Scale-Down Model Screening Study of Key Process Parameters for Anchorage-Dependent Stem Cells Using Vertical-Wheel Bioreactors

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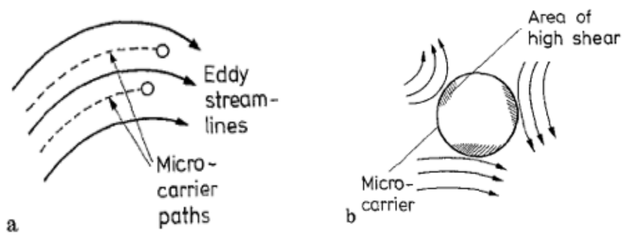
Introduction

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

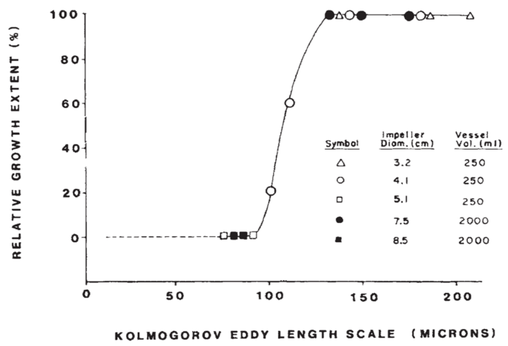
Correlation of Kolmogorov Eddy Length and Shear Stress on Microcarriers



R. S. Cherry and E.T. Papoutsakis. *Bioprocess Engineering*, 1986

Fig 1. When the Kolmogorov eddy length is large enough, microcarriers are swept along with fluid flow and do not experience shear forces. In contrast, when eddies are smaller than the size of microcarriers, cells on the surface can experience damaging shear forces.

Effect of Eddy Size on Adherent Cell Growth on Microcarriers

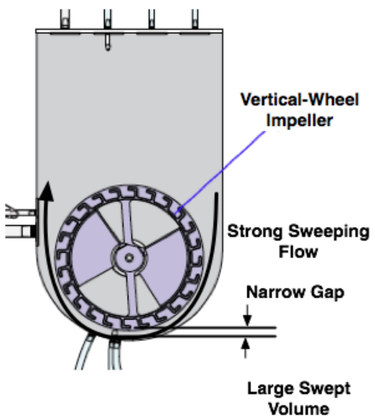


Croughan, et. al. *Hydrodynamic Effects on Animal Cells Grown in Microcarrier Cultures,* 2006

Fig 2. As eddy length becomes smaller than 2/3 the diameter of microcarriers, cell growth 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 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 (0.1L and 0.5L units) can be a representative scale-down model of larger size system and used for rapid development of microcarrier processes.



- **Combination of vertical impeller and U-shaped 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 size** produces a large swept volume, resulting in low maximum turbulent energy dissipation rate and gentle mixing

Fig 3. A Diagram and Key Features of Vertical-Wheel Bioreactors

Materials and Methods

Human bone marrow-derived mesenchymal stromal cells (hBM-MSCs) (RoosterBio) were expanded in T-225 flasks (Corning Life Sciences) using the hBM-MSC High Performance Media Kit (RoosterBio). The flasks were seeded at 3000 cells/cm^2 and incubated at 37°C in a humidified, 5% CO_2 atmosphere for 3 or 4 days. Cells were harvested by 5 minute incubation with TrypLE (Life Technologies) following a Phosphate Buffer Saline wash. PBS-MINI bioreactors of 0.1 L and 0.5 L (PBS Biotech) containing 100 mL and 300 mL culture medium, respectively, and 16 g/L collagen-coated polystyrene microcarriers (Solohill C102-1521, Pall Corporation) were then inoculated with the seed culture. Continuous agitation at 20 RPM (PBS-0.1) and 15 RPM (PBS-0.5) were used during the seeding. On the following day, the agitation speed was adjusted to the values shown in Fig 6. Two medium exchanges (50%) were performed on days 5 and 8. Cell growth was monitored after removing cells from microcarriers using TrypLE, staining with acridine orange and propidium iodide, and counting the cells using a Cellometer Auto 2000 Cell Profiler (Nexcelom Bioscience) .

Fig 4. PBS-MINI units in operation inside a humidified CO_2 incubator. PBS-0.1 units are in the foreground and PBS-0.5 are in the back.

Mixing power at various impeller speeds was measured using an apparatus that was magnetically coupled to the impeller and that was powered by a known mass falling at a constant velocity for each impeller speed. The net mixing power was calculated from this measurement by subtracting the minimum power to overcome the friction of the jig and the impeller bearing. The Kolmogorov length scale was then estimated using the following equation:

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

where,

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

Results

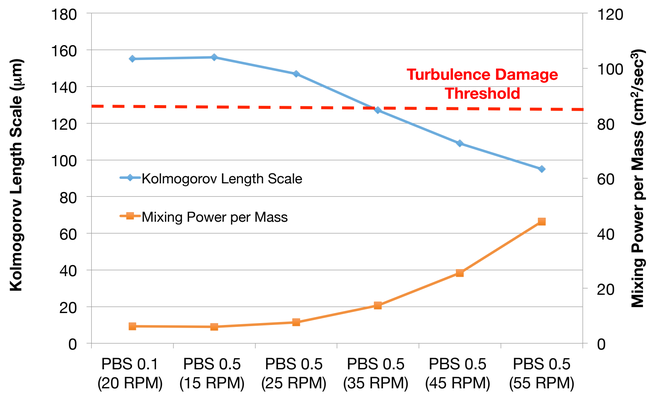


Fig 5. Measured values of mixing power per mass and calculated Kolmogorov length scale of PBS-MINI units at various agitation speeds.

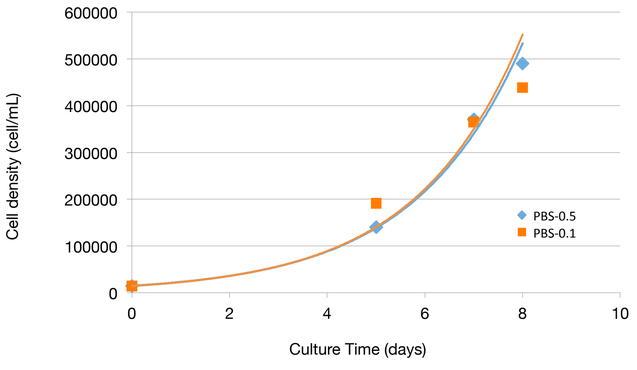


Fig 6. Both PBS-0.1 and PBS-0.5 units show equivalent cell growth rates and similar maximum densities at 20 RPM and 15RPM, respectively, with doubling times of 1.5 days. These values represent expansion ratios of 30 to 34-fold.

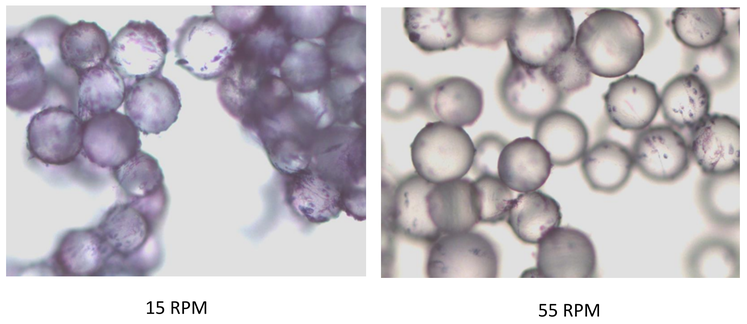


Fig 7. Representative images of hBM-MSCs on microcarriers sampled from the PBS-0.5 bioreactors on day 7 clearly illustrate the differences in cell number and extent of cell aggregation between the two agitation rates. Cells were stained with MTT prior to visualization with a light microscope at 100X magnification.

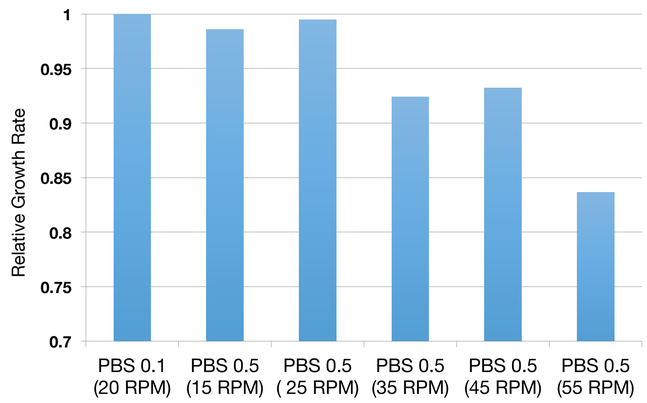


Fig 8. Increasing the power input with higher agitation speeds in PBS-0.5 units demonstrates decreased specific growth rate of hBM-MSCs on microcarriers.

Discussion

- PBS Vertical-Wheel bioreactors can provide low shear mixing environments suitable for anchorage-dependent cell growth on microcarriers and aggregates.
- As scale-down models of large size Vertical-Wheel bioreactors, PBS-MINI 0.1L and 0.5 L units were used to grow hBM-MSCs on microcarriers across a range of mixing powers and agitation speeds and can function as scale-down models for larger size Vertical-Wheel bioreactors to evaluate the effect of shear stress on cell growth rates and maximum cell densities.
- Increasing agitation speeds in PBS-MINI units confirmed the correlation between Kolmogorov eddy length and resulting damage to cells on surfaces of microcarriers.

Conclusion and Future Study

- Development and scale-up of cell culture processes involving growth on microcarriers can be challenging due to potential issues with shear stress. This study has shown that anchorage-dependent cells can be successfully grown on microcarriers in PBS-MINI units. These small-scale bioreactors are an efficient and cost-effective option for screening of various culture conditions and parameters, ranging studies, and other key process requirements.
- In addition, PBS-MINI units can serve as scale-down models as they are representative of the mixing performance and cell culture environments of larger volume Vertical-Wheel bioreactors. Thus, there is an unique potential opportunity for successful scale-up of cell culture processes using Vertical-Wheel bioreactor technology, with future work focusing on scaling up processes to 3 L, 15L, 80 L units, and beyond.