ambr® 15 cell culture Microcarriers

Application Note

Experiments using attachment dependent cells on microcarriers can be performed on the ambr 15 cell culture system

Introduction
The ambr 15 microbioreactor can be applied to adherent cell cultures such as in vaccines and regenerative medicine, using microcarrier cultures. Using new ambr 15 protocols for handling and seeding microcarriers, and exchanging media, this article demonstrates culture of Vero cells on Cytodex 1.

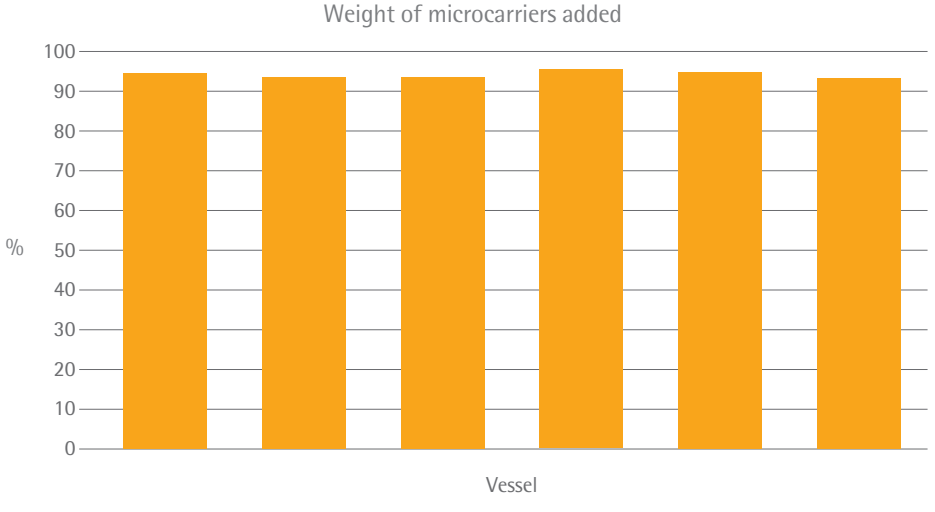
Consistent addition of microcarriers
To provide highly consistent addition of microcarriers to multiple ambr vessels, a new automated approach was devised. One of the ambr 15 vessels in each culture station was filled with a microcarrier stock suspension and stirred at 300 rpm. The desired concentration of microcarriers can then be transferred from this vessel to the other vessels within the culture station (Figure 1) with consistent results (Figure 2).

Automated media exchange Media exchange is often performed by interruption of stirring, carrier settling, media removal and media addition. To allow media exchange to take place without interruption of stirring, a new automated method of 'pipette tip settling' has been developed.

Figure 1.
Example microcarrier seeding experiment design for ambr 15 vessels.

40 g/L microcarrier Stock	2 g/L Vessel 2	3 g/L Vessel 3	4 g/L Vessel 4	5 g/L Vessel 5	6 g/L Vessel 6
2 g/L Vessel 7	2 g/L Vessel 8	3 g/L Vessel 9	4 g/L Vessel 10	5 g/L Vessel 11	6 g/L Vessel 12

Figure 2.
Consistency of microcarrier addition across 6 ambr 15 vessel replicates (CV<1%)



A sample is taken from the vessel within a pipette and the microcarriers allowed to settle for up to 3 minutes. The settled microcarriers are then dispensed to the vessel and the spent media is discarded.

Using this method, a 20% media exchange can be performed on 24 ambr 15 vessels in ~4h. As the method is fully automated, it can be performed outside of normal working hours, e.g. overnight.

Cell attachment and growth
Method: Media, Cytodex 1 microcarriers (GE) and Vero cells (ATCC) were added to ambr 15 vessels, to provide 2 g / L carriers and 1.5 x 10^5 g / L in a 6 mL volume for seeding. After 16 hours the ambr vessels were filled to 15 mL for culture.

Results: Seeding with either continuous or intermittent stirring achieved good attachment of Vero cells on Cytodex 1. Cell growth, observed by microscopy, achieved confluence by day 4 (Figure 4).

Figure 3.
The 'pipette tip settling' process

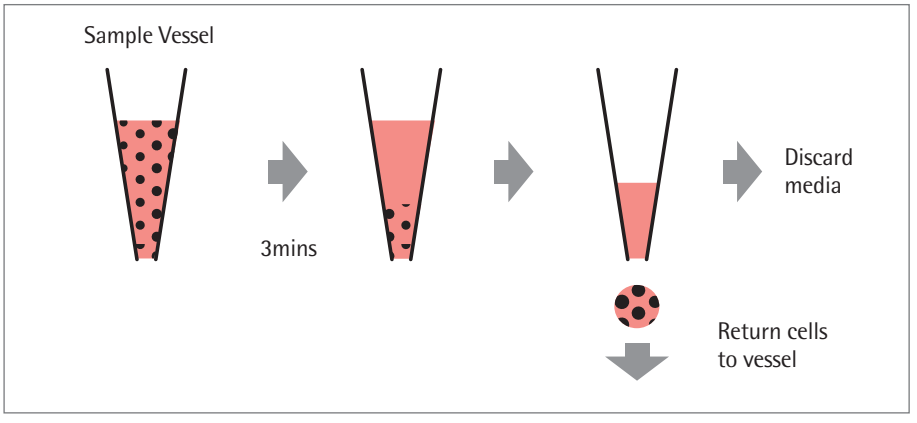
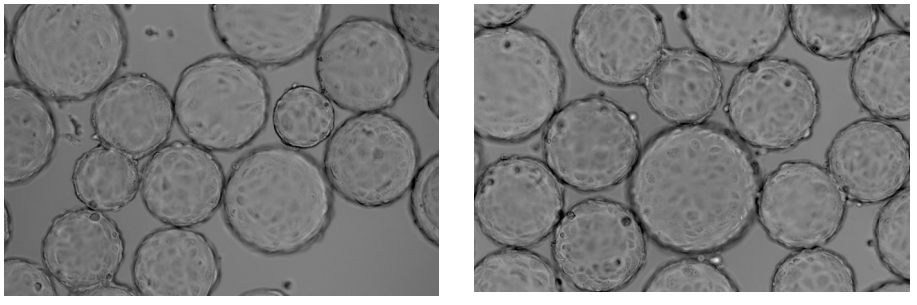


Figure 4.
Vero cells on Cytodex 1 microcarriers, day 4, in different ambr 15 vessels



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