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

Experimental Results


- 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)?
- 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/ρD³), where D = impeller diameter
- Note: Reynolds number = ND²/(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)

- 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 7. Microcarrier Suspension Study

- 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 cm²/sec, 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 2-5 STRs* (worst and best cases, respectively, from Ibrahim and Nilenow, 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 viable or non-viable, 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