A New Paradigm Of Scalable Manufacturing For Cell Therapy Products

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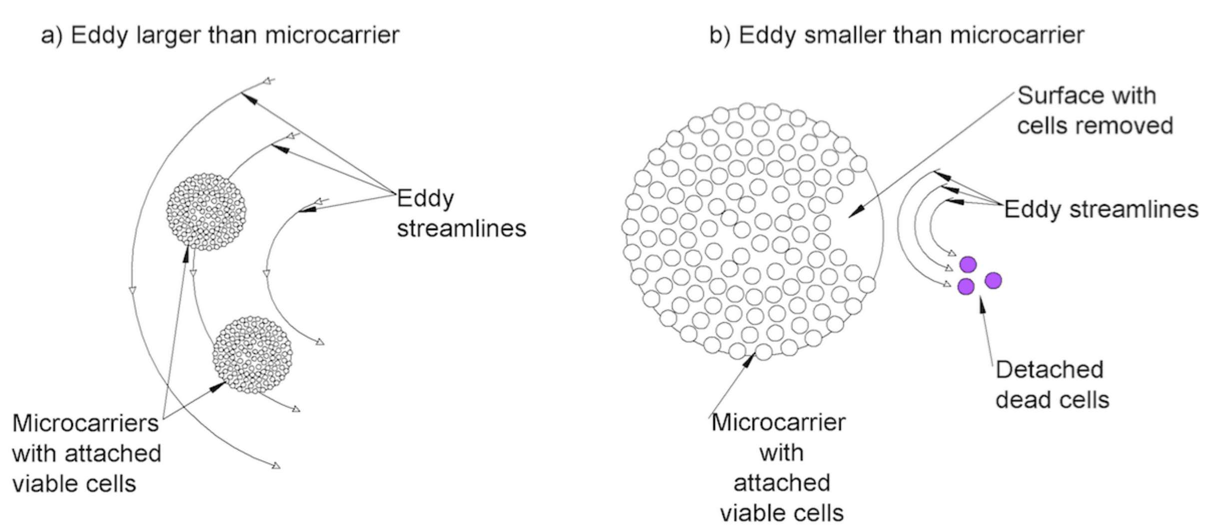
Introduction

One of the major issues regarding scale up of mammalian cell culture process is the potential negative effect of hydrodynamic shear stress on cells as vessel size increases. In particular, anchorage-dependent cells grown in aggregates or on microcarriers can be more sensitive to and damaged by shear stress.

Fig 1. Mechanism of Hydrodynamic Damage by Kolmogorov Eddy Scale

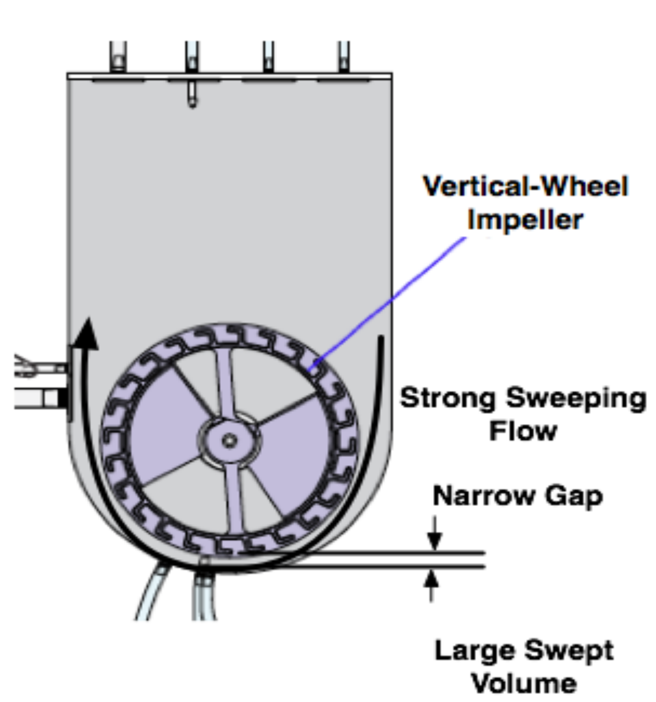


- When the Kolmogorov eddy scale is large enough, microcarriers are swept along with fluid flow and cells do not experience strong shear forces. In contrast, when eddy scale is smaller than the size of microcarriers, cells on the surface can experience damaging shear forces.
- As eddy scale becomes smaller than 2/3 the diameter of microcarriers, growth rate of human fibroblast (FS-4) cells on the surface sharply decreases due to the increasing shear forces.

Low-Shear, Vertical-Wheel™ Bioreactor Technology

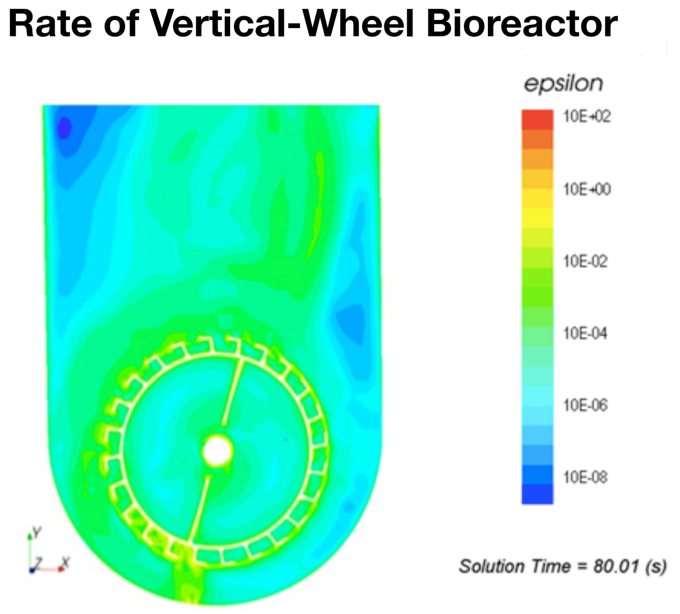
In order to suspend microcarriers uniformly, conventional stirred-type bioreactors require high power inputs and agitation speeds, resulting in increased shear stress. A family of novel PBS single-use bioreactors, with Vertical-Wheel mixing technology and a wide range of working volumes, has been introduced that can achieve homogenous mixing and microcarrier suspension with low power input and much reduced shear stress. Furthermore, the PBS-Mini (0.1 L and 0.5 L units) can be a representative scale-down model of larger size system and used for rapid development of microcarrier processes.

Fig 2. 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 3. 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

Experimental Approach and Results

Determining Mixing Power, Kolmogorov Eddy Scale and Agitation Rate for Complete Off-Bottom Suspension

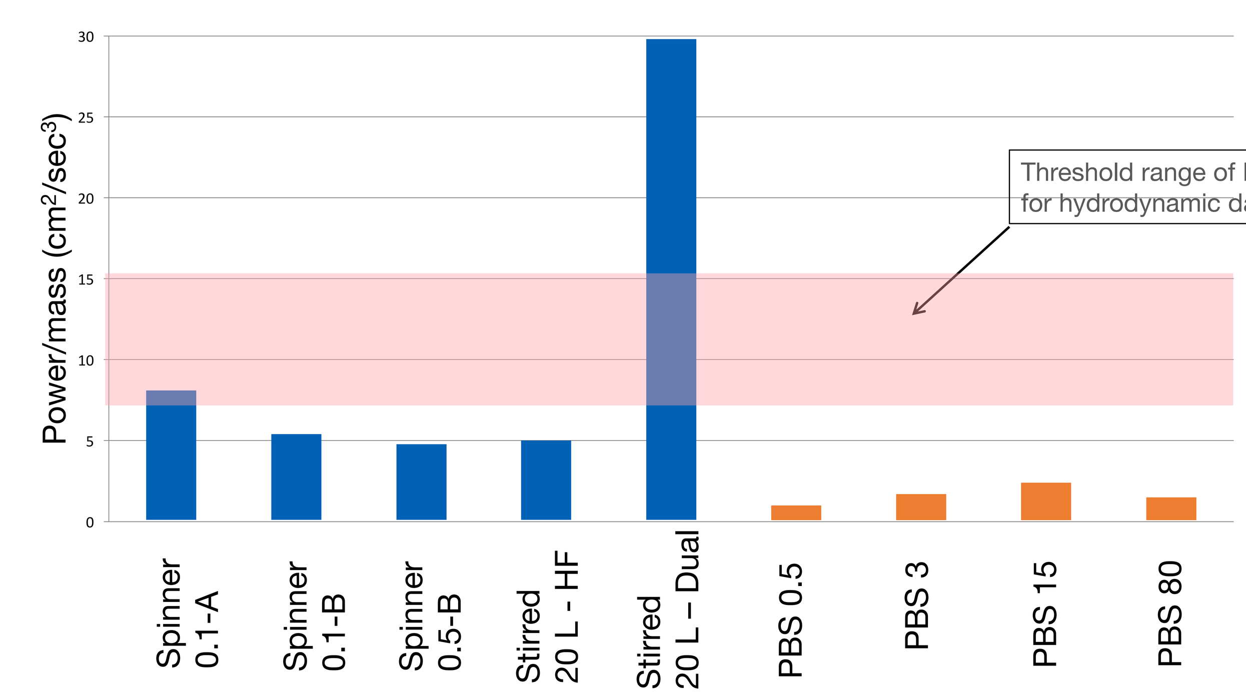
- Mixing power at various impeller speeds was precisely measured using a hydrodynamic balance
- The Kolmogorov eddy scale was then estimated using the following equation:

$$\eta = \left(\frac{\nu^3}{\epsilon}\right)^{1/4}$$

where
η = Kolmogorov eddy scale
ν = kinematic viscosity
ε = mixing power per unit mass

- The minimum agitation rate for complete off-bottom suspension of microcarriers was determined by careful visual observation with the use of a submersible bore-scope.

Fig 4. Comparison of Power/Mass (P/M) Levels at Minimum Agitation Rates Necessary for Off-Bottom Suspension in Various Type and Size Bioreactors



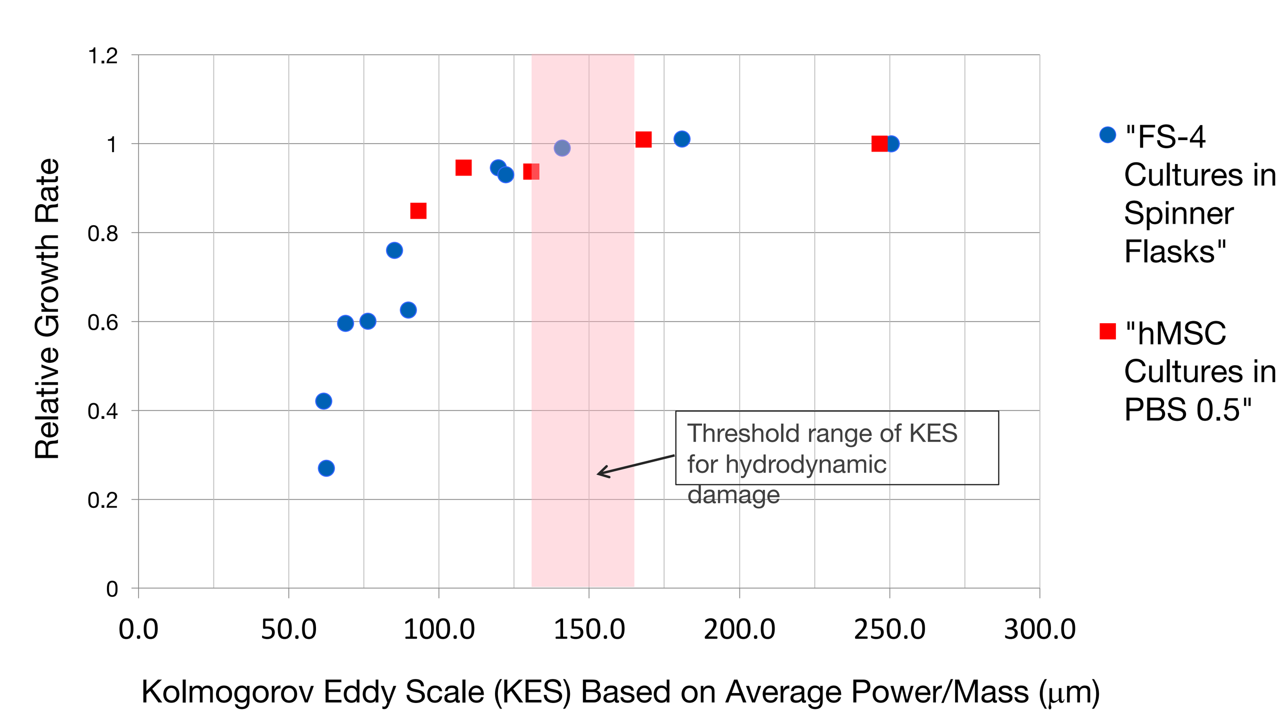
- The PBS systems have significantly more margin below the critical threshold, above which cell damage occurs.

Determining the Shear Sensitivity of hMSCs

Critical Kolmogorov eddy scales for the growth of human bone-marrow derived mesenchymal stem cells (hBM-MSCs) on collagen-coated polystyrene microcarriers were determined by growing them at various agitation rates in the PBS-0.5 bioreactors.

- Cells and Medium: hBM-MSCs and High Performance Media Kit (Rooster Bio)
- Microcarriers: Collagen-coated polystyrene microcarriers at 14 g/L, (Solohill C102-1521, Pall Corporation)
- Agitation speed: Set to 15, 25, 35, 45, and 55 RPM after seeding is complete

Fig 5. Correlation of Kolmogorov Eddy Scale and Growth Rate of Cells Grown on Microcarriers

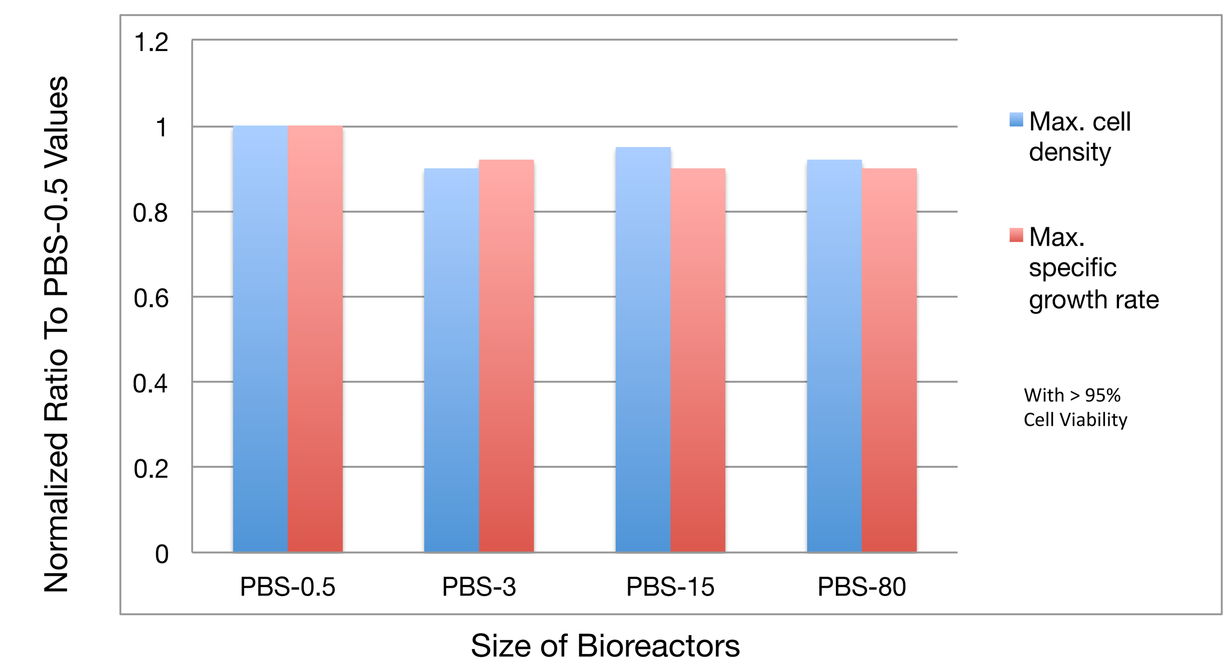


- Relative growth rate was plotted against Kolmogorov eddy scale and the threshold range of KES for hydrodynamic damage was determined to be 130-165 μm.

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

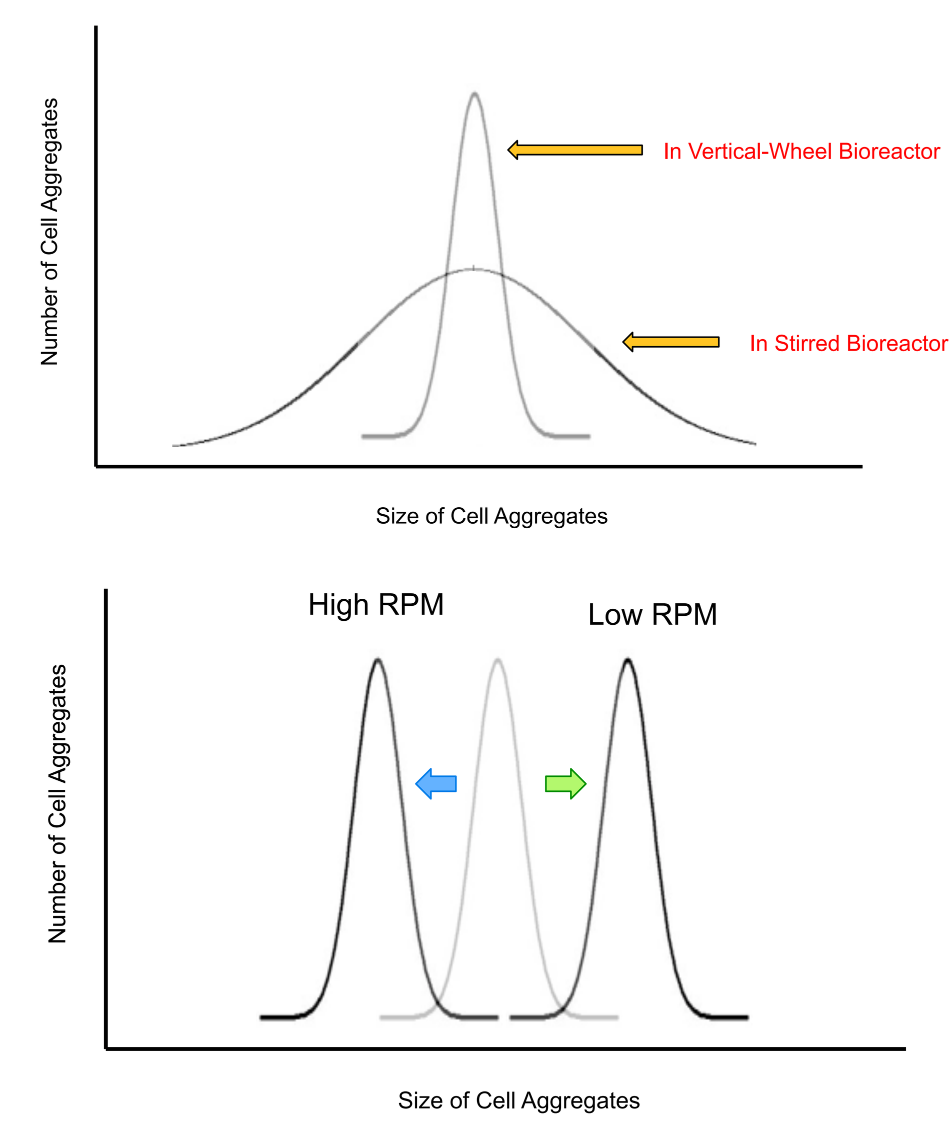
- Anchorage dependent human primary cells were grown on collagen-coated polystyrene microcarriers in serum-containing medium, in three different scales of PBS bioreactors.
- Each of the different sized PBS bioreactors was run at minimum agitation speeds necessary for full microcarrier suspension.

Fig 6. 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.

Fig 7. Uniform Size Distribution of Cell Aggregates (ES, iPS) and Control of Average Size by Adjusting Agitation Rate in Vertical-Wheel Bioreactor



Conclusion

- The minimum required power inputs necessary for complete particle suspension are well below the critical threshold of hydrodynamic damage in Vertical-Wheel bioreactors (0.5 L – 80 L).
- By using the PBS 0.5 as a scale-down model, shear sensitivity of specific cell types grown on microcarriers or as aggregates can be estimated, which helps predict process scalability in bioreactors.
- A microcarrier-based, anchorage-dependent cell culture process was successfully scaled up to 10 L working volume by achieving comparable growth characteristics in various sizes of Vertical-Wheel bioreactors.
- Narrow range of turbulent energy dissipation rates inside Vertical-Wheel bioreactor provided uniform size distribution of cell aggregates, and the desired size of cell aggregates can be controlled by agitation rate.