Experimental and Computational Fluid Dynamics Studies of Adherent Cells on Microcarriers in an ambr® 250 Bioreactor

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Introduction

Interest in microcarrier-based processes for large-scale culture of adherent cells has grown, possibly due to increasing attention towards vaccine and cell therapy applications. This opportunity drives the need for effective, high throughput, single-use, process development tools that can be translated successfully into industrial-scale systems. The automated ambr® 250 platform is one such technology, operating at a volume between 100 – 250 mL, it is both high throughput and single-use. The ambr® 250 high throughput has demonstrated significant success for suspension-based mammalian cell culture applications. However, additional investigations need to be performed on microcarrier-based processes for the culture of adherent cells.

The fluid dynamic characteristics of the stirred ambr® 250 bioreactor must be understood sufficiently to enable efficient scale-up to larger bioreactors, particularly because of the special issues arising from the presence of the solid phase.

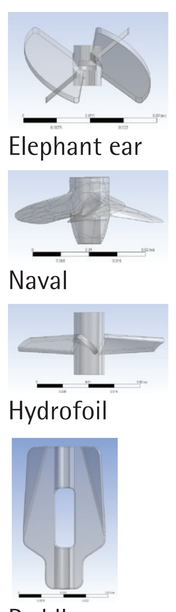
In this work some R&D prototype vessels are investigated for adherent microcarrier cell culture. The prototypes are characterized by different impellers and the vessel can be baffled or unbaffled.

Methodology

- Physical characterization of different ambr® 250 high throughput R&D prototype vessels with microcarriers, to determine power number, energy per unit volume, Reynolds number, minimum suspension speed, Kolmogorov scale.
- Computational characterization: comparison of experimental results with a Eulerian-Eulerian CFD model to investigate mixing efficacy at lower stirring speeds.
- Biological characterization: cell culture with Vero cells on Cytodex 1 microcarriers to confirm that the vessel is suitable for microcarrier cultures, scalable and consistent. Experiments with Vero cells ware performed on an ambr® 250 high throughput with 12 replicates.

List and characteristics of investigated impellers

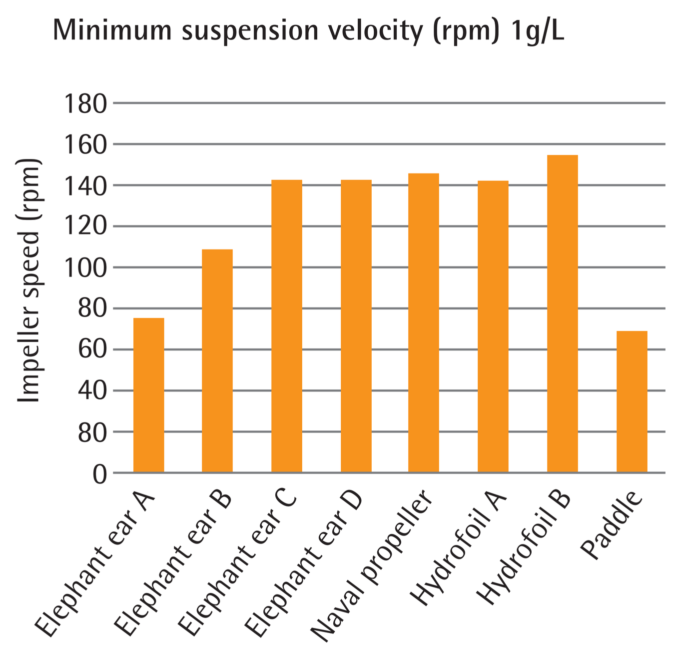
Impellers	Power number
Elephant ear A	2.069
Elephant ear B	0.811
Elephant ear C	1.370
Elephant ear D	0.613
Naval propeller	0.680
Hydrofoil A	0.376
Hydrofoil B	0.434
Paddle	5.325

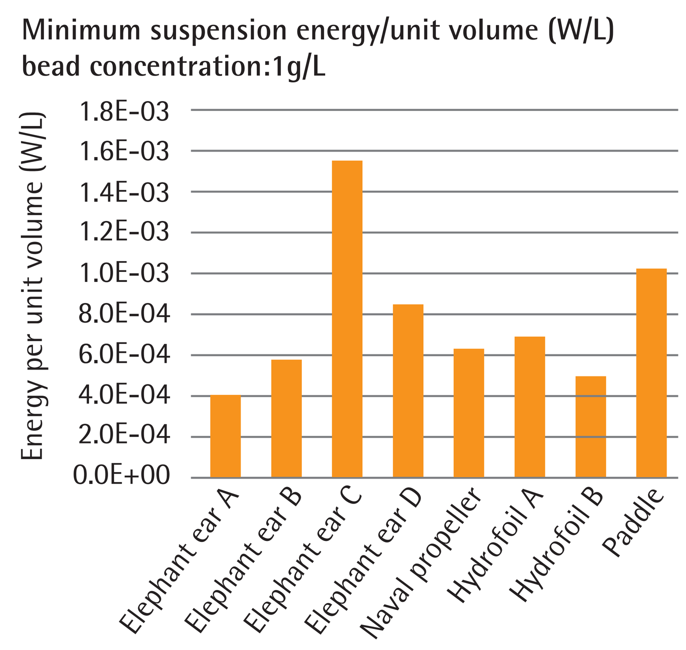


Physical Characterization

- Experimental rig with DC motor and multi-meters
- Special R&D ambr 250 high throughptut vessels
- Determination of the impeller power number (Po)
- Determination of NJS (minimum suspension speed) with Cytodex 1 microcarriers at different solid concentrations (1 to 5 g/L)

Microcarriers dyed with blue dye

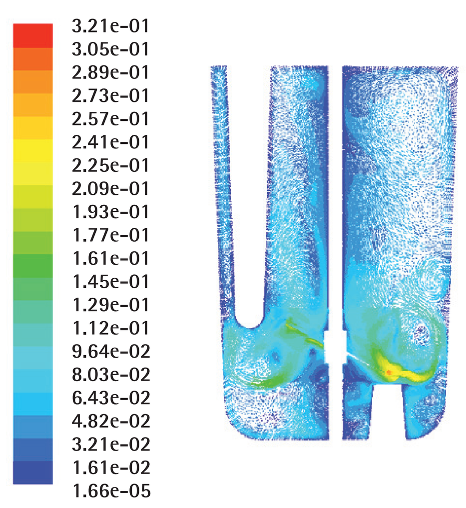




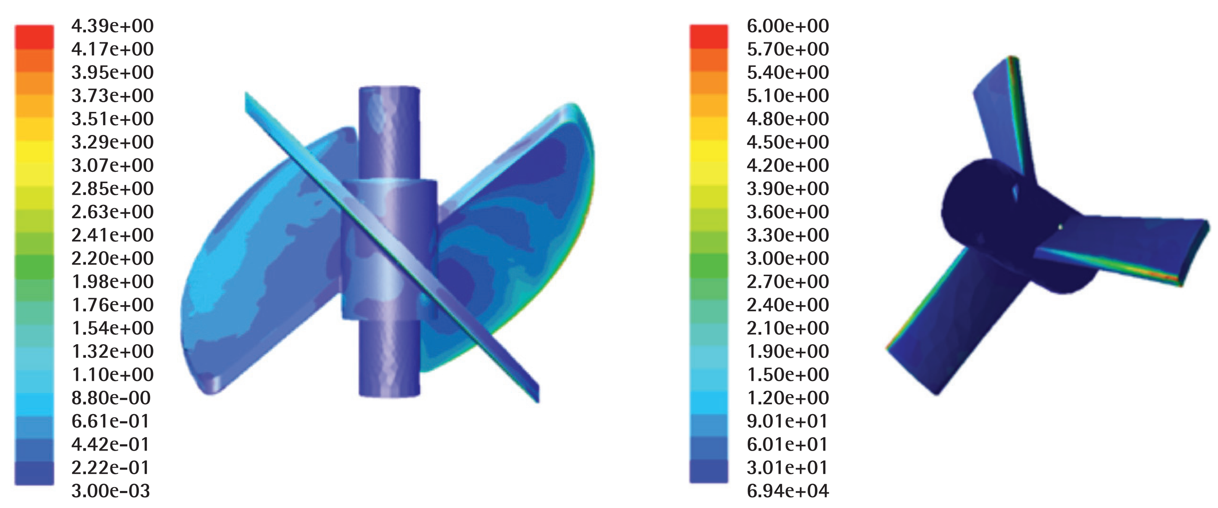
ambr® 250 high throughput vessel

Computational Characterization

- Eulerian-Eulerian CFD simulation, steady state, single liquid phase, various impellers, unbaffled and baffled vessel.
- Analyzing flow pattern, and mixing consistency throughout vessel at low stirring speeds.



Velocity flow pattern (m/s) from a CFD analysis of a Elephant ear vessel

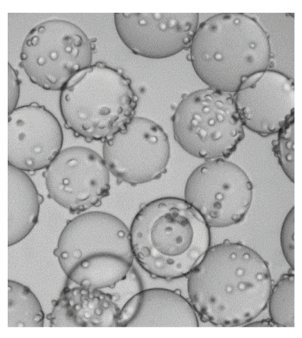


Shear stress (Pa) on Elephant ear and Hydrofoil impellers at NJS

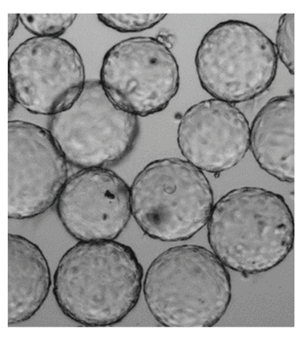
ambr® 250 high throughput

Biological Characterization

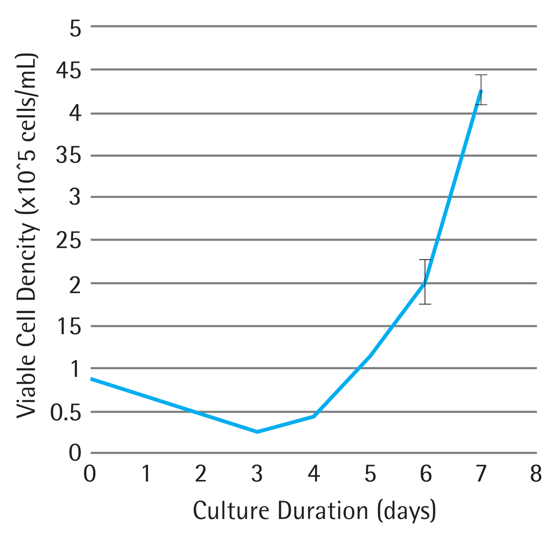
- Elephant ear impeller at 115 rpm (1.5 W m-3)
- Vero cells
- Cytodex 1 at 1 g/L concentration
- ambr 250 high throughput 12 vessel system
- DO control, pH control, 5% CO2 added, no sparging



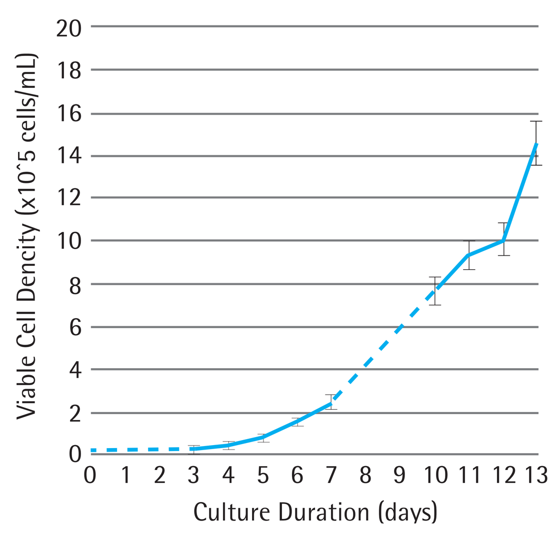
Vero cells on microcarriers, 2h after inoculation



Vero cells on microcarriers, day 7, reaching confluency



Vero cell D0 8.5x10^4 cells/mL, Cytodex 1: D0 1g/L, batch culture, N=12.



Vero cell D0 8.5x10^4 cells/mL, Cytodex 1: D0 1g/L, D7 2g/L, D11 4g/L, fed-batch culture, N=12.

Results

- Physical characterization: NJS was determined for all the impellers, along with the minimum amount of energy for solid suspension. The Elephant ear A impeller (see table) appears to be the most suitable for this (NJS without cells=80 rpm)
- Computational characterization: the elephant ear A impeller in an unbaffled vessel performs best. The area below the impeller is well mixed at low stirring speeds. Interesting results are shown for the axials impellers: at NJS they are not expressing an axial flow, but rather a radial mix of the system.
- Biological characterization: the cells were able to grow, form bridges and cover almost 100% of the available surface. Further experiments were also performed to optimize cell attachment and bed expansion with addition of more microcarriers and media exchange. Very good consistency was shown in the 12 replicates, with a very good overall growth over 13 days.

Conclusions

- A physical, computational fluid dynamics and biological analysis of an ambr® 250 process with Vero cells on microcarriers has been successfully performed.
- The ambr® 250 high throughput platform has been shown to be capable of growing cells on microcarriers.
- The new ambr® 250 vessel can have significant positive impact on process development by reducing timelines and cost for both vaccine and stem cell processes.
- New experiments are ongoing with hMSCs to investigate growth kinetics of adherent and other cell types requiring low mixing speeds in the new vessel, and to enable it for cell therapy research.
- It is also necessary to investigate comparability of new vessel with larger-scale STR systems and demonstrate that cell functionality and other quality aspects remain unaffected in new vessel.

Acknowledgments

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Knowledge Transfer Partnerships

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