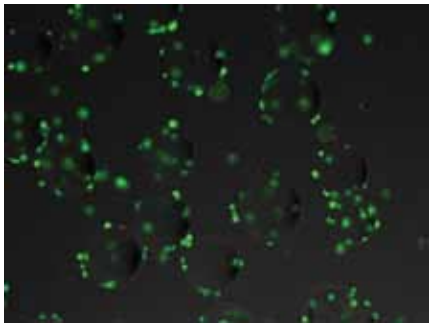
PBS BIOTECH®
Innovative Bioprocess Solutions

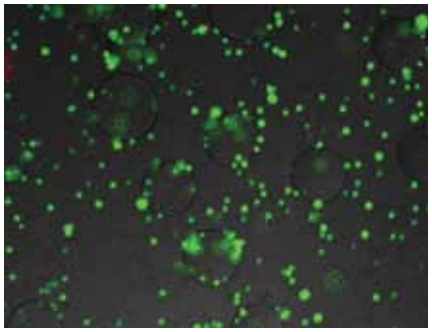
Application Note

Low shear mixing vastly improves attachment and growth of adherent cells on microcarriers

Figure 1. Post-seeding at 2 hrs

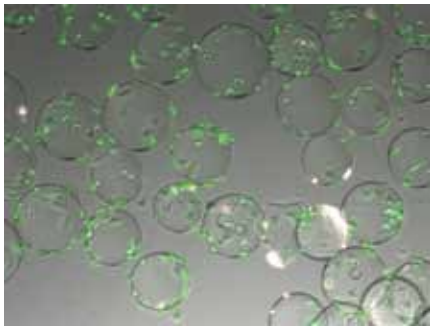


PBS3

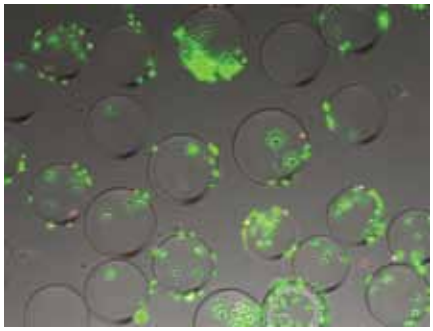


Stirred-tank

Figure 2. Post-seeding at 12 hrs



PBS3



Stirred-tank

Introduction

Microcarriers have played an important role in cell culture for the last 40 years. A number of obstacles have prevented their broader and more efficient adoption in large-scale bioprocessing of cells. One major issue affecting microcarrier cultures stems from mechanical shear forces in standard stirred-tank bioreactors; these forces hinder the attachment of cells to microcarriers, and lead to microcarrier collisions and cell damage.

Because PBS Biotech's Air-Wheel™, single-use bioreactors produce much lower shearing forces, the technology is improving the culture of adherent cell lines in bioreactors. We show the advantage of the low shear mixing of PBS Biotech® bioreactors on cell attachment and growth in a comparative study against a 200 mL traditional stirred bioreactor.

Experiment

Human alveolar adenocarcinoma (A549) cells were cultured on Cytodex-1 microcarriers for up to 150 hrs in a 200 mL standard stirred-tank bioreactor and a PBS Biotech 3L bioreactor. Invitrogen FK12 medium supplemented with 10% FBS was used. At 50 hours post-seeding the microcarriers were infected with an oncolytic adenovirus. Kinetics of attachment were followed for 0, 2, 6, 12 and 24 hours post-inoculation.

Results

Cell attachment was much faster in the PBS System. After 2 hours, almost all the seeded cells were attached to the microcarriers in the PBS system, whereas in the control, a majority of the cells remained unattached (Figure 1). After 12 hours, in the PBS system many of the cells were already taking on their spread morphology, and the seeding was very even, with very few empty microcarriers. In the control, most cells were still rounded and had not yet spread or 'put down' on the microcarriers. The faster and more even seeding of the microcarriers in the PBS systems leads to higher cell growth,

Figure 1. As early as 2 hours post-seeding, most cells in the PBS system have now attached themselves to microcarriers. The cell distribution on microcarriers is very even, and the cell morphology is already beginning to flatten. In stark contrast, in the stirred-tank control the great majority of cells have yet to attach themselves to a microcarrier and most cells remain in suspension.

Figure 2. Comparison of attachment at 12 hours. Cells cultured on Cytodex-1 in the PBS3 bioreactor are evenly distributed, well attached, and already showing the desired 'spread' morphology. Cell cultured in a stirred-tank have not yet spread (they are still rounded), and the seeding is uneven with a combination of cell clumps and microcarriers with very few cells.

cell density and virus titers. Cell density at infection time (Figure 3) and maximum virus productivity measured by $TCID_{50}$ (9.9×10^8 IP/mL vs 5.3×10^8 IP/mL) are nearly double in the PBS bioreactor.

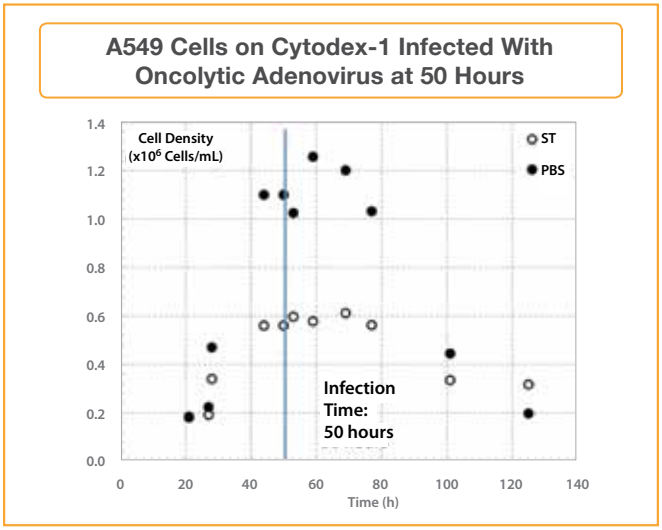


Figure 3. Growth curves for A549 cells attached to Cytodex-1 in PBS3 bioreactor versus a standard stirred-tank bioreactor. The cells cultured in the PBS3 bioreactor achieve double the density of cells cultured in a traditional bioreactor. Viral infection with oncolytic adenovirus occurred at 50 hours.

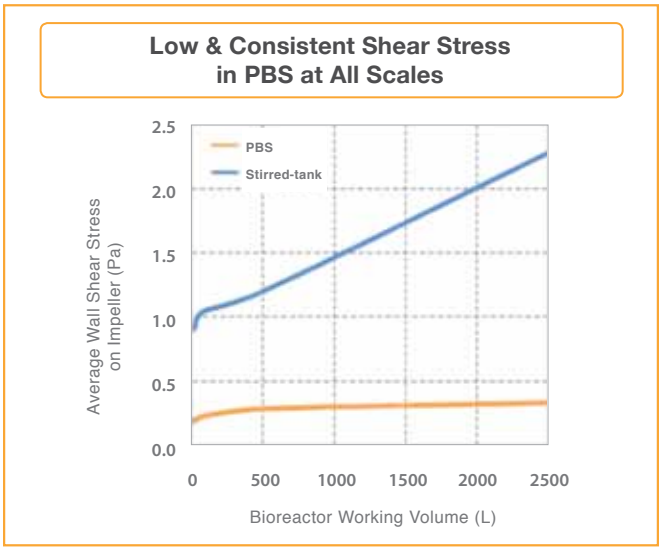


Figure 4: Average wall shear stress on the impeller for PBS systems (orange curve) and traditional stirred-tank systems (blue curve) generated by computational fluid dynamic calculations. The low shear advantage of PBS technology is even greater at larger scales.

A Clear Advantage in Cell Culture

As seen in this study, adherent cells are able to attach and grow faster and to higher densities on microcarriers in PBS bioreactors, leading to greater productivity. PBS Biotech's patented design allows for gentle, efficient mixing of cells, gases, and supplements throughout the bioreactor vessel. The fact that this gentle mixing is very scalable suggests that similar positive results can be achieved at much greater scales with the PBS systems, whereas it is well known that microcarrier scale-up is quite onerous in traditional systems.

References:

1) M. S. Croughan, J.-F. Hamel, D. I.C. Wang, Hydrodynamic Effects on Animal Cells Grown in Microcarrier Cultures Biotechnology and Bioengineering, Vol. 95, No. 2, October 5 2006.

2) G. Kretzmer, Influence of Stress on Adherent Cells Advances in Biochemical Engineering/ Biotechnology, Vol. 67 2000.

Applications and Processes

- Monoclonal antibodies
- Therapeutic proteins
- Viral expansion/expression
- Vaccines
- Stem cells
- Personalized medicine
- Biosimilars
- Batch process
- Fed-batch process
- Perfusion process
- Transient transfection expression

Product Information

- PBS 3 - Volume range of 1.5L-3L
- PBS 15 - Volume range of 7.5L-15L
- PBS 80 - Volume range of 40L-80L
- PBS 500 - Volume range of 250L-500L
- Future Release - PBS 2500 Single-use bioreactor system, (1200-2500L working volume, respectively).

Data courtesy of Instituto de Biologia Experimental Tecnologia, Portugal

Daniel Giroux
Vice President,
Research and Development

PBS BIOTECH®

www.pbsbiotech.com

Phone: +1 805-482-7272 4023 Camino Ranchero, Suite I, Camarillo, California 93012 Fax: +1 805-383-7727