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Application Note

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Ambr® 250 High Throughput to Enable Efficient Adherent Cell Culture Process Development on Microcarriers

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Introduction

The Ambr® 250 High Throughput has been proven as a reliable scale down model for suspension CHO cell cultures, producing results which predict outcomes at production scale. Developments within the platform allow for effective adherent cell culture using microcarriers, for applications such as viral vaccine production and advanced therapies.

An alternative new vessel design is combined with a wider speed range motor (100 – 4500 rpm), allowing for suspension of microcarriers with minimal power input. Additionally, an optimized script enables highly consistent media exchange via the liquid handler. Process set-up is simplified and the overall time needed for media exchange is reduced.

Find out more at:
www.sartorius.com/en/products/fermentation-bioreactors/ambr-multi-parallel-bioreactors/ambr-250-high-throughput

Microcarrier Bioreactor Vessel

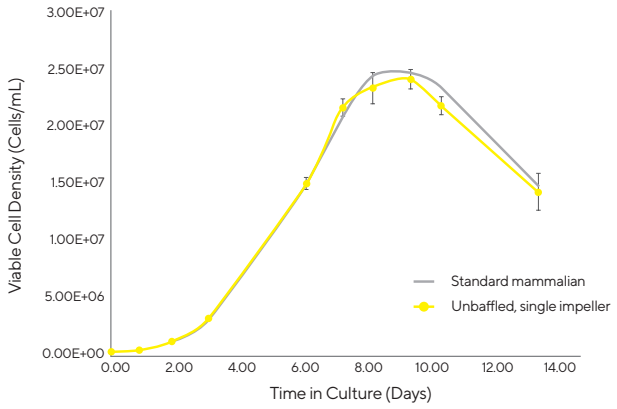
The microcarrier vessel (001-2G37) is unbaffled, with a single impeller. The 'elephant ear' impeller is designed to allow for suspension of particles at a low stirring speed and with minimal power input. The vessel can also be used for suspension cell cultures, giving more options to the Ambr® 250 High Throughput user.

Standard Suspension Cell Process

The unbaffled, single impeller vessel was compared to the standard mammalian vessel for a CHO batch experiment (Figure 1). The experiment showed no significant difference in cell growth profile or maximum cell density achieved by either vessel design. Therefore, the unbaffled, single impeller vessel is suitable not only for adherent cell culture with microcarriers but can also be used successfully with suspension cells.

Figure 1

Standard Mammalian Vessel vs Unbaffled Single Impeller Vessel For a Standard BAtch CHO Experiment



P/V Comparison for Ambr® 250 Vessels

Table 1

Power Input Reference Table for the Standard Mammalian and Unbaffled Single Impeller Vessels

Power per unit volume (W/m3)	Speed in standard mammalian vessel (rpm)	Speed in unbaffled, single impeller vessel (rpm)
1.00	144	100
1.67	175	122
2.50	200	139
19.98	400	278
67.45	600	417
159.9	800	556
312.3	1000	695
2498	2000	1390

Wide Speed Range Motor

To provide more flexibility in the variety of cell types that can be cultivated, the Ambr®250 HIgh Throughput system now offers an expanded range of stirring speeds, from 100 rpm to 4500 rpm. The enhanced lower limit enables sensitive cell line cultures at minimum power input.

Existing Ambr® 250 High Throughput systems with a standard motor (150-4500 RPM) can be upgraded to a 'wide speed range' stirrer motor for a 12 (001-8G19) or 24 (001-8G20) bioreactor system, where the Ambr® 250 High Throughput system is within warranty or a support contract. All Ambr® 250 High Throughput systems with Serial Number 141 or higher include the wide speed range motor as standard.

Vero Cell Attachment and Growth

To evaluate the suitability of the Ambr® 250 High Throughput system using the unbaffled, single impeller vessel for microcarrier appliations, an adherent Vero cell line experiment was performed with Cytodex 1 microcarriers (GE). This experiment was not optimized, but intended as a proof of concept study, to show the vessel capability and consistency of the system when applied to adherent cell culture on microcarriers.

Microcarriers were added to an Ambr® 250 vessel, to provide an initial concentration in media of 1 g/L, and allowed to equilibrate for 2 hours before inoculation with Vero cells. Cells were cultured in T-flasks before transfer to the Ambr® 250 bioreactor.

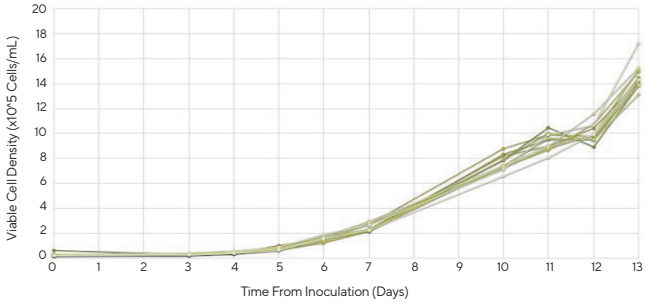
Following addition of cells to a seeding density of 8.5 x 10^4 cells/mL, agitation speed was alternated between 0 and 115 rpm at 15 minute intervals, for 2 hours. As can be seen in Figure 3A, cells are evenly distributed across the microcarriers, and at the end of the experiment microcarrier surfaces are well covered with cells with almost no bare carriers visible (Figure 3B).

Two attachment protocols were investigated using either continuous or intermittent stirring: both approaches were found to give good attachment of Vero cells.

On day 7, the total surface area for cell growth was increased by addition of microcarriers to a final concentration of 4 g/L. A 75% total volume media exchange was performed on days 7 and 11 to provide nutrients and remove waste by-products.

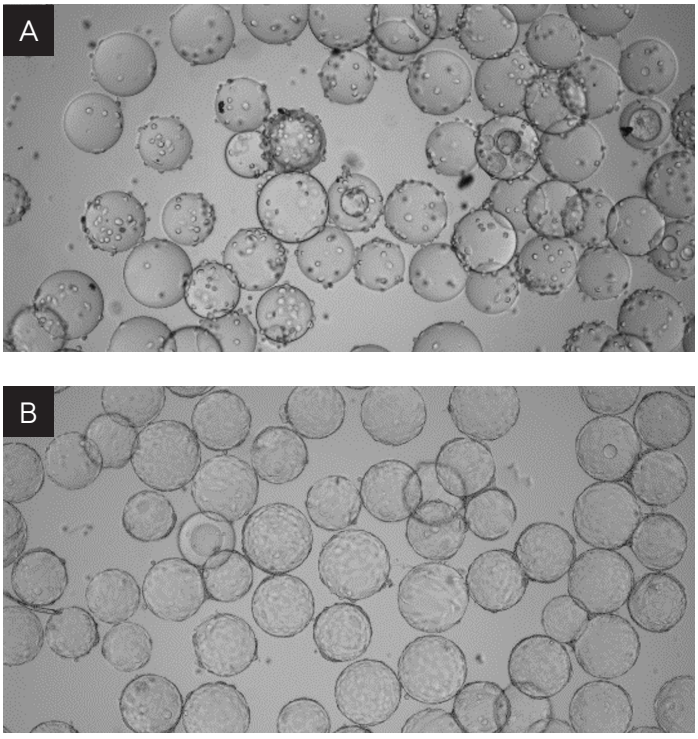
This proof of concept study showed excellent consistency (Figure 2) across 12 bioreactors. The experiment also illustrated successful bed expansion through a simple addition of carriers to the bioreactor, and maintenance of optimum growth parameters through an automated media exchange operation.

Figure 2



Note. Ambr® 250 Vero cell culture shows high consistency, N=12 bioreactors. Inoculated at 8.5 x 10^4 on 1 g/L Cytodex 1. Final cell density 1.45 x 10^6 cells/mL on 4 g/L Cytodex 1.

Figure 3



Note. Ambr® 250 Vero cell culture (a) Inoculated at 8.5 x 10^4, 1 g/L Cytodex 1; (b) Day 13 cell density 1.45 x 10^6 cells/mL, 4 g/L Cytodex 1.

Optimized Media Exchange Step

Media exchange is typically performed by interruption of stirring, which allows the microcarriers to settle, before aspiration of old media and replacement with fresh media. With the optimized Ambr® 250 High Throughput liquid handler script, it is possible to overlap the microcarrier settling and media replacement operations across multiple vessels (Figure 4) thus minimizing processing time.

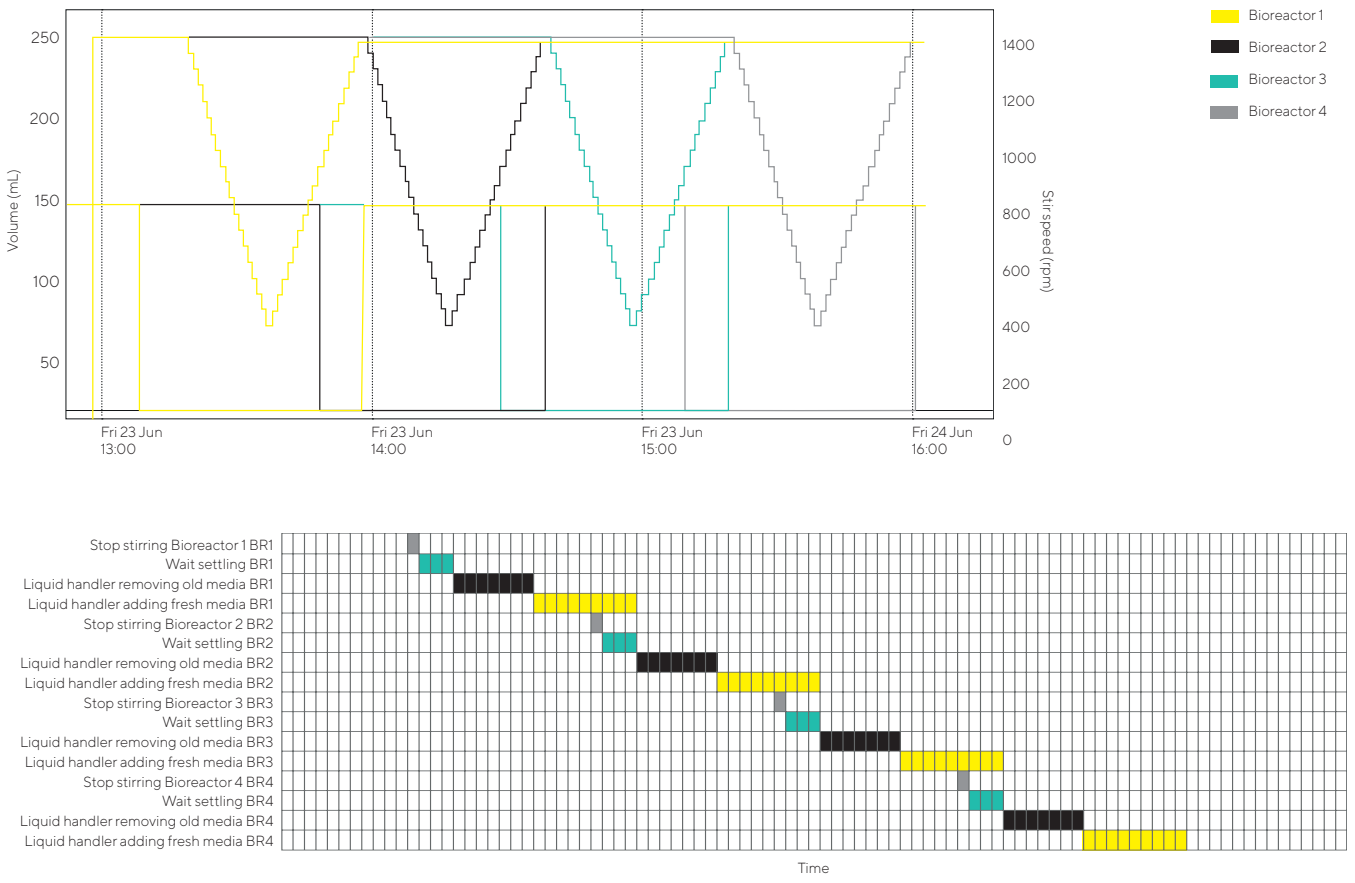
The system automates media exchange, reducing the labor required to perform multiple microcarrier experiments, and improving the consistency across replicates.

Table 2

Time taken without optimized liquid handling protocol	Time taken with optimized liquid handling protocol
4h	2h 40m

Note. Time taken for automated 75% media exchange in 12 Ambr® 250 vessels. Following 10 min settling at 0 RPM, 75% media removal by automated liquid handler, then pumped media refill.

Figure 4



Note. Overlap of the microcarrier settling periods and media replacement operations reduces overall time for media exchange for multiple bioreactors. Note: media can be replaced either by pump (Table 2) or automated liquid handler (Figure 4).

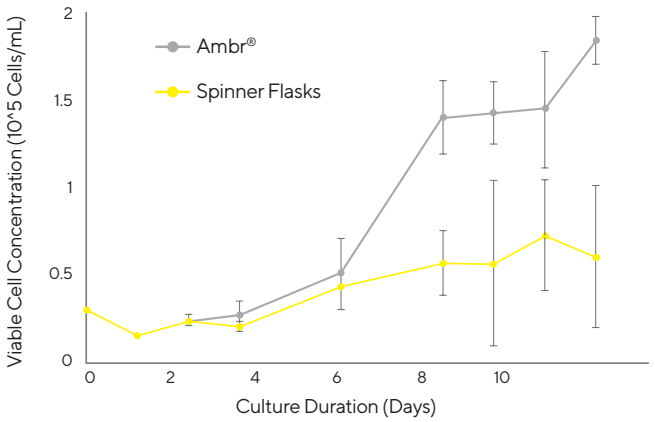
hMSC Culture and Differentiation

Human mesenchymal stem cells (hMSC, RoosterBio) were cultured on plastic microcarriers (SoloHill) to investigate growth in the Ambr® 250 system. 100 mL of hMSC culture media (PRIME-XV®, Irvine Scientific) was added to Ambr® 250 microcarrier vessels (N=2), followed by microcarriers, to provide an initial microcarrier surface area of 10 cm^2/mL. The hMSC were cultured in T-flasks, then inoculated into the Ambr® 250 bioreactors at 3000 cell/mL and attached using the protocol as for Vero cells (Figure 6A). On day 3, 100 mL of hMSC culture media was added to each bioreactor, and a 50% media exchange was performed on days 5, 7, and 9, with an additional 2.5 cm^2/mL of microcarriers added on days 5 and 9.

The Ambr® 250 microcarrier vessel enables good growth throughout the culture, achieving significantly higher cell densities compared with spinner flasks (Figure 5). The hMSC were harvested on day 10, seeded into 6-well plates and successfully differentiated to chondrogenic, adipogenic and osteogenic lineages, confirmed by appropriate staining protocols (Figure 6B-D).

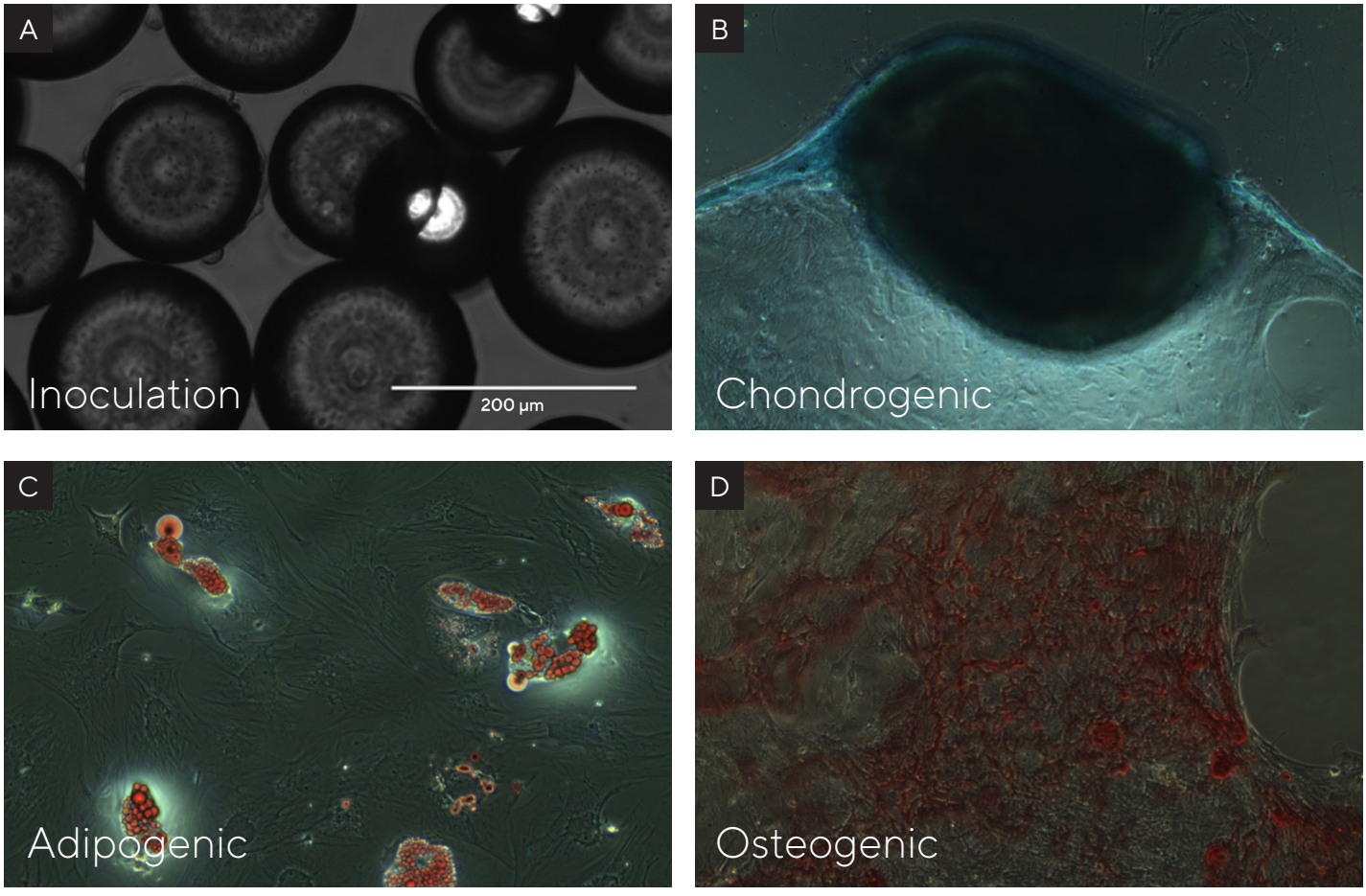
In conclusion, the Ambr® 250 High Throughput microcarrier vessel can also be successfully applied to regenerative medicine applications, with enhanced culture performance compared to spinner flasks.

Figure 5



Note. hMSC Culture on Solohill microcarriers in Ambr® 250 microcarrier vessels (N=2) and Bellco glass Spinner Flasks (N=2)

Figure 6



Note. (A) hMSC on microcarriers after the attachment protocol. (B-D) hMSC in 6 well plates, after differentiation and staining.

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