Scale-Up of Pluripotent Stem Cell Cultivation and Differentiation in A Novel, Single-Use, Vertical-Wheel Bioreactor System

Brian Lee*, Yas Hashimura, Sunghoon Jung, Francisco Rosello, Nathan Starkweather, Robin Wesselschmidt

PBS Biotech, Inc., Camarillo, CA, USA

Abstract

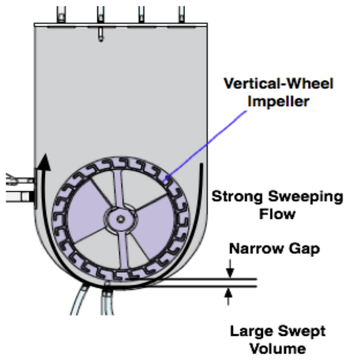
Problems with Conventional Stirred Bioreactors for the Growth and Differentiation of PSCs as Aggregates: Conventional stirred-tank bioreactors (STRs) with horizontal impellers provide inconsistent energy dissipation distribution in a culture fluid, which results in irregular size and morphology of cell aggregates. In addition, STRs require relatively high agitation power in order to fully suspend cell aggregates, which can cause high shear stress to the cells. These facts make it very challenging to achieve satisfactory cell growth and differentiation outcomes of PSCs in STRs. Moreover, these problems with STRs have been shown to become much worse as the size of bioreactor increases.

Innovative Vertical-Wheel Bioreactors at Various Scales Provide Homogeneous Energy Dissipation Distribution and Low-Sheer Environment to Enable the Generation of Uniform Size and Morphology of PSC Aggregates: A novel single-use Vertical-Wheel bioreactor system offers efficient fluid mixing to provide homogenous energy dissipation distribution enabling uniform size and morphology of cell aggregates with low power input. In addition, a desired size of cell aggregates can be achieved by controlling agitation rate under low shear environment. Since the size of cell aggregates is critical for differentiation kinetics as well as the yield and quality of final target cell type, controlling the size of cell aggregates should be crucial for optimal cell growth and differentiation process of PSCs. Furthermore, the innovative vertical mixing mechanism allows the low shear environment to remain constant across a full range of vessel sizes from 0.5 to 80 liters, offering unparalleled scalability to achieve a consistent and robust manufacturing process for shear sensitive PSC products.

Wide Applications of Vertical-Wheel Bioreactors for Scale-Up Cell Therapy Manufacturing: The single-use Vertical-Wheel bioreactors have been evaluated with several different cell types including human mesenchymal stem cells (MSCs) as well as ESCs and iPSCs.

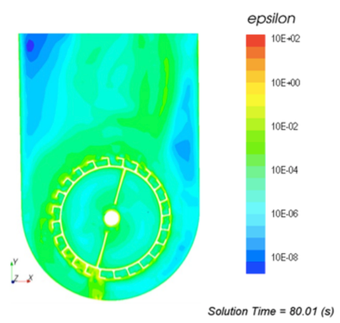
Background

Fig 1. Key Features of Vertical-Wheel Bioreactors



- **Combination of Vertical impeller and U-shape vessel** promote strong, sweeping liquid flow that results in good particle suspension
- **Oppositely-oriented axial vanes** create cutting and folding fluid flow for efficient mixing at very low power inputs
- **Large 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

Formation of Uniform Size and Morphology of PSC Aggregates and Control of Cell Aggregate Size in Vertical-Wheel Bioreactors

Fig 3. Uniform Size Distribution of Cell Aggregates (ESCs, iPSCs) and Control of Average Size by Adjusting Agitation Rate in Vertical-Wheel Bioreactor

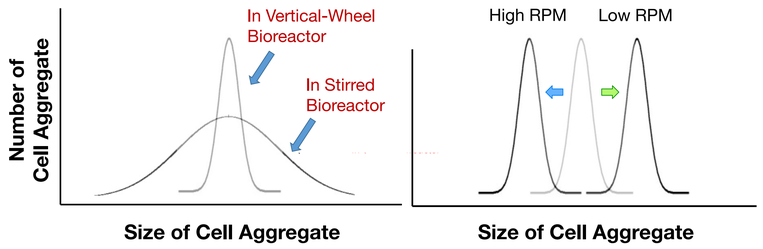


Fig 4. Size and Morphology Comparison of ESC Aggregates Grown in Spinner (Stirred) vs. PBS-Mini (Vertical-Wheel)

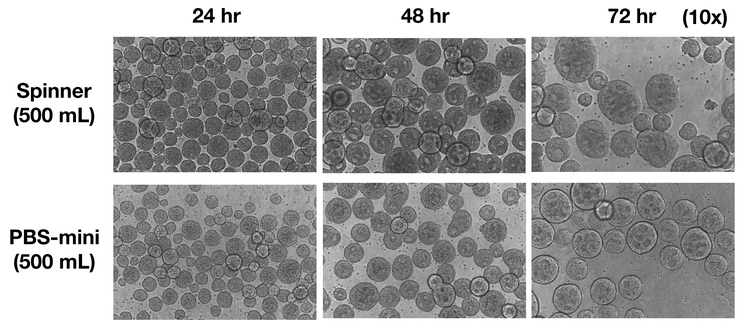


Fig 5. Comparable Size of ESC Aggregates Grown in PBS Bioreactors during Scale Up from 0.5 L to 3.0 L Working Volume

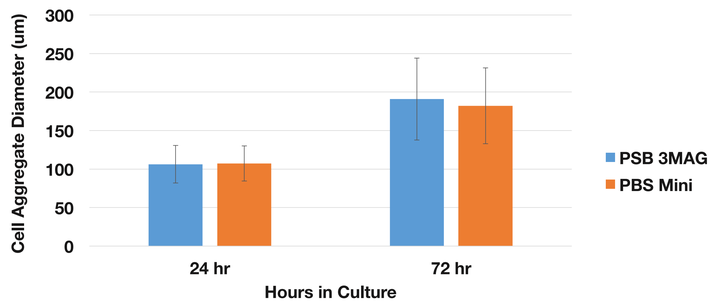
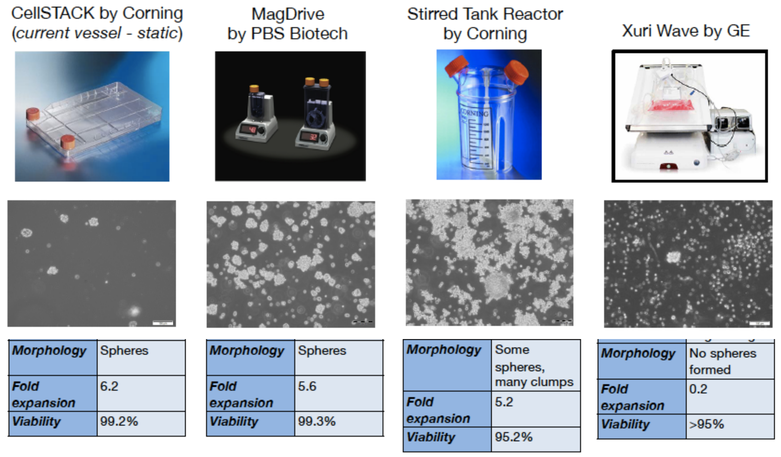


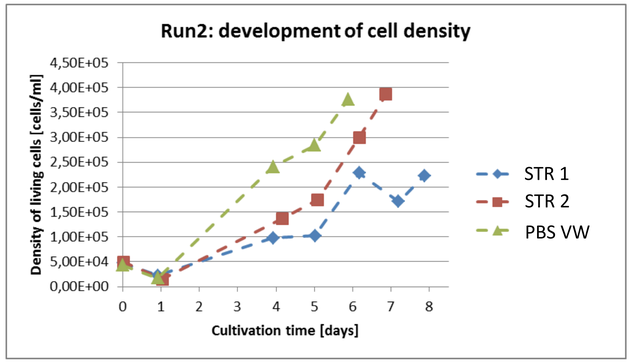
Fig 6. Comparison of Discogenic Cell Growth In Various Bioreactor Types



	CellSTACK	MagDrive	Stirred Tank	Xuri Wave
Morphology	Spheres	Spheres	Some spheres, many clumps	No spheres formed
Fold expansion	6.2	5.6	5.2	0.2
Viability	99.2%	99.3%	95.2%	>95%

Greater Cell Growth on Suspended Microcarriers in Vertical-Wheel Bioreactors with Low Sheer Environment

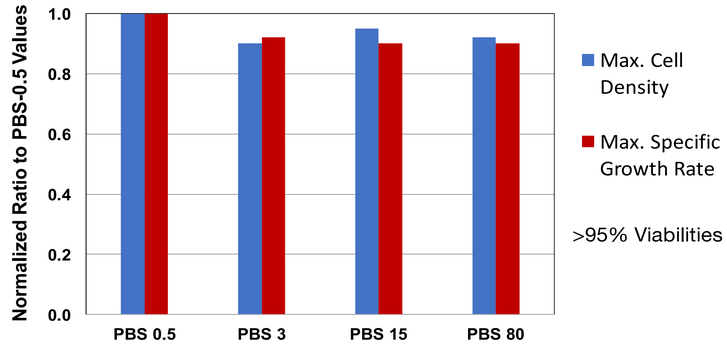
Fig 7. Comparison of MSC Growth in PBS Vertical-Wheel (VW) Bioreactors vs. Commercial Stirred-Tank Bioreactors (STR)



Scale-up of Microcarrier-Based Cell Culture Process in Vertical-Wheel Bioreactor Platform

- Anchorage dependent human primary cells were grown on collagen-coated polystyrene microcarriers in serum-containing medium, in three different scales of PBS bioreactors.
- Each of the different sized PBS bioreactors was run at minimum agitation speeds necessary for full microcarrier suspension.

Fig 8. Consistent Maximum Specific Growth Rates and Maximum Cell Densities in Various Scale Vertical-Wheel Bioreactors



- Comparable cell growth rates and maximum cell densities were achieved in PBS 0.5L, 3L, 15L, and 80L bioreactors.

Conclusions

- Homogeneous energy dissipation distribution inside Vertical-Wheel bioreactors resulted in the growth of PSCs and other cell types as aggregates with uniform size and morphology, and a desired size of cell aggregates could be achieved by controlling the agitation rate.
- The minimum required power inputs necessary for complete suspension of particles such as cell aggregates and microcarriers are well below the critical threshold of hydrodynamic damage to cells grown in Vertical-Wheel bioreactors (0.5 L – 80 L).
- Microcarrier-based, anchorage-dependent cell culture processes were successfully scaled up to 50 L working volume. The Vertical-Wheel bioreactors led to higher growth rate of MSCs and other primary cells, compared conventional stirred bioreactors

The Authors thank Semma Therapeutics, DiscGenics, Apceth Biopharma, and Kolon Life Science for providing their experimental data.