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APPLICATION NOTE

Optimising Vaccine Process Scale-Up of Attachment-Dependent Cells Using Micro Bioreactors and Microcarriers

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Abstract

The trend for increased vaccine production[1] is being driven by the requirement to produce affordable prophylactic vaccines for use in emerging markets, and also for newer types of therapeutic vaccines to treat an ever-increasing array of diseases such as cancer. These drivers, coupled with the current need to produce vaccines rapidly for pandemic threats and seasonal influenza prevention, has lead to the investigation of rapid method development for optimising the scale-up of cGMP-compliant manufacturing processes.

Introduction

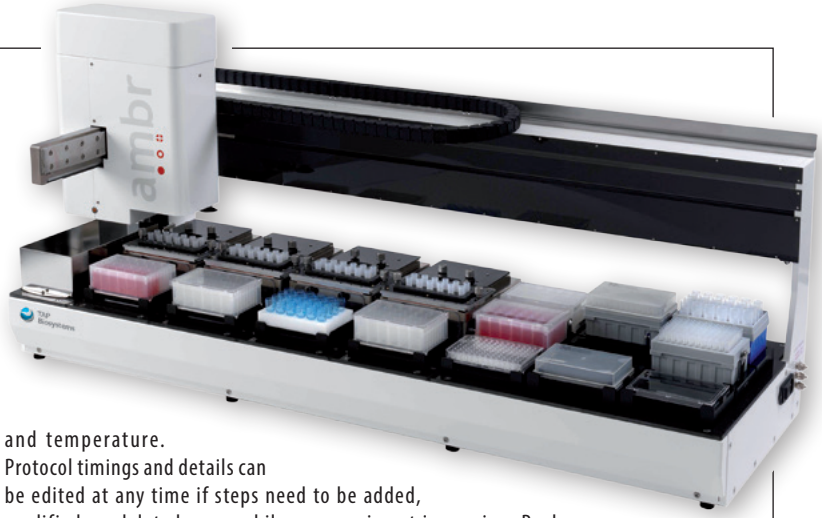
Cell culture is becoming the route of choice for manufacturing many vaccines. It offers distinct advantages over egg-based production including shorter lead times and greater process flexibility.[2]

Some of the most commonly used cell lines for vaccine production include Vero, Madin-Darby canine kidney (MDCK), PER.C6, MRC-5, and insect cells. Several cell lines (*e.g.*, Vero and MDCK) are attachment-dependent and cannot be cultivated well as suspension cultures in bioreactors.[3] Propagation in roller bottle systems is a process often used; however, process controls are limited.

There is a great deal of interest in developing vaccines using attachment-dependent cells with microcarriers in bioreactors. Better bioreactor process controls result in higher-titre vaccine production in shorter timelines.[4-6] Additionally, microcarrier processes are seen as viable options for cost-effective vaccine production at very large scales—to supply the rapidly growing demand for mass vaccination programmes in densely populated countries such as China and India.

The **ambr micro bioreactor system** has three components: (1) the disposable bioreactor (10–15 mL working volume); (2) the workstation; and (3) the software. Each bioreactor is single-use, so unlike conventional glass vessels, they do not require post-run cleaning and sterilisation. To maintain sterility, the ambr workstation is housed in a biological safety cabinet and liquid handling automation facilitates aseptic culture set-up, inoculation, feeding, and pipette tip settling, as well as sampling into vessels such as 24/96-well plates and ViCell® sample cups. These operating protocols can be configured for individual control of up to 24 or 48 reactors operated in parallel. Each bioreactor incorporates individual DO and pH sensors to enable closed-loop control of these parameters. Automated pH regulation is achieved by CO_2 sparging or alkali addition, while O_2 is sparged whenever needed to maintain a DO setpoint. There is also the option of attaching an analysis module to the workstation for at-line pH calibration, thus enabling accurate culture control.

The system is supported by software in which the user sets up a protocol of steps for the ambr to carry out. The protocol defines operating parameters such as DO/pH set points, stirrer speed, and temperature. Protocol timings and details can be edited at any time if steps need to be added, modified, or deleted, even while an experiment is running. Real-time data such as gas flow rates, volumes, and pH/DO values are logged continuously during a run. External values such as cell counts, metabolites, and vaccine titres can also be imported into the software. Data can be graphed within the software or exported for production of graphical or tabular results in spreadsheet software.



One technical problem, which is preventing wider adoption of microcarrier culture in bioreactors, is how to accurately mimic bioreactor conditions to optimise cell growth, adherence, and vaccine production with microcarrier cultures. Spinner flasks and benchtop bioreactors are currently used to define optimal media, feed, and bioprocessing conditions.[7] These types of vessels are both resource- and capital-intensive. Additionally, due to their scale, expense, and limited throughput, vaccine producers are restricted in the number of benchtop bioreactors that can be run in parallel. Typically, only a small number of parameters can be evaluated in small (2–10 L) vessels. Hence, the final process scale-up can be sub-optimal, adversely affecting outcomes such as cell density and vaccine titre. If a larger number of parameters could be studied under conditions representative of the bioreactor environment, then it would be possible to select the optimum manufacturing process quickly and shave months off of production timelines, as well as reduce production costs.

This need to conduct many parameter optimisation experiments under benchtop bioreactor conditions has resulted in the development of miniaturised, high-throughput culture technologies. The drawback with many of these approaches is that they do not mimic the stirring or sparging action of a bioreactor, so maintaining evenly distributed microcarriers (due to their fast sedimentation rate) and accomplishing media exchanges can be problematic.

In this article, we discuss how performing microcarrier culture using an automated micro-scale bioreactor system allows for even microcarrier distribution, and good cell attachment and propagation. Therefore, we will present a method of improved process development for production of a vaccine using attachment-dependent cells.

Stirred Micro Bioreactor Technology

With the need to imitate bioreactor mixing, the advanced micro bioreactor system (ambr™) was introduced by TAP Biosystems (now part of Sartorius). This stirred, sparged micro bioreactor system is now an established technology for improving therapeutic antibody production using Chinese hamster ovary (CHO) cultures.[8] This is because the ambr system mimics benchtop bioreactors, offering better environmental control, and thus, more representative culture performance than shake flasks. The ambr system has been shown to be an excellent tool for high-throughput process development.[8]

Microcarrier Distribution

An element which is key to the ambr system's success as a potential mimic for vaccine production using microcarriers is that each disposable bioreactor's contents are stirred individually by an internal impeller. Scientists can begin their experiments with uniform microcarrier samples automatically distributed from a well-mixed stock solution into the micro bioreactor cell culture vessels. In addition, the ambr's automated pipetting system enables media exchanges without interruption of stirring using an automated pipette tip settling method developed by TAP. This involves taking a microcarrier sample from the ambr bioreactor with a pipette and allowing the microcarriers to settle for up to three minutes. The settled microcarriers are then dispensed back into the bioreactor and the spent media is discarded (Figure 1). Using this method, a 20% media exchange can be performed at any time of the day or night on 24 ambr bioreactors in approximately four hours, without any manual intervention. A conventional approach can also be used where the user stops the stirring, allows the microcarriers to settle out, then removes and replaces the media (using the automated pipetting system).

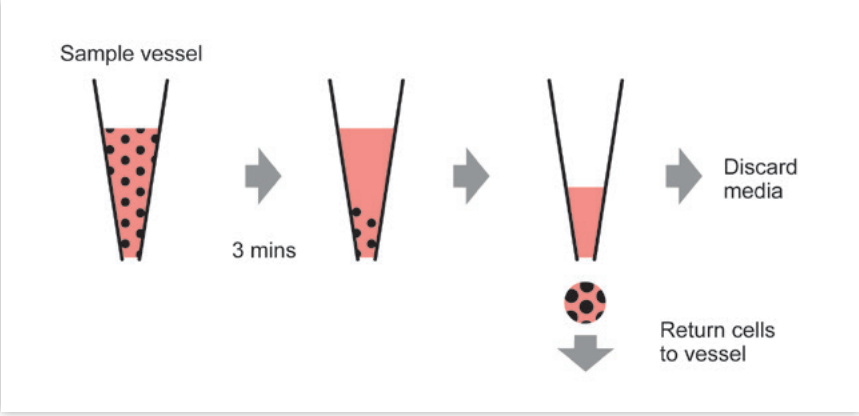


FIGURE 1. The "pipette tip settling" process.

To demonstrate that the ambr system provides uniform addition of microcarriers to multiple ambr vessels, one

of the ambr bioreactors in each workstation station was filled with a Cytodex® 1 microcarrier (GE Healthcare) stock suspension (40 g/L) and stirred at 300 rpm. The workstation was set to dispense microcarrier amounts (2, 3, 4, 5, and 6 g/L) from this microcarrier stock bioreactor to the others within the culture station. The uniformity of dispensing was measured across six ambr bioreactor replicates. The results (Figure 2) showed the weight of microcarriers dispensed is consistent with a coefficient of variation (CV) <1% of the amount programmed to be dispensed. This indicates that the ambr system can reliably and consistently dispense microcarriers from two stock vessels to the other 22 micro bioreactor vessels for the cell culture phase.

To demonstrate that the ambr system can support cell attachment and propagation on microcarriers, Cytodex 1 microcarriers and Vero cells (ATCC® CL-160™) were added to ambr bioreactors to provide 2 g/L microcarriers and 1.5×10^5/mL cells in a 6 mL volume for seeding. Vero cells were chosen, as these cells are commonly used attachment-dependent cells in the production of many prophylactic vaccines. The bioreactors were filled to 15 mL for culture and were then set to stir at low speed continuously or intermittently for 16 hours to allow initial cell attachment and spreading. Microcarriers were sampled every 24 hours and cell growth on the microcarriers was observed by microscopy. The results showed that the Vero cells achieved good attachment using continuous or intermittent stirring, and grew well on the microcarriers, attaining confluence by day four of culture. (Figure 3).

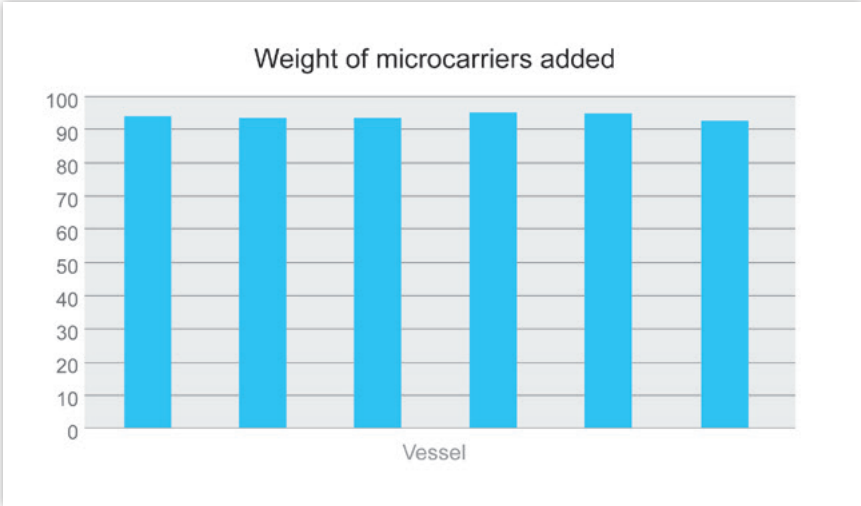


FIGURE 2. Consistency of microcarrier addition across six ambr vessel replicates (CV <1%).

These studies show that by using the ambr system's unique stirring capability and automated pipetting, microcarriers can automatically be consistently distributed across each bioreactor. Additionally, cells can attach and propagate well. This means that scientists can rapidly assess up to 24 cell-specific culture parameters in parallel, simultaneously including stirring speed, media formulation, or feed strategies to determine the optimum conditions for cell attachment, growth rate, and vaccine titre, for example.

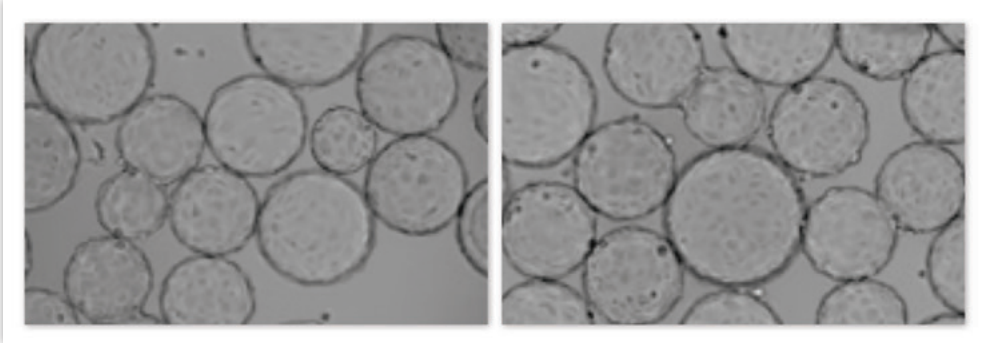


FIGURE 3. Vero cells propagated on microcarriers (day 4) in different ambr bioreactors.

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In summary, stirred, sparged micro bioreactor technology can provide a good method of developing optimal process development for propagating cells on microcarriers in vaccine production. Setting up and running benchtop vessels and spinner flasks is manually intensive, while the ambr micro bioreactor is more convenient and takes far less time to operate. This reduces the reliance on spinner flasks and benchtop bioreactors. Process development for vaccine manufacture can be performed more quickly and efficiently, as well as increasing the number of parameters that can be evaluated. Because of this, process development can often be performed in weeks rather than months, saving valuable time. Thus, utilisation of the ambr system could make a significant contribution where more affordable vaccines or a rapid response to unexpected situations such as a pandemic threat is critical.

References

[1] Kresse H, Shah M. Strategic trends in the vaccine market. *Nat Rev Drug Discov*, 2010; 12: 913-4. http://dx.doi.org/10.1038/nrd3306. PMid:21119727.

[2] Montomoli E, Khadang B, Piccirella S, Trombetta C, Mennitto E, Manini I, Stanzani V, Lapini G. Cell culture-derived influenza vaccines from Vero cells: a new horizon for vaccine production. *Expert Rev Vaccines*, 2012; 11(5): 587-94. http://dx.doi.org/10.1586/erv.12.24. PMid:22827244.

[3] Genzel Y, Dietzsch C, Rapp E, Schwarzer J, Reichl U. MDCK and Vero cells for influenza virus vaccine production: a one-to-one comparison up to lab-scale bioreactor cultivation. *Appl Microbiol Biotechnol*, 2010; 88(2): 461-75. http://dx.doi.org/10.1007/s00253-010-2742-9. PMid:20617311.

[4] Rourou S, Riahi N, Majoul S, Trabelsi K, Kallel H. Development of an *in situ* detachment protocol of Vero cells grown on Cytodex 1 microcarriers under animal component-free conditions in stirred bioreactor. *Appl Biochem Biotechnol*, 2013; 170(7): 1724-37. http://dx.doi.org/10.1007/s12010-013-0307-y. PMid:23737305.

[5] Trabelsi K, Majoul S, Rourou S, Kallel H. Development of a measles vaccine production process in MRC-5 cells grown on Cytodex 1 microcarriers and in a stirred bioreactor. *Appl Microbiol Biotechnol*, 2012; 93(3): 1031-40. http://dx.doi.org/10.1007/s00253-011-3574-y. PMid:21935589.

[6] Lohr V, Genzel Y, Behrendt I, Scharfenberg K, Reichl U. A new MDCK suspension line cultivated in a fully defined medium in stirred-tank and wave bioreactor. *Vaccine*, 2010; 28(38): 6256-64. http://dx.doi.org/10.1016/j.vaccine.2010.07.004. PMid:20638458.

[7] Hundt B, Best C, Schlawin N, Kassner H, Genzel Y, Reichl U. Establishment of a mink enteritis vaccine production process in stirred-tank reactor and Wave Bioreactor microcarrier culture in 1-10 L scale. *Vaccine*, 2007; 25(20): 3987-95. http://dx.doi.org/10.1016/j.vaccine.2007.02.061. PMid:17391818.

[8] Hsu WT, Aulakh RP, Traul DL, Yuk IH. Advanced microscale bioreactor system: a representative scale-down model for bench-top bioreactors. *Cytotechnol*, 2012; 64(6): 667-78. http://dx.doi.org/10.1007/s10616-012-9446-1. PMid:22451076, PMCid:PMC3488368.

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