Screening Study of Key Process Conditions for Anchorage-Dependent Stem Cell Cultivation in Scale-Down Model of Vertical-Wheel Bioreactors

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Abstract

Large-scale manufacturing of stem cells using microcarriers in single-use bioreactors is considered a possible way to meet the projected commercial demand for some allogeneic cell therapies. However, the high cost of stem cell media and the requirement of numerous process development and optimization runs make it difficult to develop and optimize manufacturing processes, even at 2 L bench-top scale. An innovative single-use bioreactor with Vertical-Wheel technology was recently introduced for stem cell manufacturing applications, and its scale-down models show promise for efficient and economical screening of process parameters with the goal of scaling-up to larger Vertical-Wheel bioreactors. With 100 mL and 500 mL unit sizes, these scaled down bioreactors are designed to work inside CO_2 incubators that can control temperature, pH, and dissolved oxygen levels, while the agitation rate of each bioreactor is independently controlled. A key feature of these scaled down units is that they represent the physical environments of the larger size bioreactors; all Vertical-Wheel bioreactors can suspend microcarriers uniformly with low shear stress and minimal power input. Screening and ranging studies of key process parameters can be performed in these scaled down bioreactors, permitting more facile and efficient optimization of microcarrier concentration, seeding conditions, media formulation, media exchange regime, and in-reactor cell harvest methods. Calculations between cell growth and Kolmogorov scale can also be used and applied to predict appropriate agitation speeds in order to avoid hydrodynamic shear stress damage to cells during process scale up.

Introduction

PBS Biotech produces single-use, Vertical-Wheel bioreactors that are suited for cell therapy applications such as growth of anchorage-dependent cells on microcarriers. In order to aid the transition from existing 2D to new 3D cell culture processes, PBS has created a line of scaled-down bioreactors. These PBS Mini bioreactors are simplified versions of larger, fully instrumented units and utilize the same Vertical-Wheel technology. With a working volume range from 60 ml to 500 ml, these scaled down models are designed to run in a controlled environment incubators. In order to be a truly scalable platform for process development, scaled down models must be representative of the cell culture environment in larger size units. Thus the following parameters were tested in PBS Minis to demonstrate their equivalence in larger Vertical-Wheel bioreactors:

1) Biological performance
2) Kolmogorov length scale
3) Shear rates (by Computational Fluid Dynamics)
4) Mass transfer

Fig.1. PBS Minis ~~bases~~ in operation in a humidified CO_2 incubator. PBS-0.1 units are running in the foreground while PBS-0.5 units are in the back. The cultures contain hMSCs growing on/attached to Pall (Solohill) collagen-coated polystyrene microcarriers.

Although many/some of these studies are ongoing, the importance of these parameters for a potential 3D scalable platform, as well as current hMSC culture data, will be presented.

PBS Vertical-Wheel™ Mixing Technology

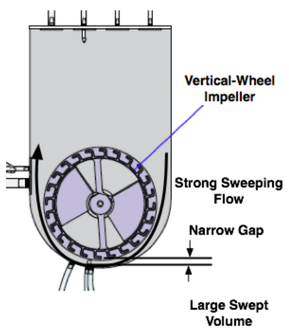
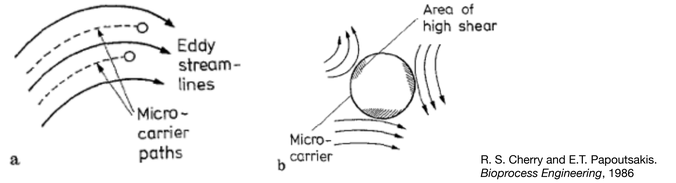


Fig.2. Vertical-Wheel Schematic

- Strong, sweeping flow created by the vertically-oriented impeller results in good particle suspension
- Oppositely-oriented axial vanes create a cutting and folding action that results in excellent mixing at very low power inputs
- Large impeller has a swept volume of 22% to 33% of the maximum working volume of the bioreactor, resulting in a very low maximum turbulent energy dissipation rate and very gentle mixing.



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

Fig 3. Illustration of how Kolmogorov length scale, or minimum eddy size, is related to damaging shear. When eddies are larger than a critical size, microcarriers get carried along and do not experience shear forces. In contrast, when eddies are 1/2 to 2/3 the size of the microcarriers, the cells can experience damaging shear forces. (Croughan, et. al. *Hydrodynamic Effects on Animal Cells Grown in Microcarrier Cultures* 2006)

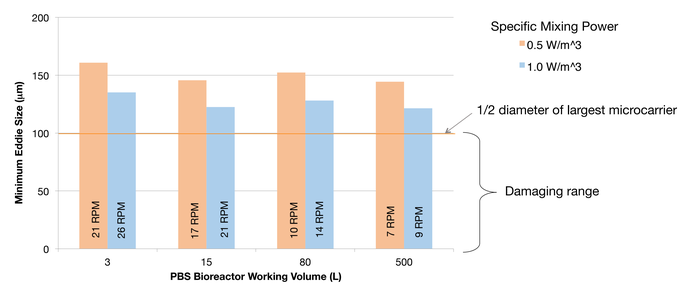


Fig. 4. Estimated minimum Kolmogorov eddy sizes of four PBS Bioreactors at two specific power inputs. The minimum mixing power required to suspend Cytodex 1 or Pall (Solohill) polystyrene microcarriers of specific density = 1.02 is less than 0.5 Watts/m^3 for all systems tested to date (PBS 3, 15 and 80). This power input corresponds to an estimated Kolmogorov length scale greater then 150 μm, which is well above the damaging range for these microcarriers.

Materials and Methods

A vial of BM-hMSCs from a working cell bank (RoosterBio, Inc.) was thawed and passaged once in T-225's (Corning Life Sciences) using the hBM-MSC High Performance Media Kit (RoosterBio, Inc.) Flasks were seeded at a density of 3000 cells/cm^2 and incubated at 37 °C in a humidified, 5% CO_2 atmosphere for 3 or 4 days. Cells were detached from the flask through a 5 minute incubation with TrypLE (Life Technologies) following a PBS wash. The bioreactors (PBS-0.5 Mini and PBS-3) as well as 500 mL single-use spinner flasks (Corning Life Sciences) were batched with medium and microcarriers (Solohill model C102-1521, Pall Corporation) and then inoculated with the single-cell hMSC suspension. The working volumes were 2L for the PBS-3 and 300 mL for the PBS-0.5 and control spinner flasks, with a microcarrier loading of 16 g/L in all cases. Continuous agitation began immediately upon adding the cells and the rates were 15 rpm for the PBS-0.5 and PBS-3 and 30 rpm for the spinner flasks. Agitation in the spinner flasks was increased to 50 rpm after 24 hours. A 50% medium exchange was performed for all cultures on days 5 and 8. Proliferation and cell yield curves were generated by withdrawing samples from the culture vessels, removing cells from microcarriers using TrypLE incubations, staining with trypan blue, and performing manual counting using a hemocytometer.

Results and Discussion

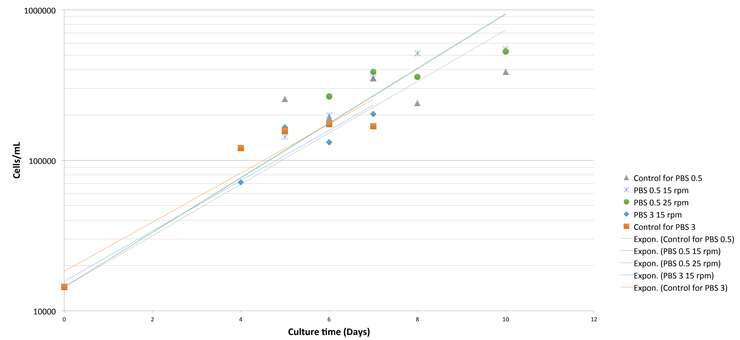


Fig. 5. Growth curves of PBS-0.5, PBS-3, and corning spinner flasks. Curves show equivalent growth rates and similar maximum densities in all cases, with doubling times from 1.7 to 1.8 days and maximum densities from 400,000 to 500,000 cells/mL. These values represent expansion ratios of 28 to 34 fold.

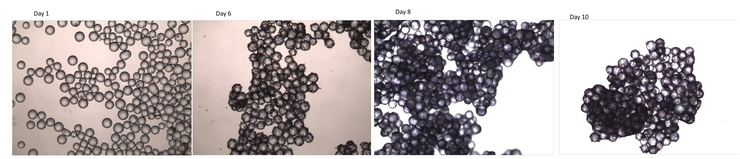


Fig. 6. Representative images of hBM-MSCs on microcarriers sampled from the bioreactors from Day 1 to Day 10. Cells were stained with MTT prior to visualization with a light microscope using a 4x objective. Close observation reveals that about 85% of all microcarriers are colonized with cells on day 1, and almost 100% colonized by day 6 . Microcarrier clumping progresses more as cells continue to grow, presumably due to the production of ECM.

Very similar growth rates and doubling times were demonstrated using the same conditions in PBS-0.5 Mini and PBS-3 bioreactors. Efforts to generate and evaluate more data regarding Kolomogorov length scale, shear stress, and mass transfer rates in PBS Minis are ongoing. There are still several important engineering and manufacturing process issues to be solved in order to create a scalable 3D process for microcarrier growth in bioreactors for cell therapy applications, with the ultimate goal being large scale commercial manufacturing. The key to true scalability is that scaled down bioreactor models must be representative and have the same geometry as larger volume units. The ability to run multiple, simple small-scale units simultaneously would greatly facilitate more efficient and economical process development efforts. This approach will greatly improve development of new processes towards clinical study and commercialization for the emerging cell therapy market by saving time and cost. PBS Vertical-Wheel bioreactor are uniquely capable of meeting these aforementioned needs, as evidenced through this study of hBM-MSC growth on microcarriers in small size units.