

Abstract

A Reliable and Scalable Bioreactor System is Required for Scale Up of Cell Therapy Manufacturing:

- Current 2D planar platforms are not adequate for commercial-scale production
- Bioreactors represent a cost-effective and scalable manufacturing platform

Conventional Stirred-Tank Reactors (STRs) Present Problems for Cells Growing on Microcarriers or as Aggregates:

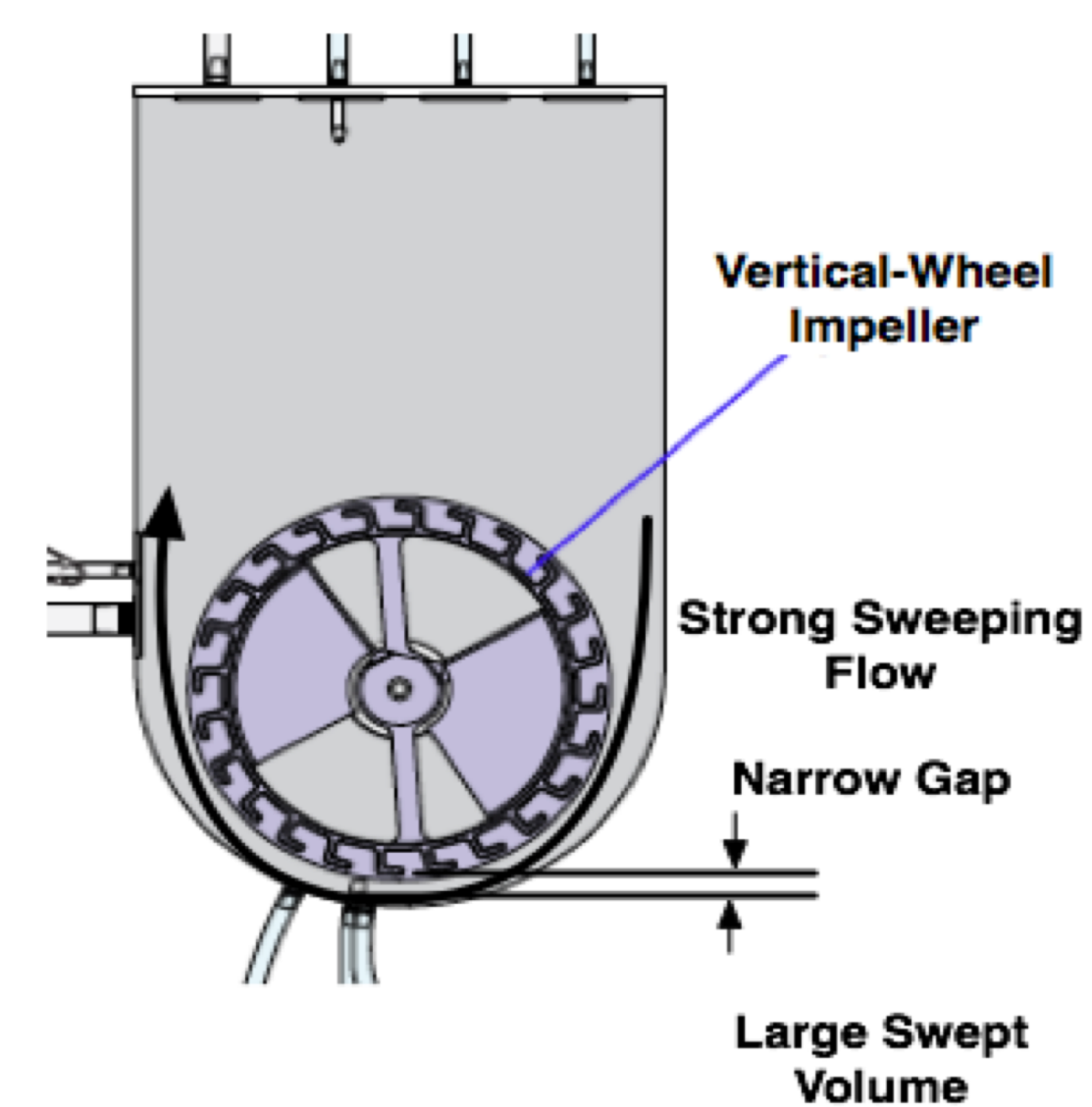
- STRs require relatively high agitation power input to fully suspend aggregates or microcarriers
- Cells growing on microcarriers or as aggregates in suspension culture are sensitive to the fluid shear stress induced by the hydrodynamic flow in STRs
- The fluid shear stress in STRs can negatively affect cell yield, differentiation efficiency, quality, and potency
- The wide gradient of energy dissipation rates that exists in STRs results in a broad variation of size and morphology of cell aggregates
- These problems with STRs become exacerbated as the size of the bioreactor increases

Scalable and Low-Shear Vertical-Wheel Bioreactors Provide Superior Growth Performance of Microcarrier and Cell Aggregate Cultures:

- Single-use, Vertical-Wheel bioreactor systems offer efficient fluid mixing with low power input, resulting in a low shear environment and unparalleled scalability across a full range of vessel sizes, from 0.1 to 80 liters
- Vertical-Wheel bioreactors also provide homogenous energy dissipation distribution, which allows for controlled production of cell aggregates with uniform size and morphology
- A built-in, adjustable height harvest valve enables rapid and efficient medium exchange and cell harvesting from large-scale microcarrier or aggregate cultures

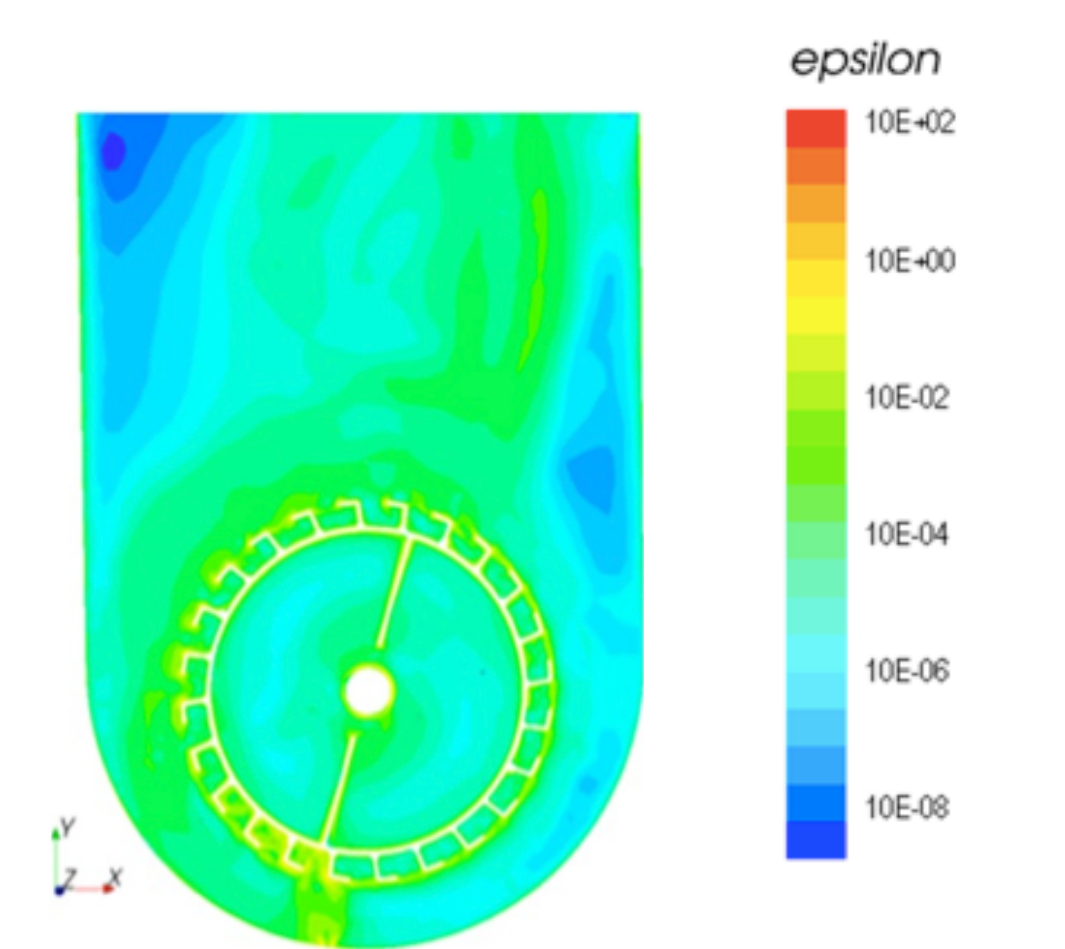
Background

Fig 1. Key Features of Vertical-Wheel Bioreactors



- **Vertical-Wheel impeller and U-shape vessel** together promote strong, sweeping liquid flow that results in homogeneous particle suspension
- **Oppositely-oriented axial vanes** create cutting and folding fluid flow for efficient mixing at very low power inputs
- **Sizeable impeller zone** has a large swept volume, resulting in a low turbulent energy dissipation rate and gentle mixing

Fig 2. Computational Fluid Dynamic Analysis for Turbulent Energy Dissipation Rate of Vertical-Wheel Bioreactor



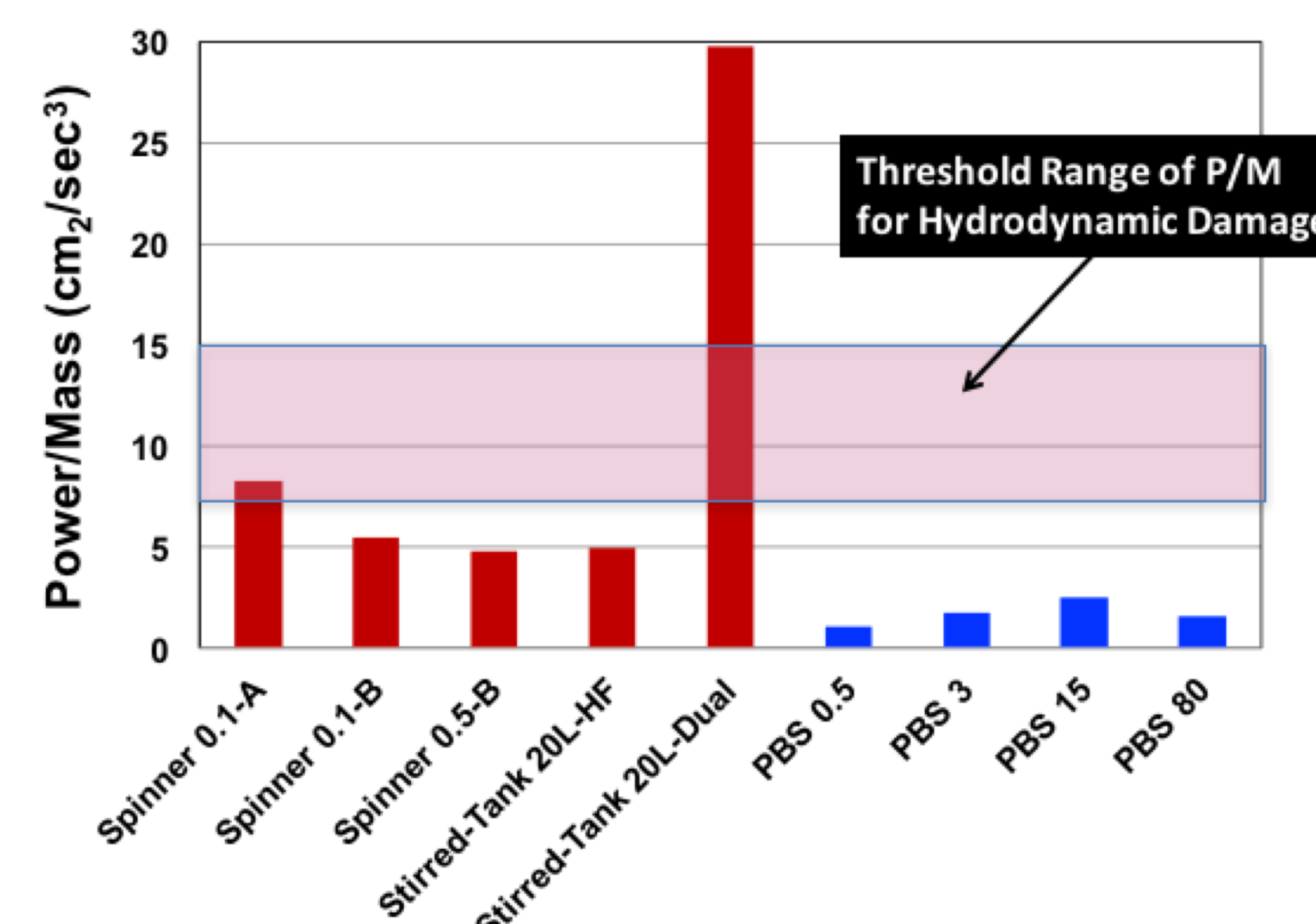
- The turbulent energy dissipation rates are shown in units of epsilon ϵ (m^2S^{-3})
- Range of turbulent energy dissipation rate is relatively narrow and uniformly distributed in the vessel
- Turbulent energy level can be adjusted by agitation rate of the Vertical-Wheel

Croughan M. et al. (2016), Stem Cell Manufacturing

Experimental Results

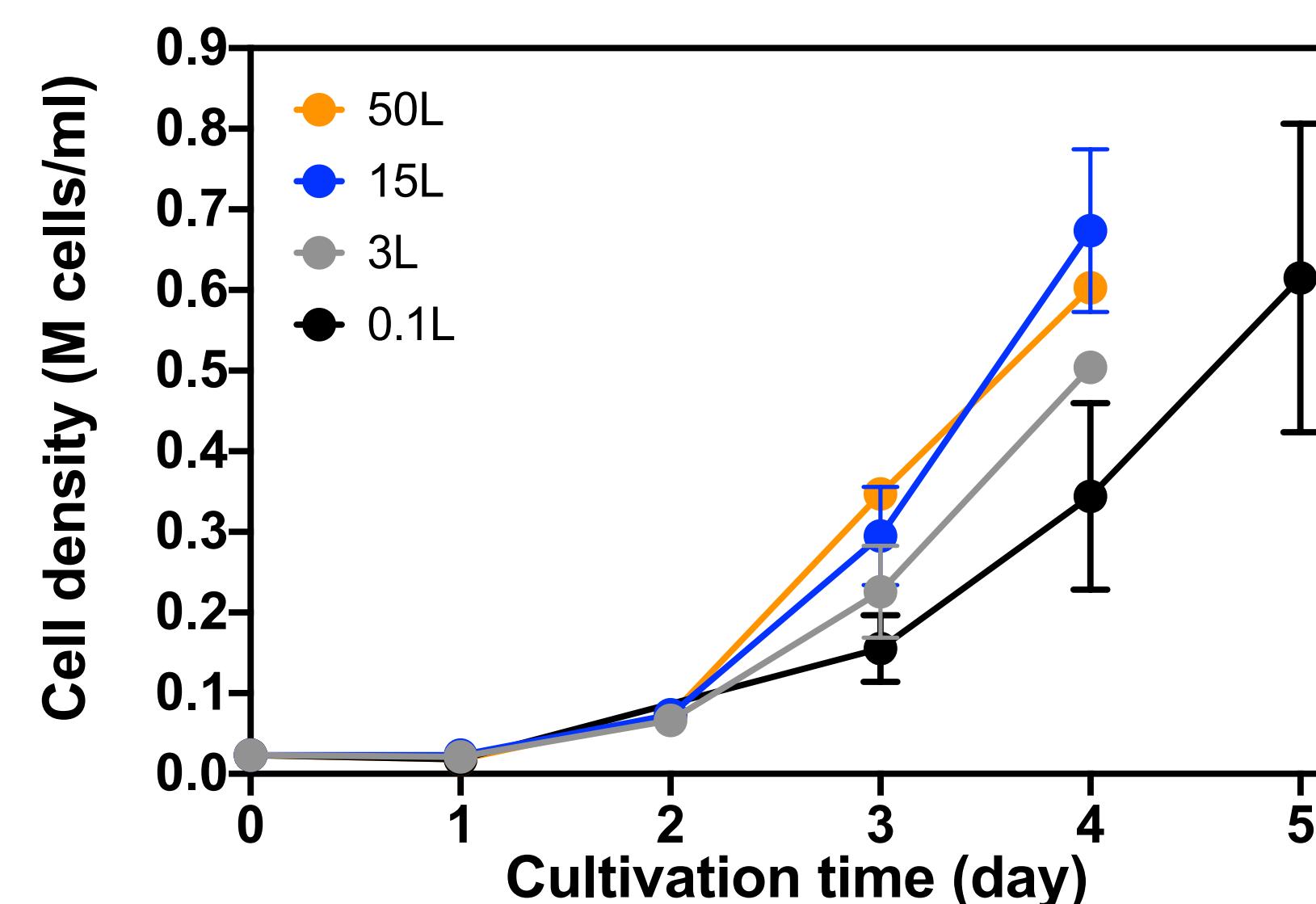
Performance of Vertical-Wheel Bioreactors on Microcarrier Culture

Fig 3. Comparison of Power/Mass Levels at Minimum Agitation Rates Necessary for Off-Bottom Suspension of Microcarriers



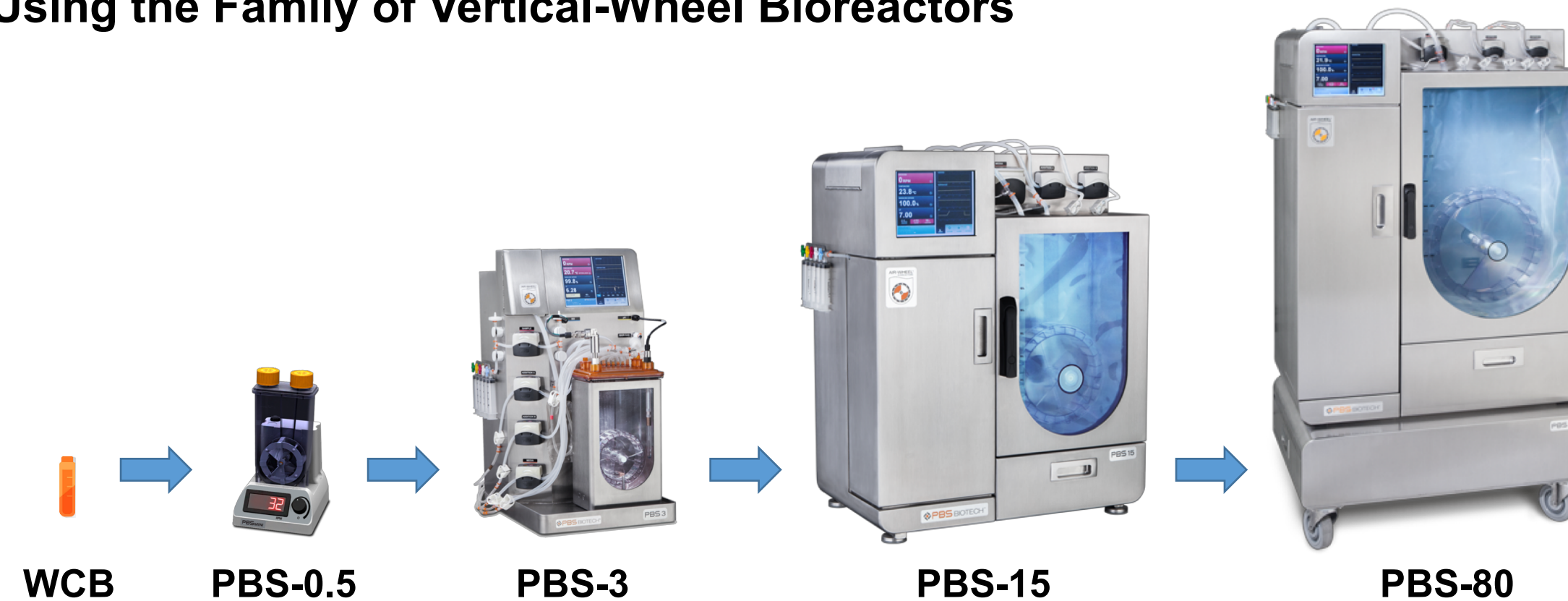
- All Vertical-Wheel bioreactors have significantly more margin below the critical threshold of power input where cell damage can occur

Fig 4. Consistent hMSC Growth in Xeno-Free Microcarrier Culture in Various Scale Vertical-Wheel Bioreactors (0.1-80L Bioreactors)



- hMSC growth in PBS-0.1 (n=2), PBS-3 (n=3), PBS-15 (n=2), and PBS-80 (n=1, 50L working volume)

Fig 5. A Representative, Large-Scale Cell Manufacturing Process Scheme Using the Family of Vertical-Wheel Bioreactors



Successful Applications of Vertical-Wheel Bioreactors Include:

- Rapid growth of freshly thawed human cells that were inoculated directly into bioreactors containing microcarriers
- Serial passaging of cells into progressively larger Vertical-Wheel bioreactors while maintaining high levels of cell growth and pluripotency
- Highly effective in-vessel dissociation of cells from microcarriers by manipulating agitation speed during enzymatic dissociation
- Rapid, efficient medium exchange and cell harvest through the use of a built-in, adjustable height dip tube (PBS-3) or harvest valve (PBS-15 & -80)

Performance of Vertical-Wheel Bioreactors on PSC Aggregate Culture

Fig 6. Uniform Size Distribution of Cell Aggregates (ESCs, iPSCs) and Control of Average Size by Adjusting Agitation Rate in Vertical-Wheel Bioreactor

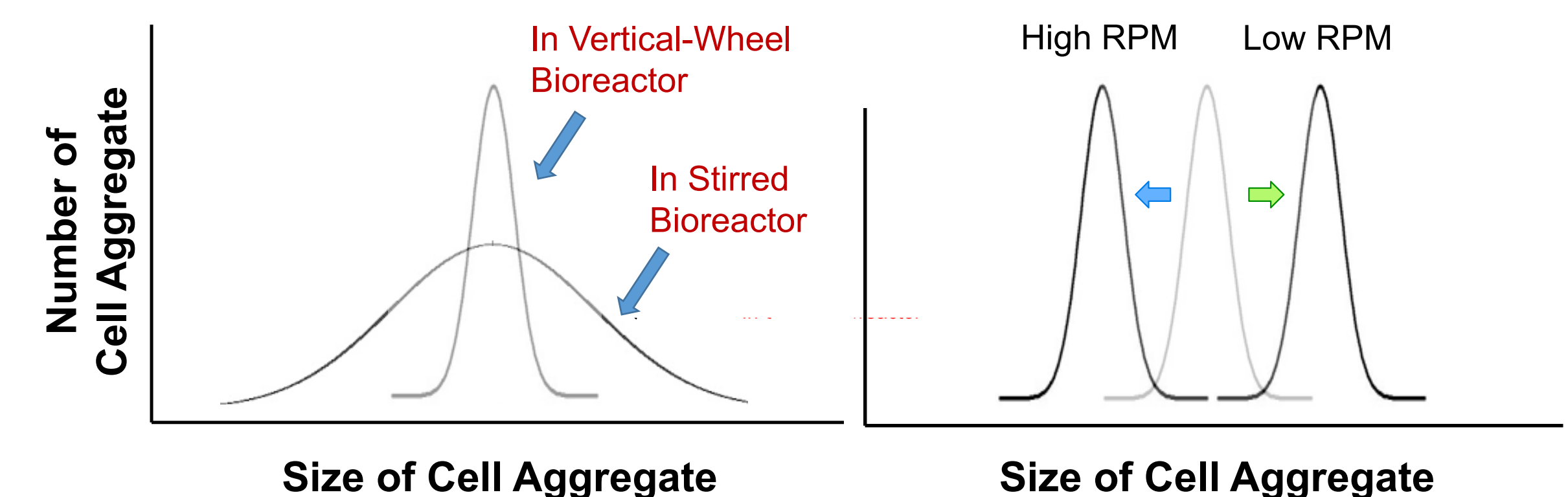


Fig 7. Size and Morphology Comparison of ESC Aggregates Grown in Spinner (Stirred) vs. PBS-0.5 and PBS-3 (Vertical-Wheel)

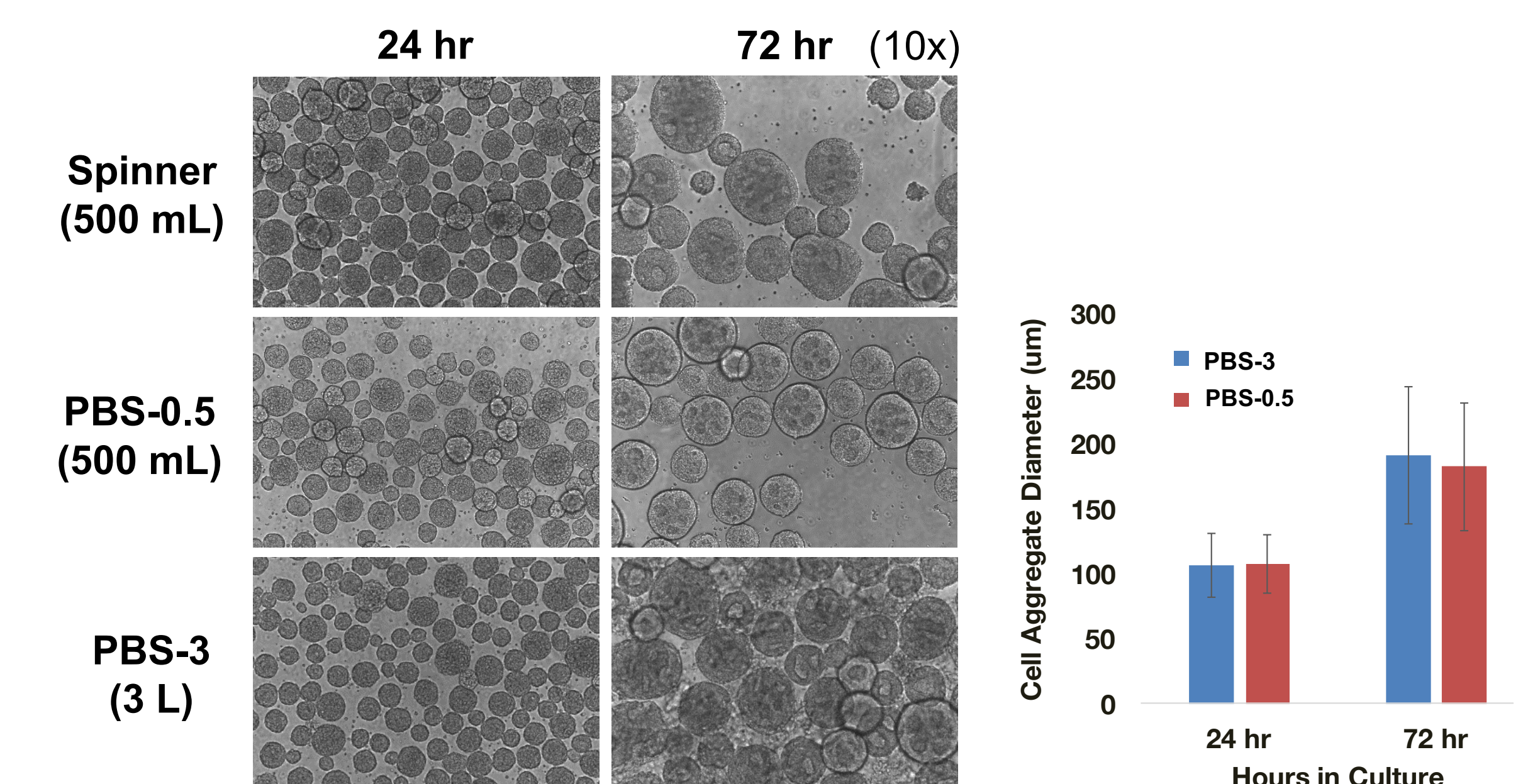
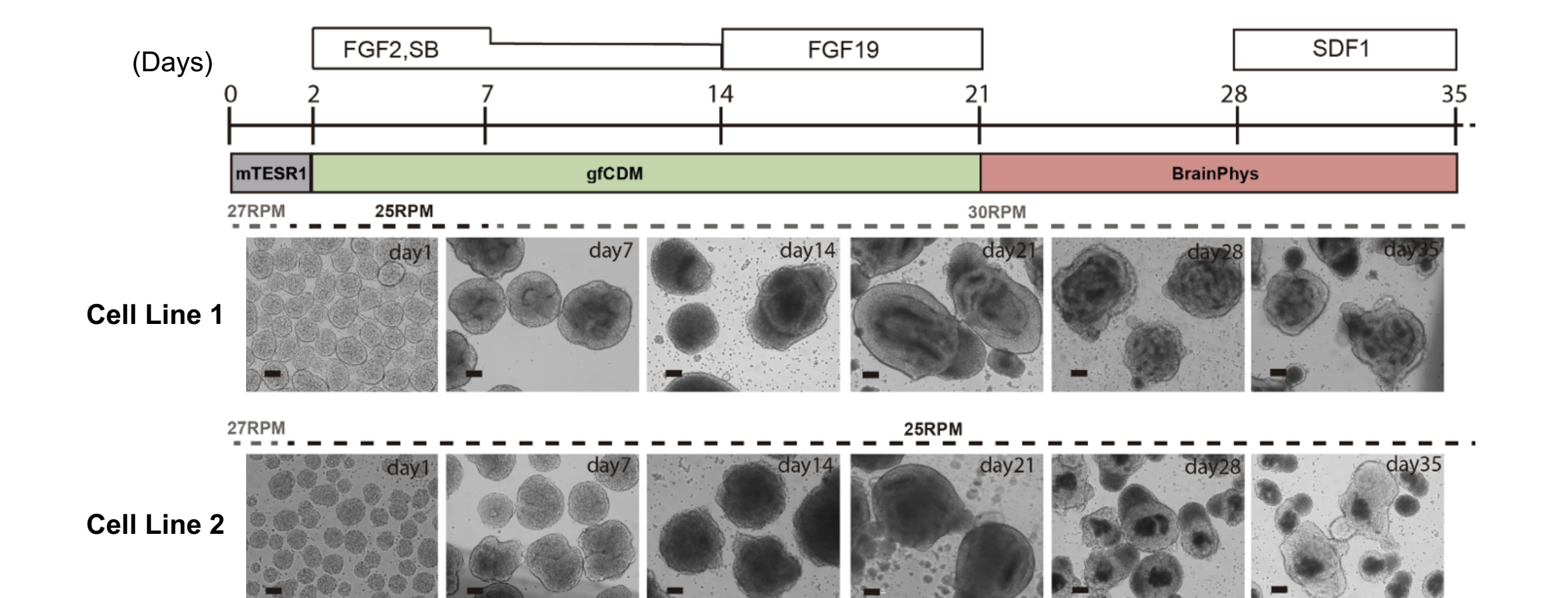


Fig 8. Directed Differentiation of Human iPSCs to Cerebellar Organoids in Vertical-Wheel PBS-0.1



- After 35 days of generation, iPSC-derived organoids were efficiently matured to GABAergic and Glutamatergic neurons (not shown) in PBS-0.1 (scale bar, 100 µm)

Conclusions

- The unique geometry and mixing mechanism of Vertical-Wheel bioreactors provide homogeneous energy dissipation distribution, fully suspended mixing with minimal power input, and low shear environments for suspension cell cultures
- For processes involving cells grown on microcarriers or as aggregates, Vertical-Wheel bioreactors provide superior performance of cell growth, control of cell aggregate size and morphology, and cell dissociation after the expansion phase
- Vertical-Wheel bioreactors have demonstrated unparalleled scalability across a full range of vessel sizes, enabling the scale up of cell therapy manufacturing for commercial production

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