ambr® 250 high throughput Microcarriers

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. A new development within the platform allows for effective adherent cell culture processing using microcarriers, for applications such as virus vaccine production and for regenerative medicine.

A 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 setup is made easier and the overall time needed for media exchange is reduced.

Microcarrier bioreactor vessel
The microcarrier vessel (001-2G37) is unbaffled, with a single impeller. The ‘elephant ear’ impeller is designed to allow for easy suspension of particles at a low stirring speed and with minimal power input. The vessel can also be used for standard suspension cell cultures, giving more options to the ambr 250 high throughput user.

Standard suspension cells process
The unbaffled, single impeller vessel was compared to the standard mammalian cell vessel design 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.
To evaluate the suitability of the ambr 250 high throughput system using the unbaffled, single impeller vessel for microcarrier applications, 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 in the bioreactor 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 $8.5 \times 10^4$ cells/mL, agitation speed was alternated between 0 to 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 and there are almost no bare carriers visible (Figure 3b).

Different attachment protocols were investigated using either continuous or intermittent stirring, both approaches were found to give a good attachment of Vero cells on Cytodex 1.

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

The results of this proof of concept study showed excellent intra-experiment 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 optimized, automated media exchange operation.

Wide speed range motor

To provide a more flexible system, the ambr 250 high throughput system will expand its stirring range to as low as 100 rpm, whilst maintaining the same maximum speed of 4500 rpm. This 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 new ambr 250 high throughput systems with Serial Number 141 or higher now include the wide speed range motor as standard.

### P/V comparison for ambr 250 vessels

Table 1. Power input reference table for the standard mammalian and unbaffled, single impeller vessels

<table>
<thead>
<tr>
<th>Power per unit volume (W/m³)</th>
<th>Speed in standard mammalian vessel (rpm)</th>
<th>Speed in unbaffled, single impeller vessel (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>144</td>
<td>100</td>
</tr>
<tr>
<td>1.67</td>
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<td>278</td>
</tr>
<tr>
<td>67.45</td>
<td>600</td>
<td>417</td>
</tr>
<tr>
<td>159.9</td>
<td>800</td>
<td>556</td>
</tr>
<tr>
<td>312.3</td>
<td>1000</td>
<td>695</td>
</tr>
<tr>
<td>2498</td>
<td>2000</td>
<td>1390</td>
</tr>
</tbody>
</table>

### Vero cell attachment and growth

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**Figure 2:** ambr 250 Vero cell culture shows very high consistency, N=12 bioreactors. Inoculated at $8.5 \times 10^4$ on 1 g/L Cytodex 1. Final cell density $1.45 \times 10^6$ cells/mL on 4 g/L Cytodex 1.

**Figure 3.** ambr 250 Vero cell culture (a) Inoculated at $8.5 \times 10^4$, 1 g/L Cytodex 1; (b) Day 13 cell density $1.45 \times 10^6$ cells/mL, 4 g/L Cytodex 1.
Schematic representation of the media exchange step

Media exchange is performed typically 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 microcarriers settling and media replacement operations (Figure 4) to provide minimal processing time across the bioreactor platform.

The total time for automated media exchange of 12 ambr 250 high throughput bioreactors is 2 hours 40 minutes. Media exchanges can be performed automatically overnight, reducing the amount of manual effort required to perform multiple microcarrier experiments and improve the consistency across replicates.

Table 2. Time taken for automated 75% media exchange in 12 ambr 250 vessels. Following 10 min settling at 0 RPM, 75% media removal by pipette, then pumped media refill.

<table>
<thead>
<tr>
<th>Time taken prior to this development</th>
<th>Time taken with optimized liquid handling scripts</th>
</tr>
</thead>
<tbody>
<tr>
<td>4h</td>
<td>2h 40m</td>
</tr>
</tbody>
</table>

Figure 4. 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 pipette (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 cm²/mL in the ambr vessels. The hMSC were cultured in T-flasks, 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 cm²/mL of microcarriers added on days 5 and 9.

The ambr 250 microcarrier vessel enables very 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.