Engineering Characterization of a Versatile Vertical-Wheel Bioreactor for Cell and Gene Therapy

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Introduction

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) Often Present Problems for Cells Growing on Microcarriers or as Aggregates:

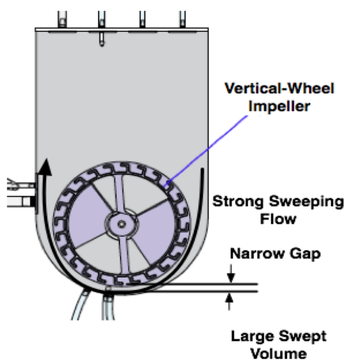
- Many STRs require relatively high agitation power input to fully suspend aggregates or microcarriers
- Cells growing on microcarriers or as aggregates in suspension culture can be sensitive to the fluid shear stress induced by the hydrodynamic flow in STRs
- The fluid shear stress in STRs can negatively 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 can 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

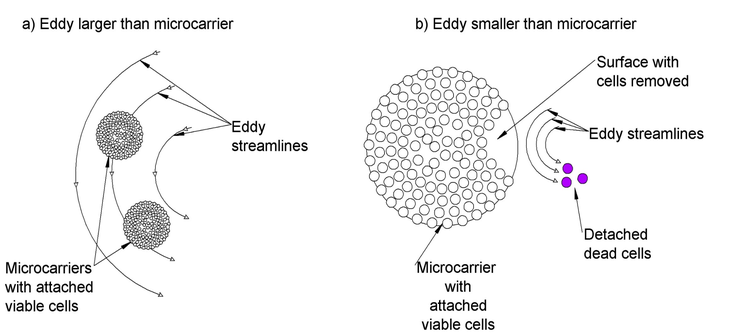
Background

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

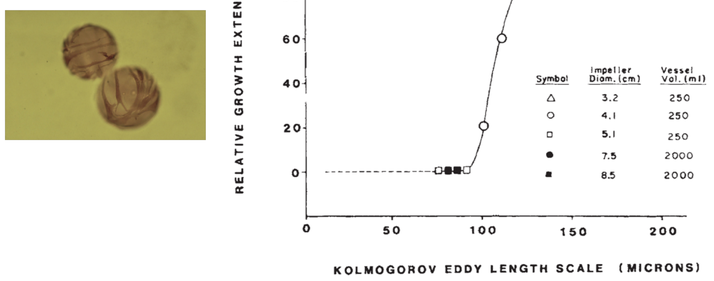
Fig 2. Effect of Kolmogorov Eddy Length on Cell Death and Detachment from Microcarriers, as Originally Proposed by Croughan et al. and Confirmed for FS-4 cells



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

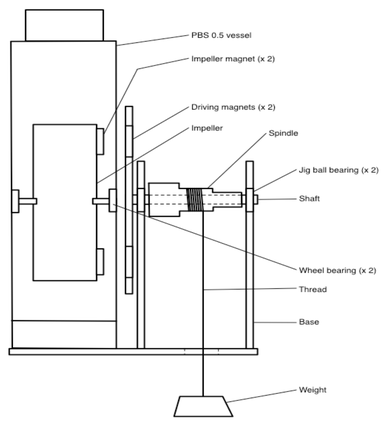
Experimental Results

Fig 3. FS-4 cells Growing on Cytodex 1 Microcarriers and Effect of Kolmogorov Eddy Length Scale on Relative Growth Extent in Stirred Spinner STRs with Various Geometries (*Croughan et al, 1985, 1987, 2000, 2006*)



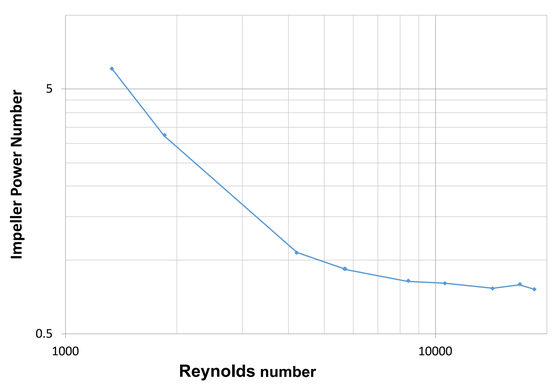
- Hydrodynamic damage becomes evident when Kolmogorov eddy length is less than 130 microns
- Does this still hold true for Vertical-Wheel Bioreactors (a new geometry)? The answer to this question is yes (Figure 6)
- To confirm, we needed power measurements for Vertical-Wheel bioreactors; Figures 4 and 5 show power measurement approach and power curve results for a Vertical-Wheel bioreactor

Fig 4. Power Measurement Approach



- Approach validated by measuring power for standard STR with Rushton impeller in turbulent regime:
- 1. Power number expected: 5 - 6
- 2. Power number measured: 5.55
- 3. Exponent on log P vs. N expected: 3
- 4. Exponent on log P vs. N measured: 3.1
- Note: P = power, N = impeller rotation rate

Fig 5. Power curve for Vertical Wheel Impeller in PBS 0.5 Mini



- Power number does not plateau until it hits about 0.8 at Reynolds numbers of approximately 8,000-10,000 or higher; this transition to a fully turbulent regime occurs at a relatively high Reynolds number versus standard STRs
- Note: Power number = $P/(N^3 D_i^5 \text{density})$, where D_i = impeller diameter
- Note: Reynolds number = $ND_i^2/(\text{kinematic viscosity})$

Fig 6. Effect of Kolmogorov Eddy Length on Relative Growth Extent for FS-4 Cells Growing on Cytodex 1 Microcarriers in Stirred Spinner STRs at Various Viscosities (*Croughan et al, 1989*), as well as MSCs on Solohill Plastic Plus Microcarriers in PBS-0.5 Bioreactors (Including Laminar Flow Regime)

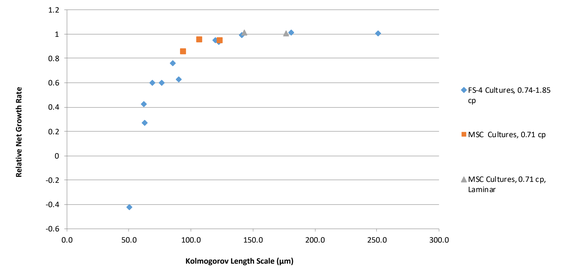
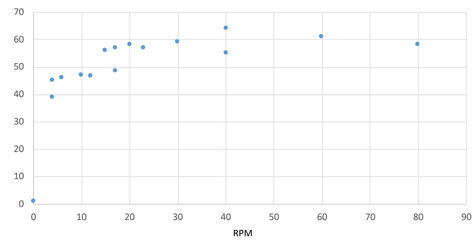
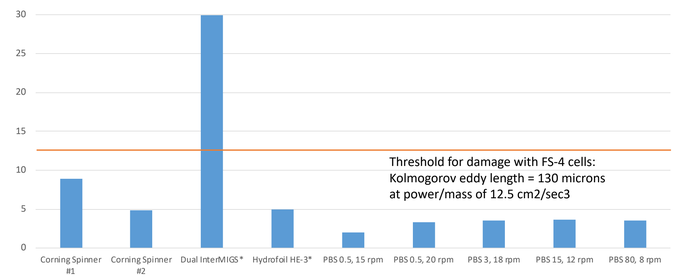


Fig 7. Microcarrier Suspension Study



- Approach: count microcarriers in suspension with increasing agitation until plateau reached; each point shown is average of counts for six 0.2 ml samples from midpoint in bioreactor
- Results: Count plateau with agitation of 20 rpm or higher, indicating all microcarriers are in suspension (also confirmed visually); range of 15- 20 rpm has been successfully used for many cultures

Fig 8. Minimum Power/Mass (cm^2/sec^3) Required For Microcarrier Suspension



- Compared to STRs, Vertical-Wheel bioreactors across all scales can fully suspend microcarriers at very low levels of power/mass, in the range of 2-3.5 cm^2/sec^3, which is far below the damage threshold for FS-4 cells and many other cell lines such as MSCs
- Data shown for Vertical-Wheel bioreactors of various sizes along with Corning spinner STRs (*Croughan et al, 1989*) and a range of 20-L STRs* (worst and best cases, respectively, from *Ibrahim and Nienow, 2004*).

Conclusions

- A system to measure the power input from Vertical-Wheel impellers was developed and validated, and the resulting power curve showed a late transition into the turbulent regime, with a plateau power number of approximately 0.8
- Vertical-Wheel impellers can be operated at sufficiently high power levels to remove cells from microcarriers, either viably or non-viably, depending upon the cells, microcarriers, and conditions; just like with STRs, hydrodynamic damage correlates with Kolmogorov eddy length scale
- Vertical-Wheel bioreactors can suspend microcarriers at very low levels of power/mass, corresponding to a Kolmogorov eddy length scale of approximately 180 microns for the larger bioreactors (3L and up) in the turbulent flow regime