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Novel Automated Micro-Scale Bioreactor Technology: A Qualitative and Quantitative Mimic for Early Process Development

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With increasing time pressures to move biological therapeutics into the clinic, bioprocessing development studies have to be limited. Currently, core studies typically involve the use of shake flasks and benchtop bioreactors to select the most productive clones, optimum media, and bioprocessing conditions.

The capacity for using benchtop bioreactors is especially limited as it is resource-intensive and has high capital equipment and infrastructure costs. Consequently, scientists frequently cannot perform full design-of-experiments (DOE) and are generally only able to take one or two of their most promising clones forward for partial DOE runs in benchtop bioreactors. These clones often perform as expected in scale-up, but scientists are never certain if they would have identified an even better performing clone if they could have screened more in benchtop bioreactors. This need to conduct large numbers of experiments under bioreactor conditions has resulted in the development and application of miniaturised high-throughput technologies for process development, e.g., monoclonal antibody production^[1] in major pharma companies.^[2]

The drawback with many current miniaturised bioreactor systems is that they do not mimic the sparged, stirring action of a bioreactor. They do not have internal impellers but use instead a gas bubble, shaking, or another method of mixing. A recent publication^[3] which describes the application of a miniaturized stirred, high-throughput bioreactor (35 mL working volume)

suggests that choosing a clone after analysis of performance in a stirred bioreactor environment is an improved method for clone selection. Many miniaturised bioreactors also have a working volume of less than 1 mL. This can make analytical testing of cell cultures problematic because there can be insufficient amounts of material for testing, particularly where multiple samples are required from a single reactor during the run.

Therefore, when designing bioreactor mimics, it is important to be able to sparge and stir the cell culture and provide enough working volume to run a full range of analytical tests on the resulting material. With these parameters in mind, The Automation Partnership (TAP) designed the advanced micro bioreactor system (ambr™) (Figure 1). This system uses 24 disposable bioreactors (Figure 2) controlled by an automated workstation. Each bioreactor has a 10–15 mL working volume and its contents are stirred by an internal impeller, and gases are supplied by sparging.



FIGURE 1. ambr advanced micro bioreactor system.

FIGURE 2. ambr disposable bioreactor.

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To determine if ambr does replicate the characteristics of classical bioreactors at micro-scale, experiments were performed to compare cell viability and antibody titre. CHO clones were then cultured in both, expressing a recombinant antibody cultured both in an ambr system and benchtop bioreactors.

Materials and Methods

Cell Line

The cell lines used were a set of three proprietary MedImmune CHO cell line clones (designated clones 1, 2, and 3) all expressing the same recombinant antibody. The cell lines were derived from a standard CHO host cell line and were screened by culturing in commercially available, chemically defined CHO media supplemented with a proprietary nutrient feed.

Culture Vessels

Bioreactor Mimic

The ambr system used as the bioreactor mimic has two components: the disposable bioreactor and the ambr workstation 24. Within the ambr workstation, there are 24 individually controlled disposable bioreactors (two sets of 12 bioreactors) and liquid handling automation to provide culture setup and inoculation, automated addition of feeds and alkali, and culture sampling. Additionally, the workstation controls the stirring speed, gas supply, and temperature. The disposable bioreactor is supplied sterilized and individually wrapped. Each individual ambr reactor has a working volume of 10–15 mL, incorporates integrated sensors for dissolved oxygen (DO) and pH, and provides individual closed loop control of these parameters. Automated pH regulation is through both control of CO₂ and liquid alkali addition. Automated DO control is through regulation of O₂ and N₂. The system also allows fully automated addition of nutrient feeds, the profiles of which are individually configurable for each vessel. Samples can be delivered into a range of vessels, including 24 or 96 well plates, Vi-CELL® (Beckman Coulter, High Wycombe, Buckinghamshire, UK) sample cups for cell counts and bar-coded cryovials. Systems with increased

numbers of bioreactors are planned and an integrated cell counter will be available later in 2010.

Benchtop Bioreactor

A 7 L benchtop bioreactor (Applikon Biotechnology, Tewkesbury, Gloucestershire, UK) was used for the bioreactor comparisons. Bioreactor data used is historical and not run in parallel with the ambr system.

Culture Media

Filter-sterilized, commercially-available and chemically-defined CHO media were used for both batch and fed batch cultures. For fed batch experiments, the batch media was supplemented with bolus additions of a proprietary nutrient feed. Bolus feed addition in both systems used the same feed-to-media ratio and feed addition timings. Feed addition was achieved via automated liquid dispensing in ambr, and by peristaltic pump in the 7 L bioreactors.

Preparing the ambr System

The pre-sterilized ambr bioreactors in the ambr workstation are divided into the two “culture stations” which each hold 12 bioreactors. The individual vessels were assigned numbers based on their location within the culture station as illustrated in Figure 3.

Inoculum Culture

CHO clones 1, 2, and 3 were expanded from frozen cell stocks using routine cell passage in shake flasks with the same chemically-defined CHO batch media used in ambr and the 7 L bioreactor.

Batch Culture

Batch culture trials were carried out to establish the vessel-to-vessel consistency of ambr. Using the liquid handling system, a single stock of CHO clone 1 cell culture at a proprietary seeding density of $< 10 \times 10^5$ cells/mL was used to inoculate the individual ambr bioreactors. Cell culture parameters for ambr were set as a ‘best estimate’ based on previous conditions in 7L bioreactors (MedImmune proprietary information) without prior optimisation. An impeller speed of 650 rpm was used in the ambr system.

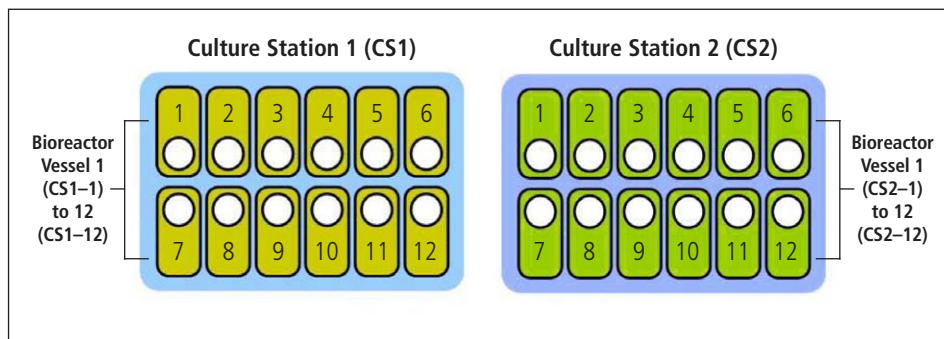


FIGURE 3. Diagrammatic illustration of the individual ambr bioreactor vessels laid out within Culture Station 1 and Culture Station 2 in the ambr workstation.

TABLE 1. Statistical analysis of viable CHO cell count in batch culture in 24 ambr bioreactors. Culture day 0 is the day of inoculation.									
Culture Day	0	1	3	5	6	7	8	9	11
Standard Deviation	0.35	0.37	1.01	2.45	2.86	3.57	3.31	4.79	11.10
% Coefficient of Variance	7.00	5.00	5.00	4.00	3.00	4.00	4.00	6.00	16.00

The cells were incubated for 11 days and automated sampling of <5 mL of culture on days 0, 1, 3, 5, 6, 7, 8, 9, and 11 were used to monitor cell growth and viability using a Vi-Cell and Trypan blue staining.

Fed Batch Culture

Fed batch culture trials were carried out to evaluate the similarity of growth profiles to 7 L bioreactors as well as additional runs to assess the capability of the system to “rank” cell lines. CHO clones 1, 2, and 3 were dispensed in 11 mL culture media at the same proprietary seeding density of 10×10^5 cells/mL and were added to each ambr bioreactor. Proprietary culture parameters for ambr were programmed with an impeller speed of 600 rpm.

The cultures were incubated for 15 days and a bolus nutrient feed was added to both systems using the same media, feed ratio, and addition timings as in the 7 L bioreactor protocol. Feed addition was achieved via automated liquid dispensing in the ambr workstation and by peristaltic pump in the 7 L bioreactors. Automated sampling of culture on days 0, 1, 3, 5, 6, 7, 8, 9, and 11 was used to monitor cell growth and viability using a Vi-Cell and Trypan blue staining. Antibody titre was determined by HPLC using a protein A column (GE Healthcare, Little Chalfont, Buckinghamshire, UK), and glucose levels were measured using an ACCU-CHEK® glucose monitor (Roche Diagnostics, Burgess Hill, Sussex, UK).

Results

Batch Culture Trials

Culturing a single clone distributed to all 24 culture vessels in a single ambr run demonstrated good consistency for viable cell number across all the 24 bioreactors, with a % coefficient of variance (CV) between 3–6% over nine days. An increase in the CV to 16% on day 11 was the result of the expected drop in cell viability to <math><10\%</math> at the end of the batch culture (Figure 4 and Table 1). No account is taken here of the variation due to the cell counter.

Fed Batch Culture Trials

For clone 1, there is good similarity between the viability profiles seen in the separate ambr vessels and in the historic 7 L bioreactor (Figure 5).

In a fed batch trial to compare productivity of different clonal cell lines in ambr and 7 L bioreactors, accumulating antibody titre was measured through the culture period and compared to that obtained in previous 7 L bioreactor runs. Four ambr bioreactors were run for each clone in each of the two culture stations within the ambr system. Data was available from single 7 L bioreactors for clones 1 and 2 and from two bioreactors for CHO clone 3. This comparison showed that clone 1 produced slightly less antibody

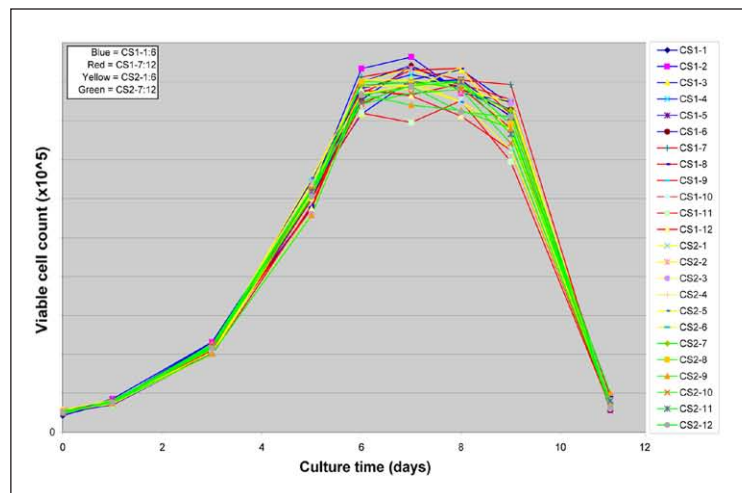


FIGURE 4. Viable cell count of a single CHO cell line cultured in 24 ambr bioreactors under identical batch conditions.

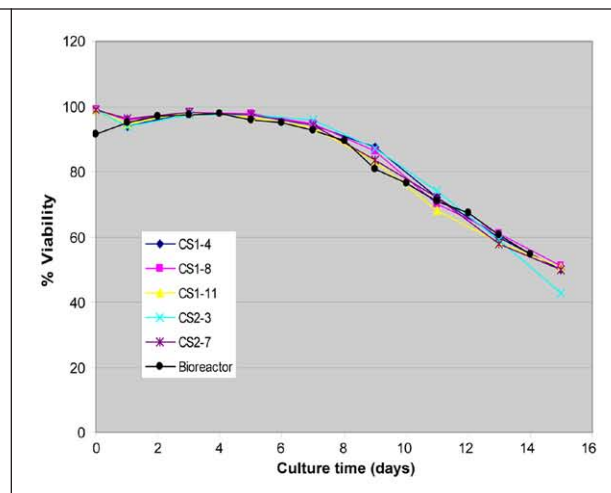


FIGURE 5. Cell viability (%) for fed batch culture of CHO clone 1 in multiple vessels of an ambr system compared to a 7 L bioreactor run in parallel.

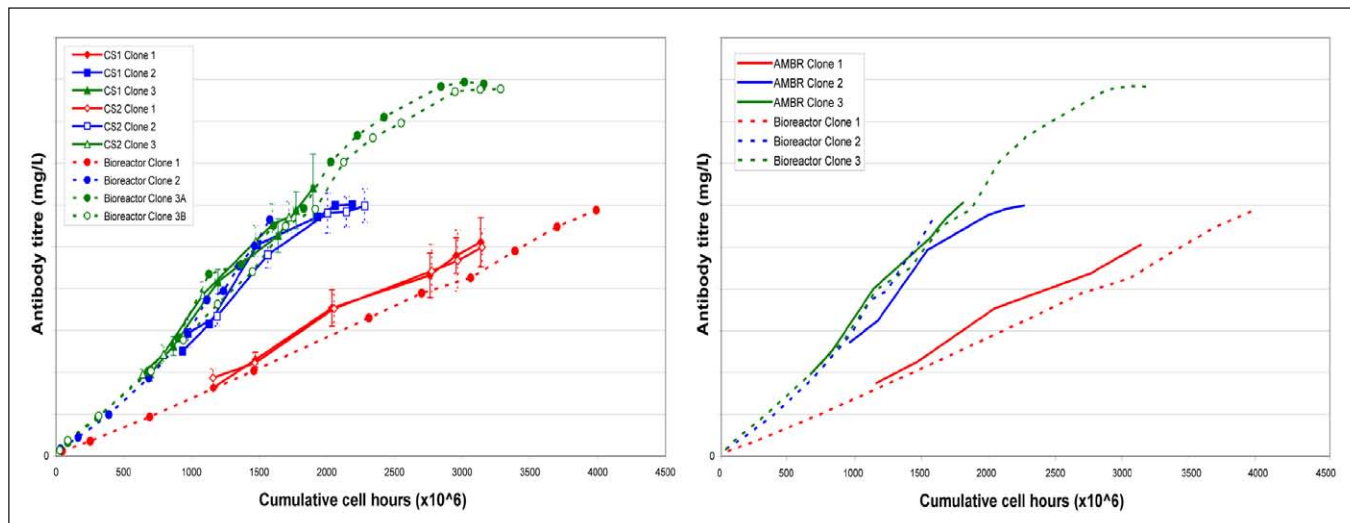


FIGURE 6. A) Antibody titre over time for three CHO cell lines cultured in an ambr system compared to historic 7 L bioreactor data. Results are shown separately for each culture station in ambr. Each culture station contained four vessels for each clone. Error bars show standard deviation. B) Same data as in FIGURE 6A showing a single mean for each clone across all ambr vessels with error bars and markers removed for clarity.

accumulation in the 7 L bioreactor than in ambr (Figure 6A and Figure 6B). Clone 1 also showed consistent antibody expression levels between individual bioreactors and between the two culture stations in the system. Clone 2 produced higher levels of antibody than clone 1 when cultured in a 7 L bioreactor and in ambr vessels. Again, clone 2 produced consistent antibody expression levels in both culture stations and individual vessels. Clone 3 showed both the highest antibody accumulation levels

and again, results in ambr demonstrated close correlation to antibody accumulation in the 7 L bioreactors.

The study indicates that ambr can help predict the clone performance in bench-scale bioreactors and may help in ranking clones and selecting the optimum clone to take forward into further process development work. Absolute antibody concentrations are not shown as these are proprietary to MedImmune.

Conclusion

The ambr system shows good vessel-to-vessel consistency from data in this study. Cell lines perform similarly in ambr and in a bench-scale bioreactors with viability profiles and antibody titres obtained in the ambr system showing good correlation with those seen in the 7 L bioreactor. This data demonstrates the potential of ambr as both a qualitative and quantitative mimic.

In summary, TAP's ambr technology offers the potential to mimic the physical characteristics of a classical bioreactor without strategic changes in the development process. It allows the rapid evaluation of multiple bioreactor cultures (24 in parallel), and is a scalable solution with extra capacity

coming from the addition of more ambr systems as demand increases. The higher capacity of ambr also facilitates the use of multiple replicates, which the classic benchtop bioreactors cannot. This could improve data quality and the implementation of more complex statistically-designed experiments for process development. Using ambr does not require infrastructure changes, major capital investments in equipment or additional staff costs, yet could enable significant savings on materials and labour. This makes ambr highly suited for use as a new, cost-effective route for cell line selection and process development.

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