aggregate size distribution across scales. Perfusion medium exchange allows the VW bioreactor to demonstrate the scalability of important hydrodynamic conditions without the aggregate size heterogeneity introduced by settled medium exchanges.

Scale-Up of MSC Microcarrier Expansion in PBS VW Bioreactor Platform

Consistency of hydrodynamic environment across scales is also important for MSCs cultured on microcarriers. The hydrodynamic profile in the bioreactor impacts cell attachment to microcarriers and microcarrier aggregation in culture. The PBS platform for scale-up of MSC expansion uses PBS VW bioreactors and commercial microcarriers to meet target lot sizes.

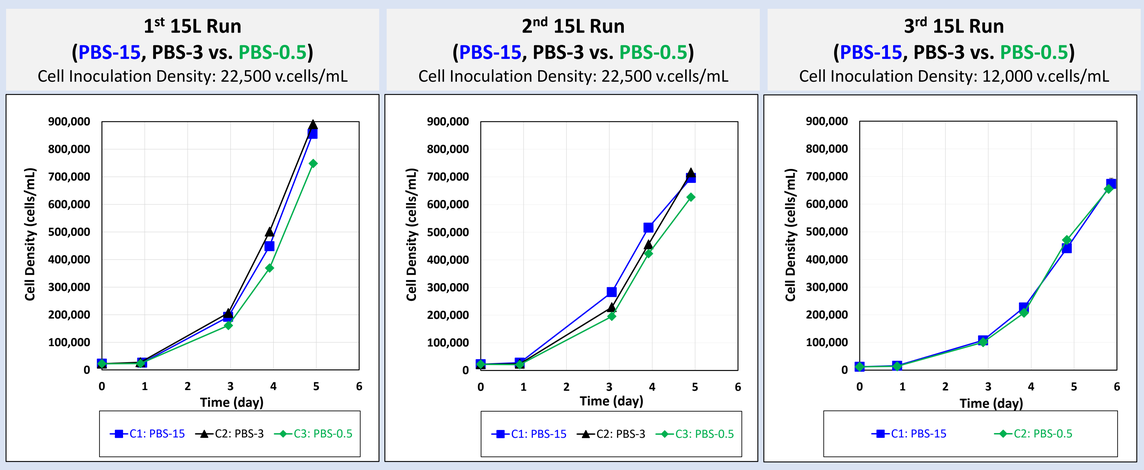


Figure 5. Scalability, robustness, and reproducibility of the PBS platform for MSC expansion on microcarriers is shown in 3 different runs, with different operators, and at different inoculation densities. In-vessel harvests were successfully performed with ~100% detachment efficiency in all runs. We have also achieved linear scale-up of microcarrier MSC expansion culture up to the 50L scale (data not shown).

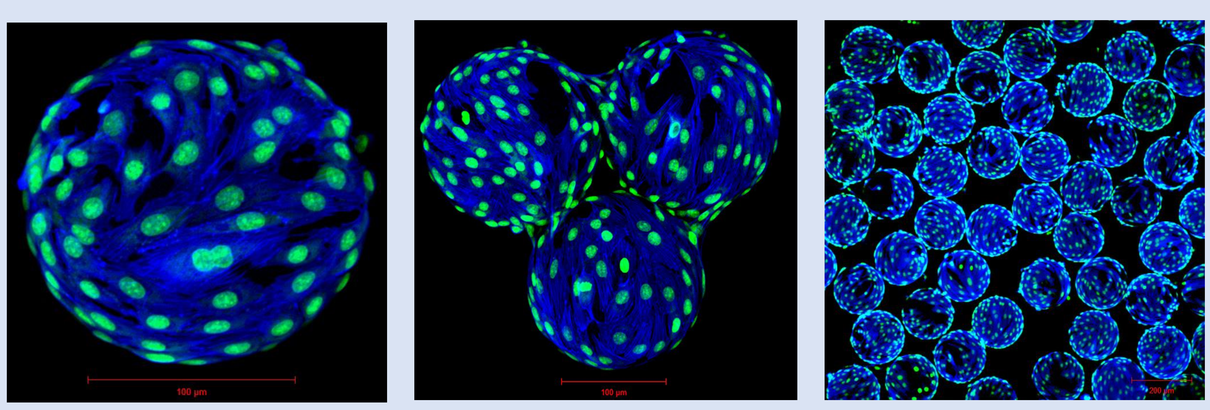


Figure 6. Confocal microscopic (Zeiss LSM 700) images of MSCs cultured in the PBS-0.1 Mini (60 rpm) on individual microcarriers, on aggregated microcarriers, and in a representative culture sample. Fluorescent staining of cell nuclei (green) and actin (blue) demonstrate uniform cell attachment on microcarriers throughout the culture.

Conclusions

- Scale-up of cell therapy processes requires not only systematic cell culture process optimization but also suitable manufacturing technologies that preserve key hydrodynamic conditions for maintaining critical quality attributes in cell therapy development such as aggregate size, and cell/aggregate morphology.
- The PBS Vertical-Wheel bioreactor platform maintains its mixing parameters such as power per mass (or volume average energy dissipation rate) across scales and the key features of this reactor eliminate technology dependent scale-up limitations with respect to hydrodynamics.
- We have presented robust and linearly scalable processes for iPSC and MSC expansion from the PBS-0.1L to the PBS-15L scales in both aggregate and microcarrier culture modalities, consistently producing over 1.5 million cells/mL by day 7 for iPSC aggregate culture and over 600,000 cells/mL by day 5 in microcarrier MSC culture.

References & Acknowledgements

[1] Borys B. S., Dang T., So T., et al. **Overcoming bioprocess bottlenecks in the large-scale expansion of high-quality hiPSC aggregates in vertical-wheel stirred suspension bioreactors**. *Stem Cell Res. Ther.* 2021;12:55.
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Header Image: J. Heggie, **Can cell therapy beat the most difficult diseases?**, *National Geographic*. (2021). Accessed April 10, 2023.
Acknowledgements
Erin Roberts and Dr. Michael Kallos from the University of Calgary's Pharmaceutical Production Research Facility for providing images in Figure 6.